Editorial
Paediatric Hospital Medicine: The Practice in Evolution
Cheung

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In the eyes of children, a visit to the hospital may already be a scary ordeal, let alone hospitalisation. The sense of vulnerability can never be greater, considering the little ones are now exposed to strangers in uniforms, environments that bear no resemblance to their cozy home, and procedures that are meant to be helpful but yet inconveniently distressing. This is the time when tender loving care from parents and hospital staff would most eagerly be sought. Those looking after the hospitalised babies may recall the elegant words of Rabindranath Tagore, "If baby only wanted to, he could fly up to heaven this moment. It is not for nothing he never wants to speak. The one thing he wants is to learn mother's words from mother's lips...... It is not for nothing he has chosen to shed tears. Though with the smile of his dear face he ever bear to lose sight of her...... It is not for nothing that he never wants to speak."

In this issue of the Journal, two articles are related to the issue of hospitalisation. Kum et al reported on the opportunity to perform developmental surveillance in hospitalised children for neurological and non-neurological problems.1 They found that up to 42.4% of the 113 hospitalised children aged 2 to 42 months had developmental difficulties, in areas of language, motor, social, emotional, play and self-help skills, in a low to moderate income country. Akyildiz and Zararsiz, on the other hand, explored the anxiety levels of parents whose children required intensive care management.2 They found that whereas the anxiety levels were similar between fathers and mothers of children who were hospitalised for acute illnesses, mothers of children with chronic illnesses experienced a higher level of anxiety. These are but only two of the different facets of issues related to the broader topic of paediatric hospital medicine.

The changing landscape of paediatric hospital medicine has become evident, not only by impression of paediatric residents and seasoned paediatricians but also from weekly review of the disease and procedural codes for hospitalised children. For those practising paediatrics in hospital settings, it has become obvious that two distinct populations of hospitalised children have emerged. The first is represented by children, who are otherwise relatively healthy, admitted for acute illnesses involving primarily a single organ system. While the organ-based stratification of childhood illnesses epitomises the practice of a reductionist clinical approach, one cannot argue against the success of the hospital-based approach in managing common respiratory illnesses including bronchiolitis, croup, and pneumonia, gastrointestinal problems including acute gastritis and gastroenteritis, problems of the urinary tract including urinary tract infection and complications of reflux, and so on. Notwithstanding, achievements in preventive
medicine and advances in medical care have shifted the management of some of these acute illnesses from hospital- to community-based care.

On the other hand, the population of hospitalised children with chronic illnesses is growing. These chronic illnesses are characterised by involvement of multiple organ systems, complexity of the underlying illnesses or undiagnosed rare conditions, existence of co-morbidities and complications, and associated mental, behavioural and psychosocial issues. It is in these children with known complex and, at times, undiagnosed rare conditions, which are neither organ- nor disease-specific, that a truly holistic care and concerted efforts of a hospital-based dedicated paediatric team with the appropriate expertise is most required.

In UK, the Great Ormond Street Hospital for Children, London, appointed a Senior Consultant with more than two decades of experience in general paediatrics to support the development of a new team of general paediatricians in 2011.3 It is worth noting the involvements of this team in the 'multi-disciplinary team meetings for children and young adults with complex problems, to contribute general paediatric input and support liaison with local services' and the running of complex care clinics to provide 'a comprehensive family centered overview of the child's or young person's needs, assist with the co-ordination of their care and liaise with all specialists involved in the child's or young person's care to help optimise their care and limit the number of hospital visits required' In US, The American Board of Medical Specialties officially recognised subspecialty certification for Paediatric Hospital Medicine in October 2016. Quoting from its press release,4 "This subspecialty certification will recognise the training that goes into preparing to care for children hospitalised with more complex conditions."

There is no doubt that the practice of paediatric hospital medicine is evolving, and which would continue to evolve given the increasing complexity of patients to be taken care of in the general paediatric ward setting. While one would not cast much doubt on the adequacy of training and the clinical competence of trained residents in the hospital management of acute, uncomplicated commonly encountered paediatric illnesses, one would beg to ask the question of the adequacy of a similar set of skills in providing complex care of children with conditions that span across different subspecialities, require special health care needs, and have multiple comorbidities that require concurrent management.

It is intuitive that an extended depth of knowledge in particular areas of clinical paediatrics and an expanded set of skills are required to cater for this increasingly complex patient population. Barrett et al recently highlighted some of the more in-depth clinical and non-clinical components.5 The clinical ones would include, amongst others, expertise in the management of children requiring special health needs and technology support, palliative care, performance of certain invasive procedures and technical skills such as insertion of central lines, ultrasonography, and chest tube placement; while non-clinical ones would include the contributions to formulating and implementing changes to improve the health care system through training and research in quality improvement, risk management, patient safety, evidence-based practice, and health information systems.

Hospital care presents not only a challenge to children, their parents and families, but also to providers of paediatric health care. As we are cognizant of the evolution and changes of the practice of hospital paediatric medicine, it is timely for us to strategise the grooming of the next generation of paediatricians to cater to the increasingly complex needs.

YF Cheung
Chief Editor

References

**Original Article**

**Hospitalisation: A Good Opportunity to Detect Developmental Difficulty in Children**

YE KUM, DG DOGAN, SK CANALOGLU, M KIVILCIM

**Abstract**

This study aimed to determine children at risk of developmental difficulty by using a developmental monitoring tool during their hospital stay. The development of 113 hospitalised children aged 2-42 months was evaluated by using expressive and receptive language, fine and gross motor, social-emotional and relational functions, play, and self-help skills areas of the Guide for Monitoring Child Development (GMCD). There were 49 (42.4%) children with developmental difficulties. Developmental difficulty was found in 72.9% of the children of mothers who expressed a concern (p<0.001). Developmental difficulties were significantly more common in children of mothers without regular prenatal follow-up (p<0.001), with low educational level (p<0.001), and who had previously suffered stillbirth (p<0.013); and in children with a birth weight below 2500 g (p<0.002), and with consanguineous parents (p<0.007).

The hospitalisation period is a good opportunity to identify children at risk of developmental problems and refer them for further assessment and early intervention.

**Key words**

Child; Development; Hospitalisation

**Introduction**

The developmental disability rate in children is reported to be 10-15% in the United States of America.¹² The number of studies on developmental problems in children aged 3 years and below in the world is inadequate, but it is obvious that these problems are more common in low and moderate income (LAMI) countries¹ with increased risk factors that influence early childhood development such as malnutrition, infectious disease, iron deficiency and low birth weight.⁴ High-income countries have healthcare systems that enable prevention, early diagnosis and management of developmental difficulties in young children² whereas LAMI countries have only recently started to focus on these issues.³ It is reported that children with mild or moderate problems are not diagnosed until they start school in these countries although routine surveillance is required to quickly identify these children and prevent any loss of their potential with early intervention.⁶ The American Academy of Pediatrics has suggested every healthy child to be screened for development with a relevant instrument at 9, 18, 24 or 30 months even if the parents or caregivers have no concerns⁷ but such instruments are not in common use.⁸⁻¹⁰

The health care system does not routinely monitor and prevent the risk factors related to developmental problems in most LAMI countries. The families actually contact the health care system at a young age for vaccination, monitoring of growth and acute or chronic diseases.¹ Hospitalisation
Hospitalisation and Developmental Difficulty

can provide an important opportunity for paediatricians to perform screening for developmental difficulties in countries where vaccination and monitoring for growth are not generally performed by paediatricians.

The fact that children with confirmed developmental problems are more frequently hospitalised indicates that this may also be valid for children with as yet undetected developmental problems. Some studies have found a higher incidence of developmental disabilities in hospitalised children. Such monitoring of hospitalised children for developmental problems may be especially important in developing countries where it may not be possible to monitor all children.

The purpose of this study was to determine the developmental difficulty prevalence in hospitalised children using a developmental monitoring tool, Guide for Monitoring Child Development (GMCD). The term 'developmental difficulty' was used to mean conditions that could put a child at risk for suboptimal development, or result in a developmental deviance, delay, disorder or disability.

Methods

Participants

A total of 113 children aged 2-42 months who were hospitalised in a subspecialty ward (cardiology, gastroenterology, endocrinology, haematology, neurology, nephrology, respiratory disease) of the Turgut Ozal Medical Center in Malatya, Turkey for treatment of an acute disorder for more than 48 hours were included in this cross-sectional observational study. The centre is a tertiary hospital with 150 beds and serves children from Eastern Turkey. We invited 125 mothers accompanying their children to the study and included them after obtaining informed consent if they agreed (12 mothers refused). The study was approved by the Inonu University Ethics Committee.

Procedure

The medical information of the children was obtained from patient charts. A semi-structured questionnaire was used to collect information from the caregivers about sociodemographic variables such as mother's age, educational level, and family structure with a face-to-face interview. The GMCD was then administered with an open-ended, precoded, 10-minute interview with the primary caregiver by an investigator with relevant training. Based on the interview, the children's developmental status was evaluated and recorded. The developmental support and managing parts of the instrument was not included in this study. Patients with suspected developmental difficulty were then referred to the developmental paediatrics department for follow-up and formal evaluation.

Measures

Guide for Monitoring Child Development (GMCD)

The GMCD was developed by Ertem et al in 2008 in Turkey with the aim of monitoring development in children aged under 42 months. It is administered with an open-ended, precoded, 10-minute interview with the primary caregiver. The sensitivity is 88% (CI % 95:0.69-0.96) and the specificity is 93% (CI % 95:0.83-0.97). A large, NIH-supported multinational study on the international standardisation, validation and efficacy of the GMCD is currently being conducted in Argentina, India, South Africa and Turkey.

GMCD has three components as developmental monitoring, developmental support, and management of developmental difficulties. The parents are first asked whether they have any concerns and these are investigated further if the answer is positive. Otherwise the clinician explains why it is important to know how the child functions and asks the open-ended questions on the following developmental domains: expressive language, receptive language, fine and gross motor functions, social-emotional and relational functions, play, and self-help skills (for children older than 12 months). The responses are checked against specific milestones and additional questions are asked as necessary. A child that has reached all milestones at the required age is said to have a GMCD result that is "appropriate for age" whereas otherwise the result is "not appropriate for age" and "need for further evaluation with or without intervention". We defined children with a GMCD result that was not appropriate for age as "has developmental difficulty" in this study.

Data Analyses

The SPSS 17.0 software (SPSS Inc., Chicago) and chi-squared analysis were used for statistical analysis of the data. The chi-squared test was used to evaluate whether there was a significant difference between children with and without developmental difficulty regarding variables such as maternal age, education, and consanguinity.
Results

The age range of the 113 children was 2-42 months with a mean age of 11.4±9.6 months. There were 56 (49.6%) males and 57 (50.4%) females. The reasons for admission were neurological (31.0%), gastrointestinal (15%) or respiratory (17.7%) problems. 91.2% were born at term and 19.5% were born with a birth weight less than 2500 g. Only 30 (26.5%) were the first child of the family. The mean maternal age was 29.0±6.0 years. The educational level was secondary school or below in 77 (68.1%) mothers. Most of the mothers were housewives (90.3%). The socioeconomic characteristics of the study population are shown in Table 1.

GMCD evaluation of the children revealed 44 (39.9%) children with and 69 (61.1%) without a developmental difficulty. The male/female ratio was 0.91. The prevalence of children with a developmental difficulty only in one area was 13.6%. Many children had more than one difficulty. The difficulty was related to gross and fine motor skills in 37 (32.7%), verbal language skills in 33 (29.2%), receptive language in 28 (24.8%) personal/social skills in 31 (27.4 %), and the play area in 39 (34.5%). We evaluated 45 children older than ten months for self-care activity and 17 (37.8%) had self-care problems. The prevalence of developmental difficulties according to developmental milestones is shown in Table 2. Among the 44 children with a developmental difficulty and acute problems, 8 children were already under follow-up (6 for Down syndrome and 2 for dysmorphic syndrome). This decreased the prevalence of children diagnosed with a developmental delay for the first time during hospitalisation to 31.8%.

A developmental concern was mentioned by 32 (28.3%) mothers. Most concerns were regarding gross and fine motor development (59.3%), speech (40%), nutrition and weight gain (15.6%). A developmental difficulty was found in 72.9% of the children of mothers who expressed a concern. Mothers of children with a developmental delay reported a statistically significantly higher number of concerns (p<0.001).

Table 1 Sociodemographic characteristics

<table>
<thead>
<tr>
<th>Gender</th>
<th>n=113 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>56 (49.6)</td>
</tr>
<tr>
<td>Male</td>
<td>57 (50.4)</td>
</tr>
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<table>
<thead>
<tr>
<th>Age (month)</th>
<th>n=113 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-6</td>
<td>43 (38.0)</td>
</tr>
<tr>
<td>7-12</td>
<td>31 (27.4)</td>
</tr>
<tr>
<td>13-18</td>
<td>14 (12.4)</td>
</tr>
<tr>
<td>19-24</td>
<td>12 (10.6)</td>
</tr>
<tr>
<td>25-42</td>
<td>13 (11.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mother age (year)</th>
<th>n=113 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19-25</td>
<td>35 (31.0)</td>
</tr>
<tr>
<td>26-35</td>
<td>61 (54.0)</td>
</tr>
<tr>
<td>36-48</td>
<td>17 (15.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mother education</th>
<th>n=113 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illiterate</td>
<td>16 (14.2)</td>
</tr>
<tr>
<td>Primary school graduates</td>
<td>48 (42.5)</td>
</tr>
<tr>
<td>Secondary school graduates</td>
<td>13 (11.5)</td>
</tr>
<tr>
<td>High school graduate</td>
<td>28 (24.8)</td>
</tr>
<tr>
<td>University</td>
<td>8 (7.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mother working status</th>
<th>n=113 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Housewife</td>
<td>102 (90.7)</td>
</tr>
<tr>
<td>Working</td>
<td>11 (9.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of siblings</th>
<th>n=113 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sibling</td>
<td>30 (26.5)</td>
</tr>
<tr>
<td>One</td>
<td>39 (34.5)</td>
</tr>
<tr>
<td>Two</td>
<td>23 (20.4)</td>
</tr>
<tr>
<td>Three and more</td>
<td>21 (18.6)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Family structure</th>
<th>n=113 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear family</td>
<td>92 (81.4)</td>
</tr>
<tr>
<td>Extended family</td>
<td>21 (18.6)</td>
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</tbody>
</table>

Table 2 Developmental difficulty prevalence

<table>
<thead>
<tr>
<th>Developmental milestones</th>
<th>Expressive language</th>
<th>Receptive language</th>
<th>Gross and fine motor</th>
<th>Relationship</th>
<th>Play</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=113 (%)</td>
<td>n=113 (%)</td>
<td>n=113 (%)</td>
<td>n=113 (%)</td>
<td>n=113 (%)</td>
<td>n=113 (%)</td>
</tr>
<tr>
<td>Detected</td>
<td>33 (29.2)</td>
<td>28 (24.8)</td>
<td>37 (32.7)</td>
<td>31 (27.4)</td>
<td>39 (34.5)</td>
</tr>
<tr>
<td>Difficulty</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detected</td>
<td>80 (70.8)</td>
<td>85 (75.2)</td>
<td>76 (67.3)</td>
<td>82 (72.6)</td>
<td>74</td>
</tr>
<tr>
<td>No Difficulty</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Developmental difficulties were significantly more common in children of mothers without regular prenatal follow-up (p<0.001), with low educational level (p<0.001), and who had previously suffered stillbirth (p<0.013); and in children with a birth weight below 2500 g (p<0.002), and with consanguineous parents (p<0.007).

Factors such as gender, maternal age, family structure, and the child being the result of an unwanted pregnancy did not influence any of the outcomes.

Discussion

This observational study is the first to determine the developmental difficulty prevalence in hospitalised children in a LAMI country. We found that our sample of children aged 2-42 months hospitalised for acute illnesses had a higher incidence of developmental difficulty than the general population. The disability rate in the general population is 12.29% according to the Turkish Statistical Institute. The large number of neurological disorders and genetic disorders such as Down syndrome in this study sample has increased the rate of children with difficulties. However, this is also a solid indicator of children with developmental difficulties being hospitalised more often.

There are a few western studies on the incidence of developmental problems in hospitalised children. Although the sampling and study methods are different than our study, their reported incidences were also high. The first study by Feldman et al in 1993 reported that the development of children aged less than 3 years monitored for more than 1 month in a tertiary hospital could be evaluated in 61% of the patients and a developmental problem was found in 78% of this subgroup. Petersen et al reported a significant prevalence of developmental problems in hospitalised patients in their 2006 and 2009 studies.

Parents' concerns are thought to be effective in directing the primary caregiver to the early detection of behavioural and developmental problems. Glascoe used parental concerns as a screening device named Parents Evaluation of Developmental Status "PEDS" and found that motor, language and global/cognitive area concerns were able to identify 79% of children aged 21-84 months and thus showed high sensitivity. The first question of the GMCD is also about parental concerns and all the concern-related questions within PEDS. The question is: "By development I mean her learning, understanding, communicating, relationships, her behaviour and emotions, how she uses her fingers and hands, legs and body, her hearing and vision. Do you have any concerns about your child's development in any of these areas?" The evaluation of each developmental state together with the query about parental concerns increases the sensitivity of GMCD in determining developmental difficulties.

A child may be confronted by many risk factors within a certain period or during development. This situation, called double jeopardy, is clearly demonstrated in this study. For example, the rates of specific developmental risk factors for children under 3 years of age such as consanguinity, low birth weight, and low maternal educational level were significantly higher in children with developmental difficulty. This shows once again the importance of evaluating biological and psychosocial risk factors as well during interventions.

The hospitalisation period provides an opportunity to determine the presence of developmental problems and appropriate referral, especially in LAMI countries where it is not easy for children to access health care services. However, hospitalisation may be the only time that children are evaluated by a paediatrician and a developmental problem can be recognised early in some countries.

This study had some limitations. First of all, the study was conducted at a single hospital so it is difficult to generalise our findings. The patients were hospitalised in subspecialty wards, with a high number of neurology patients, leading to a higher rate of developmental problems in the sample.

Children from LAMI countries have a higher risk of developmental difficulties and other medical problems. Children presenting with acute problems may not have other surveillance opportunities in the community and it may be possible to detect a subgroup of such children with developmental difficulties at this time. Hospitalisation can be an excellent opportunity to perform structured developmental assessment by interviewing the caretaker. Children presenting to acute settings may not have too many opportunities for surveillance in the community and an additional cohort of children with developmental difficulties could be detected by this method.

Declaration of Interest

There's no declaration of interest.
References

**Parental Anxiety During PICU Admission: A Single Centre Experience from Turkey**

**B Akyildiz, G Zararsiz**

**Abstract**

**Objective:** The aim of this study was to assess anxiety levels in parents of children admitted to a paediatric intensive care unit (PICU) and to identify influencing factors at admission. **Method:** One hundred and seventy critically ill children and their parents were enrolled in this study. The patients' demographic data, Paediatric Risk of Mortality score (PRISM III), Paediatric Logistic Organ Dysfunction score (PELOD), reason for admission, comorbidities, mechanical ventilator procedure, PICU stay, and survival were recorded from the medical chart. The Beck Anxiety Inventory (BAI) was used to evaluate the parents' anxiety. Additionally, the parents' sex, age, time of admission, admission place, education level, and family income were recorded. **Results:** A total of 170 children and their parents were enrolled in this study. Ninety-two patients had a prior chronic illness. The family members of children in a PICU experience moderate anxiety during admission. The BAI was statistically higher in mothers than in fathers (p=0.009). According to the children's medical history, both parents' median BAI were not significantly different in acute illness (p=0.52). On the other hand, the BAI of mothers was higher than that of fathers who had children with chronic illness (p=0.03). We found two variables that significantly contributed to the anxiety of parents associated with the PICU admission. These were the time of admission (OR 1.05, 95% CI 1.00-1.10) and a mechanical ventilation procedure (OR 5.93, 95% CI 2.66-10.53). **Conclusions:** While the anxiety is prominent for both parents of acutely ill children, the mothers of chronically ill children need more emotional support to create a robust patient-physician relationship during PICU admission. During this period, determining parental needs or the sources of stress may contribute to both the short- and long-term improvement of parents' mental health.

**Key words**

Anxiety; Children; Paediatric intensive care unit; Parents

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**Introduction**

Having a child in the paediatric intensive care unit (PICU) has been shown to be a stressful experience for both mothers and fathers. This crisis is amplified by the stress felt by the parents in the PICU environment. The intensive care setting is a busy and frightening place dominated by sick children, medical personnel, advanced medical equipment, bright lights, and shrill monitors. Additionally, the child's uncertain outcome, the disruption of the parents' role and their separation from the child, the
Appearance of the child, and the fear of the child suffering from painful procedures contribute to stress.2-6 During this period, health professionals are traditionally trained to focus on the needs of the patient, and the anxiety of the family may be overlooked.1,2 On the other hand, it is very difficult to evaluate parents' anxiety because of limited communication. The Beck Anxiety Inventory (BAI) is a simple and brief method to assess parents' anxiety in the PICU.7 It has been widely used since 1988 and was adopted by Ulusoy et al in 1998 for Turkey.8,9 The present study aimed to evaluate the anxiety of parents whose children were admitted to the PICU and the factors influencing this situation in Turkey.

**Material and Methods**

This study was conducted in the PICU of the Erciyes University Faculty of Medicine, a 12-bed PICU, and was approved by the local ethics committee of Erciyes University. A total of 170 patients and their parents were enrolled in this study. The patients' demographic data, Paediatric Risk of Mortality score (PRISM III), Paediatric Logistic Organ Dysfunction score (PELOD), reason for admission, comorbidities, time of admission, admission place, mechanical ventilator procedure, PICU stay, and survival were recorded from the medical chart. The BAI was used to evaluate the parents' anxiety. Additionally, the parents' sex, age, education level, family income, and experience of losing a child were recorded. Inclusion criteria were defined as having a child hospitalised in the PICU, willingness to participate, and no previous diagnosis of psychiatric disease. Participants who did not meet these criteria were excluded.

**Beck Anxiety Inventory**

The parents' anxiety levels were evaluated with BAI by questioning either mother or father of the patient. This scale is a self-report measure of anxiety. The total score is calculated by finding the sum of the 21 items. Each item scored between 0 and 3 points.7 A score of 0-21 = low anxiety, a score of 22-35 = moderate anxiety, and a score of 36 and above = potentially concerning levels of severe anxiety.

Patients were classified on the basis of socioeconomic status: monthly income less than 1,500 Turkish Lira (TL) was regarded as poor socioeconomic status, 1,500-3,000 TL was moderate socioeconomic level, and above 3,000 TL was considered good socioeconomic status. The parents' education levels and whether they had previously lost any children were also recorded.

**Statistical analysis** was performed using SPSS version 22.0 (IBM, Armonk, NY, USA). The normality of data was tested by the Shapiro-Wilk test. Numerical variables were expressed as mean ± SD or median (minimum, maximum) where appropriate. The comparison between groups for data with a normal distribution was performed using Student's t-test, and the comparison between groups for data that did not show a normal distribution was performed using the Mann-Whitney U test. The bivariate and partial correlation tests were used to analyse the correlations between parameters. Two-tailed p values of 0.05 were used to indicate statistical significance. Categorical variables were compared by means of a χ² test. Univariate and multiple binary logistic regression analyses were performed to estimate influencing effects of multiple variables on BAI. Significant variables at p<0.25 were included to the multiple model, and forward elimination method was used via likelihood ratio statistic, to identify the independent risk factors.

**Results**

A total of 170 children (79 boys [46.5%] and 91 girls [53.5%]) and their parents (92 mothers [54.1 %] and 78 fathers [45.9%]) were enrolled in this study. The children's median age was 36 months (1-216 months). Admission diagnoses included the following: 72 patients had a respiratory failure, 7 patients had an infectious disease, 23 patients had a neurological disease, 20 patients had a haematology/oncology disease, 9 patients had a cardiac disease, 13 patients had a trauma, and 18 patients had an intoxication. The subsequent mortality rate was 16.5%. Ninety-two patients (54.1%) had chronic illness previously. Baseline characteristics and demographic data are summarised in Table 1. The median age was 31 years (19-51 years) for mothers and 32 years (23-50 years) for fathers (p=0.273). The BAI was statistically higher in mothers than in fathers (median BAI in mothers 26 (min.5-max.45) vs median BAI 22 (min.1-max.43) in fathers, p=0.009). No significant correlation was found among PRISM, PELOD, or BAI (p=0.68, r=0.08; p=0.32, r=0.03). However, there was a low but significant correlation between child age and BAI (p=0.04, r=0.151). The BAI was not statistically significant in terms of the sex of the children (p=0.86).
According to the medical history, both parents' median BAI was not statistically significant (median BAI level for mothers 25 [min.6-max.43] vs. median BAI for fathers 24 [min.7-max.45], p=0.52) in acute illness. On the other hand, the BAI of mothers was higher than the BAI of fathers who had children with chronic illness (median BAI level for mothers 23 [min.1-max.45] vs. median BAI for fathers 18 [min.5-max.42], p=0.03). Based on the medical history, the BAI of both parents is illustrated in Figure 1. On comparison of parents' demographic properties, the educational and socioeconomic statuses of mothers were lower than those of fathers (p=0.01). Eight (3 fathers and 5 mothers) parents had lost a child before. There were no differences in terms of the experience of losing a child between parents (median BAI level for mothers 27 [min. 12-max. 39] and median BAI for fathers 25 [min.10-max. 37], p=0.352). Detailed analysis of the parents is presented in Table 2. We evaluated the influencing factors of BAI. Using univariate logistic regression analysis, parent's sex, (Odds ratio (OR) 1.52, 95 CI 0.83-2.79), child's age (OR 1.04; 95% CI 0.99-1.09), time of admission (OR 1.59, 95% CI 0.86-2.95) and mechanical ventilation practice (OR 4.73, 95% CI 2.45-9.16) were all independently associated with BAI. With multiple logistic regression analysis, only two factors; time to admission (OR 1.05, 95% CI 1.00-1.10) and mechanical ventilation practice (OR 5.93, 95% CI 2.66-10.53) were independently associated with BAI severity (Table 3).

Table 1  Basic characteristic of patients admitted in PICU

<table>
<thead>
<tr>
<th>Patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, months, median</td>
<td>36 (1-216)</td>
</tr>
<tr>
<td>Male</td>
<td>79 (46.5%)</td>
</tr>
<tr>
<td>Length of PICU days</td>
<td>16 (2-124)</td>
</tr>
<tr>
<td>Survival</td>
<td>138 (75.2%)</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>100 (58.8%)</td>
</tr>
<tr>
<td>PRISM III</td>
<td>11 (1-41)</td>
</tr>
<tr>
<td>PELOD</td>
<td>11 (1-41)</td>
</tr>
<tr>
<td>Admission diagnosis</td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>72 (42.4%)</td>
</tr>
<tr>
<td>Infectious</td>
<td>7 (4.1%)</td>
</tr>
<tr>
<td>Neurologic</td>
<td>23 (13.5%)</td>
</tr>
<tr>
<td>Haematology/oncology</td>
<td>20 (11.8%)</td>
</tr>
<tr>
<td>Cardiologic</td>
<td>9 (5.3%)</td>
</tr>
<tr>
<td>Trauma</td>
<td>13 (7.6%)</td>
</tr>
<tr>
<td>Intoxication</td>
<td>18 (10.6%)</td>
</tr>
<tr>
<td>Child with medical history</td>
<td></td>
</tr>
<tr>
<td>Chronic</td>
<td>92 (54.1%)</td>
</tr>
<tr>
<td>Acute</td>
<td>78 (45.9%)</td>
</tr>
<tr>
<td>Previous PICU admission</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>71 (31.1%)</td>
</tr>
<tr>
<td>No</td>
<td>99 (68.9%)</td>
</tr>
</tbody>
</table>

Data are expressed as n (%) or median (minimum-maximum). PRISM III: Paediatric Risk of Mortality; PELOD: Paediatric Logistic Organ Dysfunction; PICU: Paediatric Intensive Care Unit

Table 2  Comparison of parents' properties

<table>
<thead>
<tr>
<th></th>
<th>Mother</th>
<th>Father</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>31 (19-51)</td>
<td>32 (23-50)</td>
<td>0.273</td>
</tr>
<tr>
<td>BAI</td>
<td>26 (5-45)</td>
<td>22 (1-43)</td>
<td>0.009</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;High school</td>
<td>72 (80%)</td>
<td>45 (56.3%)</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>16 (17.8%)</td>
<td>34 (42.5%)</td>
<td>0.01</td>
</tr>
<tr>
<td>University</td>
<td>2 (2.2%)</td>
<td>1 (1.2%)</td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>79 (87.8%)</td>
<td>51 (63.8%)</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>10 (11.1%)</td>
<td>24 (30%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Good</td>
<td>1 (1.1%)</td>
<td>5 (6.2%)</td>
<td></td>
</tr>
<tr>
<td>BAI with experience of child lost</td>
<td>27 (12-39)</td>
<td>25 (10-37)</td>
<td>0.352</td>
</tr>
</tbody>
</table>

BAI: Beck Anxiety Inventory. Data are expressed as n (%) or median (minimum-maximum).
Discussion

It is very important for all staff personnel to know about parental stress and to understand how parents are feeling and how to best to deal with them during admission. This can facilitate and improve communication between parents and medical professionals during this stressful time.1,2

This is the first study to evaluate the anxiety of both parents and contributing factors in a PICU in Turkey. According to our results, it can be said that the mean BAI level of parents is at a moderate level. Additionally, mothers were significantly more stressed than fathers. These results were different from other reports showing equal stress for both mothers and fathers.10,11 The mean BAI level of the parents was also significantly higher among those with children with acute illness than those with children with chronic illness for both mothers and fathers. In acute illness, the parental reactions are associated with many factors, such as the child’s sudden illness, lack of knowledge, and uncertainties regarding with long-term outcomes.12 On the other hand, the mean BAI level was only statistically higher in mothers who had chronically ill children. In Turkey, mothers are more involved in the care of their child, while fathers are mainly responsible for home income and take less part in directly caring for their children. Additionally, the mothers get stressed because of extra energy and time requirement, not being able to deal with the other child. Tensions rise in human relations, particularly in marital and emotional relationships. Inappropriate reactions to this traumatic situation and feelings of guilt in the family may lead to depression in the mother.13,14 Similarly, van Oers et al reported that parents of a chronically ill child, especially mothers, had high levels of anxiety and depression.14 We also noted that all parents of children who were admitted for the first time were more stressed. This period was described by parents as a shock or disbelief at the situation; guilt and blame were avoidance and escape strategies.15 These findings are the same as those of Kumar and Avabratha’s study.16

As we know, the loss of a child is a significant stressor on parents. The risk of a child’s death may yield an undesirable reaction, such as refusing treatment or an initial aggressive attitude. In our study, 8 (3 fathers and 5 mothers) parents had lost a child before. Interestingly, their BAI levels were not statistically different from each other. The reason may be due to the sociocultural and religious differences from other countries. Prayer can often be comforting and helpful to decrease anxiety levels for parents.17

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate logistic regression</th>
<th>Multiple logistic regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95 % CI  p</td>
<td>OR 95 % CI  p</td>
</tr>
<tr>
<td>Parent gender</td>
<td>1.52  0.83-2.79  0.176</td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>1.09  0.51-2.33  0.822</td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>1.94  0.34-10.96  0.403</td>
<td></td>
</tr>
<tr>
<td>Age of patient</td>
<td>1.02  0.75-1.07  0.358</td>
<td></td>
</tr>
<tr>
<td>Gender of child</td>
<td>1.09  0.56-1.99  0.783</td>
<td></td>
</tr>
<tr>
<td>Age of child</td>
<td>1.04  0.99-1.09  0.141</td>
<td></td>
</tr>
<tr>
<td>Admission place</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emergency service</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Inner hospital</td>
<td>1.05  0.532-2.088  0.880</td>
<td></td>
</tr>
<tr>
<td>Outer hospital</td>
<td>1.25  0.542-2.881  0.600</td>
<td></td>
</tr>
<tr>
<td>Time of admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 am to 4 pm</td>
<td>1.59  0.86-2.95  0.138</td>
<td>1.05  1.00-1.10  0.07</td>
</tr>
<tr>
<td>4 pm to 8 am</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previously child lost</td>
<td>1.54  0.37-6.86  0.537</td>
<td></td>
</tr>
<tr>
<td>Mechanical ventilation procedure</td>
<td>4.73  2.45-9.16  0.001</td>
<td>5.93  2.66-10.53  0.001</td>
</tr>
</tbody>
</table>
We also evaluated the influencing factors of BAI. Using univariate logistic regression analysis, parent's sex, child's age, time of admission and mechanical ventilation practice were all independently associated with BAI. With multiple logistic regression analysis, we found two variables that significantly contributed to the anxiety of parents associated with the PICU. These were the time of admission and a mechanical ventilation procedure. Previous studies have revealed that parental stress is affected by children's intubation status. Needle et al also highlighted that the parents interpreted mechanical ventilation as a more invasive procedure, and these procedures cause higher parental anxiety during admission. Mechanical ventilation was found to be a more significant stressor in our study.

Another factor in our study related to the anxiety level was the time of admission. We discovered that there is only one study evaluating the relationship between time of admission and anxiety. Despite the study indicated no statistical difference related to anxiety level; the parents' anxiety was higher at admission between 4 PM and 8 AM in our study. The opinion that there are less medical personnel during this period compared to the period between 8 AM and 4 PM could explain this result.

Consequently, the family members of a child in the PICU experience moderate anxiety during admission. While the anxiety is more prominent for both parents of an acutely ill child, the mothers of chronically ill children may need more emotional support to create a robust patient-physician relationship during PICU admission. During this period, determining parental needs or the sources of stress may contribute to both the short- and long-term improvement of parents' mental health.

**Conflict of Interest**

All authors have no conflict of interest regarding this paper.

**References**

19. Erdem Y. Anxiety levels of mothers whose infants have been cared for in unit level-I of an intensive care unit in Turkey. J Clin Nurs 2010;19:1738-47.
Feeding Practices Among Romanian Children in the First Year of Life

CA Becaeanu, IF Tinca, Re Smădeanu, G Leșanu

Abstract

Background: The impact of early nutrition habits is of extreme importance for future development and nutritional status. Many aspects of infants' nutrition are based upon traditions and popular beliefs of the societies. This study was aimed to assess complementary feeding influencing factors in correlation with the socio-economic circumstances from Romania. Methods: A longitudinal study was carried out in the Ambulatory of "Grigore Alexandrescu" Emergency Children's Hospital from Bucharest, the capital of Romania, evaluating toddlers at their check-up visit at 1 year old. Data collection included interview questionnaires with parents upon multiple outcomes regarding first year of life nutrition practices and socio-demographic aspects. Results: A total of 382 parents completed the questionnaire, with a response rate equivalent to 85.29%. A percentage of 68.1% of infants were breastfed since their first day of life, while only 41.6% were exclusively breastfed for four to six months. Regarding timing, 85.6% initiated complementary feeding between 4 and 6 months, while 8.9% were prematurely weaned, and 5.5% experimented this after 7 months of age. Multivariate analyses showed that rural areas, low income families and low level of educations mothers are among risk factors for inappropriate complementary feeding practices (p<0.05, CI 95%). Conclusion: The underprivileged population which is more frequently exposed to mistakes in complementary feeding should represent the target audience of programs consisting of material support and easily accessible information. An appropriate mix of informational politics for both parents and healthcare providers may improve the rates of breastfeeding and complementary feeding practices in our country.

Key words

Complementary feeding; Breastfeeding; Dietary habits; Nutrition

Introduction

Timing of complementary feeding introduction is a period of critical vulnerability in children's development. This period is not only associated with growth and changes in nutritional needs, but also with the infant's physiological and neurological maturation. During this period of time, the infant's diet undergoes a major transition from liquid diet to solid food, with an increased nutrition diversity being necessary in order to meet the infant's growth requirements. The transition period from exclusive breastfeeding to two years is a critical window for optimal growth and development of the child. Therefore, strong emphasis must be placed on the timely introduction of solid foods that are...
appropriate for the child's dietary changes, so as to promote
good health, adequate nutritional status and balanced
growth for babies and toddlers. Complementary feeding
has been strongly influenced by various factors such as
culture, family and economic conditions. In some countries,
baby feeding practices are based mostly on traditions and
speculations and not on scientific evidence.

Some of the previous studies indicated that early feeding
patterns in terms of timing, amount and content might
influence metabolic changes and imbalances over the
adulthood period. Thus, there is an increasing research
interest upon understanding adequate early feeding patterns
that can prevent obesity.

In order to avoid confusion, the European Society for
Paediatric Gastroenterology, Hepatology and Nutrition
(ESPGHAN) issued new recommendations in 2008 on
complementary feeding, which can be adapted to local
infant feeding conditions and practices. Exclusive or full
breastfeeding for about 6 months is a desirable goal and
complementary feeding should not be introduced before
17 weeks and not much more later than 26 weeks, according
to these recommendations.

Delaying introduction of solid food is also undesirable
because it promotes suboptimal acquisitions of zinc, protein,
iron, and vitamins B and D leading to growth suppression
and feeding disorders.

Given the available evidence, a proper knowledge of
local and national nutritional patterns and their determinants
might promote opportunities to make optimal changes in
order to improve solid food introduction habits. This study
was aimed to assess complementary feeding influencing
factors in correlation with the socio-economic
circumstances from Romania. The goals of this research
were to identify the prevalence of breastfeeding, timing
and patterns of complementary feeding among the paediatric
population aged 11 to 13 months, with different social,
educational and economic background. We hope that our
results might help implementing proper intervention to
sustain exclusive breastfeeding practices all over the country
as well as improving national complementary feeding
guidelines.

Methods

Population Under Study and Data Collection
This pilot study was conducted in the Ambulatory of
"Grigore Alexandrescu" Emergency Hospital for Children
in Bucharest, the capital of Romania. The unit provides
general and specialised paediatric care services for the
population within the metropole and surroundings regions.
The inclusion criteria were parents raising children aged
12±1 month and who sought child health check-up for their
infants in the above mentioned paediatric unit. There was
only one exclusion criteria, represented by parents' refusal
to participate in the survey. The sample size calculation
was performed by the statistician with a 95% confidence
level and a 5% margin of error. Initially, a total of 423
parents agreed to participate, but the responses of 382 of
them were used in the final analyse, as only these subjects
had complete medical records with accurate information.

Social and Nutritional Investigation
The survey instrument, e.g. questionnaire applied to
parents, was designed by specialised paediatricians, based
on validated questions used in previous studies that proofed
to be important in determining early nutritional habits.

The data obtained during the interview regarding
maternal socio-demographic condition included
information of: age, residence (urban vs. rural), level of
education (Higher: higher education; secondary/college:
high-school, 8 classes; None/primary: none or 4 classes),
living standard (the average income per family member)
throughout the previous six months were taken into
consideration: low (<90 euro/family member), medium
(>90 euro and <180 euro/family member), high (>180 euro/
family member), according to the evidence provided by the
National Statistics Institute with respect to the national
average wage, marital status: married/cohabiting or single/
divorced. Obstetrical details were noted in terms of birth
type: vaginal/cesarean, as well as gestational age (GA) at
birth: full term/preterm or post term.

We assessed breastfeeding initiation, duration and the
overall prevalence. Exclusive breastfeeding (EBF) criteria
named breast milk as the only element in infant diet, without
any other liquids or solids, except vitamins, minerals,
medicines and oral rehydration formulas, according to
World Health Organization (WHO) criteria. Mothers were
asked to state weather they nourished their newborn
within the first hour after birth or afterwards.

To evaluate introduction of solid foods, the interviewees
were asked about the age in months at the onset of
complementary feeding and about the type of the first solid
food offered to their infants.

Ethical Considerations
The study was submitted for review and finally approved
by the institution's ethics committee. Parents were presented
the study protocol and had several opportunities to ask questions during the follow up period. After going through the survey protocol, they were asked to sign an informed consent regarding the study.

**Statistical Analyses**

Questionnaire responses were collected and analysed using SPSS (version 13.0). Chi-square tests were used to evaluate relationships between different selected variables (e.g., to find association between maternal education and duration of breastfeeding or early weaning and socio-economic characteristics). The critical value for significance was set at p<0.05 for all analyses. Univariate ordinal logistic regression was used to assess relationships between each family/infant variable and the timing of complementary foods. The odds ratios with 95% confidence intervals were calculated in order to evaluate the risk of independent variables. Cases with missing data on any of the study variables were excluded.

**Results**

**Sample Characteristics**

Initially, a total of 423 legal guardians of children aged 11 to 13 months filled the questionnaire, but only data from 382 of the children were used in the study, as only these subjects had complete medical records with accurate information. The distribution of individual, demographic and parental characteristics of the selected group are given in Table 1. A high proportion of infants belong to families with higher education levels (more than 80%) and living standards (more than 70%). The mean age of mothers was 24.3±1.6 (standard deviation), and they mainly raise their children in families being married or cohabiting in 76.70% of cases.

**Exclusive Breastfeeding Rate**

About 68.1% of mothers interviewed in the study declared that they have initiated breastfeeding within the first days of life. However, no more than 59.7% continued exclusive breastfeeding at the age of 1 month, and the proportion continued to decrease over time, so by the months six after birth, only 29.9% of the sample population were still being breastfed. It is important to note that 1% of the study population was fed with cow milk starting early after discharge until the day of the inclusion in the study; in the meantime, none of them used milk from another woman, instead of formula. Women who delivered vaginally were more likely to continue breastfeeding their infants (67.6%) compared to those who delivered using caesarian section (53.1%, p=0.05), showing that encouraging vaginal delivery increases the likelihood of practicing exclusive breastfeeding 1.64 fold (Table 2).

**Introduction of Solid Foods**

As shown in Table 3, infants were weaned variously concerning the age. The median age of introduction of solid foods was 17.9 weeks (IQR 16.2). Most of the subjects reported introducing solid to their infants in the recommended age window of 4-6 months (85.6%), 8.9% of them were prematurely given solid foods, prior to the age of 4 months. For the remaining 5.5% of the sample, weaning was performed when they were older than 7 months. Living in rural areas, low level of maternal education, decrease family economic level were associated with introducing solids early rather than within the 4-6 month window. There were no important association regarding late introduction of solid food, over 7 months (Table 3).

**Type of Solid Food According to Age**

Overall, the first solid food option offered to the infants enrolled in the study was vegetables (50.7%), a third of them

---

Table 1  Demographic and nutritional characteristics of 1-year-old Romanian infants enrolled during the study period (n=382)

<table>
<thead>
<tr>
<th>Attribute</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median (SD))</td>
<td>11.80±0.3</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(male / female)</td>
<td>237/145</td>
<td>62.04/37.9</td>
</tr>
<tr>
<td>Mother's age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median (SD))</td>
<td>24.3±1.6</td>
<td></td>
</tr>
<tr>
<td>Living environment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(rural / urban)</td>
<td>125/257</td>
<td>31.72/67.27</td>
</tr>
<tr>
<td>Mother's level of education (n (%))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None / primary</td>
<td>63</td>
<td>16.5</td>
</tr>
<tr>
<td>Secondary / college</td>
<td>160</td>
<td>41.9</td>
</tr>
<tr>
<td>Higher</td>
<td>159</td>
<td>41.6</td>
</tr>
<tr>
<td>Living standard (n (%))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>93</td>
<td>24.3</td>
</tr>
<tr>
<td>Medium</td>
<td>203</td>
<td>53.1</td>
</tr>
<tr>
<td>High</td>
<td>86</td>
<td>22.5</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married / cohabiting</td>
<td>293</td>
<td>76.70</td>
</tr>
<tr>
<td>Single / divorced</td>
<td>89</td>
<td>23.29</td>
</tr>
</tbody>
</table>
received fruits (30.1%) and the rest experienced cereals as the first complementary dietary compound (14.3%). Table 4 represents the food groups recommended by WHO and recorded by our survey according to age group.

**Discussion**

This is the first study aimed to assess early nutrition habits within the socio-economic, geographical and ethnic conditions specific to Romania, on a representative population aged 11 to 13 months. The socio-economic transformations that occurred throughout the last 25 years were generated mostly by the integration of the former communist countries into the European Union, focused on improvement in the living standard in our country. Health politics managed to provide financial support for families with many children, including financial subsidies or free formulas, the 2-year maternal leave, as well as the possibility of intra-family decision making with respect to the number of children by legalising abortion. In addition, easy access to free or private healthcare, alongside an efficient communication of information on all media channels, led to the improvement of awareness and specialised supervision of child development during the first years of life. The paucity of such studies in our country make this one even more important considering the

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Factors associated with breastfeeding rates for at least 4 months among the 382 participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exclusive breastfeeding for 4 months</td>
</tr>
<tr>
<td></td>
<td>Total (N)</td>
</tr>
<tr>
<td><strong>Mother</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>18-25</td>
<td>108</td>
</tr>
<tr>
<td>26-32</td>
<td>274</td>
</tr>
<tr>
<td><strong>Living environment</strong></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>125</td>
</tr>
<tr>
<td>Urban</td>
<td>257</td>
</tr>
<tr>
<td><strong>Mother’s education</strong></td>
<td></td>
</tr>
<tr>
<td>None / primary</td>
<td>63</td>
</tr>
<tr>
<td>Secondary / college</td>
<td>160</td>
</tr>
<tr>
<td>Higher</td>
<td>159</td>
</tr>
<tr>
<td><strong>Living standards</strong></td>
<td></td>
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<td>Low</td>
<td>93</td>
</tr>
<tr>
<td>Medium</td>
<td>203</td>
</tr>
<tr>
<td>High</td>
<td>86</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
</tr>
<tr>
<td>Married / cohabiting</td>
<td>263</td>
</tr>
<tr>
<td>Single / divorced</td>
<td>119</td>
</tr>
<tr>
<td><strong>Birth</strong></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>279</td>
</tr>
<tr>
<td>Cesarean</td>
<td>103</td>
</tr>
<tr>
<td><strong>Initiation of breastfeeding</strong></td>
<td></td>
</tr>
<tr>
<td>Within the first hour after birth</td>
<td>63</td>
</tr>
<tr>
<td>After the first hour after birth</td>
<td>319</td>
</tr>
<tr>
<td><strong>Gestational age (GA) at birth</strong></td>
<td></td>
</tr>
<tr>
<td>Full term</td>
<td>273</td>
</tr>
<tr>
<td>Preterm or post term</td>
<td>109</td>
</tr>
</tbody>
</table>

EBF: exclusive breastfeeding
implication for improving optimal early feeding practices by targeted interventions.

Classically, younger mothers are considered less likely to initiate and continue breastfeeding their infants because they are more often single mothers, they want to continue attending the school and they have low educational levels. This study showed no strong influence of those factors on the cessation of EBF before age of four months, although we could speculate that being younger than 18, living in rural environment, having low educational level or low family income together with cesarion section delivery might be a risk for discontinuation of EBF. Our results conclude that exclusive breastfeeding was the initial option for 68.1% of the mothers, but only a percentage of 41.6±8% children have been exclusively breastfed for four to six months. Comparing these results to previous ones from similar studies revealed that the concern for initiating breastfeeding is higher in countries such as Italy, with a recorded percentage of 91.1%, Spain 81.7%, Germany 82%. On this research, the prevalence of EBF for the first 4 months was higher in the full term new-borns and in the vaginal delivery group. Supportive and promotive actions to initiate and continue EBF in various parts of the society might lead to improving EBF rates in our population. It seems necessary to continue the campaign promoting breastfeeding carried out during the last few years in Romania, in order to raise awareness with respect to the benefits of breastfeeding among young mothers with low level of education. This study confirms that education is the only criterion for determining correct breastfeeding, and an educated mother who knows the benefits of maternal milk wishes to breastfeed and make all necessary efforts, so as to maintain breastfeeding for as long as possible (Table 2).

Complementary feeding was correctly introduced in compliance with ESPGHAN's current recommendations between four and six months in 85.6% of the cases. A study published in 2010 assessing complementary feeding in five

Table 3  Reported age of solid food introduction by household variables

<table>
<thead>
<tr>
<th>Age in months at introduction of solid foods (%)</th>
<th>Association of determinant with solid foods introduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before 17 weeks</td>
</tr>
<tr>
<td>Mother's age (years)</td>
<td>NS</td>
</tr>
<tr>
<td>18-25</td>
<td>13.5</td>
</tr>
<tr>
<td>26-32</td>
<td>28.8</td>
</tr>
<tr>
<td>Living environment</td>
<td>*</td>
</tr>
<tr>
<td>Rural</td>
<td>15.2</td>
</tr>
<tr>
<td>Urban</td>
<td>5.8</td>
</tr>
<tr>
<td>Mother's education</td>
<td>*</td>
</tr>
<tr>
<td>None / primary</td>
<td>22.2</td>
</tr>
<tr>
<td>Secondary / college</td>
<td>10.0</td>
</tr>
<tr>
<td>Higher</td>
<td>2.5</td>
</tr>
<tr>
<td>Living standards</td>
<td>*</td>
</tr>
<tr>
<td>Low</td>
<td>19.4</td>
</tr>
<tr>
<td>Medium</td>
<td>7.4</td>
</tr>
<tr>
<td>High</td>
<td>1.2</td>
</tr>
<tr>
<td>Breastfeeding for any period</td>
<td>NS</td>
</tr>
<tr>
<td>Yes</td>
<td>17.8</td>
</tr>
<tr>
<td>No</td>
<td>25.5</td>
</tr>
</tbody>
</table>

NS: Not significant (P≥0.05)
* P<0.05
European countries revealed a tendency towards early complementary feeding in Belgium, Poland, Spain, Italy and Croatia. A percentage of 8.9% of the assessed infants were given early complementary feeding before 4 months, and 9.5% after 9 months. The proper time of solid food introduction has both nutritional and developmental consequences. Therefore, infants will receive less human milk which is required as unique nutrient up to the sixth month of life after birth. Mother's low level of education, low socio-economic status and living standards in rural areas are more likely to early initiate complementary feeding. The same tendency was noticed in other similar studies. Therefore, it would be beneficial to provide training for family physicians and develop educational programmes on the issue of complementary feeding, especially for underprivileged groups. There are differences in the levels of awareness of families with respect to appropriate infant complementary feeding but unfortunately, similar national data in the Romanian infants are not available. As in some previous studies, initiation and the duration of breastfeeding appears to protects against early introduction of complementary feeding in infants. Nevertheless, our research indicate that being breast-fed early couldn't stop early initiation of solid food introduction and breastfeeding for more than 4 months is associated with delay timing of complementary feeding. A key factor for optimal timing of complementary feeding might be represented by initiation and supportive breastfeeding during the first months of life.

On this study, over half of the children were given vegetables as their first solid food and a third of them received fruits and/or cereals to initiate complementary feeding. A very small percentage, 3%, consisted of children that were introduced to adult food as complementary feeding. Studies carried out in other European countries showed that foods most often introduced at the onset of complementary feeding are fruits and vegetables in Italy, potatoes in Sweden and rice in England. ESPGHAN indicates that practices related to solid food introduction are the results of tradition and local customs, as well as cultural and economic factors. Also, there was a tendency towards the late introduction (after 7 months) of allergenic foods, according to older recommendations suggesting that the risk for allergic or autoimmune diseases may increase in cases of early exposure to allergenic foods. Documented studies carried out during the past years conclusively proved the opposite, which led to the issue of new ESPGHAN recommendations. It seems that the fight against this old preconception must be continued, in parallel with a campaign consisting of scientific information for family physicians and discussions supported by statistic evidence from the parents.

Our study has several limitations mainly regarding the fact that participants were the one attending the Ambulatory of our hospital as their 1 year follow-up visit. Although we included subjects coming from all social classes, most of them belong to population living in urban areas, with higher education level and higher incomes. Therefore, EBF prevalence and complementary feeding practices might be different from other samples of families all over the country.

### Conclusions

This research concludes that it is necessary to keep promoting breastfeeding through regular campaigns focusing on the superior benefits of human milk in comparison with formulas. Although the rate of proper solid food introduction was good, some specialised debates and informational meetings organised by qualified professionals should take place in the society.

The underprivileged population which is more frequently exposed to mistakes in complementary feeding should represent the target audience of programs consisting of material support and easily accessible information.

### Table 4  Infant feeding practices among the 382 participants

<table>
<thead>
<tr>
<th>Food groups</th>
<th>Age of child</th>
<th>4-6 Months</th>
<th>7-9 Months</th>
<th>10-12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n [%]</td>
<td>n [%]</td>
<td>n [%]</td>
<td></td>
</tr>
<tr>
<td>Cereals</td>
<td>54 [14.3]</td>
<td>366 [95.9]</td>
<td>374 [98]</td>
<td></td>
</tr>
<tr>
<td>Vegetables</td>
<td>193 [50.7]</td>
<td>338 [88.5]</td>
<td>382 [100]</td>
<td></td>
</tr>
<tr>
<td>Dairy products</td>
<td>0 [0]</td>
<td>121 [31.67]</td>
<td>354 [92.8]</td>
<td></td>
</tr>
<tr>
<td>Flesh food</td>
<td>0 [0]</td>
<td>152 [39.8]</td>
<td>309 [80.9]</td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>0 [0]</td>
<td>304 [79.7]</td>
<td>325 [85.2]</td>
<td></td>
</tr>
</tbody>
</table>
References

Vitamin D Deficiency and Lower Respiratory Tract Infections in Newborn Infants

MM Gharehbaghi, R Ghergherechi, B Karimi

Abstract

Purpose: There are reports that suggest vital and complex role of vitamin D in immune system function and regulation. The consequences of mild forms of vitamin D deficiency are less known. An association of subclinical vitamin D deficiency and acute lower respiratory infection in non-rachitic children has been reported. This study was conducted to determine serum concentrations of 25 hydroxy vitamin D in non-rachitic neonates with lower respiratory infections. Methods: This case control study was conducted in a university referral hospital. Forty admitted neonates with diagnosis of lower respiratory tract infection were enrolled in study as case group. Control group consisted of 40 healthy newborn infants who were seen in outpatient clinic without any respiratory symptoms. The serum 25(OH)D3 was measured using a chemiluminesence immunoassay. Results: The mean concentration of vitamin D in neonates with pneumonia was 9.6±6.8 ng/ml and 14.7±9.3 ng/ml in control group (p=0.02). Vitamin D deficiency was determined in 37 neonates (92.5%) in case group and 26 neonates (65%) in control group (p=0.005). There was vitamin D deficiency in 36 (90%) mothers in case group and 23 (57.5%) mothers in control group (p=0.002). The concentration of vitamin D was below 10 ng/ml in 26 patients (65%), 10-20 ng/ml in 11 neonates (27.5%); and above 20 ng/ml in 3 cases (7.5%) of neonates in case group (p=0.001). Conclusion: This study shows vitamin D deficiency is a common problem in our community and infants with acute respiratory infections had lower vitamin D concentrations. Therefore, future studies needed to determine how to effectively improve vitamin D status and achieve its optimal function.

Key words Lower respiratory tract infections; Neontate; Vitamin D

Introduction

The biochemical structure of vitamin D was discovered in the 1930. Vitamin D is a steroid hormone and plays a major role in regulation of calcium and phosphorus haemostasis, bone metabolism and bone development.1,2 Sunshine exposure is important for endogenous synthesis of vitamin D. Vitamin D is also absorbed from dietary sources in the duodenum and jejunum. Both of them hydrolysed in the liver to form 25 hydroxy vitamin D, which is the most abundant vitamin D metabolite. Circulating 25(OH)D3 concentrations provide a useful index of vitamin
D status that reflects both dietary intake and sunshine exposure. Its serum half-life is long (approximately 3 weeks). The measurement of 1,25 dihydroxy vitamin D provide little additional data about vitamin D status because it is tightly controlled by physiologic secondary hyperparathyroidism in a vitamin D deficient status. There is new information about the vitamin D role on glucose haemostasis, immune system, cardiovascular diseases and cancer. A severe vitamin D deficiency causes impaired mineralisation of bone tissue. Vitamin D deficiency is associated with increased risk of many common cancers, type 1 and 2 diabetes, rheumatoid arthritis and multiple sclerosis in adults.6

There are reports that suggest vital and complex role of vitamin D in immune system function and regulation. The 1,25 dihydroxy vitamin D acts to promote the innate immature response to pathogens.7 Clinical vitamin D deficiency that manifests as rickets was associated with pneumonia in different countries including Ethiopia, Yemen and Kuwait.9 The consequences of mild forms of vitamin D deficiency are less known. Vitamin D insufficiency and deficiency have been associated with type 1 diabetes mellitus, allergies and atopic diseases in infants and children.11 Subclinical vitamin D deficiency as identified by low serum concentration of 25 (OH) D3 has been associated with increased risk of tuberculosis in adults. An association of subclinical vitamin D deficiency and acute lower respiratory infection in non-rachitic children was reported by Wayse in Indian children.13 Epidemiologic studies have identified a link between inadequate vitamin D concentrations and respiratory infectious disease. There is little studies in this field in neonatal period. This study was conducted to determine serum concentrations of 25 hydroxy vitamin D in non-rachitic neonates with lower respiratory infections.

Methods

Study design: This case control study was conducted in a university referral hospital (children hospital, Tabriz, Iran) during March-September 2014.

Setting and sample: Forty admitted neonates of age 7 to 30 days with diagnosis of lower respiratory tract infection were enrolled in study as case group. Control group consisted of normal newborns infants who were seen in outpatient clinic without any respiratory symptoms. Acute lower respiratory tract infection (ALRI) was defined as the combination of clinical symptoms such as lower chest in drawing with respiratory rate more than 60 per minute and radiologic findings. Neonates with respiratory distress who diagnosed as transient tachypnea of newborn, respiratory distress syndrome, congenital pneumonia were excluded from study. Other exclusion criteria were chronic diseases and glucocorticoid therapy. In our country, it is recommended to start multivitamin supplementation for term newborns at age of 15 days that contains 400 IU vitamin D in each ml of droplet.

Ethical consideration: Ethic committee of Tabriz University of medical sciences approved the study. Written informed consent was obtained from parents.

Measurements: Venous blood specimens were collected from all enrolled neonates and their mothers. Collected specimens were stored at -20°C until analysis. The serum 25(OH) D3 was measured using a chemiluminescence immunoassay. Vitamin deficiency is typically defined as circulating 25(OH) D3 concentrations less than 20 ng/ml (50 nM/L).14,15 No consensus on optimal serum 25(OH) D3 exists. We considered both cut off 10 and 20 to define vitamin D deficiency.

Data analysis: Data were analysed using SPSS software version 13.0 for windows and presented as mean ± standard deviation (SD). The difference between mean values of both groups was compared using student’s t test. Quantitative variables were compared by using the chi-square test. A p value of 0.05 or less was considered statistically significant.

Results

The neonates in case and control groups were similar with respect to gestation age and birth weight. Demographic characteristics of patients in both groups are showed in Table 1.

None of the neonates had clinical signs of rickets including craniotabes. The mean concentration of vitamin D was 9.6±6.8 ng/ml in neonates of case group, and 14.7±9.3 ng/ml in control group (p=0.02). There was vitamin D deficiency in 37 (92.5%) neonates in case group and 26 (65%) in control group. The concentration of vitamin D was below 10 ng/ml in 26 patients (65%), 10-20 ng/ml in 11 neonates (27.5%); and above 20 ng/ml in 3 cases (7.5%) of neonates in case group. There was vitamin D deficiency in 26 neonates (65%) in control group. It was less than 10 ng/ml in 11 neonates (27.5%), 10-20 ng/ml in 15 neonates (37.5%); and above 20 ng/ml in 13 (32.5) patients in control group, p=0.001 (Table 2).

The mean serum total calcium (laboratory reference range
Table 1  Demographic characteristics of patients

<table>
<thead>
<tr>
<th></th>
<th>Case group (neonates with ALRI)</th>
<th>Control group (healthy neonates)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=40</td>
<td>N=40</td>
<td></td>
</tr>
<tr>
<td>Gestation age, wk</td>
<td>38.7±1.7</td>
<td>39.3±0.9</td>
<td>0.16</td>
</tr>
<tr>
<td>Birth weight, gr</td>
<td>3168±503</td>
<td>3082±507</td>
<td>0.54</td>
</tr>
<tr>
<td>Current body weight, gr</td>
<td>3437±639</td>
<td>3547±676</td>
<td>0.55</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>23 (57.5)</td>
<td>22 (55)</td>
<td>0.82</td>
</tr>
<tr>
<td>Age, days</td>
<td>16.6±7.3</td>
<td>13.3±7.6</td>
<td>0.11</td>
</tr>
<tr>
<td>Apgar score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 minute</td>
<td>8.85±0.49</td>
<td>8.95±0.22</td>
<td>0.43</td>
</tr>
<tr>
<td>5 minutes</td>
<td>9.94±0.23</td>
<td>10</td>
<td>0.28</td>
</tr>
<tr>
<td>Delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean section, n (%)</td>
<td>21 (52.5)</td>
<td>20 (50)</td>
<td>0.82</td>
</tr>
<tr>
<td>Living location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industrial cities</td>
<td>24 (60)</td>
<td>25 (62.5)</td>
<td>0.81</td>
</tr>
<tr>
<td>Birth order</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First child</td>
<td>27 (67.5)</td>
<td>22 (55)</td>
<td>0.36</td>
</tr>
<tr>
<td>Maternal age, yr.</td>
<td>24.3±5.6</td>
<td>27.0±4.5</td>
<td>0.07</td>
</tr>
<tr>
<td>Maternal weight, kg</td>
<td>66.8±11.8</td>
<td>73.1±15.7</td>
<td>0.09</td>
</tr>
</tbody>
</table>

ALRI=acute lower respiratory tract infection

Table 2  Neonatal biochemical parameters and vitamin D status in mothers and neonates

<table>
<thead>
<tr>
<th>Vitamin D concentration (ng/ml)</th>
<th>Case group (neonates with ALRI)</th>
<th>Control group (healthy neonates)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=40</td>
<td>N=40</td>
<td></td>
</tr>
<tr>
<td>Infants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 10</td>
<td>26 (65%)</td>
<td>11 (27.5%)</td>
<td>0.003</td>
</tr>
<tr>
<td>10-20</td>
<td>11 (27.5%)</td>
<td>15 (37.5%)</td>
<td>0.63</td>
</tr>
<tr>
<td>More than 20</td>
<td>3 (7.5%)</td>
<td>14 (35%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Mothers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 10</td>
<td>25 (62.5%)</td>
<td>8 (20%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10-20</td>
<td>11 (27.5%)</td>
<td>15 (37.5%)</td>
<td>0.47</td>
</tr>
<tr>
<td>More than 20</td>
<td>4 (10%)</td>
<td>17 (42.5%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total calcium, mg/dl</td>
<td>8.98±1.52</td>
<td>9.15±2.14</td>
<td>0.86</td>
</tr>
<tr>
<td>Serum phosphorus, mg/dl</td>
<td>5.87±1.17</td>
<td>5.88±1.13</td>
<td>0.95</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/Lit</td>
<td>585±191</td>
<td>685±374</td>
<td>0.19</td>
</tr>
</tbody>
</table>

ALRI=acute lower respiratory tract infection
8-10.8 mg/dl), phosphorous (5-7.8 mg/dl) and alkaline phosphatase levels (146-600 U/L) in neonates of two groups are shown in Table 2. Exclusice breast feeding was noted in 35 neonates (87.5%) in case group and 30 neonates (75%) in control group. The mean concentrations of vitamin D was 24.58±8.3 ng/ml in mothers of control group and it was 17.23±10.5 in mothers of case group (p=0.69). There was vitamin D deficiency in 36 (90%) mothers in case group and 23 (57.5%) mothers of neonates in control group, p=0.002 (Table 2). Vitamin D supplementation by multivitamin drops was started in 13 neonates (32.5%) in case group and 20 neonates (50%) in control group (p=0.17). White blood cell count was 10632±4256 in case group and 8190±2095 in control group, p=0.02. Platelet count was not significantly different between two groups. C reactive protein was positive in 95% patients with pneumonia. Blood culture was positive in 3 patients that were coagulase negative staphylococcus in 2 cases and staphylococcus aureus in one patient. Neither patient needed respiratory support with ventilator.

Discussion

In our study, vitamin D deficiency was determined in 92.5% in case group and 65% in control group. Few studies have published about the prevalence of vitamin D deficiency in newborn infants. One study in Netherlands, reported a high prevalence of vitamin D deficiency in newborn infants of dark skinned or veiled mothers. Their cut-off value for vitamin D deficiency was 25 nmol/L. It is necessary to multiply 25(OH) D concentrations in nano gram per mili litter by 2.496 to convert its concentrations to nano moles per liter. The reported prevalence of vitamin D deficiency was 63.3% and they had higher alkaline phosphatase concentrations that suggest increased bone turn over.16 There is other report of severe vitamin D deficiency in non-European infants17 without data on their pigmentation. Sachan found similar high prevalence in newborn infants and pregnant mothers in India, a country with abundant sunlight.18

In the study of Merewood and co workers, vitamin D deficiency was present in 58% of the newborns and 35.8% of the mothers at Boston.19 In one study from Boston, vitamin D deficiency (cut off 12 ng/ml) was determined in 65% of neonates.20 In another study from Pennsylvania, vitamin D deficiency was reported in 9.7% and vitamin D insufficiency in 56.4% of white neonates. Neonates were classified as vitamin D deficient (less than 15 ng/ml), vitamin D insufficient (15-32 ng/ml) or vitamin D sufficient (>32 ng/ml) in their study. Deficiency was more common in white neonates born in spring than those born in summer. Among black neonates, vitamin D deficiency and insufficiency were found in 45.6% and 46.8% of patients without seasonal effect.21

Sunlight exposure during spring, summer and fall for 5-15 minutes (from 10 AM to 3PM) provides adequate cutaneous vitamin D synthesis. Black persons and individuals with darker skin require 5-10 times longer sunlight exposure.22,23

In one study in Turkey, the serum 25(OH) D3 levels less than 10 ng/ml was significantly more common in newborns with ALRI.24 Vitamin D concentration was significantly lower in their mothers. In one study in Indian, 150 neonates aged 2-60 months were enrolled that 80 cases had ALRI. Serum 25 (OH) D3 increased significantly with age and levels less than 22.5 nmol/L was significantly more common in children with ALRI. They obtained similar results when a 50 nmol/L cut off was used for normal vitamin D status.13 A study in New Zealand found increased risk of respiratory infection by 3 months of age among infants who had cord blood 25(OH) D3 concentrations less than 25 nmol/L.25

In our study, although serum vitamin D levels were low in most of neonates in both groups, it was significantly lower in patients with ALRI.

The vitamin D concentration in human milk is lower than its levels in regular formulas (20-60 IU/L vs. 400-600 IU/ L). If breast feeding infants don't receive vitamin D supplementation, their vitamin D stores could be depleting within 8 weeks after birth.26,27 Breast milk is ideal for enteral caloric source for infants. Infants born to mothers with vitamin D deficiency are at an increased risk to develop vitamin D deficiency if they have not supplementation with vitamin D. Most of studied neonates were exclusively breast feeding in our study. Low serum vitamin D was common in mothers of studied neonates. Breast milk may not provide enough vitamin D for neonates. The current recommendation for daily vitamin D supplementation may be inadequate, since over one third of studied neonates received vitamin D 400 IU/day from 15 days of birth. Future studies with larger number of patients and longer duration of patient follow up are recommended to determine the optimal daily vitamin D supplementation for prevention of vitamin D deficiency.
Conclusion

Our study results show vitamin D deficiency is a common problem in our community and infants with acute respiratory infections had lower vitamin D concentrations. Therefore, future studies needed to determine how to effectively improve vitamin D status and achieve its optimal function.

Conflict of Interest

None

References

Case Report

Diagnosing Infections from the Peripheral Blood Smear

Abstract

Examination of the peripheral blood smear is probably an under-utilised laboratory investigation in clinical practice. Three illustrative cases are presented in which reading of the blood smear provides important clues that directly lead to the identification of the infective agents. In the first case, a male neonate with thrombocytopenia at birth manifested polymorphic atypical lymphocytosis. Perinatal cytomegalovirus infection was suspected and was confirmed when the viral DNA was detectable in the plasma. In the second case, a 7-month-old girl was admitted with respiratory distress and signs of pneumonitis. Lymphocytosis with atypical cells showing convoluted nucleus strongly indicated pertussis, which was confirmed when Bordetella pertussis DNA was found from the nasopharyngeal swab. In the third case of a 16-year-old boy who presented with a 3-month history of fever and diarrhoea, the finding of atypical lymphocytosis with abundant large granular lymphocytes pointed to the diagnosis of infectious mononucleosis. This was confirmed when Epstein-Barr virus DNA was found in the plasma. With appropriate management, all recovered from their primary symptoms/signs. Examination of the peripheral blood smear can be a powerful adjunct to guide diagnostic tests and to refine the use of antibiotics in the management of childhood infections.

Key words

Complete blood count; Cytomegalovirus; Infectious mononucleosis; Peripheral blood smear; Pertussis

Introduction

Complete blood count (or full blood count) is the most commonly ordered blood test in clinical practice, especially in hospitalised children. Modern automated blood cell counters can accept as little as 0.2 mL of anticoagulated blood and produce a detailed report on red cells, white cells and platelet, with differential white blood cell counts. A drop of the remaining blood is then smeared and stained. Depending on the hospital or laboratory policy, peripheral blood smears are only read if there are qualifying clinical indications, the automated blood cell counts are abnormal, or if there are alarming flags raised by the automated counter.

In the evaluation of a febrile child or when an infection is suspected, the automated complete blood count report provides a total white blood cell (WBC) count and a 5-cell differential. The differential includes neutrophil, lymphocyte, monocyte, eosinophil and basophil. In addition, there is an extra column of large unstained cell (LUC) that contains cells the automated counter fails to differentiate. Based on the proportion and absolute counts of the neutrophil and lymphocyte, the clinician may be able to grasp the likelihood of a bacterial versus a viral illness. The automated report, however, does not provide clues for any specific infection.

The peripheral blood smear can provide further details about the qualitative changes in the circulating blood cells. Some of these changes, such as left shift in the granulocytes and cytoplasmic changes in the neutrophil, may strongly
favour a diagnosis of bacterial sepsis. Other changes in the lymphocytes may provide important hints to more specific bacterial or viral pathogens.

Case Reports

Case 1
A term infant with a birth weight of 2.2 kilogram was born to an Indian couple. He was admitted to the special baby care unit because of transient tachypnoea. Besides the small size, the clinical examination was unremarkable. The complete blood count done shortly after birth showed normal haemoglobin and total WBC but thrombocytopenia (platelet, $44 \times 10^9$/L). Examination of the peripheral blood smear revealed 20% of atypical lymphocytes with various morphology and atypical mononuclear cells with cytoplasmic vacuoles. The haematological findings were most suggestive of perinatal cytomegalovirus (CMV) infection. Quantitative polymerase chain reaction for CMV-DNA confirmed the diagnosis with $1.53 \times 10^5$ copies/mL in the plasma. Chorioretinitis was absent but hearing screen by otoacoustic emissions was not successful. He was discharged on day 10 of life and referred to an otorhinolaryngologist for hearing assessment.

Case 2
A 7-month-old Italian girl residing in Jakarta was medically evacuated to our hospital because of progressive respiratory distress and the finding of marked leukocytosis on complete blood count. The child had not been well for a week. She was admitted to the local hospital because of tachypnoea and blood tests revealed a total WBC of $98 \times 10^9$/L. Blood dyscrasia was suspected and hence the emergency transfer of the child. Intravenous ceftriaxone was started prior to evacuation. On admission, her temperature was $38^\circ$C. Fine crepitations were audible from both sides of the lungs. Lymphadenopathies were not found. The liver and spleen were just palpable below the costal margins, which were probably secondary to hyper-inflation of the lungs. The complete blood count showed Hb 12.4 g/dL, WBC $77.14 \times 10^9$/L, platelet $812 \times 10^9$/L. Under the microscope, 10% of the WBC were atypical lymphocytes. Fifteen percent of the lymphocytes showed abnormal nuclear folding with convolution (Figure 1). A few showed radial segmentation. No malignant cells were seen. These changes, in addition to absolute lymphocytosis ($34.7 \times 10^9$/L), were typically seen in pertussis infection. Oral azithromycin was added. The report of the nasopharyngeal swab came back later positive for Bordetella pertussis DNA. A slow but complete recovery ensued.

Case 3
A 16-year-old American boy was residing in Java with his missionary family. For the past 3 months, he had been noted to be lethargic from time to time with repeated febrile episodes and temperatures as high as $39^\circ$C. Blood tests and chest radiograph were unrevealing. His symptoms persisted despite several courses of oral antibiotics. In the preceding month, he started to have diarrhoeal symptoms and some subjective weight loss. His condition was stable on examination. No significantly enlarged lymph node, liver or spleen could be found. There was no obvious focus of infection either. An inflammatory bowel disease was initially suspected. The stool was watery with some leukocytes, but bacteria, rotavirus, and parasites were not found. Stool calprotectin was not increased. The complete blood count, however, showed, Hb 14.6 g/dL, WBC $12.7 \times 10^9$/L and platelet $178 \times 10^9$/L. The peripheral blood film revealed 25% of atypical lymphocytes. Half of these were large granular lymphocytes (Figure 2). The haematological findings were consistent with infectious mononucleosis. Epstein-Barr virus DNA at a concentration of 21,000 copies/mL plasma was found. Antibiotic treatment was stopped and attention was directed to fluid and nutritional support. His symptoms completely resolved a month later.

Figure 1  Atypical lymphocytes in the 7-month-old girl (Case 2) showing nuclear clefts and convolutions (marked by arrow).
Discussion

With manual spread of a drop of blood on a glass slide and automated staining system, the preparation of a peripheral blood smear is a simple and fast procedure in the laboratory. Yet the morphological information it provides is rich. For certain haematological disorders, for instance leukaemia, examination of the peripheral blood smear can be diagnostic. For others, the peripheral blood smear provides important clues in the clinical management and determines which diagnostic tests are indicated.\(^2\)

Clinicians often refer to the complete blood count with differentials when evaluating patients with fever and infectious diseases. The blood smear provides qualitative, morphological changes that are not obvious on the automated report from the analyser. Similar to haematological conditions, the peripheral blood smear can be diagnostic of parasitic infections.\(^3\) Morphological changes in the granulocytes are often regarded as important, though nonspecific, clues for an occult bacterial infection. The appearance of immature precursors, often referred to as "left shift", or cytoplasmic changes in the neutrophils such as toxic granules, vacuoles, and Dohlé bodies are often regarded as signs of bacterial sepsis. Fragmented red cells or schizocytes are the hallmark of microangiopathy, and may be a sign of disseminated intravascular coagulation in severe sepsis.

In contrast to the granulocytes, morphological changes in lymphocytes may give more specific clues as to the infecting agents. Atypical lymphocytes are reactive changes often found in viral infections and immune disorders.\(^4\) When present in significant proportion (more than 10%), it is coined infectious mononucleosis and is characteristically seen after infection with the herpesviruses. Epstein-Barr virus infection is the classical example and CMV infection is another, as illustrated in Cases 1 and 3 in this report. In immunocompromised patients, large granular cells spread at the feathered end of the blood smear have been shown to be CMV-infected cells.\(^5\) In contrast to other bacterial infections, Bordetella pertussis induces both neutrophilia and lymphocytosis. The quantitative changes in lymphocyte count are secondary to lymphocytosis-promoting factor, one of the several toxins produced by the bacteria. The morphological changes of the reactive lymphocytes that include nuclear clefts and convolutions, however, are poorly understood.\(^6,7\)

In most laboratories, the reading of the peripheral blood smear is no longer routine. Criteria that incorporate clinical information, numerical deviations in the automated counts, and flags raised by the analyser are often employed to determine which smears are to be read manually. Guidelines from the International Society for Laboratory Hematology are a useful reference.\(^8\) Hence, clinicians looking after children with infectious diseases have to inform the laboratory for the manual examination of the peripheral blood smear if they want to look for morphological clues.

In summary, examination of the peripheral blood smear or film is a useful adjunct in the laboratory evaluation of infectious diseases, but administrative and technical constraints may limit its application.

Declaration of Conflicts of Interest

None

References

Case Report

Hypertension as the Presentation of Disseminated Rhizopus in an Infant with Acute Myeloid Leukaemia

FL Huang, CF Lin, PY Chen, TK Chang

Abstract

Rhizomycosis is an angiotropic and life-threatening infection in immunocompromised patients. We report an infant with acute myeloid leukaemia who suffered from an intra-abdominal mass with progressive hypertension due to left renal, superior mesenteric and splenic arterial occlusions associated with Rhizopus infection, which was diagnosed by multidetector-row computed tomography and tissue biopsy. She underwent intensive management including surgery and liposomal amphotericin-B administration, but she died of progressive leukaemia. To the best of our knowledge, simultaneous infarction of three obstructed arteries in an infant with Rhizopus spp. infection has not been reported in the literature. We conclude rhizomycosis is a life-threatening infection in a child with leukaemia who has neutropenic status, and Rhizopus spp. infection may be associated with multiple arterial infarctions. Hence, early diagnosis, aggressive surgery, and adequate treatment are suggested.

Key words

Hypertension; Infarction; Leukaemia; Rhizopus

Introduction

Mucormycosis is a rare opportunistic infection and an angio-invasive mycosis with high morbidity and mortality rates caused by Mucorales, an order of the Zygomycetes class of fungi. The most common mucormycosis genera are Rhizopus and Mucor. Mucormycosis has emerged as an increasingly important fungal infection during the past decade in haematopoietic stem cell transplant recipients and patients with haematologic malignancies. After primary infection in the respiratory tract, gastrointestinal tract or mucocutaneous infection, mucormycetes may cause disseminated, metastatic disease through mycotic emboli with invasion of blood vessel walls by the hyphae. The angio-invasive disease causes vascular thrombosis, tissue infarction, and destruction with subsequent ischaemia, necrosis, and suppurative pyogenic reactions. Herein, we report an infant with acute myeloid leukaemia who suffered from intestinal fungal infection and progressive hypertension due to multiple arterial occlusions in the abdomen with Rhizopus infection, which was diagnosed via tissue pathology, fungal culture and multidetector-row computed tomography.

Case Report

A 7-month-old girl had a history of full-term birth, with birth weight of 3880 gm, was in good health, and had met
Multiple Arterial Occlusions with Rhizopus

fair development milestones. She became febrile (38.5°C) and some blood-tinged material in her diaper and gross haematuria were noted. Multiple petechiae over her trunk and face were also found. She was brought to Taichung Veterans General Hospital for examination. A complete blood count showed a haemoglobin level of 6.9 g/dL, a white blood cell count of 197.7x10^9 cells/L, and a platelet count of 10x10^9 cells/L. The peripheral blood smear revealed 49% of white blood cells were blasts. The surface markers study of bone marrow blasts showed CD33 was 94%, CD15 was 93% by flow cytometry, and acute myeloid leukaemia was diagnosed. Idarubicin (9 mg/m²/day for 3 days) and cytosar (100 mg/m²/day continuous intravenous drip for 7 days) under the Taiwan paediatric oncologic group acute myeloid leukaemia protocol were administrated on 8 November 2012.

Her body temperature elevated to 38.7°C and her absolute neutrophil count was 0.3x10^9 cells/L on 16 November 2012. Broad spectrum antibiotics including meropenem and vancomycin were given due to febrile neutropenia. She also had received granulocyte colony-stimulating factor (5 mcg/kg/day) since the first day of febrile neutropenia as well as empiric antifungal agent oral fluconazole (5 mg/kg/day) and nystatin 3 days later. However, she suffered from progressive abdomen distension and vomiting during the following week. The abdominal X-ray revealed a distended abdominal wall and subcutaneous emphysema over the left lower quadrant area. Abdominal computed tomography showed massive ascites, crowding of the bowel loop over the central portion of the abdomen and dilatation of the bowel loop over the left middle abdomen. The liver, spleen, and kidneys revealed no significant abnormal finding. Intestinal obstruction with ascites was diagnosed and the girl underwent abdominal surgery on 26 November 2012. Gangrenous change of the proximal jejunum and ischaemia of the splenic flexure of the colon (Figure 1) were found during surgery. Thus, 20 cm of the jejunum and 5 cm of the colon were excised. The pathologic report of intestinal specimens showed extensive fungal infection and marked

Figure 1  (a): Photograph showing the gangrene change of the proximal jejunum. (b, c): Histological tissue sections of the jejunum illustrating irregular-sized hyphae, where (b) (eosin/H&E stain) shows invasion of the blood vessel and haemorrhagic infarct. (c) (eosin/H&E stain) showing the blood vessels of resection margins invaded by fungal hyphae. (d): Lactophenol blue wet-mount microscopy illustrating the broad, ribbon-like hyphae with absence of transverse septa and root-like rhizoids typical of Rhizopus species.
haemorrhagic infarctions were noted in the jejunum and colon (Figure 1). Liposomal amphotericin-B (5 mg/kg/day) was administered. Four sets of bacterial and fungal cultures from blood samples were sterile. Her serum also tested negative for Aspergillus galactomannan. Two sets of fungal cultures from the intestinal tissue specimens were positive within 24 hours for a cotton-like, white-gray fungus without reverse pigment growing at 28°C and 37°C. Growth was inhibited by cycloheximide. Microscopically, the isolate had broad aseptate hyphae, a white to gray-brown thallus formed from stolons with long unbranched sporangiophores, which were produced by root-like hyphae (rhizoids) (Figure 1). These characteristics were used to identify the fungus as Rhizopus spp. and thus the patient was diagnosed with intestinal rhizomycosis infection.

The girl's general condition was stabilised after the operation but progressive and persistent tachycardia (average heart rate of more than 180 beats/minute) and hypertension (average blood pressure above 187/110 mmHg) were found 8 days after surgery. Echocardiography and electrocardiogram were done and normal heart function was noted, except for sinus tachycardia. Renal artery obstruction was suspected, so abdominal multidetector-row computed tomography was performed to assess the abdominal vascular anatomy. The images showed total occlusion of the left renal artery, superior mesenteric artery, and splenic artery with infarctions of the left kidney and spleen (Figure 2). The renal functions were checked and showed normal results. The girl received antihypertension agent (intravenous Labetalol) treatment, but catheterisation and thrombolytic agents were withheld due to refractory thrombocytopenia. Chemotherapy was also withheld owing to the invasive fungal infection and unstable vital signs. Her hypertension and tachycardia were brought under control after one week. Unfortunately, progressive hyperleukocytosis with blasts was found by peripheral blood smear, and multiple progressive small nodules were noted over the girl's scalp, trunk, and limbs, which were diagnosed as leukaemia infiltration by mass biopsies. The girl died of refractory leukaemia and invasive fungal infection on 20 December 2012, despite liposomal amphotericin-B treatment for three weeks.

**Discussion**

According to a systematic review and analysis of reported cases conducted by Zaoutis et al., high-risk factors for developing mucormycosis include young age, infantile acute myeloid leukaemia under chemotherapy, neutropenic status, and usage of broad spectrum antibiotics. All of these factors were found in the present case. Zygomyces infection is characterised by tissue infarction and necrosis due to angio-invasive hyphae. Once established, the disease is rapidly progressive and is often fatal, as in the present case. The portals of entry of zygomycetes are usually the respiratory tract, the skin, and, less frequently, the gut when fungal spores are inoculated, inhaled, or ingested. Gastro-intestinal mucormycosis is considered a rare manifestation. It is mainly described in premature neonates, where it presents as necrotising entero-colitis. After primary infection, zygomycetes may cause disseminated, metastatic disease through mycotic emboli. Characteristically, invasion of blood vessel walls by the hyphae occurs and contributes to necrotic and ischaemic appearance of tissue.

Clinical diagnosis of mucormycosis is difficult, and is often made at the last stage of the disease or post-mortem. Unlike aspergillosis, there is no available biomarker such as galactomannan to aid in early diagnosis. Histologically, the diagnosis of mucormycosis is relatively easy in the case of rhino-orbital and mucocutaneous involvement. However, when deep tissues are invaded, samples are difficult to acquire, and therefore it may be particularly challenging to obtain the correct diagnosis. Even with vessel invasion, zygomycetes are not isolated easily from blood culture, as was shown in our patient. de Mol and Meis reported an adult who suffered from the left renal artery obstruction-induced kidney infarction caused by Rhizopus. To our knowledge, the present study is the first reported case of an infant with simultaneous obstruction of three arteries and Rhizopus spp. infection.

The treatment of mucormycosis requires a rapid diagnosis, correction of predisposing factors, surgical resection, debridement, and administration of appropriate antifungal agents. Surgery appeared to improve the outcome, except in the case of gastrointestinal zygomycosis in children. Survival increased to 70% for patients treated with a combination of surgery and antifungal therapy compared to 57% for those treated with surgery or medication alone. Among patients treated with antifungal therapy, liposomal amphotericin B was the most commonly used for zygomycosis with less nephrotoxicity compared with amphotericin B deoxycholate. Other antifungal posaconazole or caspofungin can be used in combination with amphotericin B in case of treatment failure or as a substitute for serious side effects. Posaconazole has largely been used as salvage therapy or in cases of intolerance to amphotericin B, with a success rate of 60%.

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Most azoles, including fluconazole and voriconazole, are ineffective against zygomycetes. Unfortunately, our patient initially received fluconazole and nystatin as empiric antifungal agents to treat her febrile neutropenia. The role of iron chelation (e.g., deferasirox) is controversial. It has been reported that the iron chelator deferasirox protects mice against mucormycosis; however, a recent double-blinded, phase 2 study on deferasirox with liposomal amphotericin B treatment for mucormycosis failed to demonstrate a benefit of combination treatment. According to a report by the Working Group on Zygomycosis of the European Confederation of Medical Mycology, the overall mortality rate of zygomycosis was 47%. In high-risk patients, including haematopoietic stem cell transplantation recipients, with mucormycosis, the disease-related mortality rate has been shown to be least 75%. Our patient received aggressive surgery with adequate dosage of an antifungal agent to treat rhizomycosis. Her hypertension and tachycardia were brought under control after treatment; however, she died from progressive leukaemia.

We conclude that rhizomycosis is a life-threatening infection in an infant with leukaemia who has neutropenic status and may be associated with multiple arterial infarctions; hence, early diagnosis, aggressive surgery, and adequate treatment are suggested.

Figure 2  (a) Multidetector-row computed tomography of the abdomen revealed occlusions in the left renal artery (b), superior mesenteric artery (c), and splenic artery (d) with infarction of left kidney and spleen.
Conflict of Interest

We declare that we have no conflict of interest.

References

A Rare Case of Spontaneous Intestinal Perforation at a Paediatric Emergency Department

JJ Chen, CH Lien

Abstract
Abdominal pain is a major complaint at the paediatric emergency department. Intestinal perforation is rare, and it is usually caused by other intestinal diseases or appears in patients with risk factors. We present a case of spontaneous intestinal perforation in a completely healthy patient. The present case is a reminder that spontaneous intestinal perforation can occur in healthy people without any underlying diseases or other risk factors.

Key words
Abdominal pain; Intestinal perforation; Peritonitis; Pneumoperitoneum

Introduction
Abdominal pain is a major complaint at the paediatric emergency department. Diseases that require immediate surgical intervention or are life-threatening are extremely critical, and one of such situations is intestinal perforation. Intestinal perforation is rare, occasionally difficult to diagnose, and can lead to septic shock or even death without proper treatment. Usually, it occurs when patient has previous medical conditions, such as acute enterocolitis. In this article, we present a paediatric patient suffered from spontaneous intestinal perforation without any underlying disease or previous medical condition.

Case
A 3-year-old girl suffered from a sudden onset of abdominal pain without vomiting or diarrhoea after waking up in the morning. She was brought to the emergency department at Hsinchu Mackay Memorial Hospital in Taiwan. She was healthy without any discomfort before the previous night. Underlying disease or surgical history was denied. At the emergency department, her body temperature was 39.2°C. Physical examination revealed a flat abdomen with hypoactive bowel sound. However, abdominal tenderness, mass, or peritoneal signs could not be evaluated using palpation because she was uncooperative and crying persistently during examination. When walking, she bent her body forward slightly. Erect abdominal radiography revealed abundant stool in the colon with local air collection in the right and left upper abdomen. We arranged for a lateral decubitus abdominal radiography, but no radiological evidence of pneumoperitoneum was found. Abdominal ultrasound found diffuse gaseousness without evidence of appendicitis, intussusception, or intestinal obstruction. Hard stool passed after an enema. She then felt more comfortable despite the persistence of abdominal pain. We suggested staying at the emergency department for further observation, but her parents requested discharge and went home. At home, the abdominal pain progressed and high fever persisted. No vomiting or respiratory tract infection symptoms were noted. She passed loose stool twice. In addition, she refused to walk and asked her parents to carry her all day. The next morning, she was brought back to our emergency department. Abdominal tenderness, mass, or peritoneal signs again could not be evaluated because she was uncooperative during examination.
Blood sampling revealed the white blood cell count to be 21,700/µL and serum C-reactive protein to be 28.69 mg/dL. Repeated standing and lateral decubitus abdominal plain film didn't reveal any radiologic signs of pneumoperitoneum, such as continuous diaphragm sign, Rigler's sign, falciform ligament sign, football sign, or Telltale triangle sign (Figure 1). Abdominal ultrasound revealed bowel dilatation without obvious peristalsis. Abdominal CT showed free air under the diaphragm (Figure 2). The patient received surgical intervention, and a perforation hole was seen in the descending colon. No foreign body was found. After surgical repair of the perforation and antibiotics treatment, she recovered and was discharged with a favourable general condition. Pathologic results illustrated necrotic tissue with infiltration of acute inflammatory cells. No other specific sign of neoplasm was found.

Discussion

Intestinal perforation has various aetiologies including appendicitis, foreign body ingestion, diverticulitis, bowel ischaemia, Crohn disease, neoplasm, anorectal malformation and iatrogenic causes. Trauma or child abuse can also cause intestinal perforation. In neonates, necrotising enterocolitis may complicate intestinal perforation. Moreover, idiopathic spontaneous intestinal perforation is a clinical entity distinct from necrotising enterocolitis. Necrotising enterocolitis and idiopathic spontaneous intestinal perforation occur mainly in premature infants, particularly those with very low birth weight. Chen et al discussed spontaneous intestinal perforation in children, but all of those patients had symptoms of acute enterocolitis such as fever and diarrhoea. Therefore, intestinal perforation is usually caused by other intestinal diseases or appears in patients with risk factors. In the present case, the patient was completely healthy before the onset of abdominal pain and fever. She had no underlying diseases or other gastrointestinal symptoms or signs before colonic perforation. Under this condition, considering intestinal perforation in the differential diagnosis is difficult. The only clues that indicated the possibility of intestinal perforation were the patient's inability to walk normally and the need to bend her body forward when moving. When these signs appear, a patient usually has acute abdomen that requires immediate treatment or even surgical management. That was the reason we arranged for more image studies, including abdominal CT, to locate any conditions requiring surgery and diagnose this patient quickly.

Figure 1  Lateral decubitus abdominal plain film didn't find any evidence of free intra-abdominal air.

Figure 2  Abdominal CT illustrated free intraperitoneal air (black arrow) under the diaphragm.
When intestinal perforation is suspected, several examinations should be performed to make a diagnosis. Typically, blood sampling shows leukocytosis and high C-reactive protein level. Plain abdominal radiography to detect free intra-abdominal gas is the first option when intestinal perforation is suspected. With a carefully executed radiographic technique, plain abdominal radiography can demonstrate as little as 1 mL of free gas on an erect chest or left lateral decubitus abdominal film. However, the sensitivity of plain abdominal radiography in detecting pneumoperitoneum has varied from 51% to 96% in various studies. When plain abdominal radiography does not reveal pneumoperitoneum, ultrasonography is a useful diagnostic modality. Intestinal perforation should be considered if ultrasonography reveals direct signs (e.g., an increased echogenicity of a peritoneal stripe associated with multiple reflection artifacts and characteristic comet-tail appearance) or indirect signs (e.g., thickened bowel loop and air bubbles in ascitic fluid or in a localized fluid collection, bowel or gallbladder thickened wall associated with decreased bowel motility or ileus). However, ultrasonography is less useful in the absence of direct or indirect findings of pneumoperitoneum. In this situation, CT can be used to confirm pneumoperitoneum. CT is considerably more sensitive than plain radiography is in detecting free intraperitoneal air. In addition, CT may provide direct visualisation of the perforation site.

Intestinal perforation requires early recognition and a prompt surgical intervention. The present case is a reminder that spontaneous intestinal perforation can occur in healthy people without any underlying diseases or other risk factors. If a patient with abdominal pain bends his or her body forward when walking or even refuses to walk, then spontaneous intestinal perforation should always be considered.

Conflicts of Interest Statement

The authors have no conflicts of interest relevant to this article.

References

A Chinese Family with Dominant Deafness-onychodystrophy Syndrome

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Abstract
Dominant deafness-onychodystrophy syndrome (DDOD syndrome; MIM 124480) is a rare disorder characterised with congenital sensorineural hearing loss accompanied by dystrophic or absent nails. We herein report a family with two members with congenital sensorineural hearing loss and dystrophic or absent nails via autosomal dominant transmission. Genetic analysis showed an ATP6V1B2 mutation (c.1516C>T (p.Arg506X)) in the two patients. The phenotypes described in our family were similar with those reported families. Our findings supported the evidence that ATP6V1B2 gene mutation may cause DDOD syndrome; and this family's conditions were within the spectrum of DDOD, which will provide valuable hints on diagnosis of this disease.

Key words Dominant deafness-onychodystrophy syndrome; Hearing loss; Mutation

Introduction
Dominant deafness-onychodystrophy syndrome (DDOD syndrome; MIM 124480) is a rare disorder, which is characterised mainly by congenital sensorineural hearing loss accompanied by dystrophic or absent nails.1 Up to now, ten families with DDOD syndrome in various ethnic populations have been reported.1-3 Yuan et al1 reported three unrelated Chinese DDOD pedigrees with identical phenotypes including severe congenital sensorineural hearing loss, absence of nails and aplasia of the middle phalanx in the fifth fingers. They identified a de novo mutation (c.1516C>T (p.Arg506X)) in ATP6V1B2 as the cause of DDOD syndrome in the affected patients. It's the first report on the genetic cause of DDOD syndrome.

Herein, we report a family with two members with congenital sensorineural hearing loss and dystrophic or absent nails via autosomal dominant transmission. Genetic analysis showed an ATP6V1B2 mutation (c.1516C>T (p.Arg506X)) in the two members. Clinical characteristics and molecular analysis results were described, and this report will add to the knowledge of DDOD syndrome diagnosis.

Case Report
This study has been approved by the Institutional Review Board of Children's Hospital, Zhejiang University. We have obtained a written consent for reporting this pedigree from the family members. The pedigree's data were collected from March 2014 to August 2014. The proband is a girl aged five months with hearing loss and hypoplastic nails on fingers and toes. Her mother had congenital deafness with unknown cause; her father was 23 years old with congenital hearing loss and similar hypoplastic nails on fingers and toes. Both of her parents had normal intelligence and facial appearance. The proband was born vaginally at term (38 weeks) without birth asphyxia after an uneventful
pregnancy. Her birth weight was 2600 g (P50) and length 50 cm (P65). She had normal hearing screening results at birth, however without response to sound. Audiograms revealed a severe hearing loss (DBHL>80). Physical examination showed absence of nails of the first and fifth fingers and all the toes; all fingers and toes were short and small, and aplasia of the terminal phalanx in the fifth toe was found (Figure 1). X-ray showed the distal phalanx of the second, third and fourth fingers appearing to be thinner and smaller than normal, and the distal phalanx of all fingers and all the toes; all fingers and toes were short and small, and aplasia of the terminal phalanx in the fifth toe was found (Figure 1). X-ray showed the distal phalanx of the second, third and fourth fingers appearing to be thinner and smaller than normal, and the distal phalanx of all fingers and all the toes; all fingers and toes were short and small, and aplasia of the terminal phalanx in the fifth toe was found (Figure 1). X-ray showed the distal phalanx of the second, third and fourth fingers appearing to be thinner and smaller than normal, and the distal phalanx of all

Figure 1  The girl was found with absence of nails on the first and fifth fingers and on all the toes; all fingers and toes were short and small, and aplasia of the terminal phalanx in the fifth toe was found (A&B); her father had similar features (C&D); X-ray on the daughter (E&F) and father (G&H) showed small phalanges in the first and fifth fingers, distal phalanx of the second, third and fourth fingers appeared to be thinner than normal and small phalanges of all five toes except the big toe.
five toes were all small except the big toe (Figure 1). Neuropsychiatric exam on the proband showed normal physical and intellectual development. The proband's father was found deafness since infancy, who had similar presentations with toenails completely absent with short terminal phalanges; all fingers and toes were also short and small, and small terminal phalanx in the fifth toe was also found (Figure 1). The proband's uncle was healthy with normal hearing. Both the proband's grandparents were normal and healthy. They denied deafness in the other family members including the grandmother's five siblings and grandfather's two siblings. The proband's mother's family members were all normal with normal hearing.

Genomic DNA from the two patients and four healthy members of this family was isolated from peripheral blood; molecular analysis was done, and all exons and intron-exon boundaries of *ATP6V1B2* (NM_001693.3) were amplified by PCR from genomic DNA. The variant was tested by Sanger sequencing. A heterozygous c.1516 C>T (p.Arg506X) mutation in *ATP6V1B2* was found in the proband and her father. The other four healthy members showed normal results (Figure 2).

**Discussion**

"Deafness and onychodystrophy" (DOD) is a rare congenital disease with either autosomal dominant or recessive inheritance. The recessive form, DOOR syndrome (deafness, onychodystrophy, osteodystrophy, mental retardation and seizures; MIM 220500), was more severe. Differentiated from DDOR syndrome, patients with DDOD syndrome had normal intelligence and no seizures. Yuan et al. firstly reported the aetiology of DDOD in three Chinese pedigrees. They identified an *ATP6V1B2* c.1516 C>T mutation in three independently identified DDOD patients.

![Figure 2](image-url) A heterozygous c.1516 C>T (p.Arg506X) mutation in ATP6V1B2 was found in the proband (A) and his father (B). The other four healthy members showed normal results (C).
which provides evidence that defect in \textit{ATP6V1B2} is the genetic aetiology for DDOD syndrome. A cochlea-specific \textit{Atp6v1b2}-knockdown mouse model revealed that \textit{Atp6v1b2} deficiency leads to severe sensorineural hearing loss.\textsuperscript{1} Therefore, we highlighted \textit{ATP6V1B2} gene in our pedigree and found the same mutation, c.1516 C>T.

The probands in Yuan's report displayed identical phenotypes including severe congenital sensorineural hearing loss, absence of nails and aplasia of the middle phalanx in the fifth fingers. None showed inner ear malformation and intellectual disability.\textsuperscript{1} Our patients had very similar features with those three families. They all had hearing loss, absence of nails and aplasia of the phalanx in the fingers. Absent/hypoplastic nails and deafness were the characteristic manifestations of DDOD, which were reported in all the ten families. Bulbous swelling of terminal phalanges were found in four families, which was not found in our patients; Our family had short distal phalanges which was reported in other two DDOD families.\textsuperscript{2,3} Three families reported dental anomalies (late dentition, small or conical teeth, oligodontia),\textsuperscript{2,4,5} which was not found in our family as well as Yuan's three families. White and Fahey also reported dysmorphic facial features, aplasia cutis and epilepsy.\textsuperscript{2} Vind-Kezunovic et al indicated that when hearing loss and nail abnormalities coexist in three family members, a common cause is to be suspected; as the skin and the nails, together with the membranous labyrinth of the inner ear, are derived from the embryonic ectoderm.\textsuperscript{3} In the present pedigree, both father and daughter have same presentations of hearing loss and nail abnormalities. However, the other family members were all normal. We suggested that the father may have a \textit{de novo} mutation and the daughter inherited from the father under an autosomal dominant way.

In summary, we identified a mutation (c.1516 C>T (p.Arg506X)) in \textit{ATP6V1B2} as the cause of DDOD syndrome in the family. The mutation was consistent with those reported in the other three Chinese families. The phenotypes described in our family were similar with those reported families though with some variants in other families. Our findings supported the evidence that \textit{ATP6V1B2} gene mutation may cause DDOD syndrome.

**Ethical Statement**

This study has been approved by Institutional Review Board of Children's Hospital, Zhejiang University. We have obtained the written consent for reporting this pedigree from the family members. The consent has been documented and reviewed by the Institutional Review Board and stored at the Institutional Review Board office. A copy of the English version of the approval has been uploaded for reviewing.

**Funding Statement**

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**Conflict of Interest**

None

**Acknowledgement**

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**References**


Oral Presentation

Prediction of Attention Deficit Hyperactivity Disorder (ADHD) Risk Using an Infant Measure: Externalising Symptoms at 12 Months and Risk of ADHD at 54 Months

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Background and Aims: Attention Deficit Hyperactivity Disorder (ADHD) is generally diagnosed at the start of school age. By finding a reliable measure of externalising symptoms during infancy to predict risk of ADHD, early intervention may be started before school age.

Methods: Using the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) cohort, we obtained externalising symptoms using the Infant Toddler Social Emotional Assessment (ITSEA) tool at 12 months and ascertained risk for ADHD using the Computerized Diagnostic Interview Schedule for Children (C-DISC) at 54 months (N=163). Nonparametric descriptive statistics compared low and intermediate risk of ADHD based on early ITSEA externalising scores. Binary logistic regression models examined the relationships between the ITSEA externalising score and risk of ADHD, after controlling for child and family factors.

Results: At 12 months, the mean externalising score was 0.580±0.278. At 54 months, 41.2% were intermediate risk and 58.8% were low risk. Mann-Whitney U test showed that the mean externalising score of children with intermediate risk of ADHD was higher than those with low risk of ADHD (U standardized 3.984, p<0.001). After controlling for gender, birth weight, 3-month postnatal State-Trait Anxiety Inventory (STAI) score, 12-month child media use, and ITSEA 12-month internalising raw score, the model showed that high externalising scores at 12 months increased the prediction of ADHD risk at 54 months by the odds of 13.70 times (OR 13.70, 95%CI 2.62-71.50, p=0.002). for final model and included covariates.

Conclusions: An infant measure at 12 months has predictive validity of risk of ADHD in preschool years. Thus, clinicians should consider administering this infant measure when concerns are raised about externalising symptoms and high risk infants should be followed up regularly for behavioural assessments. Future studies should look at how this infant measure correlates with actual ADHD diagnosis in later childhood.

Prognostic Accuracy of Parent-reported Ages and Stages Questionnaire in Assessing the Developmental Outcome of Preterm Infants

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Background and Aims: Preterm birth and low birth weight are associated with developmental delay. Given these risks, early identification and intervention are imperative. However, standardised tests to assess development are often time-consuming and require trained personnel. In contrast, the Ages and Stages Questionnaire (ASQ) is a parent-completed questionnaire which can be used for developmental screening in children up to 5 years of age. Thus, an assessment of the prognostic accuracy of the ASQ compared to standardised assessment tools is essential. The aim of the study is to compare the prognostic agreement between the ASQ (3rd Edition) and standardised assessment tools.

Methods: This was an observational study of preterm infants presenting to the Neonatal Neurodevelopmental Clinic at Singapore General Hospital from January 2014-June 2017. At follow-ups, the ASQ was completed by parents, and standardised assessment tools were administered: Peabody Developmental Motor Scales 2nd Ed (PDM) by the physiotherapist (6, 12 months), Bayley-
Dynamics of the Gut *Bifidobacterium* Microbiota During the First Three Years of Life: A Quantitative Bird’s-eye View

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**Background and Aims:** Bifidobacteria represent a major element of infant gut microbiota and impart significant beneficial effects on infant’s gut, immune and metabolic health. We investigated the fecal carriage of eight signature *Bifidobacterium* species in healthy infants prospectively from first day to 3 years of life, and examined the effect of factors.

**Methods:** The study included healthy term Japanese infants (n 89; M 49; F 40). Fecal samples (=1 g) at age 1 day, 7 days, 1, 3 and 6 months, and 3 years were collected *Bifidobacterial* groups and species viz. were enumerated by using sensitive reverse-transcription-quantitative PCR assays targeting bacterial 16S rRNA molecules. The study was approved by the Institutional ethical committee, and prior written informed consent was obtained from the parents.

**Results:** About 21% of babies carried bifidobacteria at first day of life (mean bacterial count: 6.1±1.8 log10 cells/g; feces); but this count (and prevalence) gradually increased to 7.7±2.3 (62%), 8.3±2.1 (76%), 9.2±1.9 (97%), and 9.6±1.7 cells/g (99%) at age 7 days, 1, 3 and 6 months, respectively. At 3 years, all babies carried bifidobacteria (mean count: 9.7±1.0 cells/g). *B. longum*, *B. breve*, *B. catenulatum* and *B. bifidum* were the first colonisers (detected at day 1). *B. infantis*, *B. dentium* and *B. adolescentis* appeared at day 7 whereas *B. angulatum* was detected only at 3 years. In terms of count as well as prevalence, *B. longum*, *B. breve*, and *B. catenulatum* remained most dominant bifidobacterial clades throughout the study period. Compared to vaginally-born babies, cesarean-born babies had significantly or insignificantly lower carriage of bifidobacteria from age 7 days to 3 months, with difference being most prominent for *B. catenulatum*. Interestingly, within vaginally-born babies, those who started formula-feed as early as first week of life had higher carriage of bifidobacteria during first 6 months as compared to those who were exclusively breast-fed during first 3 months.

**Conclusions:** Our study presents a quantitative bird’s-eye view of the age-related dynamics of typical infant-associated *Bifidobacterium* species in infant gut during the critical developmental window of life. The data further demonstrate the effect of various factors such as birth mode, feeding type, gender etc. on bifidobacterial colonisation during infancy and early childhood, besides displaying the correlation pattern of bifidobacteria with other gut microbes. Given the fundamental role of gut microbiota in numerous aspects of infant’s long-term health, these data should prove to be informative and important for prospective studies on paediatric microbiota and gastroenterology.
Characteristics of Patent Ductus Arteriosus in Congenital Rubella Syndrome

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Background and Aims: A large-scale rubella outbreak occurred throughout Vietnam between January and July 2011 and many congenital rubella syndrome (CRS) cases emerged. This study aimed to determine characteristics of CRS-associated cardiac complications, particularly morphology and hemodynamics of patent ductus arteriosus (PDA) as compared to those in non-CRS patients.

Methods: (1) Retrospective descriptive study: we reviewed medical records of [1] Laboratory/clinically confirmed CRS cases admitted to Children’s Hospital 1 (CH1) between December 2010 and December 2012, identified by the previous published study and [2] Clinically diagnosed CRS cases who had PDA transcatheter occlusion therapy in the Department of Pediatric Cardiology at CH1. (2) Comparative study of PDA: We compared characteristics of PDA between children in (1) with PDA treated by transcatheter closure (CRS-PDA) and those with PDA treated by transcatheter closure at CH1, between July 2014 and December 2015, after rubella outbreak was over (non CRS-PDA).

Results: (1) A total of 109 children with CRS were enrolled. Among them, 48% were boys, 67% had laboratory confirmed CRS, and the mean birthweight was 2129 g. Cardiac defects (99%), cataract(s) (70%), and hearing impairment (4%) were detected and 17% died at discharge. (2) Fifty-one CRS-PDA and 248 non CRS-PDA cases were analysed. Compared with non CRS-PDA, those with CRS-PDA had lower median age at closure (p=0.0081), less mean birthweight (p<0.001), more pulmonary stenosis (p<0.001), more aortic stenosis (p<0.001), more pulmonary hypertension measured by catheter (mean main pulmonary artery pressure ≥25 mmHg, p=0.006), more main pulmonary artery pressure and more aortic pressure both in systole and diastole (mean systolic pressure of main pulmonary artery; 48.8 vs 36.3 mmHg, p<0.001, mean systolic pressure of aorta; 93.1 vs 73.6 mmHg, p<0.001). Also, mean aorta side diameter of PDA was larger (p=0.0115) and proportion of tubular type of PDA was more in CRS-PDA (27.5% vs 10.2%, p=0.014).

Conclusion: Tubular type, which is difficult-to-treat by transcatheter closure, and pulmonary hypertension are commoner in PDA in CRS and it needs intervention earlier compared with PDA in non-CRS.

Unmanipulated Haploidentical Stem Cell Transplantation with Post-transplant Cyclophosphamide in Children with Severe Thalassaemia with Good Outcome and Rapid Immune Reconstitution

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Background and Aims: Thalassaemia disease can be treatable by allogeneic marrow transplantation. However, HLA-matched donors are difficult to find. HLA-haploidentical haematopoietic cell transplantation (Haplo-HCT) is an alternative transplant strategy for patients without an HLA-matched donor. Recently, expanding the number of patients treatable by haplo-HCT especially in Thailand. Conversely, lack of the study supported the immune reconstitution outcome. The aim of this study was to evaluate the haplo-HCT in Thai children with thalassemia.

Methods: This retrospective study was conducted at Ramathibodi Hospital, including patients with severe thalassaemia who received haplo-HCT in which collected from their parents. Fludarabine, busulfan and anti-thymocyte globulin were used as a conditioning regimen. In addition, all patients were received fludarabine plus dexamethaxone at 3 and 6 weeks prior to conditioning regimen. All patients received unmanipulated haematopoietic cell products. Graft-versus-host disease (GvHD) prophylaxis regimen consisted of cyclophosphamide, mycophenolate mofetil, and calcineurin inhibitor. Moreover, patients with positive donor specific HLA were received rituximab additionally.

Results: Fourteen patients, at the median age of 11.8 (3.7-18.8) years, enrolled in the study. Thirteen patients were diagnosed as beta-thalassaemia/haemoglobin E disease and 1 patient had beta-thalassemia major disease. Eleven patients received stem cells from their mothers. The average stem cells dose was 10.1 x 10^6 cells/kg of recipient body weight. The median time of neutrophil and platelet engraftment were 14 and 26.5 days after transplantation, respectively. Eight patients developed mucositis; 3 patients had engraftment syndrome. Three patients developed hepatic veno-occlusive disease. In our study, 10 patients developed grade I-II acute
GvHD. Mild chronic GvHD was found in 7 patients. Ten patients encountered viral infections especially BK virus (n=8), adenovirus (n=2), and cytomegalovirus (n=3). Secondary graft failure was found in 1 patient. At the median follow-up time of 15.5 (3.3-36.0) months, the one-year event free survival and the one-year overall survival rates were 92.86% and 100%, respectively. Moreover, the number of NK cell, B cell, and T cell decreased to the lowest point (111.5, 5.083 and 442.0 cells, respectively) at one month then the number of B cell and T cell gradually increased to the peak at 1 year after transplantation. Interestingly, the serum immunoglobulin G level decreased gradually to the lowest point (6.358 mg/ml) at three month after transplantation whereas the number of immunity cells were lowest at the first month.

**Conclusion:** Our haplo-HCT with post-transplant cyclophosphamide gives a good outcome with high rate of viral infection. Immune reconstitution study showed rapidly increase of number of immune cells and immunoglobulin levels.

**Upregulated Genes of Intracellular Salmonella Typhimurium After Invasion Into Human Intestinal Epithelial Cells**

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**Background and Aims:** *Salmonella* pathogenicity island-1 (SPI-1) and SPI-2 genes account for bacterial invasion into host cells and survival of intracellular *Salmonella*, respectively. Whether additional *Salmonella* genes after internalisation into human intestinal epithelium contribute to bacterial intracellular survival remains unknown. Therefore, we aimed to investigate which genes of *Salmonella* Typhimurium were significantly regulated after *Salmonella* invades human intestinal epithelium.

**Methods:** Caco-2 cells were infected with *S. Typhimurium* SL1344 (MOI=5) for 2 hours. Then, the cells were treated with gentamicin for 1 hour and remained incubated for an additional 15 hours. After 18-hour incubation, the infected cells were lysed to obtain intracellular bacteria. Total RNAs of extracellular and intracellular *S. Typhimurium* SL1344 were isolated from two independent infections, reverse-transcribed to cDNAs, and subsequent cRNAs were processed for RNA microarrays (Agilent Custom Salmonella GE 8 x 15K Microarray), which were scanned, quantified, and analysed. Transcriptomes of extracellular and intracellular *S. Typhimurium* SL1344 were compared using the Student’s t test to determinate the p values. A p value <0.05 with > 1 log2 or < -1 log2 fold change was considered statistically significant. Then, several significantly upregulated genes were selected for validation using RT-PCR.

**Results:** Compared to extracellular *S. Typhimurium*, a total of 1249 genes of intracellular *S. Typhimurium* within Caco-2 cells were significantly regulated, including 831 genes upregulated and 418 genes downregulated. Most of the plasmid genes were significantly upregulated (54 P1 genes and 23 P2 genes; e.g. 3.707 log2 fold-change in SL1344_P1_0060) except for 4 significantly downregulated genes (traF, traE, pilL, and SL1344_P1_0073). In addition to some SPI-2 genes (sse, ssc, and ssa genes), the genes associated with synthesis of biotin (bioC, bioA, bioB, bioF, and bioD), enterobactin (entD, entE, entA, entB, entC, entF), ferric bactin (fepA, fepG, fepC, fepB, fepD, fepE), colicin (cirA and imm), and bacteriophage shock protein (pspC, pspA, pspD, pspB, and pspE) were significantly upregulated in intracellular *S. Typhimurium* SL1344. Most of the significantly downregulated genes include those encoding invasion-associated secreted proteins (e.g. sopE, hilA, OrgA, flgE, fliC, and fliB) and SPI-1 genes (e.g. inv, sip, spa, and prg genes). The RT-PCR analysis validated mRNA expression of the selected significantly upregulated genes from microarrays, including P2_0016, bioC, entD, fepA, cirA, and pspC, in intracellular *S. Typhimurium* relative to extracellular *S. Typhimurium* (all p<0.05).

**Conclusions:** The majority of plasmid genes and the genes associated with synthesis of biotin, enterobactin, ferric bactin, colicin, and bacteriophage shock protein are important for host-induced bacterial virulence and survival after invasion of *S. Typhimurium* into human intestinal epithelial cells.

**Efficacy and Safety of Infliximab in Juvenile Idiopathic Arthritis and Juvenile Ankylosing Spondylitis: A Randomised, Double-blind, Controlled Study**

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**Objective:** The randomised double-blind method was designed to observe the efficacy and safety of infliximab for juvenile idiopathic arthritis (JIA) and juvenile ankylosing spondylitis (JAS).
Methods: The 45 cases of this study were allocated to treatment group and control group using the randomised, double-blind method. The treatment group was divided into JIA subgroups and JAS subgroup. The test group received MTX combined with infliximab intravenous infusion (JIA group: 3 mg.kg⁻¹; JAS group: 5 mg.kg⁻¹); the control group received MTX combined an equal volume of placebo intravenous infusion.

Results: The treatment groups of this study included 12 JIA cases and 7 JAS cases while the control group included 18 JIA cases and 8 JAS cases. The ASAS 20 response rate of JAS treatment group after two weeks was 85.7%, which was far higher than 25%, the rate of the control group (p=0.04). The ASAS 20 response rate in the treatment group at the endpoint was 100%, while the rate of control group was 37.5% (p=0.07). The total number of infliximab injection was 124, including 24 JIA and JAS cases. One JIA case of penicillin anaphylaxis appeared with systemic wheal -like rash during the 4th injection, and the rash subsided one hour later with the oral phenergan treatment.

Conclusion: This study shows that MTX combined with infliximab can quickly alleviate joint pain and reduce inflammatory markers compared with single MTX in the treatment of juvenile idiopathic arthritis and juvenile ankylosing spondylitis.

Genome-wide DNA Methylation Analysis Identifies the crucial role of β-catenin (CTNNB1) in the Pathogenesis of Kawasaki Disease

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Background: Kawasaki disease (KD), a form of acute febrile vasculitis syndrome, is the most frequent cause of cardiac illness in children under five years old. While KD's aetiology is largely unknown, genome-wide studies in recent years have indicated that epigenetic factors may play a vital role in its pathogenesis.

Methods: We enrolled 24 KD patients and 24 non-KD controls to access their DNA methylation status using HumanMethylation450 BeadChips. Results were confirmed using pyrosequencing at CpG methylation sites according to the array data. Furthermore, another 34 KD patients and 62 control subjects were enrolled for expression validation. Functional study was performed using knockdown target gene expression in endothelial cells.

Results: Of the 3193 CpG methylation regions with a methylation difference ≥20% between KD and controls, 3096 CpG loci revealed hypomethylation, with only 3% (97 CpG loci) being hypermethylated. Pathway buildup by sub-network analysis identified 11 networked genes among hypermethylated regions, including four transcription factors nuclear factor of activated T-cells 1 (NFATC1), v-ets avian erythroblastosis virus E26 oncogene homolog 1 (ETSI), runt related transcription factor 3 (RUNX3), retinoic acid receptor gamma (RARG), and the activator β-catenin (CTNNB1). Ten of these network-selected genes demonstrated a considerable mRNA decrease in KD patients. Furthermore, β-catenin knockdown in endothelial cells with venous (HUVEC) or arterial (HCAEC) origins drastically increased expression of CD40 and CD40L.

Conclusion: This study is the first to identify network-based susceptible genes of hypermethylated CpG loci, their expression levels, and the functional impact of β-catenin which could be involved in both the cause and development of KD.

Significance Statement: This is the first study to identify network-based susceptible genes of hypermethylated CpG loci through HumanMethylation450 BeadChips, their expression levels, and the functional impact of β-catenin which could be involved in both the cause and development of KD. β-catenin also provide future treatment potential for Kawasaki disease. und and Society for Relief of Disabled Children.

Unsafe Environment Puts Slum Children in Peril: A Cross-sectional Study on Microbial Contamination of Complementary Food and Water in Dhaka, Bangladesh

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Background and Aim: Under-nutrition accounts for 3.5 million deaths and 35% of disease burden among children of less than five years of age. In developing countries, inappropriate or inadequate introduction of complementary food causes decline in child's growth after the age of 6 months resulting in high prevalence of malnutrition. Complementary food prepared without maintaining proper hygienic practices exposes the child to enteropathogens. As a result food and water borne infectious disease like diarrhea has a negative impact on the nutritional status of these children. We
conducted a cross-sectional study to examine microbial contamination of complementary food and water, status of household food access, water, sanitation, hygiene practice and nutritional status of children 0-59 months old in four slums of Dhaka, Bangladesh.

Methods: This cross-sectional study took place from December 2015 to May 2016. A total of 360 children aged 0-59 months and their mothers/caregivers participated in the study. Household food security, socio-economic context, nutritional status, hygiene and feeding practices were recorded. Complementary food and water samples were collected from 72 households and tested for microbial contamination. Logistic regression (backward step-wise) model was fit to identify the factors that were significantly associated with malnutrition. Strength of association was determined by calculating adjusted odds ratios (aOR) and their 95% confidence intervals (CI). Probability of <0.05 was considered statistically significant.

Results: Among the 360 under 5 children, 63.3% (CI 0.58-0.69) were malnourished as evident either by weight-for-height, weight-for-age or by height-for-age. Yeast and moulds, and coliform were detected in 85.7% (CI 0.74-0.93) and 73.2% (CI 0.59-0.84) of complementary food samples, respectively. About 82.5% of the households had food insecurity. Logistic regression shows that malnutrition was associated with lack of hand washing after cleaning the child’s bottom following defecation (OR 2.04; 95% CI 1.27-3.29), low birth weight as perceived by mothers (OR 1.96; 95% CI 1.11-3.47) and exclusive breast feeding (OR 0.44, 95% CI 0.27-0.70).

Conclusion: Slum children under the age of five were most often stunted. Complementary food and water samples were contaminated which could be attributed to poor quality of water, sanitation and food preparation practices. Integrated efforts may promote healthy complementary feeding practices in the low income settlements.

Acknowledgement: The paper originates from EcoPoor, research programme, which is funded by the UK Government’s ESPA (www.espa.ac.uk) programme (NE-L001616-1).

Background and Aims: Calcineurin inhibitors (CNIs) have been used off-label for the treatment of refractory Kawasaki disease (KD). However, it remains unknown whether CNIs show protective effects against the development of coronary artery lesions in KD patients. The aim of this study is to investigate the effects of CNIs on coronary arteries and the mechanisms of their actions on coronary arteritis in a mouse model of KD.

Methods: We performed experiments with FK565, a ligand of nucleotide-binding oligomerisation domain-containing protein 1 (Nod1) in wild-type, SCID, CARD9−/−, and MyD88−/− mice. We also performed in vitro studies by using vascular and monocyteic cells, and vascular tissues.

Results: Histopathological analyses showed that both cyclosporin A and tacrolimus exacerbated the Nod1-mediated coronary arteritis in a dose-dependent manner. Cyclosporin A induced the exacerbation of coronary arteritis in mice only in high doses, while tacrolimus exacerbated it within the therapeutic range in humans. Similar effects were obtained in SCID and CARD9−/− mice but not in MyD88−/− mice. CNIs enhanced the expression of adhesion molecules by endothelial cells and the cytokine secretion by monocyteic cells in our KD model. These data indicated that both vascular and monocyteic cells were involved in the exacerbation of coronary arteritis.

Conclusions: The present study revealed the exacerbation of coronary arteritis by CNIs in a Nod1-mediated KD murine model. Activation of MyD88-dependent inflammatory signals in both vascular cells and macrophages appears to contribute to their adverse effects. Particular attention should be paid to the development of coronary artery lesions when using CNIs to treat refractory KD.

Using Paired-end Whole Genome Sequencing (WGS) to Investigate Complex Chromosome Rearrangements (CCRs) Associated with Congenital Anomalies and Neurodevelopmental Disorders

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Objective: Our aim is to apply paired-end whole genome sequencing (WGS) to study a patient with congenital anomalies and developmental delay and complex chromosomal rearrangements (CCRs). Specifically we would like to improve the resolution of breakpoint analysis and to
identify the underlying causes of the clinical manifestations.

**Case Report:** A 10-month-old girl was referred for trigonocephaly and metopic synostosis. She was also found to have global developmental delay. Karyotyping showed a de novo type IV complex chromosomal rearrangement, 46, XX,t(5;12),t(8,10,18)dn, and chromosomal microarray revealed a 8q23.3 deletion. However, these cytogenetic results can only explain part of her clinical features.

**Method:** We hypothesized that the unexplained clinical features are related to the CCRs. We aim to map the exact breakpoints using WGS. We performed a high-depth (i.e. 60X coverage) pair-ended WGS followed by bioinformatics analysis. Breakpoints for the CCRs were investigated using MANTA as the structural variant caller, followed by manual visualisation of the raw data, with reference to the cytogenetic findings. The results were confirmed by Sanger sequencing with custom designed primers. The breakpoints were further analysed with clinical correlations to see if they can explain the clinical features via (a) direct gene disruption; (b) cryptic genomic imbalance and/or (c) disruption of topologically associated domains (TADs).

**Results:** WGS confirmed all the known cytogenetic findings and provided a single nucleotide resolution for the CCR breakpoint. It confirms that the 8q23.3 deletion results in haplo-insufficiency of TRPS1 which is known to cause Tricho-Rhino-Phalangeal syndrome. In addition, the CCR breakpoint at chromosome 5 has directly disrupted CTNND2 at intron 2, a gene known to be critical in causing intellectual disability in patients with Cri-du-chat syndrome. No additional cryptic genomic changes or TAD alternation involving other known disease-causing genes was identified. Micro-homology signatures were identified at the CCR breakpoints, providing insights into the mechanism underlying these complex rearrangements.

**Discussion:** Using WGS, we are able to identify the breakpoints of CCRs at single nucleotide level in this patient. The findings revealed that she actually has at least the dual diagnoses of trichorhinophalangeal syndrome and CTNND2-related intellectual disability. Compared to conventional methods, WGS has improved the diagnostic precision for CCRs, empowering a more individualized approach for both genetic counselling and management of this child and the family.

**Acknowledgement:** The study is supported by (i) Seed Fund for Basic Research (HKU201611159197) and (ii) the Society for the Relief of Disabled Children.

**Molecular Diagnosis of Hepatic Glycogen Storage Disease by Gene Panel-based Next-generation Sequencing: Results in 108 Cases**

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**Background and Aim:** To evaluate the utility of molecular diagnosis for liver-affecting GSD of large sample volume by targeted gene panel-based NGS assay.

**Methods:** This work reports the use of gene panel-based next-generation sequencing to diagnose patients with hepatic glycogen storage disease. Sequence variants were matched against biochemical and clinical hallmarks.

**Results:** Altogether 108 Chinese paediatric patients clinically diagnosed with liver-affecting GSD were enrolled in the study. The most common related genes were AGL (GSD type III), PHKA2 (GSD type IXa), G6PC (GSD type Ia), and PYGL (GSD type VI). The molecular diagnosis rate for NGS was 88.0% (95/108) in total. In our study, 168 variations were detected, the majority of which had previously been associated with the phenotype of the disease. Prevalent examples include: a missense mutation (c.648G>T, p.L216L) in G6PC, occurred in 61.5% (16/26) of GSD type Ia alleles; a splicing-site mutation (c.1735+1G>T) in AGL, accounted for 6.9% (4/58) of GSD type III alleles; and a missense mutation (c.884G>A, p.R295H) in PHKA2, made up nearly 12.5% (3/24) of GSD type IXa alleles. Furthermore, we detected 69 variations that have never been reported before; most were either frame-shift or nonsense mutations (31 frame-shift mutations, 25 splicing-site mutations, 22 nonsense mutations, and 3 non-frame-shift deletions), and thus lead to a complete loss of gene function.

**Conclusions:** Our results clearly indicate that this method is an accurate, prompt, and cost-effective tool for clinical diagnosis of complex diseases with genetic heterogeneity such as GSD, providing a mutation search from large CNVs to SNVs and small indels in a single platform, thus facilitating diagnosis confirmation, appropriate medical care, and genetic counselling.
Early Food Allergy and Symptoms of Airway Allergy March on the Risk of Attention Deficit Hyperactivity Disorder in Chinese Children: A Cross-sectional Study

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Background and Aims: Few studies investigated the effects of food allergy and the symptoms of allergy march on ADHD in children. We aim to investigate the effects of early food allergy and symptoms of allergy march on the prevalence of attention-deficit/hyperactivity disorder (ADHD) in school-age children.

Methods: This cross-sectional study was conducted in school-age children in grade 1-6 in elementary schools in China using cluster-stratified methods from nine cities across China between November and December 2005. A family and social environmental questionnaire including the diagnosis history of ADHD and allergic diseases (food allergy, allergic rhinitis and bronchial asthma), as well as general information of the children were completed by the parents of school-age children. The children were grouped as: no food allergy group, single food allergy group (FA group), food allergy complicated with one airway allergy march symptom group (FA+AR/BA group), and food allergy complicated with two airway allergy march symptoms group (FA+AR+BA group) according to the diagnosis history of airway allergic diseases.

Results: The prevalence of allergic rhinitis (20.4%) and asthma (11.6%) in the food allergy group were both significantly higher than in the non-food allergy group (9.0% and 2.8%, respectively) (both p<0.001). The multivariable analysis showed that single food allergy (OR=1.53, 95%CI: 1.13-2.05, p=0.005), food allergy complicated with allergic rhinitis or asthma (OR=3.36, 95%CI: 2.19-5.14, p<0.001), and food allergy complicated with allergic rhinitis and asthma simultaneously (OR=4.08, 95%CI: 2.05-8.11, p<0.001) were independently associated with the increased risk of ADHD.

Conclusions: Early exposure to food allergen is a risk factor of ADHD in school-age children. The symptoms of airway allergy march resulted in a synergism with a higher risk of ADHD in children with food allergy. Monitoring food allergy in early life could provide information for the early prediction and intervention for the consequent allergy march and ADHD in children.

Diet Glycemic Index Change During Pregnancy is Associated with Placenta Insulin Related Gene DNA Methylation Variation

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Background and Aims: Low glycemic index (GI) diet is proved to be a new effective approach to help pregnant women manage body weight and improve the health of offsprings however the mechanism remains unclear. Our study analysed the association between the change of diet GI during gestation and newborns' DNA methylation.

Methods: The study sample was from a randomised controlled trial. Two different education programs were provided to overweight pregnant women to achieve glycemic index reduction of their diet. The information of their diets in 3 trimesters was collected by 24 hour diet records and the corresponding GIs were calculated. Placentas tissue were collected to extract DNA. Twelve subjects whose diet GI from 1st trimester to the last trimester decreased most remarkably were chose as the case group; 12 subjects whose diet GI increased most were chose as the control group. The genome wide methylation level of two groups was examined by Illumina Human Methylation 450K Bead Chip. Genome-wide differential methylation analyses were performed and followed by various bioinformatics analysis such as Gene Ontology, KEGG Pathways. The probes discovered by significant differential methylation region (DMR) were verified by pyro sequencing.

Results: The diet GI decreased 24.3 (20.1-26.2) averagely in the case group and increased 19.6 (15.2-29.1) averagely in the control group. According to the genome wide methylation analysis, 2259 MVPs were found. Among all the MVPs, the methylation level of 1499 (66.4%) positions increased and 760 (33.6%) positions decreased when the case group was compared with the control group. 108 differentially methylated regions were found and were related to 595 positions and 95 genes such as PLIN1, PRKZ, IER3. Nine genes (SLC6A5, PTPRN2, PLIN1, ASPSCR1, GALNT2, CPT1B SSTR4, CIDEA, KLF15) and 40 sites with differential methylations related to insulin resistance were discovered. Seven of which located in regulatory region were verified by pyrosequencing. The methylation level of cg0509389 in 3'UTR of PLIN, cg14631053 in 5'UTR of CPT1B, cg17586860, cg18197392 in SSTR4 and cg20950011 (CIDEA) CPG island was lower than in control group by 0.1-0.2.

Conclusions: The change of diet glycemic index change
during gestation may have impact on offspring insulin resistance level through varying placenta tissue insulin resistance related gene methylation. The findings needs further study to validate.

**Identification of Potential Transcriptomic Markers in Developing Asthma: An Integrative Analysis of Gene Expression Profiles**

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**Background and Aims:** Asthma represents a chronic respiratory disorder characterised by airway inflammation, airflow obstruction, and bronchial hyperresponsiveness to stimuli. Airway epithelial cell (AEC) dysfunction plays an important role in asthma, hence systematic screening is required to identify AEC abnormalities and improve the diagnosis and treatment of asthma. Rapid growth of high-throughput transcriptomic data largely enables gene expression profiling and diagnostic targets identification in disease nowadays. In the past decade, several studies have focused on the transcriptional profiling of asthma using microarrays to identify candidate genes involved in asthma. Analysis of multiple transcriptomic datasets has the likelihood of discovering robust candidates for diagnosis and treatment. Therefore, to identify potential transcriptomic markers in developing asthma, we investigated gene expression patterns in AEC between asthma patients and healthy controls by an integrative analysis of multiple public microarray datasets in this study.

**Methods:** R software and bioconductor packages were used for data pre-processing, differentially expressed (DE) genes identification, and support vector machine (SVM) model training. Enrichment analysis and co-expression network construction were also performed using DAVID and Cytoscape software, respectively.

**Results:** 3 microarray datasets (192 cases and 91 controls in total) were collected for this analysis. 62 DE genes were identified in asthma, among which 43 genes were up-regulated, 19 genes were down-regulated, and a set of them were not studied in asthma previously. Enrichment analysis revealed that those DE genes strongly associated with proteolysis, retina homeostasis, humoral immune response, and salivary secretion. A co-expression network of DE genes was also constructed using highly correlated DE gene pairs (correlation coefficients >0.8), which included 20 nodes and 18 edges. Recursive feature selection of all DE genes showed that 44 genes were sufficient to achieve close to 95% prediction accuracy, including the clusters of co-expressed DE genes. Using the selected features, a SVM classifier (asthma versus healthy control) was trained. The performance of the SVM classifier was evaluated using 10-fold cross-validation and the cross-validation error was 0.079.

**Conclusions:** In conclusion, we identified consistently DE genes in asthma that could potentially serve as transcriptomic markers. GO and pathway analyses revealed that those candidates strongly associated with proteolysis, retina homeostasis, humoral immune response, and salivary secretion. A SVM classifier (asthma versus healthy control) was also trained based on candidate transcriptomic markers in this study. These results provide novel insights into the pathogenesis of asthma, and promote the generation of diagnostic gene sets.

**Distinctive Cytokine Profile Between Acute Focal Bacterial Nephritis and Acute Pyelonephritis in Children**

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**Background and Aims:** Acute focal bacterial nephritis (AFBN) is a severe form of upper urinary tract infection (UTI) with neurological manifestations and focal renal mass lesions on computed tomography (CT). Prolonged antibiotic therapy may improve the renal outcome, but the early differential diagnosis of AFBN from acute pyelonephritis (APN) is challenging. We searched for effective biomarkers of AFBN based on the pathophysiology of upper UTIs.

**Methods:** Of 52 upper UTI cases treated at Yamaguchi University between 2009 and 2016, 38 paediatric patients with AFBN (n=17) or APN (n=21) who underwent ultrasonography and/or CT were enrolled. The clinical data and serum cytokine concentrations were analysed to differentiate AFBN from APN.

**Results:** AFBN patients tended to be older, and have a higher body temperature, longer febrile period, more frequent neurological symptoms, higher immature neutrophil count, lower lymphocyte count, higher procalcitonin and urine β₂-microglobulin levels. AFBN
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patients showed higher serum levels of IFN-β, IL-6, IL-10 and soluble TNF-receptor 1 (sTNFR1) (all p<0.05). Although levels of the cytokines were variably correlated among each other, multiple logistic regression analysis revealed that combination of IFN-β and IL-6 levels were most relevant for distinguishing AFBN from APN. The discriminant power of the logistic equation was 0.86 in terms of the area under the curve by the ROC analysis.

**Conclusions:** In AFBN, serum levels of 4 out of 7 cytokines examined were higher compared with those in APN. For distinguishing AFBN from APN, IFN-β and IL-6 were most relevant.

The Tetraspanin CD9 Is an Adverse Prognostic Factor in Paediatric B-precursor Acute Lymphoblastic Leukaemia and Can Be Effectively Targeted by Neutralising Antibody in Preclinical Animal Models

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**Background:** B-precursor acute lymphoblastic leukaemia (B-ALL) is the most common childhood malignancy. Despite advances in risk-directed chemotherapy, high-risk paediatric B-ALL remain associated with poor clinical outcomes, underscoring the need for development of novel targeted therapy. CD9 has been associated with metastasis and progression of various types of malignancies, but its prognostic relevance and therapeutic potential in paediatric B-ALL remain largely unknown.

**Aims:** This study was designed to (1) characterise the expression of CD9 in a cohort of paediatric B-ALL patients and its association with 5-year clinical outcomes; and (2) evaluate the efficacy of using a CD9 antibody for treatment of B-ALL in preclinical animal models.

**Methods:** Cell surface expression of CD9 on leukaemic blasts in paediatric B-ALL patients was characterised by flow cytometry. The effects of targeting CD9 with a neutralising antibody as a single agent or in combination with chemotherapy on inhibition of B-ALL progression were evaluated in the NOD/SCID mouse xenograft model.

**Results:** CD9+ patients had significantly lower overall and relapse-free survival (RFS) rates compared with CD9- cases. Subgroup analysis revealed remarkably poorer outcomes in CD9+ patients of the high-risk group. A similar trend was also observed in patients of the intermediate-risk group but not in the standard-risk group. In univariate analysis, CD9 positivity, age <1 year, white cell count ≥100 x 10^9/L and poor prednisone response were associated with lower RFS rate. In multivariate analysis, CD9 positivity remained as an independent prognostic factor for adverse survival outcomes. Administration of CD9 antibody substantially reduced leukaemic burden and prolonged survival of animals xenografted with the intermediate-risk 697 (TCF3-PBX1+) and high-risk RS4;11 (MLL-AF4+), but not the standard-risk Reh (ETV6-RUNXI+) cell lines. Similarly, CD9 antibody treatment significantly decreased B-ALL progression in patient-derived xenografts with a wide spectrum of genetic and disease features, including TCF3-PBX1+ and MLL-AF4+ cases and those with relapsed/refractory diseases. Importantly, CD9 antibody in combination with conventional chemotherapy consisting of vincristine, dexamethasone and L-asparaginase further prolonged animal survival, when compared to animals treated with CD9 antibody or chemotherapy alone.

**Conclusions:** Expression of CD9 in paediatric B-ALL patients was associated with adverse survival outcomes and could be used for refinement of clinical risk group stratification. CD9 blockade, in adjunct to chemotherapy, was highly effective for suppressing B-ALL progression in preclinical animal models and could be developed as a novel and promising strategy for treatment of high-risk paediatric B-ALL.

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Prospective Study of Risk Factors for Wheezing Phenotypes in Hong Kong Children

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**Background and Aims:** Given the importance of early-life wheezing as a strong risk factor for subsequent asthma, the identification of early-life determinants for wheezing phenotypes can improve our prediction for the development of childhood asthma. Ten asthma loci were identified in the genome-wide association study by GABRIEL Consortium. Nonetheless, the relevance of these loci on early-life wheezing remains unclear. This birth cohort study characterised both environmental and genetic determinants for early-life wheezing.
**Methods:** Early-life factors, environmental factors and occurrence of wheezing phenotypes of 149 healthy Chinese neonates born in September 2012 were prospectively assessed at baseline and 9 and 24 months. Buccal swab samples were genotyped for single-nucleotide polymorphisms (SNPs) reported by GABRIEL using TaqMan genotyping assays. Linear regression and binary logistic regression were used to identify the genetic and environmental risk factors for wheezing.

**Results:** Adjusted for gender and family history of asthma/eczema as covariates, presence of household smokers (odds ratio [OR] 5.89, 95% confidence interval [CI] 1.02-33.84; p=0.047) and furry pet exposure (OR 6.47, 95% CI 1.21-34.69; p=0.029) at birth were risk factors for current wheeze at 9 months. These exposures were also associated with increased risk for wheeze ever both at 9 months (OR 3.99, 95% CI 1.35-11.84; p=0.012 and OR 3.51, 95% CI 1.06-11.59; p=0.040) and 24 months (OR 2.68, 95% CI 1.03-7.00; p=0.044 and OR 3.81, 95% CI 1.31-11.08; p=0.014). Besides, visible mould or dampness at home increased the risk for current wheeze at 9 months (OR 10.53, 95% CI 1.17-94.47; p=0.035). Concerning the genetic factors, rs2284033 in **IL2RB** was weakly associated with current wheeze at 9 months (OR 9.32, 95% CI 1.22-71.08; p=0.031), while rs11650680 in **TOP2A** was associated with wheeze ever at 9 months (OR 0.27, 95% CI 0.08-0.91; p=0.035) and rs1295686 in **IL13** with wheeze ever at 24 months (OR 2.51, 95% CI 1.01-6.26; p=0.049). All other SNPs were not associated with any wheezing traits.

**Conclusions:** This study identifies exposures to passive smoking, pet keeping and visible mould or dampness as risk factors for wheezing phenotypes at 9 and 24 months. **IL2RB**, **TOP2A** and **IL13** appear to be candidate genes for early-life wheezing, which should be replicated in larger cohorts.

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**Haploidentical Stem Cell Transplantation for Primary Immunodeficiency Disorders in Children: Challenges and Outcome from a Tertiary Care Centre in India**

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**Background:** We describe our experience in the use of haploidentical stem cell transplantation (haploSCT) in children with PID.

**Patients and Methods:** We performed a retrospective study at the blood and marrow transplant unit at Apollo Cancer Institutes, Chennai including children up to 18 years diagnosed to have PID who underwent haploSCT from 2008 to 2016.

**Results:** Of the total 17 paediatric haploSCTs for PID, 7 were for severe combined immunodeficiency, 2 - Wiskott Aldrich syndrome, 2 - primary HLH, 1 each with Chediak Higashi syndrome and Griscelli's syndrome with accelerated HLH, 2 - MSMD, 1 - Hyper IgM syndrome. Haplograft was from a sibling donor in 5, parent donor in 12 children. PBSC was used in 13, bone marrow in 4. Techniques of T depletion used were 1 each with CD 34 selection and Campath in the bag, TCR alpha/beta depletion 3, CD3/19 selection 1 and post transplant cyclophosphamide (PTCy) in 10 children. 14/17 (82.3%) transplants resulted in engraftment by Day 16-21 post HSCT with sustained complete chimerism in 11 children (64%). Hyper IgM syndrome and MSMD were 2 conditions where primary rejection resulted in autologous reconstitution. One child with WAS dropped his chimerism to 77% around D+90 post HSCT and was salvaged with a donor lymphocyte infusion. One child with ADA deficient SCID has mixed chimerism 5 months post HSCT and remains infection free. Acute skin and gut GVHD responsive to steroids of grade 2-3 was noted in 3/15 (20%), CMV reactivation in 6/15 (40%) children. Overall mortality was found to be 5/17 (29%). Two deaths among infants receiving PTCy were due to sepsis and severe ARDS. Campath use resulted in refractory CMV disease and death. Two children where TCR α/β depletion and CD3/19 selection was used died of progressive leukoencephalopathy probably of viral etiology.

**Conclusions:** HaploSCT is a feasible option for cure in children with PID where no compatible family or matched unrelated donor has been found with engraftment rates of 82%, durable graft in 64% and overall survival of 70%. In our series, we have had superior outcome with the use of PTCy compared to ex vivo T depletion with survival rates of 80% in this group. The cost of the monoclonal antibodies alone is about 1200,000 Indian rupees making this procedure manifold expensive compared to PTCy. Careful patient selection will improve outcomes using this simple but cost effective method of treating children with PID in the future.
Reference Values for Peripheral Blood Lymphocyte Subsets of Healthy Children in China: A Multi-centered Study

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Background and Aims: Defining abnormality of peripheral blood lymphocyte distribution is the key to suspect primary immunodeficiency and numerous other immune disorders in childhood. Use of domestic reference values is known to improve the accuracy of flow cytometric analysis by integrating local variation due to race, gender, and age. As there were no published estimates, we now report establishment of reference values for peripheral blood lymphocyte phenotypes applicable to the healthy childhood population in China.

Methods: Blood samples were taken from 1075 children aged 0-18 years, and 20 peripheral blood lymphocyte subsets were determined by means of seven color-flow cytometry. Relative and absolute sizes of each subset were calculated. When absolute numbers were concerned, a dual platform approach was used.

Results: Reference values for age-related lymphocyte subsets in seven age groups of T-, B-, NK-cell subsets were estimated, including naïve, central memory, effector memory, terminally differentiated of helper T cell (Th), and cytotoxic T cell, TCRαβ double negative T cell, γδT cell and naïve, memory, transitional, plasmablasts of B cell. The distributions of lymphocyte subsets changed by age. Naïve CD4 T-cells showed a gradually decrease relative size while the percentage of memory CD4 T-cells increased. As for the CD8 T-cells, similar pattern of changes was observed. Both CD4 and CD8 TEMRA cells showed a low frequency in newborns and a dramatic increase during the first year of life. The absolute numbers of CD4 and CD8 T-cell subsets had parallel changes to the relative numbers. The frequency of TCRαβ+DNT cells showed a gradually increase while the absolute number range changed a little. γδT cells percentage gradually increased and the absolute number of γδT cells had a wide range related to ages. Naïve B-cells (CD19+ CD27+ IgD-) composed the greatest B cell subsets in all age groups, while memory B-cells gradually increased. Transitional B-cells showed obvious age-related variations while plasmablasts did not in both relative and absolute sizes.

Conclusions: This study provides a largest scale research project on peripheral blood lymphocyte subsets analysis of healthy children, which is multicentered and multiparametered. Based on the statistical methods, the reference values reflect the continuous maturation of lymphocyte subsets during childhood. And localised reference values of peripheral blood lymphocytes subsets may be more suitable for clinical evaluation of immune abnormalities for Chinese children.

Effects of Parent-implemented Early Start Denver Model on Chinese Toddlers with Autism Spectrum Disorder: A Non-randomised Controlled Trial

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Background and Aims: It has been a consensus that early screening, diagnosis and intervention can effectively improve the prognosis of autism spectrum disorder (ASD). The trinary screening network and 2-tiered referral system have been primarily established in Shanghai and several other cities. As a result, the disparity between the staggering increase in the prevalence of ASD toddlers and the lack of a systematic intervention approach for them has become increasingly obvious in China.

Objectives: To evaluate the effects of a 26-week, low-intensity, parent-implemented Early Start Denver Model (P-ESDM) intervention on developmental outcomes, ASD severity of ASD toddlers, and on parental stress of their parents.

Methods: The present study is a non-randomised controlled trial. Subjects in P-ESDM group (n=23) were recruited from 1.5-2.5-year toddlers who were screened positive on Checklist for Autism in Toddlers (CHAT-23) in Xuhui and Minhang District and diagnosed with ASD by DSM-V in developmental and behavioural clinic of Children’s Hospital of Fudan University. ASD toddlers in the community group (n=20) were recruited from age-matched ASD toddlers coming from other districts or provinces with the same diagnosing procedure as P-ESDM group. Parents and children of P-ESDM group attend 1.5-hour therapy per week for 26 weeks. Children of community group received any interventions available from communities or private services. Assessments were completed at baseline (T1) and 26 weeks later (T2).

Results: After adjusting for baseline differences between the two groups, compared with community group, P-ESDM
group demonstrated improvement that was more significant in general development, especially in Personal-Social, Language and Eye-Hand Coordination domains. The both groups did not have much improvement in ASD severity using standardised Autism Diagnostic Observation Schedule (ADOS), but P-ESDM group showed greater improvement in parent-reported social communication and symbolic play than community group did. Although parents in P-ESDM group experienced decreased parenting stress while those in community group showed an opposite trend, the difference was not significantly related to the group assignment.

**Conclusions:** Parent-implemented Early Start Denver Model on Chinese toddlers with ASD for longer duration have shown some potentials in improving their developmental outcomes as well as social communicational skills reported by parents. Our results also supported the importance of early detection and intervention for ASD.

**A TBX5 3'UTR Variant Increases the Risk of Congenital Heart Disease in the Han Chinese Population**

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**Background and Aims:** TBX5 is a vital transcription factor and it contributes to cardiac development in a dosage-dependent manner. But little is known about the potential association of TBX5 regulatory variations with congenital cardiac malformations. This study aimed to investigate the relationship between TBX5 3' untranslated region (UTR) variants and risk for congenital heart disease (CHD) susceptibility in two Han Chinese populations, and to reveal its molecular mechanism.

**Methods:** The relationship between TBX5 3'UTR variants and CHD susceptibility was examined in 1,177 CHD patients and 990 healthy controls in two independent case-control studies. Following the association study, Quantitative real-time PCR and Western blot analysis were performed to confirm TBX5 expression in CHD heat tissues of different genotypes. In addition, luciferase reporter assays, surface plasmon resonance analysis and zebrafish experiments were applied to reveal the function of TBX5 3'UTR variants.

**Results:** Variant rs6489956 C>T was found to be associated with increased CHD susceptibility in both cohorts. The combined CHD risk for the CT and TT genotype carriers was 1.83 times higher than that of CC genotype, while the risk for CT or TT genotype was 1.94 times and 2.31 times higher than that of CC carriers, respectively. Quantitative real-time PCR and Western blot analysis showed that T allele carriers exhibited reduced TBX5 mRNA and protein levels in CHDs tissues. Compared with C allele, T allele showed increased binding affinity to miR-9 and miR-30a in both luciferase assays and surface plasmon resonance analysis. Functional analysis confirmed that miR-9 and miR-30a down-regulated TBX5 expression at the transcriptional and translational levels, respectively. The assays in zebrafish model were in support of the interaction of miR-9/30a and TBX5 3’UTR (C and T allele).

**Conclusions:** We concluded that TBX5 3’UTR variant rs6489956 increased susceptibility of CHD in the Han Chinese population because it changes the binding affinity of two target miRNAs that specifically mediate TBX5 expression.

**Effects of a Group-based Acceptance and Commitment Therapy Versus Asthma Education for Training Parents to Manage Their Children with Asthma: A Randomised Controlled Trial**

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**Background and Aims:** Parents of children with asthma face many psychological difficulties that can adversely affect their asthma care. Acceptance and Commitment Therapy (ACT) is a contextual behavioural therapy to help parents to better accept their psychological difficulties and work towards achieving better health outcomes for their children. This study aimed to examine the efficacy of a parental training program using group-based ACT for childhood asthma management in comparison with an asthma education talk, as measured by the children’s use of healthcare services due to asthma exacerbations at six months after the intervention.

**Methods:** An assessor-blinded, two-armed randomised control trial was conducted. Parents and their children aged 3-12 years were consecutively recruited in a public hospital in Hong Kong from January to July 2016. The parents were randomly assigned either to four weekly sessions of a group-based ACT intervention (ACT group), or to an asthma education talk as the usual care plus three weekly telephone
reminders (Control group). The goal of ACT was to enhance the psychological flexibility of the parents in caring for a child with asthma in the following ways: (i) to be aware of the present moment with thoughts and feelings, (ii) to accept and adapt flexibly to challenging situations, and (iii) to take actions to achieve valued goals in childhood asthma management. The primary outcomes were the number of visits to emergency departments (EDs), outpatient clinics, or hospital admissions due to asthma exacerbations at six months after the intervention. Changes in these outcomes between groups were examined using generalised estimating equations.

**Results:** 168 parents (age M=38.4, 88.1% mothers) and their children with asthma (age M=6.8) participated. One hundred and sixty-two (96.4%) parent-child dyads successfully adhered to the follow-up assessments up to six months. When compared to the control group, children whose parents were allocated in the ACT group showed a significant drop in ED visits (adjusted (incidence rate ratio) IRR=0.22, 95% CI [0.08, 0.59], p=0.003), and in outpatient clinic visits (adjusted IRR = 0.27, 95% CI [0.08, 0.84], p=0.024) due to asthma exacerbation. There was no significant effect on the hospital admissions between groups (p=0.455).

**Conclusions:** The results suggest that parents' active commitment to engaging in meaningful activities with their children with asthma and accepting the related psychological difficulties might help them to better manage their children's asthma, thereby producing better health outcomes for their children.

**Integrated Genomic Analysis of Paediatric Germ Cell Tumours**

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**Background and Aims:** Germ cell tumours (GCTs) arise from primordial germ cells, which migrate during embryogenesis from the yolk sac through the mesentery to the gonads. GCTs include several histological subgroups, such as yolk sac tumour and teratoma. Since they derive from the same cell origin, primordial germ cells, GCTs may have common genetic alterations. The isochromosome of 12p is a common genomic alteration in adult testicular GCTs, and TP53 or KIT mutation has been frequently found in adult testicular GCTs. However, genetic basis of paediatric GCTs is still to be elucidated due to its rarity.

**Methods:** Forty-six paediatric GCT samples, which included 9 teratomas, 6 dysgerminomas, 2 embryonal carcinomas, 6 mixed germ cell tumours (MGCT), and 23 yolk sac tumours (YST) were used in this study. We applied genome-wide analysis for genetic abnormalities using SNP array analysis to 46 cases, whole-transcriptome sequencing (WTS) to 39 cases, and methylation array analysis to 26 cases. To validate gene mutations detected by WTS, targeted deep sequencing was also conducted.

**Results:** SNP array analysis revealed that chromosomal gains were predominantly detected rather than chromosomal losses in paediatric GCTs. Among the recurrent gains, 12p gain was the most frequent genetic alteration in this study. Nine samples with 12p gain have been speculated to have the isochromosome of 12p. Based on the consensus clustering of expression data, GCTs were divided into 3 clusters. Each cluster showed a distinct pattern of gene expression and characterised by histological subgroups. Cluster 1 includes all teratomas. Cluster 2 includes all ECs and most of DGs. Cluster 3 includes most of YSTs. This result enlighted each histological subtypes of GCTs have clearly characteristic gene profiles. Intriguingly, high expression of KIT or CXCR4 pathway genes were commonly detected in all clusters. Activating mutations of KIT (n=2) and NRAS (n=1) were detected in group 2 and group 3, respectively. Consensus clustering of methylation array analysis divided GCTs into two clusters, which were consistent of histological subtypes, teratomas and yolk sac tumours (YSTs), respectively. We also detected several DMRs between two clusters. Cluster of YSTs had hypermethylation of RASSF1, which is known as a tumour suppressor gene.

**Conclusions:** We identified specific gene expression profiling of paediatric GCTs and upregulation of KIT or CXCR4 signaling in GCTs. Since KIT and CXCR4 signaling is essential for migration and proliferation of primordial germ cells, these findings suggest that KIT or CXCR4 signaling have a potential role in the pathogenesis of GCTs, and might be novel therapeutic targets for GCTs.
Haploidentical Stem Cell Transplantation with Post-transplant Cyclophosphamide in Paediatric High-risk Acute Leukaemia with Good Disease Controls and Immune Reconstitution
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Background and Aims: Acute leukaemia is the most common childhood cancer worldwide. Most of the patients, suffering from relapsed or refractory disease, need allogeneic stem cell transplantation; however donor availability is the main obstacle. To solve this dilemma, haploidentical stem cell transplantation has been recently introduced. In this study, we would like to analyse the results and the immune reconstitution of this novel approach at Ramathibodi Hospital.

Methods: We retrospectively reviewed medical record of 23 paediatric patients with high-risk or relapsed ALL and AML, whose age less than 18 years old, between August 2012 and February 2017. All patients were in the remission state of disease at the time of the study. HLA studying of the patients and their parents were done by high-resolution technique searching for HLA subunit A, B, C, DR and DQ. Parents with greater HLA matching were selected as a haploidentical donor for their sibling. Before receiving conditioning regimen, patient’s blood was obtained to measure serum IgG and WBC level and then continuously evaluate at 1, 3, 6, 12, 24 month post-transplantation for immune reconstitution analysis. All patients received conditioning regimen, thiotepa based or TBI based, prior to transplantation. Then patients received unmanipulated haploidentical stem cell products at the day 0, then received post-transplantation cyclophosphamide, cyclosporine or tacrolimus, and mycophenolate mofetil as the graft-versus-host-disease prophylaxis. The median follow-up time of this study is 17.1(0.6-51.3) months.

Results: Twenty-two patients had engraftment. One patient, who was not engrafted, deceased due to severe infection. One patient was suffered from grade III-IV acute GVHD; three patients developed moderate-to-severe chronic GVHD. Thirteen patients encountered viral infections especially cytomegalovirus (8 cases), BK virus (6 cases) and adenovirus (4 cases). No patient had primary graft failure. Two patients died of relapsed disease while three patients died of severe infection. The one-year event-free survival and the one-year overall survival were 75.3 and 79.3%, respectively. The relapse rate was 19.1 (0.5-59.4) % meanwhile the transplant-related mortality was 15.0 (0.2-54.9) %. T, B and NK cell numbers were at the lowest values at one month after transplantation. Interestingly, CD4+ T cell was reduced but not to the critical point while memory helper T-cell received little effect.

Conclusions: Our haploidentical transplantation with Post-transplant cyclophosphamide gives a satisfied outcome. But viral reactivation is the major morbidity in our study. This conditioning regimen would not reduce CD4+ T cell to the critical point and be safe for memory helper T-cell.

Turning Weakness to Strength: Lessons Learnt in Delivering Cure for Primary Immune Deficiency Disorders in India
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Aim: Haematopoietic stem cell transplantation (HSCT) is the only form of cure in children with primary immune deficiency (PID). We present our experience in cure for PID and lessons learnt.

Patients: All children less than 18 years of age with PIDs who were transplanted at Apollo Cancer Institutes, Chennai from 2008 to 2016 were included in the study where factors affecting morbidity and mortality were analysed.

Results: A total of 62 PID transplants have been performed at our centre till December 2016 - 37 T cell defects, 3 B cell defects, 11 Phagocytic defects, 10 primary HLH, 2 MSMD. The conditioning regimen was myeloablative in children with Wiskott Aldrich Syndrome, Hyper IgM, and Chronic Granulomatous Disease with fludarabine/busulphan. All children with SCID, HLH, CVID, Leucocyte Adhesion Defect were treated with a reduced intensity conditioning using fludarabine/treosulphan. Co-morbidities at the time of HSCT namely infections, disseminated BCG infection and failure to thrive was noted in 62.5% of the SCID babies. All children with WAS and Hyper IgM syndrome had eczema, bloody diarrhea or pneumonia and refractory immune cytopenias. Children with CGD had previous fungal granulomas or tuberculosis, with CVID had bronchiectasis and with LAD had non-healing ulcers. Among children with SCID, most common cause of death was bacterial sepsis whilst children with WAS died due to immune cytopenias. GVHD was the cause of death in 2 children, CMV in 3 children. Primary graft failure was seen in 2 children. Overall survival rate is 62%.

Conclusion: This is the first series in India with survival rates of 62% in children with PID. Conditioning regimens need to be chosen based on the genotype of an individual.
child. The pre-engraftment phase is critical in babies with SCID due to maximum mortality risk due to bacterial sepsis during this phase. Wiskott-Aldrich syndrome poses unique challenges due to immune dysregulation and these children need to be monitored for late immune cytopenias affecting mortality in over 50%. GVHD is a predominant problem in children with CGD with a risk of 80%. In children with primary HLH and less than 6 months of age, acute pulmonary haemorrhage is a risk factor affecting mortality. In all these children, CMV viral load needs to be monitored and treated early. Haploidentical HSCT is a feasible option in children with no matched family donors with success rates on par with unrelated donor HSCT.

**IVIG Replacement Is Essential for DOCK8 Deficiency Patients**

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**Background and Aims:** DOCK8 immunodeficiency syndrome (DIDS) is a combined immunodeficiency characterised by recurrent viral infections, severe atopy, and early onset malignancy. Immunological abnormalities include lymphopenia, defective antibody function, and variable serum immunoglobulin levels. Here, we analyse the B cell receptor repertoire (BCR) characteristics and antibody avidity of DOCK8 patients, and attempt to understand the possible mechanisms of humoral immunity dysregulation, and to provide the scientific basis for intravenous immunoglobulin (IVIG) replacement therapy of these patients.

**Methods:** Three patients with DIDS were enrolled in the study. Analysis of BCR characteristics including somatic hypermutation (SHM) frequency was performed by using deep sequencing on multiplex PCR products of BCR heavy chain CDR3s. The antibody avidity of human tetanus and haemophilus influenza B antibodies was determined by ELISA using thiocyanate elution. IVIG replacement treatment and infection condition were retrospectively investigated.

**Results:** For individual samples, means of 63,635 to 72,384, and 50,048 to 106,868 unique CDR3 sequences were generated in DOCK8 deficiency patients, and healthy controls. Regarding the gene usage frequencies, the usage of IGHV1-2_IGHJ3, IGHV3-3-IGHJ2, IGHV7-4-1_IGHJ4 decreased in patients with DIDS compared to healthy controls, while the usage of IGHV1-8_IGHJ6, IGHV2-5_IGHJ6, IGHV3-53_IGHJ5, IGHV4-39_IGHJ4, IGHV4-61_IGHJ4 increased. Negatively charged amino acids in patients with DIDS were decreased compared to healthy controls. The SHM frequency of IGHV3 gene and IGHV4-55 gene were decreased in patients with DIDS. The antibody avidity of human tetanus antibody in one patient with DIDS was reduced compared to healthy control (2.871 mol/L vs 5.871 mol/L). The antibody avidity of haemophilus influenza B antibody in two patients with DIDS was reduced compared to healthy controls (0.302 mol/L vs 2.027 mol/L, 0.369 mol/L vs 2.326 mol/L). Patients received regular IVIG therapy with reduced frequency of infections and improved severity of infections.

**Conclusions:** Our results reveal skewing of BCR repertoire and decreased antibody avidity in patients with DIDS. Although IgG level is normal in DOCK8 patients, the IVIG replacement therapy is still necessary.

**ORMDL3 May Participate in the Pathogenesis of Bronchial Epithelial-mesenchymal Transition in Asthmatic Mice with Airway Remodelling**

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**Background and Aims:** Asthma is a common chronic respiratory disease in children and is caused by a complex interaction of genetic and environmental factors. Orosomucoid-like 3 (ORMDL3) is a candidate gene that has been strongly linked to asthma, but the underlying mechanisms are unknown. ORMDL3 regulates the expression of metalloproteinases and TGF-β, and ORMDL3 transgenic mice exhibit increased airway remodelling. Hence, ORMDL3 may be associated with airway remodelling. We attempted to examine the relationship between ORMDL3 and the severity of airway remodelling in asthmatic mice and to determine whether ORMDL3 induces epithelial-mesenchymal transition (EMT) in the bronchial epithelium.

**Methods:** BALB/c mice were randomly assigned to control and asthma groups. Lung tissues were collected on days 3, 7, and 14 of ovalbumin (OVA) challenge. We observed airway remodelling in asthmatic mice by hematoxylin and eosin (HE) and Masson staining. Morphological changes in the bronchial epithelium were assessed by transmission electron microscopy. The EMT-related indicators E-cadherin (E-cad), fibroblast-specific protein 1 (FSP1), and Vimentin (VIM) were assessed by western blotting and real-time PCR at different time points.
of airway remodelling in asthmatic mice to detect the EMT trend. Then, the localisation of ORMDL3 was observed by immunohistochemistry, and its protein and mRNA expression was examined by western blotting and real-time PCR, respectively. Furthermore, the bronchial epithelial cell line 16HBE14o- was transfected with an ORMDL3-expressing plasmid, and changes in E-cad, FSP-1, and VIM were detected by immunofluorescence, western blotting and real-time PCR, and cell invasive ability was assessed by microscopy.

**Results:** ORMDL3 expression in the bronchial epithelium was correlated with airway remodelling and EMT progression in vivo. Transfection of ORMDL3 into 16HBE14o- cells in vitro induced EMT.

**Conclusions:** ORMDL3 may regulate EMT in the bronchial epithelium, thereby affecting airway remodelling in asthma.

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**Long-term Prognosis and Genotype-phenotype Correlations of Patients with Left Ventricular Noncompaction**

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**Background and Aims:** Left ventricular noncompaction (LVNC) has since been classified as a primary genetic cardiomyopathy, but the genetic basis is not fully evaluated. The aim of the present study was to identify the genetic spectrum using next- generation sequencing (NGS) and to evaluate genotype-phenotype correlations in LVNC patients.

**Methods:** We targeted and sequenced 73 genes related to cardiomyopathy in 102 LVNC patients using NGS. Clinical evaluation consisted of clinical presentation and symptoms; a personal and family history; arrhythmia; thromboembolism; electrocardiogram (ECG); two-dimensional Doppler, and color Doppler echocardiography.

**Results:** A total of 102 patients enrolled in this study; 54 male and 48 female, aged from fetus to 12 years old. We identified 43 pathogenic variants (39 were missense, 1 deletion, 1 nonsense, and 2 splice site variants) in 16 genes in 39 patients (38%), 28 were novel variants. Sarcomere gene variants accounted for 63%, variants in genes associated with channelopathies accounted for 12%. Overall, MYH7 was most commonly mutated (n=19, 44%), followed by TAZ, and rare variant collapsing analysis showed variants in these two genes contributed to the risk for LVNC. There was only one pathogenic variant in each of MYBPC3, TNNC1, LMNA, ANK2, KCNH2, KCNE3, JUP, HCN4, BMPRIA, and TBX5. Patients with pathogenic variants had early age of onset, and more severely decreased LV ejection fractions. Double heterozygous variants were identified in four patients, all of whom presented with congestive heart failure during the fetal or neonatal periods, and died before their first birthday. Survival analysis revealed that patients with double variants showed worst prognosis compared to patients with single variant and without variants. There were no differences between the age onset, heart failure onset, LVEF and family history and between the sarcomere and non-sarcomere groups, but survival analysis showed that the prognosis of patients with non-sarcomere variants was worse than patients with sarcomere variants. Patients carrying MYH7 and TAZ pathogenic variants displayed different phenotypes. Adverse events were noted in 17 patients, including 13 deaths, 3 heart transplantation and one implantable cardioverter-defibrillator insertion. Congestive heart failure at diagnosis and pathogenic variants were independent risk factors for these adverse events.

**Conclusions:** NGS revealed a wide spectrum of genetic variations and a high incidence of pathogenic variants in LVNC patients. These pathogenic variants were independent risk factor for adverse events. Patients harboring pathogenic variants showed poor prognosis and should be closely followed.

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**A Gain-of-function SYK Mutation in a Very Early Onset Inflammatory Bowel Disease Patient**

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**Background and Aims:** Severe forms of inflammatory bowel disease that develop in very young children are often caused by single gene defects. This study is aimed to determine the causative mutation in novel gene and pathways by whole exome sequencing (WES) and define precision medicine approaches based on the underlying genetic defect.

**Methods:** We performed WES analysis in the parent-child trio samples and confirmed by Sanger sequence. We used luciferase assay, western blot and immunoprecipitation to determine the different effects on NF-κB and MAPK signaling pathway between wild type and mutant group. We also used the inhibitor R406 to investigate its role on the activation of NF-κB and MAPK signaling pathways.
**Results:** We identified an infantile IBD case who presented in the first two weeks of life with severe colitis and fistulising disease with a novel *de novo* autosomal dominant S550Y mutation in the Spleen Tyrosine Kinase (SYK). Functional studies demonstrated that the mutation resulted in hyper-tyrosine phosphorylation of SYK both in vitro and in PBMCs isolated from the patient. The S550Y SYK mutation resulted in enhanced auto-phosphorylation of Y525/526 and subsequent activation of both NF-κB and MAPK signaling through stabilising the binding of SYK to TAB2 and TRAF3/6. These effects could be partially reversed using the SYK inhibitor R406.

**Conclusions:** The novel de novo SYK mutation is the first SYK mutation identified to cause human disease and functional studies suggest targeting SYK may be beneficial for treating patients with arthritis and colitis.

Sleep Duration is Negatively Associated with Carotid Intima-media Thickness in Adolescents

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**Background and Aims:** Sleep plays an essential role in maintaining metabolic homeostasis, ensuring memory consolidation and body restoration. However, sleep deprivation is an increasing phenomenon, and in adults, emerging evidence suggests chronic sleep deprivation can lead to adverse cardiovascular events. Sleep pattern tracks from young age and whether sleep deprivation in adolescents is associated with any cardiovascular risks remains unknown. In this study, we aimed to investigate the relationship between sleep duration and carotid intima-media thickness (CIMT) in adolescents. We hypothesised that short sleep duration was associated with increased CIMT in this paediatric population.

**Methods:** Healthy subjects aged 10-18 years old were recruited from a school-based cohort established to examine the prevalence of obstructive sleep apnoea in Hong Kong. All subjects underwent anthropometric measurements, overnight polysomnography (PSG) and CIMT assessment. Mean sleep duration was obtained from a prospective 7-day sleep diary. Subjects who were overweight or with an obstructive apnoea-hypoapnoea index (OAHI) ≥5 were excluded from the analysis. The subjects were divided into groups according to their mean sleep duration for comparisons, regression analysis was used to assess the association between CIMT and sleep duration and other possible correlates.

**Results:** One hundred and forty one subjects completed the assessments. Male subjects tended to have shorter sleep duration than females. There were no significant differences in age, BMI, tanner stage and parental history of hypertension between groups of different sleep durations. Subjects with shorter sleep duration had higher CIMT ($r=-0.267$, $p=0.001$). Sleep duration was an independent parameter negatively associated with CIMT.

**Conclusions:** Sleep duration was found to be negatively associated with CIMT in adolescents. Adult adverse cardiovascular events may take its origin from adolescence as a result of chronic sleep deprivation. Our study endorsed the importance of adequate sleep duration in adolescents, a critical period when various physiological changes are taking place.

Establishment of the Nasal Microbiota in the First 18 Months of Life: Correlation with Early Onset Rhinitis and Wheezing

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**Background and Aims:** We hypothesise that the dynamic establishment of the nasal microbiota in early life influences local mucosal immune responses and the susceptibility to develop respiratory disorders in childhood. The aim of this study was to monitor, evaluate and compare the development of the nasal microbiota of healthy infants with those who developed respiratory symptoms in the first 18 months of life.
**Methods:** From a larger birth cohort (n=1,237), Growing Up in Singapore Towards healthy Outcomes (GUSTO), a subcohort of subjects (n=122) was selected and further categorised according to respiratory outcomes in the first 18 months into (1) rhinitis alone (symptoms of sneezing, runny and/or blocked nose lasting for at least four weeks in the first 18 months) (n=28), (2) rhinitis with concomitant wheeze (n=34) and (3) controls (n=60). Controls were selected with similar characteristics (mode of delivery, age, birth order and day-care attendance) and had negative responses for rhinitis and wheeze. Anterior nasal swabs were collected at seven time points. Nasal microbiota signatures were analysed via 16S rRNA multiplexed pair-end sequencing. Statistical analysis included: univariate and longitudinal multivariate to evaluate bacteria abundance over time and correlation analysis to analyse relationship between bacteria groups.

**Results:** Overall bacterial diversity (Shannon Diversity Index) increased over time in control infants (p=0.026), but decreased in rhinitis infants (without wheeze: p=0.046; with wheeze: p=0.034). The relative abundance of two main dominant phyla showed significant differences in trends between the three clinical groups. *Proteobacteria* was significantly increased in rhinitis with wheeze (p=0.026) with a corresponding decrease in *Actinobacteria* in both rhinitis groups compared to controls (rhinitis alone p<0.01, rhinitis with wheeze p<0.05). These differences in phyla were related to *Oxalobacteraceae* family for *Proteobacteria* phyla, and *Corynebacteriaceae* family and *Corynebacterium* spp. for *Actinobacteria* phyla. No differences were observed in the abundance of *Firmicutes* phylum between clinical groups, however, an increased in abundance of *Alloiococcus* spp. (*Aerococcaceae* family) was associated with rhinitis and concomitant wheeze (p<0.01). In only rhinitis and concomitant wheeze group, *Corynebacteriaceae* showed strongest negative correlation with those of *Oxalobacteraceae* (Rs=-0.6286) and *Aerococcaceae* (Rs=-0.7412) (p<0.01). There was no correlation between *Aerococcaceae* and *Oxalobacteraceae*.

**Conclusions:** The nasal microbiome profiles differed between healthy and rhinitis in the first 18 months of life, especially in those with concomitant wheeze. These results support the hypothesis that nasal microbiome plays a role in the development of respiratory disorders in children. This study provides insights into the development of novel strategies for the treatment and management of these disorders.

**Liver and Spleen Stiffness for Predicting the Presence and Severity of Esophageal Varices in Children with Chronic Liver Diseases**

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**Background and Aims:** Esophageal varices (EV) caused by portal hypertension among children with chronic liver diseases can lead to significant morbidity and mortality from variceal bleeding. Esophagogastroduodenoscopy (EGD) is currently the most accepted tool to diagnose EV but it is considered invasive. Recent studies have shown that spleen stiffness by using transient elastography (TE) can predict EV in adults. However, studies of spleen stiffness in children are limited and its cutoff for the presence of EV has not been reported. We aimed to determine the correlation of liver and spleen stiffness by TE for the presence and grading of EV and identify the cutoffs of stiffness data for predicting presence of EV.

**Methods:** Children aged <15 years with chronic liver disease and portal hypertension were invited to enroll for EGD (to evaluate EV) and TE (FibroScan®). Data on liver stiffness measurement (LSM) and spleen stiffness measurement (SSM) were collected. Information on the LSM and SSM was blinded to the endoscopists, and vice versa. LSM and SSM were then analysed for the potential cutoff values, sensitivity and specificity of the presence and grading of EV.

**Results:** We studied 40 patients (65% female, 70% with biliary atresia, median age of 20.5 months). Median (interquartile range) of LSM and SSM were 69 (19-75) and 37 (14-75) kilopascal (kPa), respectively. EV grade I, II and III were noted in 10, 9, and 10 patients, respectively. Both LSM and SSM had significant correlations with the presence of EV (r=0.53, p=0.001 and r=0.43, p=0.007). Furthermore, significant correlation was noted between SSM and EV grade II-III vs. grade 0-I (r=0.43, p=0.008) but not for LSM (r=0.26, p=0.12). Area under the ROC for LSM and SSM with the presence of EV was 0.83 (95%CI 0.66-0.99) and 0.77 (95%CI 0.61-0.93). The combination of LSM and SSM by applying the cutoff of 18.8 kPa for LSM and 16.9 kPa for SSM provided 83% sensitivity and 82% specificity for the presence of EV.

**Conclusion:** Transient elastography defining LSM and SSM can be considered to use as a non-invasive screening method for the presence of EV and of large EV.
SHANK3 Deletion and Related Phenotypes in Chinese Children with Autism and Shank3-KO Zebrafish Display Autistic-like Behaviours

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Background and Aims: Autism spectrum disorder (ASD) is well known as a heritable, debilitating neurodevelopmental disorder manifesting in early development. A mount of studies showed that SHANK3 gene had a strong causal relationship with ASD and/or 22q13.3 deletion syndrome. However, the data of Chinese ASD patients with SHANK3 deletion is insufficient and the mechanism is not clear.

Methods: MLPA and Sanger sequencing were carried out to confirm the SHANK3 deficiency of Chinese children. Moreover, systematic and comprehensive evaluations were performed to Chinese-specific features. In addition, shank3 was knock-out (KO) using a CRISPR/Cas9 system in zebrafish to build a transgenic zebrafish model.

Results: As to the patients, six participants lacked the whole gene of SHANK3 with 22q13.3 deletions ranging in size from 55 kb to 4.8 Mb and three participants with de novo SHANK3 mutation were included. They were characterised by high rates (100%) of ASD, developmental delay, hypotonia, several dysmorphologies and perception abreaction. New and rare features were also viewed in this study: ectropion of nostril sparse hair, ankle deformity, whole-body hairy, hanked-3-lap arms, snaggletoothed or extra teeth and unusual-dehydrated skin, and extreme hyperactivity/self-stimulation. As to the zebrafish model, the shank3-KO zebrafish displayed varying degrees of developmental retardation compared with the wild-type zebrafish, such as ventral curled body, less melanin, less somites and so on. Moreover, the homozygous zebrafish were more significant than the heterozygotes. What’s more, in zebrafish social interaction test, shank3-KO zebrafish showed less interest exploring conspecific zebrafish both in swimming distance ratio and swimming time ratio. Furthermore, in zebrafish social preference test, shank3-KO zebrafish displayed reduced polarisation of fish shoals, looser and larger schools, and higher percentage of fish leaving the group and spending time outside the shoal which implied a disorganised social structure. In addition to social deficits, the trace pattern analysis of zebrafish found several obvious behavioural stereotypes, such as repetitive, stereotypic “repeated self-rotation” swimming behaviour.

Conclusions: In our study, the severity of intellectual, hypotonia, and speech impairments were seen in SHANK3 deficiency which highlighted the prominence of SHANK3 in ASD. Zebrafish, a typical animal model, will play a critical role in further studying the relationship between phenotype and genotype of ASD and insighting into the molecular mechanisms underlying the clinical heterogeneity of ASD.

Myocardium Specific Gene Therapy Can Partly Rescue Cardiac Troponin T Deficiency Related Cardiomyopathy

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Background and Aims: Cardiac troponin T (cTnT) gene mutations can lead to cardiomyopathy. Gene supplement therapy is the most direct and viable option for diseases caused by a single structural protein deficiency. We plan to generate a transgenic zebrafish line to study the feasibility of myocardium specific gene therapy on cTnT gene mutated-related cardiomyopathy.

Methods: Firstly, we generated to a transgenic zebrafish line of temporal and spatial specific cTnT gene expression by injecting a Tol2-based knock-in vector carrying the cardiac specific promoter, cmle2, the Tet-on system, and red fluorescence fusion labeled tnnt2a cDNA sequence. Then, we screened F1 and crossed it with tnnt2a mutant zebrafish (obtained from our lab), the in situ dosage-induced expression transgenic therapy zebrafish tnnt2a mutated model was obtained. Lastly, Dox induction test and RNA-seq analysis were performed to observe whether myocardium specific gene therapy can be feasible and find underlying causes.

Results: The Tg (cmle2-tetone-tnnt2a-p2A-mKate2; cmle2; EGFP; tnnt2a+) was eventually obtained, which was proved by fluorescence reaction and genome sequencing. The phenotype of the Tg zebrafish assembles dilated cardiomyopathy without supplement therapy. Moreover, DOX induction test showed that the partial cardiac abnormal phenotypes of tnnt2a mutant could be rescued, such as atrium and ventricle enlargement, no heart beats. However, the optimisation of multiple doses and induction time did not completely restore the heart shape. What’s more. That the dysregulation expressions of the valve and outflow tract associated genes had not been rescued efficiently through transcriptomics analysis would be the underlying cause of partial rescue.

Conclusions: Gene supplement therapy was feasible for cardiomyopathy caused by cTnT gene mutant. However, for the treatment of TNNT2-relevant cardiomyopathy, it was
necessary to take into account the gene supplementation of myocyte, valve and outflow tract cell.

**The Construction of SENP1 Specific Lentiviral Vector and Its Effects on Apoptosis of Alveolar Epithelial Cells Induced by Hyperoxia**

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**Objective:** To construct a SENP1-RNAi lentivirus vector and establish human type II alveolar epithelial cells (HEPApiC) that stably express SUMO specific protease 1 (SENP 1). To investigate the relationship between SENP1 and apoptosis induced by oxidative in HEPApiC after exposing to hyperoxia.

**Methods:** This experiment was divided into two parts. Part one: Preparing for virus particles with constructed plasmid LV3-SENP 1-RNAi to infect the HEPApiC cells, and detecting the expression of SENP 1 in different groups by qRT-PCR and Western blot. Part two: the experiment was based on SENP 1 stably silenced HEPApiC cells and the model of lung injury was induced by mixture gas formed with \( O_2 \) (900 ml/L) and \( CO_2 \) (50 ml/L). Cells were randomly divided into 6 groups: control group, empty vector infected group, experimental group, hyperoxia group, hyperoxia and empty vector infected group, hyperoxia and experimental group. After culturing for 12h, 24h and 48h, cells would be collected and the indicators would be detected. Obtaining cells apoptosis situation through flow cytometry instrument after 24h. Then testing the transposition of SIRT1 by immunofluorescence technique after 24h. Measuring the expression of SENP1, SIRT1 separately in cytoplasm and nucleus, P53 and AC-P53 by Western Blot after 24h.

**Results:** Part one: LV3-SENP 1-RNAi was successfully constructed and virus was correctly packaged. Part two: the apoptosis rate increased when cells were dealt with by hyperoxia 24h later according to the results of flow cytometry instrument. Furthermore, the apoptosis rate in hyperoxia and experimental group was lower than another two groups disposed by hyperoxia. Immunofluorescence results showed that SIRT1 protein translocated more in those groups dealt with hyperoxia and the difference between six groups was statistically significant \( (\chi^2=99.34, p=0.000 <0.05) \). Finally, compared with control group, at the level of protein, the expression of SENP1, SIRT1 in cytoplasmic, AC-P53 expressed less as SIRT1 in nucleus expressed more than that in hyperoxia group, but both were failed to meet the level of control group \( (p<0.05) \).

**Conclusion:** SENP1 and SIRT1 were involved in the oxidative stress induced by hyperoxia in preterm infants. Hyperoxia led to higher expression of SENP1. Then the translocation of SIRT1 increased. SIRT1 decreased in nucleus and increased in cytoplasm, which induced acetylation of P53 and apoptosis.

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**MiR-29b Regulates Cardiomyocytes Proliferation via Targeting NOTCH2**

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**Background and Aims:** Tetralogy of Fallot (TOF) is a developmental cardiac manifestation with an incidence of 3 per 10,000 live births. Recent study have showed that TOF is relevant to altered proliferation, migration, or differentiation of the precardiac cells of the secondary heart field. Researches have addressed the role of microRNA (miRNAs) in cardiac development. The role of miR-29b in adult cardiovascular diseases including cardiac fibrosis, dilated cardiomyopathy and myocardial ischaemia-reperfusion injury has been studied widely, yet the involvement of miRNA-29b in TOF remains unclear. Our aim is to explore the effect and mechanisms of miR-29b on contributing to TOF pathogenesis.

**Methods:** A total of 13 TOF patients and 7 normal controls were included in our study. All tissue samples were obtained from the right ventricular outflow tract (RVOT) immediately after surgical resection or autopsy. Real-time RT-PCR and Western Blot were used to quantify genes expression. Tg Cmlc2: GFP reporter zebrafish embryo were microinjected with miR-29b to explore its role in cardiac development in vivo. Dual-luciferase reporter assay was designed to validate the target gene. CCK-8, EdU incorporation assay and flow cytometry were performed to evaluate cardiomyocyte proliferation.

**Results:** (1) We observed that miR-29b-3p was up-regulated in the RVOT of TOF patients when compared with normal controls. (2) Zebrafish injected with miR-29b-3p mimics exhibited abnormal heart morphology and function. The proliferation rate of zebrafish cardiomyocytes was also reduced in vivo. (3) CCK-8 and EdU incorporation assay showed that miR29b-3p mimics potently inhibited cardiomyocytes proliferation in vitro. Conversely, inhibition
of miR29b-3p substantially induced cardiomyocytes proliferation. (4) A higher proportion of cells in G2/M stage in miR29b-3p mimics group was observed, which suggest that miR29b-3p could arrest cardiomyocytes in G2/M stage. Positive cell cycle regulators, such as cyclins, catenin beta 1 and PCNA, were down-regulated in miR29b-3p mimics group. (5) We observed that NOTCH2 were significantly decreased RVOT of TOF patients. DLR assay identified NOTCH2 was a direct target of miR-29b-3p. (6) Transfection of NOTCH2 siRNA significantly decreased cardiomyocytes proliferation. Moreover, the promoting effect of miR-29b-3p inhibitor on proliferation were partly abrogated by Notch2 siRNA in cardiomyocytes.

**Conclusions:** miR-29b-3p functions as a novel regulator of cardiac development and inhibits cardiomyocytes proliferation via direct targeting of the NOTCH2, which provides groundwork for a new therapy approach to TOF.

**Asthma Diagnosis Was Associated with Single-nucleotide Polymorphisms of the Gene Encoding Human Rhinovirus-C Receptor in Children**

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**Background and Aims:** Asthma is a common obstructive lung disease in children. Rs6967330 of CDHR3, being the gene encoding human rhinovirus-C (HRV-C) receptor, was reported to be a risk factor for severe asthma exacerbations in Danish preschoolers. Recent data suggested that the mutant of this single-nucleotide polymorphism (SNP) increased bronchial epithelial susceptibility to HRV-C infection, but the relevance of CDHR3 for asthma diagnosis remains unclear. This study investigated the association between CDHR3 and childhood asthma diagnosis and subphenotypes.

**Methods:** Ten tagging SNPs located within 5-kb both upstream and downstream from rs6967330 were selected by HaploView 5.0 based on 1000 Genomes database searched at pairwise $r^2 \geq 0.8$ for linkage disequilibrium (LD) for all SNPs with minor allele frequencies (MAFs) $\geq 0.01$ in Southern Han Chinese (CHS). These tagging SNPs were genotyped by TaqMan assays on QuantStudio 12K Flex real-time PCR system. Genetic associations between these SNPs and categorical and quantitative variables were analysed by logistic and linear regression, respectively, fitted for recessive and co-dominant models.

**Results:** 903 Chinese children with asthma and 1205 non-allergic controls were recruited, with their mean (SD) age in years being 11.0 (4.1) and 13.6 (4.5). Atopy, defined as having at least one positive skin prick test or aeroallergen-specific immunoglobulin E, occurred in 75.3% of patients and 37.9% of controls ($p<0.0001$). The overall genotyping efficiency was $\geq 95\%$. MAFs of tested SNPs were comparable to those published for CHS, except rs448025 and rs543085868 which were monomorphic. Asthma diagnosis was significantly associated with rs6967330 under additive model (odds ratio [OR] 1.34 and 95% confidence interval [CI] 1.00-1.80; $p=0.049$) and dominant model (OR 1.40 and 95% CI 1.03-1.90; $p=0.032$). This SNP, however, was not associated with atopy or spirometric indices. None of the other SNPs was associated with asthma diagnosis or subphenotypes.

**Conclusions:** Rs6967330 of CDHR3 is associated with asthma susceptibility in Hong Kong Chinese children, but none of the SNPs is associated with patients' lung function. Whereas these results may reflect the importance of HRV-C infection in modulating asthma susceptibility, prospective studies with larger sample size are needed to confirm this genetic association.

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A Plausible Biomarker to Predict Relapsed Disease in a Very High-risk Group of Paediatric Acute Lymphoblastic Leukaemia

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Background: Acute lymphoblastic leukaemia (ALL) is the most common paediatric oncologic disease worldwide. While the incidence rate in Thailand was 2.88 per 100,000 children per year, the 5-year overall survival rate was 64.9%. Mortality usually related to relapsed disease. Predictive biomarker of relapsed ALL is therefore one of the unmet clinical needs. Proteomics is a promising technology for biomarker discovery by elucidating differential expression of 100-1,000 proteins between disease states and conditions. Proteomic profiling of relapsed and newly diagnosed leukaemic cells of the very high risk group may provide predictive biomarkers of relapsed ALL in paediatric patients.

Objectives: (1) To identify the differences of the proteomics profiles of relapsed leukaemic cells with newly diagnosed leukaemic cells in a very high-risk group of ALL patients. (2) To analyse the correlation of proteomics profiles to clinical information of relapsed very high risk ALL patients.

Study Designs: Cross-sectional study

Material and Methods: Paediatric ALL patients diagnosed during 2008-2014 at Ramathibodi Hospital were enrolled. Very high risk ALL patients whose bone marrow samples had collected were included, while those with poor quality of bone marrow samples were excluded. Clinical information, including dates (birth, diagnosis, relapse and last follow up), disease status (initial WBC count, serum lactate dehydrogenase, immuno-phenotype, molecular genetics, cytogenetic and organ involvement) and chemotherapy protocols was collected. Samples of the very high risk group ALL patients were categorised into two groups (relapsed vs. newly-diagnosed) and then submitted to proteomic analysis, consisted of 2-D gel electrophoresis (2-DE), protein spot matching, in-gel tryptic digestion, MALDI-TOF/TOF protein identification and validation using western blot analysis.

Results: A total of 195 ALL cases were enrolled. Among these, fourteen cases were classified as the very high risk group. Only eight BM specimens were qualified for proteomic analysis, which then divided into relapsed and newly-diagnosed groups (2 relapsed and 6 newly-diagnosed). Proteomic analysis showed 22 protein spots had significantly different intensities between relapse and newly-diagnosed group (p<0.05) but there were only 3 protein spots (i.e., ATPB; ATP synthase subunit beta, TUBB1; Tubulin beta-1 chain and PHB; prohibitin) that had pattern of protein expressions same as previous reports, related to leukaemia. Of these, the decreased expression of ATPB was validated by western immunoblotting.

Conclusions: This study employed proteomic analysis to elucidate ATPB as a candidate biomarker of relapsed disease. Further validation study using an independent cohort is required to demonstrate clinical applicability of ATPB in the very high risk group of paediatric ALL.

Paediatric T-cell Acute Lymphoblastic Leukaemia (T-ALL): An Integrated Genetic and Epigenetic Analysis

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Background and Aims: Paediatric T-cell acute lymphoblastic leukaemia (T-ALL) is an aggressive haematologic malignancy accounting for 10% to 15% of newly diagnosed paediatric ALL cases. Although genetic basis of paediatric T-ALL has been well characterised by several reports including our recent research, no
A comprehensive study has yet explored the epigenetic profiles and their potential contribution to clinicopathological features of T-ALL.

**Methods:** To describe epigenetic landscape of T-ALL, we conducted methylation array analysis using Illumina HumanMethylationEPIC array, whole transcriptome sequencing (WTS), and targeted-capture sequencing for 158 ALL-related genes in a cohort of 48 cases with T-ALL. Following analyses were performed using R (v3.3.0) and ChAMP package.

**Results:** After normalisation, 3210 probes were selected to identify the most variable methylated probes, when a standard deviation of the beta-values across the samples was above 0.35. Unsupervised consensus clustering using standard deviation of the beta-values across the samples was above 0.35. Unsupervised consensus clustering using selected 3210 probes clearly identified 4 distinct sample clusters. Combined analyses with expression and fusion data from WTS revealed that these 4 clusters were characterised by 438 methylation, high methylation, respectively.

**Conclusions:** Based on the DNA methylation profiles, paediatric T-ALL is clustered into 4 distinct subtypes, which exhibited remarkable correlation with fusion gene status, gene expression patterns, genetic signatures, and clinical outcomes. Especially ALDH1A2 was significantly upregulated in M1 cluster, suggesting that activation of retinoic acid signaling pathway has a potential role in the pathogenesis of T-ALL. Although our cohort in the current study is very limited, our results suggested that the biological phenotype of T-ALL is mediated by both genetic and epigenetic regulations. Therefore, explorations for aberrant DNA methylation along with genetic alterations might be helpful for development a new therapeutic strategy for T-ALL.

**Association of Factor VIII and Factor IX Mutations, HLA Class II, Tumour Necrosis Factor-α and Interleukin-10 on Inhibitor Development Among Thai Haemophilia A and B Patients**

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**Objectives:** To investigate the association of factor VIII and IX mutations, HLA-DRB1, TNF-α and IL-10 influencing the risk of inhibitor development among Thai patients with haemophilia A and B.

**Methods:** A total of 100 patients with haemophilia A (severe 84, moderate 16 cases) and 36 with haemophilia B with a mean age of 14.4 years were enrolled. All patients received on-demand treatment for episodic bleeding and home treatment for early bleeding. Plasma derived products of cryoprecipitate, cryoprecipitate-removed plasma, FFP, fresh dried plasma, heat-treated lyophilised cryoprecipitate and factor concentrate were given exclusively.

**Results:** The occurrence of high inhibitor among patients with severe haemophilia A was 29.7% (25/84). Factor VIII mutations were identified in 97% (97/100) including intron 22 inversion (n=53), large deletion (n=5), nonsense (n=23) and missense mutations (n=16). Eleven were novel. The risk of high inhibitor among patients with severe degree was found in intron 22 inversion, 34.7% (17/79); large deletion, 40% (2/5) and nonsense mutation, 25% (5/20) similar to other studies. However, neither of patients with missense mutation developed high inhibitor. Factor IX mutations were identified in all 36 studied patients including missense (n=24), nonsense (n=7), splicing site (n=3) and promoter (n=2). Seven were novel. Unfortunately, inhibitor was found in one patient with severe haemophilia B whose mutation was a point mutation at exon 2: c.223C>T, p.R75X. No significant difference among frequencies of HLA-DRB1*15, TNF-α-308A and IL-10-1082G alleles in haemophiliacs with and without inhibitor, was found.

**Conclusion:** Thai patients with severe haemophilia A receiving exclusively plasma-derived products of FFP, cryoprecipitate, and small amount of factor concentrates on episodic treatment plus home treatment for early bleeding episodes, possessed a 29.4% chance of high inhibitor. Factor
VIII and factor IX mutations showed a significant contribution for inhibitor development. The HLA-DRB 1*15, TNF- \( \alpha \)-308A and at the IL-10-1082G were of lesser impact.

The Safety and Efficacy of Plasma-derived Factor VIII Concentrate Produced by the National Blood Center, Thai Red Cross Society

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Objectives: To study the safety and efficacy of factor VIII concentrate produced by the National Blood Center, Thai Red Cross Society among Thai patients with haemophilia A.

Methods: Previously-treated patients with haemophilia A for more than 150 days were enrolled in the study. After 5-day wash-out period, they received 500 units of locally-produced factor VIII concentrate. The vital signs and clinical manifestations were closely monitored before and at 1, 2 and 4 hours. Factor VIII clotting activity (FVIII:C) was determined before and 30 minutes after factor VIII concentrate administration. Then, 500 units of factor VIII concentrate twice weekly were prescribed as a low dose prophylaxis regimen for 3 months. In cases of any bleeding episodes, the patients received only locally-produced factor VIII concentrate. The infectious markers of HIV, hepatitis A, B and C viruses were screened before and after 3 months of factor VIII administration. Also, the pharmacokinetics of FVIII:C were studied among 10 patients.

Results: In total, 37 patients with haemophilia A (severe 33, moderate 4) were enrolled in the study. Altogether, 32 patients completed the study. Their mean (SD) age was 20.4 (6.9) years old. The mean (SD) incremental FVIII:C after receiving one unit of factor VIII concentrate per BW (kg) was 2.3% (0.5). The pharmacokinetics analysis revealed the half-life of FVIII:C at 12.7 hours. The efficacy of bleeding control among 42 episodes found in 18 patients was 2.3% (0.5). The pharmacokinetics analysis revealed the half-life of FVIII:C at 12.7 hours. The efficacy of bleeding control among 42 episodes found in 18 patients was 2.3% (0.5). The pharmacokinetics analysis revealed the half-life of FVIII:C at 12.7 hours. The efficacy of bleeding control among 42 episodes found in 18 patients was 2.3% (0.5).

Conclusion: Factor VIII concentrate produced by the National Blood Center, Thai Red Cross Society showed safety and efficacy in bleeding control among Thai haemophilia A patients with severe and moderate degrees.

Integrated Genetic and Epigenetic Analysis of Neuroblastoma Utilising the Open Dataset

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Background and Aims: Neuroblastoma (NBL) is the most common extracranial solid tumour in children and often associated with a poor prognosis. Besides few known abnormalities such as ALK mutation and MYCN amplification, molecular features of NBL are still poorly understood. Many genetic/epigenetic datasets are currently available as open data, which are of use in the research of relatively rare paediatric tumours. We re-analysed an open data, focusing on high-risk NBL to disclose whole picture of genetic/epigenetic basis of high-risk NBL.

Methods: We analysed 94 samples with high-risk NBL from the open data of "TARGET NBL" from National Cancer Institute, which contains all the three-platform data; whole exome sequencing, RNA sequencing, and DNA methylation array analysis.

Results: Consensus clustering based on DNA methylation status revealed 5 distinct clusters, which were independent of the pathological findings. Instead, these clusters exhibited remarkable correlation with genetic lesions. Cluster 1 had lower frequency of copy number alterations and gene abnormalities compared to the other clusters. Cluster 2 was characterised by 11q deletion combined with 3p and 4p deletions. ATRX alterations were also enriched in this cluster. 11q deletion was relatively
frequent in both Clusters 3 and 5, but 3p or 4p deletion was less common in these clusters. Clusters 3 and 5 were also characterised by hyperdiploid with frequency of around 80%. Among the 12 cases with ALK mutations, 5 cases were grouped into Cluster 3 and 4 cases with MYCN amplification were included in Cluster 4. Cluster 4 was strongly linked to MYCN amplification and 1p deletions without 11q and 4p deletions. Furthermore, consensus clustering of 51 samples with 11q deletion based on DNA methylation status revealed another 2 distinct clusters. Cluster A was strongly linked to 4p deletion. Event-free-survival of Cluster B was significantly worse than that of Cluster A (p=0.00025).

Conclusions: Although further validation of these insights in additional cohort would be necessary, our results highlight the close relationship with DNA methylation and genetic alterations in high-risk NBL and revealed genetic/epigenetic catalogue of this disease.

Clinical Features and Treatment of Oncologic Emergencies
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Background and Aims: The survival rates of paediatric malignancies continue to improve over the past several decades. This dramatic increase in survival has been accomplished due to new therapeutic strategies and enhanced supportive care. On the other hand, the paediatric oncology patients may present with variety of life-threatening situations resulted from structural or functional compromise of the critical organs, leading to early cancer-related death. Thus, there is a need to better understand oncology emergency in paediatric cancers in order to optimise treatment strategy and further improve the survival rate. Here we report oncologic emergency cases for 8 years in our institute.

Methods: We retrospectively reviewed paediatric oncology cases in our institution from January 2009 to March 2017 and extracted by chief complaints or symptoms at the presentation. We defined eligible symptoms as airway obstruction, respiratory failure, shock and disturbance of consciousness. We investigated age, time to diagnosis from the first physician contacted, the primary lesions, examinations, treatments and outcomes.

Results: In total 230 paediatric oncology cases were assessed, and 8 cases were identified as oncology emergency cases (3 neuroblastoma cases, 1 rhabdomyosarcoma case, 1 unclassifiable sarcoma case, 1 T-LBL/ALL case, 1 aplastic anaemia case and 1 anaplastic ependymoma case). The median age at the diagnosis was 3.5 years (2 to 12 years). The median time to diagnosis was 0.75 months (7 days to 2 months), and 6 patients admitted to paediatric intensive care unit. Emergency surgery was needed in 3 cases, and emergency endotracheal intubation was performed in a case. All patients subsequently received chemotherapy. Currently, 5 cases are alive and 3 cases died, however there was no dying case in the acute phase.

Conclusion: For most paediatric cancers, prognosis generally depends on the biology of the tumour, however, time to diagnosis relates to outcome in paediatric oncologic emergency cases. With prompt recognition and treatment initiation in the emergency department involved by paediatric oncologists, surgeons, radiation oncologists and other medical specialists, lives can be saved and quality of life maintained.

Transcriptomic and Epigenetic Analyses of Hepatoblastoma Identify Subgroups with Different Clinical and Biological Features
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Background and Aims: Hepatoblastoma (HBL) is the most common paediatric liver tumour. Despite intensive multimodal therapy, the prognosis of high-risk HBL remains poor, and this underscores the importance of understanding HBL pathogenesis and developing novel
therapeutic modalities. However, as the mutation rate in HBL is relatively low and molecular targets except for Wnt/beta-catenin pathway have not been established, our understanding of the molecular basis of HBL is still limited.

**Methods:** To obtain a comprehensive set of the molecular lesions in HBL, we performed methylation array analysis, single nucleotide polymorphism (SNP) array-based copy number (CN) analysis and RNA sequencing (RNA-seq) on 31 diagnostic biopsy samples of HBL.

**Results:** SNP array analysis showed frequent whole-arm CN gains in multiple chromosomes, especially in 1q, 2, 5, 6, 7, 8, 17q and 20. Uniparental disomy/trisomy (UPD/UPT) of chromosome 11p was observed in 6 cases (19%). Gene expression data obtained from RNA-seq showed upregulation of Wnt signaling pathway in HBL. Consensus clustering based on methylation data indicated the presence of 3 distinct clusters. Cluster 1 exhibited low expression of \(NQO1\) (encoding a cytoplasmic reductase associated with detoxification pathways) with the promoter hypermethylation, and less frequent CN alterations. Patients with lower serum AFP levels at diagnosis were enriched in this cluster. Being consistent with low expression of \(NQO1\), known as a poor prognostic factor in several kinds of tumours, patients in cluster 3 showed better survival rate, although not reaching statistical significance (log-rank test, \(p=0.07\)). In contrast, cluster 2 exhibited higher expression of \(NQO1\) with the promoter hypomethylation. In addition, all but one case in cluster 2 had 1q/2q gains. Cluster 3 was characterised by high expression of \(GSTP1\) (encoding a glutathione S-transferase that plays an important role in detoxification) associated with the promoter hypomethylation and marked by low age at diagnosis. Intriguingly, cluster 3 included most of the cases with UPD/UPT of chromosome 11p.

**Conclusions:** Our integrated genome-wide study of HBL identified methylation subgroups well correlated with biological and clinical features. It may be useful for clinical risk stratification and searching for new molecular targets.

**Combination of SAHA and Bortezomib Counteracts Anti-apoptotic function of EBNA-3C in Wp-restricted Endemic Burkitt Lymphoma a**

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**Introduction:** Induction of cell death synergism of Burkitt lymphoma (BL) with type III or Wp-restricted latency was found to be achieved by the combination of suberoylanilide hydroxamic acid (SAHA) and bortezomib (SAHA/bortezomib). Nevertheless, this synergistic cell death was not observed in BL with type I latency by this drug combination. Since Epstein-Barr virus nuclear antigen (EBNA)-3A, -3B and -3C proteins are expressed in type III or Wp-restricted latency, but not in type I latency, it was hypothesised that the anti-apoptotic functions of EBNA-3 proteins for the survival of BL cells are offset by SAHA/bortezomib.

**Methods and Results:** The effect of SAHA/bortezomib against the survival of BL cell lines infected with EBNA-3A, -3B or -3C knockout EBV or with their revertant EBV was investigated. Isobologram analysis indicated that the cell death synergism induced by SAHA/bortezomib on EBNA-3C revertant BL was decidedly greater than that on EBNA-3C knockout BL. Nonetheless, the cell death synergism in either BL with type I latency, EBNA-3A or -3B revertant versus their knockout pairs was approximately identical. Furthermore, it was demonstrated that SAHA/bortezomib triggered weaker expression of p-cdc25c (inducer of G2/M arrest), whereas stronger expression of p21\(^{\text{WAF1}}\) (inducer of apoptosis) in EBNA-3C revertant BL than EBNA-3C knockout BL. Moreover, the levels of cyclin B1 and p-cdc2 proteins were found to be increased in EBNA-3C knockout BL, but not in EBNA-3C revertant BL by SAHA/bortezomib treatment. Therefore, G2/M checkpoint arrest induced by SAHA/bortezomib was almost disappeared in EBNA-3C revertant BL which was subsequently prone to apoptosis. On the contrary, noticeable G2/M arrest induced by SAHA/bortezomib was found in EBNA-3C knockout BL. In vivo test by using SCID mice showed that the growth of EBNA-3C revertant xenografts were suppressed more significantly by SAHA/bortezomib, compared with that of EBNA-3C knockout xenografts. Additionally, it was also found that the expression of p21\(^{\text{WAF1}}\), cleavage of PARP and caspase-3 were markedly decreased by N-acetylcysteine, which is a reactive oxygen species (ROS) scavenger, proposing that the induction of apoptosis of EBNA-3C revertant BL was ROS-dependent.

**Conclusion:** It was justified that SAHA/bortezomib counteracted the functions of EBNA-3C and induced apoptosis, which is ROS-dependent, of Wp-restricted Burkitt lymphoma through G2/M checkpoint arrest.
Investigation of the Immunomodulatory Effect on Human Mesenchymal Stem Cells Derived iPSCs and Its Derived Neural Progenitor Cells

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Background and Aims: Induced pluripotent stem cell (iPSC) is conventionally reprogrammed from fibroblast, but other cell types have been explored in order to improve the efficiency and accessibility. Mesenchymal stem cell (MSC) is a somatic multipotent stromal cell with potent immunosuppressive function and can evade from HLA-barrier, making it an attractive adjuvant for transplantation and regenerative medicine applications. We aim to generate iPSCs from MSCs by genetically transducing a panel of stem cells transcription factors. We also would investigate whether the immunomodulatory effects of the reprogrammed MSCs can be retained.

Methods: MSCs isolated from human bone marrow were reprogrammed by Sendai virus-based delivery of Oct3/4, Sox2, Klf4, and c-Myc genes. Colonies of reprogrammed cells were expanded and selected following with characterisations of a panel of stem cells and neural cells markers including SSEA4, TRA1-60, SOX2, NANONG, OCT3/4, SOX1, PAX6, NESTIN and TUJ1 by using immuno-staining, qPCR and flow cytometry. The pluripotency was examined by embryoid body (EB) formation in vitro and teratoma formation in vivo. The immunomodulatory effect was evaluated by co-culture experiments of iPSCs derived-neural progenitor cells with human lymphocytes in vitro. CD3+ T cells stained with CFSE were selected and quantified after activation with specific surface markers CD69 or CD25.

Results and Conclusion: iPSCs were successfully generated from human MSCs. Human MSCs-derived iPSCs (hMSCs-iPSCs) expressed the same transcriptional factors and surface markers as those derived from human embryonic stem (ES) cells. hMSCs-iPSCs also formed embryoid bodies and teratoma, which showed the pluripotency of the cells. Neural progenitor cells derived from the hMSCs-iPSCs highly expressed PAX6, which is a key regulatory gene in brain development. In the co-culture system with lymphocytes, these neural progenitor cells showed lower CD3+ T cells activation and proliferation compared to those derived from ES cells. This may suggest an advantage of iPSCs with MSC origin. Nevertheless, it is necessary and essential to have further research on the underlying mechanisms and potential clinical applications of hMSCs-iPSCs.

Novel Mutations in Patients with Hereditary Red Blood Cell Membrane Disorders Using Next-Generation Sequencing

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Background and Aims: To identify the diagnosis and investigate genotype-phenotype relation of intractable hereditary red blood cell (RBC) membrane cases.

Methods: We have utilised next-generation sequencing (NGS) to provide a high-throughput, highly sensitive assay. Three unrelated families including 15 individuals were analysed with a panel interrogating 600 genes of haematopathy disorders. Where possible, inheritance patterns of pathogenic mutations were determined by sequencing of other relatives.

Results: We found 2 novel mutations in ANK1 (Y216X and E142X) responsible for hereditary spherocytosis (HS), which were stop gain single nucleotide variants (SNV). Furthermore, novel SPTA1 mutation (H54P) has been identified which was a nonsynonymous SNV and associated with hereditary elliptocytosis (HE). In addition, patients who coexist with erythropoiesis gene mutations, showed a more severe disease phenotype.

Conclusions: NGS panel provides a fast and accurate method at a molecular diagnosis in patients with intractable hereditary RBC membrane disorders. An integrated approach of medical history, clinical and molecular test, and pedigree analysis, is beneficial to these patients and families.

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The Study of METTL3 and METTL14 Expression in Childhood ETV6/RUNX1-positive Acute Lymphoblastic Leukemia

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Background and Aims: This study was aimed to explore the METTL3 and METTL14 expression in children with ETV6/RUNX1 (E/R)-positive acute lymphoblastic leukemia (ALL) and investigate the relation between the METTL3 and METTL14 expression with clinical features.

Methods: This study included 37 newly diagnosed E/R-positive ALL children and 6 controls in Institute of Haematology and Blood Disease Hospital. The CD10+CD19+ cells were sorted by flow cytometry (FCM) and mRNA was extracted from these cells. Real-time fluorescent quantitative PCR was used to detect the expression level of METTL3 and METTL14.

Results: Among the 37 cases, 51.35% (n=19) were boys and 48.65% (n=18) were girls and the median age was 4.72 (1.72-11.99) years. Among the 6 controls, 50% (n=3) were boys and 50% (n=3) were girls and the median age was 5.24 (1.53-13.17) years. The expression level of METTL3 and METTL14 in E/R-positive ALL patients were lower than in controls (p<0.05). Although the difference of METTL3 and METTL14 expression level between the relapse patients with non-relapse patients did not have statistical significant (p=0.171, p=0.150, respectively), the two gene expression levels in relapse patients were lower than in non-relapse patients. At last, the METTL3 and METTL14 expression were not correlated with blast percentage, white blood cell count and the level of lactate dehydrogenase (LDH).

Conclusions: The expression level of METTL3 and METTL14 were much lower in E/R-positive ALL patients than in controls and much lower in relapse patients than in non-relapse patients. Thus, METTL3 and METTL14 may play an important role in the pathogenesis and relapse mechanism of paediatric E/R-positive ALL patients.

Intracranial Haemorrhage in Children with Inherited Bleeding Disorders: A Single Center Study in China

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Background: Intracranial haemorrhage (ICH) is a life threatening condition in children. Inherited bleeding disorders (IBD) may cause ICH.

Aim: This single center study aim to identify the incidence, risk factors, and neurological outcome in children with IBD.

Method: From 2005 to 2016, 240 children with inherited bleeding disorders from Nanfang Hospital, department of paediatrics, were evaluated. The ICH episodes were identified by medical history, general physical examination, detailed neurological examination, and CT or MRI exam. The risk factors, location of ICH, management strategies, surgical intervention and outcome were noted.

Results: ICH was confirmed in 54/240 children with IBD. The overall risk of ICH among children with IBD was 22.5% (95% CI: 17.2, 27.8%). 52/54 (96.2%) (95% CI: 91.1, 99.9%) were haemophilic patients. The median age was 30 months (0-204) and 18/54 (33.3%) (95% CI: 20.3, 46.3%) had an ICH in the first year of life. 6/52 (11.5%) (95% CI: 2.6, 20.5%) haemophilic children had multiple episodes of ICH in which 4 were inhibitor positive. 9/52 (17.3%) (95% CI: 6.7, 27.9%) haemophilic children were inhibitor positive while in 24/52 (46.1%) (95% CI: 32.1, 60.2%) children the inhibitor was not assessed. 13/54 (24%) (95% CI: 12.3, 35.9%) had positive family history of IBD. Twenty-two (36%) (95% CI: 23.7, 48.5%) out of 61 ICH episodes were caused by trauma and 39 (63.9%) (95% CI: 51.5, 76.3%) were non-trauma related. Subdural haematoma was most frequently observed. Mortality risk from ICH in children with IBD was 5/54(9.2%) (95% CI: 1.3, 17.2%). Eleven (22.4%) (95% CI: 10.3, 34.6%) of 49 survivors had known neurological sequelae while 38 (77.5%) (95% CI: 65.4, 89.7%) had no documented evidence of neurological impairment.

Conclusion: Haemophilia is the most common disease among IBD leading to ICH. Risk and consequences of ICH in IBD were high during the first year of life while in older children better outcome may be expected. The optimal management of ICH depends on immediate recognition and prompt replacement therapy.
Development and Application of a Rapid Multiplex Molecular Detection Method of Children Leukaemia Fusion Genes
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Background and Aims: Fusion gene detecting is widely used in the diagnosis and treatment of leukaemia. This study develops a rapid detecting method of eight common fusion genes in leukaemia of children.

Methods: The approach need only one step RT-PCR mediated by universal primers after obtained total RNA from bone marrow specimens, then acquired the size of the amplified fragment after analysed by capillary electrophoresis assay.

Results: A total of 122 patients with positive leukaemia fusion genes were examined by this technique, 121 cases were detected successfully. Respectively, 21 cases were detected with CBRB-MYH11 fusion gene, 13 cases were detected with SIL-TAL1 fusion gene, 16 cases were detected with TEL-AML1 fusion gene, 16 cases were detected with E2A-PBX1 fusion gene, 15 cases were detected with PML-RARa fusion gene, 14 cases were detected with AML1-ETO fusion gene, 13 cases were detected with MLL-AF4 fusion gene, except for 1 case was not detected. This method proves to be with high accuracy and detection rate.

Conclusion: Therefore, one step multiple RT-PCR combining capillary electrophoresis analysis system can be used as an important tool for the clinical diagnosis, treatment and prognosis of paediatric leukaemia.

Genetic Variations of GWAS-identified Genes and Neuroblastoma Susceptibility in Chinese Children
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Background and Aims: Neuroblastoma is one of the most commonly diagnosed solid cancers of the early childhood, in the development of which genetic factors may play an important role. Previous genome-wide association studies (GWASs) have identified nine genes associated with neuroblastoma susceptibility for Caucasians. With the purpose to find whether genetic variations in these genes are also associated with neuroblastoma susceptibility for Southern Chinese children, we genotyped 25 polymorphisms within these genes by Taqman method in 256 cases and 531 controls.

Methods: Odds ratios (ORs) and 95% confidence intervals (CIs) were used to evaluate the strength of the associations. We then performed meta-analysis to further evaluate the associations. Furthermore, we calculated the area under the receiver-operating characteristic curves (AUC) to assess which gene/genes may be better in the predictive for neuroblastoma risk.

Results: We confirmed that CASC15 rs6939340 A>G, rs4712653 T>C, rs9295536 C>A, LIN28B rs221634 A>T, and LMO1 rs110419 A>G were associated with significantly altered neuroblastoma susceptibility. We also confirmed that rs6939340 A>G (G vs. A: OR=1.30, 95% CI=1.13-1.50) and rs110419 G>A (A vs. G: OR=1.37, 95% CI=1.19-1.58) were associated with an increased neuroblastoma risk for all subjects. We also found that the combination of CASC15, LIN28B, and LMO1 may be used to predict neuroblastoma risk (AUC=0.63, 95% CI=0.59-0.67).

Conclusions: Overall, we verified that five GWAS-identified polymorphisms were associated with neuroblastoma susceptibility in a Southern Chinese population, which needs further validation in larger sample size studies.

Novel GD2 Aptamer Selectively Delivers Cytotoxic Agent to Neuroblastoma Tumour Cells in vitro
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Background and Aims: Chemotherapy is a major treatment for late stage Neuroblastoma (NB), but its efficacy is often limited by the adverse effects of cytotoxic agents. There is an urgent need to develop novel targeting therapy for NB. Aptamers are single-strand oligonucleotides that can bind to target molecules with high affinity and specificity because of its specific spatial structure. Aptamers possess distinctive advantages as targeting ligand: high affinity for binding to most molecules, limited synthesis cost, low-immunogenicity, and small size that allows it to penetrate solid tumours. Due to these advantages, aptamers have been employed as novel targeting ligands in tumour targeted therapy. Ganglioside GD2 exits in membrane of NB tumour cell is a promising target for targeted therapy, so far, however, GD2 aptamer has not been reported in literature. Since here in this project, we plan to develop a novel functional GD2 aptamer for NB targeted therapy.

Methods: GD2 aptamer was selected by SELEX.
technology from a random DNA library \textit{in vitro}. This DNA library was 90-base in length. After each SELEX round, the flow cytometry was carried out to assess the enrichment of clones capable of targeting GD2 molecule. The binding specificities and affinities of GD2 aptamer were evaluated by flow cytometry. To detect whether GD2 aptamer could decrease the adverse effects of cytotoxic agents, an aptamer-doxorubicin complex (Apt-Dox) was formulated by intercalating doxorubicin into the DNA structure of GD2 aptamer. GD2+ NB cell line IMR32 and GD2- cell line EL4 were either incubated with Apt-Dox, free Dox, and PBS. Then the cell viabilities were assessed by MTS test.

\textbf{Results:} After the 9th SELEX round, the abundance of enrichment was the furthest. We tested 102 clones and found a clone showed relatively strong binding specificity to GD2, whereas had low binding to BSA. This clone was termed as GGDL1. Further, the binding affinity of GGDL1 to GD2 was 56.98nM. The cell viability of GD2+ cell line IMR32 treated with Apt-Dox was 30.8%, with free Dox was 28.9%. However, the cell viability of GD2- cell line EL4 treated with Apt-Dox was 87.9%, with free Dox was 36.7% (p<0.01).

\textbf{Conclusions:} GD2 aptamer may have potential utility as a targeting ligand for selective delivery of cytotoxic agent to GD2-expressing NB tumour cells.

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High Frequency of NUDT15 Variant Among Chinese Paediatric Patients with Acute Lymphoblastic Leukaemia in Hong Kong
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\textbf{Background:} Thiopurine is an essential treatment of childhood acute lymphoblastic leukaemia (ALL). Thiopurine S-methyltransferase (TPMT) mutation is a well-known mutation affecting metabolism of thiopurine. Recently, a variant in NUDT15 gene [p.Arg139Cys(R139C)] is identified in Asian population with increased risk of thiopurine-induced leucopenia. We started screening for TPMT and NUDT15 mutation in Chinese ALL children to determine their prevalence.

\textbf{Method:} All new cases of paediatric ALL were screened for TPMT and NUDT15 variant in a tertiary referral centre in Hong Kong. Sanger sequencing of TPMT exons 5, 7 and 10 and NUDT15 exons 1 to 3 were performed.

\textbf{Results:} From August 2015 to June 2017, 45 Chinese children were screened with 28 male and 17 females. Among them, 42 subjects were treated with Chinese Children Cancer Group (CCCG) ALL 2015 study and 3 infants were treated with Interfant-ALL 2006 protocol. Thirteen subjects (28.8%) were found to carry NUDT15 variant, 11 being heterozygous (24.4%) and 2 (4.4%) were homozygous. The commonest variant was NUDT15 R139C (Haplotype *3) detected in 9 subjects. Four had p.Gly17_Val18dup and p. Arg139Cys variant (Haplotype *2). Two subjects (4.4%) were heterozygous for TPMT variant, 1 being double heterozygous with NUDT15 R139C variant. Among 42 subjects treated with CCCG ALL 2015 protocol, 33 had completed 8 weeks of consolidation therapy with 6-mercaptopurine (6MP) and methotrexate. 6MP starting dose is reduced in subjects with known TPMT or NUDT15 variants. 6MP would be withheld during severe leucopenia (WCC <1.5 or ANC <0.5). In subjects with wild-type NUDT15 and TPMT allele (n=22), median dose of 6MP given in consolidation therapy was 1380mg/m$^2$ (range 987 mg/m$^2$ to 1695 mg/m$^2$) and it was 695 mg/m$^2$ (range 254 mg/m$^2$ to 846 mg/m$^2$) in subjects with heterozygous NUDT15 variant (n=8). Two subjects with homozygous NUDT15 and double heterozygous NUDT15 and TPMT variants received 210 mg/m$^2$ and 249 mg/m$^2$ of 6MP, respectively. Despite the reduction of 6MP starting dose in subjects with NUDT15 or TPMT variants, 7 out of 11 (63.6%) subjects had severe leucopenia during consolidation therapy leading to interruption of 6MP and further dose reduction. In comparison, only 3 out of 22 subjects (13.6%) with wide type allele had 6MP interruption and dose reduction.

\textbf{Conclusion:} NUDT15 variant is occurring at high frequency among Chinese population in Hong Kong while TPMT variant is infrequently encountered. Screening for NUDT15 variant should be performed for all newly diagnosed Chinese ALL patients. Patients with NUDT15 and TPMT variant are susceptible to thiopurine side effects and prone to develop severe leucopenia.

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Influence of Photoirradiation on the Measurement of Direct Bilirubin by the Vanadic Acid Method
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\textbf{Introduction:} Our team has been conducting research on bilirubin with emphasis on bilirubin photoisomers (BPs)
in neonatal hyperbilirubinaemia. (ZZ)-Bilirubin is photochemically converted into various water-soluble BPs. This mechanism is utilized for phototherapy. The standard laboratory test sometimes detects high direct bilirubin (DB) levels in neonates after phototherapy. Several methods of DB measurement are affected by BPs, but it is not known whether BPs affect the vanadic acid (VA) method, which is commonly used in Japan.

**Objectives:** To elucidate the involvement of BPs in DB measurements by the VA method after photoradiation in vitro.

**Methods:** DB levels were measured before and after 15-s or 60-min blue light-emitting-diode photoradiation of bilirubin/albumin mixtures. The VA method determines the DB level from the difference in absorbance at 450 nm between the time of mixing a tartrate buffer with a sample (point A) and the time of adding a VA solution to the resulting mixture (point B). BPs were measured by high-performance-liquid-chromatography (HPLC) method reported by Itoh et al. (J Chromatogr A 1999). HPLC chromatograms were recorded at 455nm to measure BPs at point A and point B. The measurement was repeated five times in each session.

**Results:** The DB level increased by 0.37 mg/dL after 15-s photoradiation and by 1.12 mg/dL after 60-min photoradiation compared with before photoradiation. Before photoradiation, BPs were not detected and only (ZZ)-bilirubin was detected at both point A and point B. After 15-s photoradiation, (ZE)-bilirubin and (ZZ)-bilirubin were detected at point A, while only (ZZ)-bilirubin was detected at point B. After 60-min photoradiation, (EE)-cyclobilirubin, (EZ)-cyclobilirubin, (ZE)-bilirubin, (EZ)-bilirubin and (ZZ)-bilirubin were detected at point A, while only (ZZ)-bilirubin was detected at point B.

**Discussion:** The VA method calculates the DB level from the difference in absorbance between before and after addition of VA; this difference increased after photoradiation. Before photoradiation, only (ZZ)-bilirubin was observed at both point A and point B, and not converted into BPs. Therefore, the increase in DB after photoradiation was thought to correspond to the changes in the HPLC chromatograms, suggesting that the increased DB level after photoradiation was due to the influence of BPs that disappeared after addition of VA. Also, it became clear that at least (ZE)-bilirubin was involved as DB, because (ZE)-bilirubin was the first BP formed after the start of photoradiation.

**Conclusion:** After photoradiation, DB values calculated by the VA method include BPs.

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**Different Feeding Strategies for Very Low Birth Weight Infants**

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**Background and Aims:** To determine the effect and safety of different feeding strategies for very low birth weight infants (VLBWIs).

**Methods:** A comparative study was carried out to compare two different feeding strategies for VLBWIs. The first strategy is to feed VLBWIs with a "slow" increment speed (15-20 mL/kg/d), while the other is to choose a relative "fast" increment speed (25-35 mL/kg/d). All the VLBWIs were fed by own mother's human milk (HM) (or fortified HM). When HM was not available, preterm formula (PF) would be the choice. Feeds would not be increased if feeding intolerance (FI) occurred. Parenteral nutrition (PN) would be admitted before the full enteral feeding (FEF) of 160 mL/kg/d was achieved.

**Results:** The average gestational age (GA) of the VLBWIs in "fast" group (n=75) was smaller that of the "slow" group (n=76) (30.38±1.84 weeks versus 31.10±2.08 weeks, p<0.05); while the other demographic background (birth weight [BW], gender, delivery pattern, ratio of small for gestation age [SGA], ratio of artificial feeding, and Apgar score at 5 min) of both groups were similar (p>0.05). The incidence of necrotising enterocolitis (NEC) (Bell stage ≥II) was similar in both groups (slow 7.89% and fast 1.33%, p=0.116). The incidence of FI was also similar in both groups (slow 51.43% and fast 45.95%, p>0.05). Although VLBWIs in the fast group attained FEF earlier (median days [25th and 75th percentile]: slow 15.00 [11.00, 22.25] days and fast 9.00 [7.00, 14.00] days, p<0.05) and the time of central venous line was also shorter in the fast group (slow 296.00 [216.00, 428.50] hours and fast 216.00 [161.75, 283.25] hours, p<0.05), their ages at discharge were not statistically different (slow 34.43 [33.46, 35.47] weeks and fast 34.86 [33.86, 36.04] weeks, p=0.062). Neither were their time to regain their BW (slow 5.00 [2.00, 7.00] days and fast 4.00 [0.00, 6.00], p=0.107) and the rate of weight gain (slow 22.08±2.59 g/kg/d and fast 21.96±1.03 g/kg/d, p=0.896).

**Conclusions:** A "fast" rate of feeding increment (25~35 mL/kg/d) did not increase the incidence of NEC ≥ stage II and FI, which would be tolerated well in VLBWIs and therefore reduce their exposure time of PN and central venous.
Comparison of Effects of Bubble Nasal Continuous Positive Airway Pressure and Nasal Continuous Positive Airway Pressure for Neonatal Respiratory support

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Background and Aims: To compare the effects and safety of bubble nasal continuous positive airway pressure (BNCPAP) and nasal continuous positive airway pressure (nCPAP) for neonatal respiratory support.

Methods: We retrospectively analysed the data of the neonates who accepted BNCPAP or nCPAP treatment in the neonatal ward of West China Second University Hospital of Sichuan University during March 2016 and October 2016. Neonates were divided into BNCPAP group and nCPAP group according to the ventilation strategies. Demographics data of both groups were documented, including gender, birth weight (BW), gestational age (GA), underlying diseases, Apgar score, delivery pattern, as well as whether pulmonary surfactant (PS) was administrated. The outcome measures included: death, failure of intervention, and bronchopulmonary dysplasia (BPD). Multiple administration of PS, duration of respiratory support, days in hospital and retinopathy of prematurity (ROP) were also documented. The safety data were also analysed. All the data were analysed by SPSS 19.0.

Results: A total 47 infants were included in the nCPAP group and 60 infants in the BNCPAP group. There were more males in the nCPAP group (57.45% in the nCPAP group versus 31.67% in the BNCPAP group, p<0.05). While other demographics data were of no significant difference between two groups (p>0.05). Mortality rate in BNCPAP group and nCPAP group were of no significant difference (0.00% versus 2.13%, p>0.05); neither was rate of failure of intervention (0.87% versus 2.21%, p>0.05). The durations of respiratory support were of no significant difference in both groups (p>0.05). Meanwhile, rate of pneumothorax and/or pneumomediastinum were of no significant difference in both groups (p>0.05).

Conclusion: There is no evidence to support that significant difference of effects and safety is existed between BNCPAP and nCPAP for neonatal respiratory support. However, BNCPAP might be more suitable for neonatal transport system and/or basic hospitals with limited medical support ability because of its easy and cheap.

Attenuated SUMOylation of SIRT1 in Premature Neonates with Bronchopulmonary Dysplasia

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Background and Aims: To investigate the effects of hyperoxia on the expressions of small ubiquitin-related modifier (SUMO) and sirtuin 1 (SIRT1) proteins, and to examine interactions between these proteins in premature neonates with bronchopulmonary dysplasia (BPD).

Methods: In this prospective study, peripheral blood mononuclear cells (PBMCs) were isolated from residual venous blood samples of 20 premature infants with BPD and 20 gender-matched premature infants without BPD (non-BPD group). Expressions of SUMO and SIRT1 proteins in PBMCs were assessed by Western blot analysis, and their interactions in PBMCs were detected by immunoprecipitation assay. Based on the fraction of inspired oxygen (FiO2) administered, neonates were divided into normal- (FiO2=21%), low- (21% <FiO2 <30%), medium- (30% ≤FiO2 <40%), and high-oxygen (FiO2≥40%) groups.

Results: Expression levels of SUMO1 and SUMO2/3 proteins in the normal-oxygen group were significantly lower than those in the medium- or high-oxygen groups, but were comparable to those in the low-oxygen group. SIRT1 expression in both the medium- and high-oxygen groups was significantly lower than that in the normal-oxygen group. In the BPD group, the expression of SIRT1 protein was lower, and its interaction with SUMO1 and SUMO2/3 was attenuated as compared to that in the non-BPD group.

Conclusion: Oxygen therapy Supplemental oxygen with FiO2≥30% was associated with upregulation of SUMO1 and SUMO2/3 expressions and downregulation of SIRT1 expression.

NBO Intervention Improving Maternal Exclusive Breastfeeding with Perinatal Anxious Symptoms

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Background and Aims: The present study aimed to determine whether Neonatal Behavioral Observation (NBO) intervention could ameliorate maternal perinatal anxious symptoms, and further to explore the efficacy of
the NBO in improving the rate of exclusive breastfeeding breastfeeding.

Methods: The 14-item Hamilton Anxiety Scale (HAMA) were used to assess the hospitalised pregnant women waiting for delivery within 37-42 weeks of gestation. The number of normal group was 100, 105 subjects were diagnosed with anxious symptoms according to the score of HAMA>14. All of the subjects were divided into two groups: control group (n=40) and NBO intervention group (n=65) which was presented NBO video before delivery, and NBO operation within 3 days postpartum. The number of NBO intervention is 6 times from 3 days to 42 days postpartum, once every week. The beginning time of milk secretion, frequency of breastfeeding and breastfeeding rate were recorded.

Results: (1) The HAMA scores in the NBO intervention group were lower than those of the control group within 15 days postpartum (p<0.05). There was no significant difference in the HAMA scores between the NBO intervention group and normal group within 42 days postpartum (p>0.05). (2)The beginning time of milk secretion in the NBO intervention group were earlier than those of the control group (p<0.05). The frequency ≥10 of breastfeeding within 24h in the first 3 day postpartum in the NBO intervention group was more than those of the control group (p<0.05). (3) Within 3, 15, 42 days postpartum, the rate of exclusive breastfeeding (58.5%, 61.5%, 63%) in the NBO intervention group were higher than those of the control group (37.5%, 35%, 40%), respectively (p<0.05).

Conclusions: NBO intervention could ameliorate maternal perinatal anxious symptoms, and further to improve exclusive breastfeeding rate.

Vitamin D Ameliorates Necrotising Enterocolitis via Down-regulating TLR4-mediated Intestinal Cell Apoptosis

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Background and Aims: Necrotising enterocolitis (NEC) is a severe complication of preterm births, in which the toll-like receptor 4 (TLR4) had been reported to play an important role. As an established regulator of TLR4, vitamin D had been demonstrated to be intestinal-protective in many studies. Since preterm births were often accompanied simultaneously by vitamin D deficiency and NEC, there might be some association between vitamin D status and the incidence or severity of NEC. This study aimed at investigating the vitamin D status in preterm births with and without NEC and comparing them with term subjects. The influence of vitamin D on NEC was further studied in a rat model to find out the association between vitamin D/VDR pathway and TLR4 in NEC.

Methods: Level of serum 25(OH)D was tested in 15 preterm births with NEC, 12 preterm births without known serious complications at the time of dismiss as well as 20 healthy term births. Neonatal Wistar rats were grouped and NEC was induced the formula feeding and cold/asphyxia stress method. Vitamin D treatment was administered to some subgroups and compared with those receiving the vehicle only. Microscopic structure, apoptotic protein expression, intestinal permeability, inflammatory cytokine expression and TLR4 expression were investigated and compared statistically.

Results: Preterm births with NEC had significantly lower vitamin D levels than those without NEC and healthy subjects. Vitamin D increased the survival rate, lowered the Nadler's scores in NEC pups. Enterocyte apoptosis and local inflammation, which was very prominent in NEC rats, was greatly reduced by vitamin D. Tight junction function was maintained, as demonstrated by normalised expression of tight junction proteins and decreased intestinal permeability. The increase in the expression of TLR4 in NEC models was also suppressed by vitamin D.

Conclusions: Vitamin D may increase the survival rate, alleviate structure damage and preserve intestinal barrier function. These were achieved partly through restoration of VDR expression and suppression of TLR4. Therefore, pro-inflammatory cytokines release and enterocyte apoptosis was largely inhibited. Since TLR4 activation is a typical pathological change of NEC, vitamin D therapy may be an effective prevention of NEC.

Beta-ketothiolase Deficiency (BKD) Presented with Ketoacidosis and Variable Glucose Levels: Case Report of 3 Chinese Patients in Hong Kong

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Introduction: Beta-ketothiolase Deficiency (BKD) is a metabolic disease affecting ketone body metabolism and isoleucine degradation. Clinically, it is characterised by intermittent ketoacidotic events that are frequently associated with febrile illness, gastroenteritis, poor feeding or prolonged fasting. Clinical symptoms vary widely,
range from vomiting, tachypnoea or lethargy to altered consciousness, seizures, coma or death. Diagnosis of BKD can be made by urinary organic acid analysis, plasma acylcarnitine profile, enzymatic analysis or mutational analysis of ACAT1 gene. It is a rare condition and its prevalence was estimated to be less than 1 in one-million newborns. 

Case Report: We reported three unrelated Chinese patients who were presented to our hospital at age 12 to 24 months with similar history of fever and poor oral intake for 2-4 days before attending emergency department. All of them were noted to have labored breathing and two of them were intubated shortly after admission. They were all found to have severe metabolic acidosis (pH <7.1 and BE >-20) with significant ketosis. All three cases were subsequently confirmed to have BKD due to mutations in ACAT1 gene. Ketoacidosis in BKD can occur with normo-, hypo- or hyperglycaemia which could mimic other medical conditions such as ketotic hypoglycaemia or diabetic ketoacidosis. Such variations in blood glucose levels were also seen in our cases during the acute metabolic decompensation with ketoacidosis. BKD has been described as an IEM with good prognosis. Avoidance of prolonged fasting and modest dietary protein restriction usually lead to a favourable outcome. None of our cases had further ketoacidotic attacks.

Learning Point: The diagnosis of BKD can be challenging because of wide heterogeneity of clinical presentations. During acute metabolic decompensation, ketoacidosis may occur with highly variable blood glucose levels, ranging from hypo-, normo- to hyper-glycaemia in different patients. With early diagnosis and appropriate treatment of BKD, most patients remain asymptomatic afterwards. Clinicians should therefore be alert to this diagnosis in young children presenting with acute ketoacidosis. In addition, implementation of expanded newborn screening which could identify BKD in presymptomatic stage can also improve the prognosis and outcome of BKD patients.

Association Between Maternal Dietary Diversity and Nutritional Status of Under Two Years Age Children: Results from a Case-control Study in an Urban Care Setting of Dhaka

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Background: Mothers have the prime responsibility of selecting, preparing, and serving nutritious foods to support their children. However, the diets of mothers are often overlooked along with potential impact of poor diet on health and nutrition of both mother and their children.

Objectives: Objective of this study was to identify association between maternal dietary diversity and nutritional status of under-2 years old children attending a diarrhoeal disease treatment hospital in Dhaka, Bangladesh.

Methods: This study was a hospital-based age and sex matched case-control study conducted among under-2 years old children attending the short-stay unit of Dhaka Hospital of icddr,b from November 2016 to February 2017. Stunted children, having a length-for-age z-score (LAZ) <-2 were selected as cases. Controls were defined as children who were not wasted or underweight (weight-for-height and weight-for-age z score ≥-2) and have LAZ ≥-1. Maternal dietary diversity was assessed using Guidelines for Minimum Dietary Diversity for Women (MDD-W) where mothers were asked about their recall of consumption of ten defined food groups on the previous day of the interview (24-hour recall). After collecting all the data, they were analysed using STATA software for Windows (version 13).

Results: Total 296 children (148 cases and 148 controls) were enrolled in this study. Each group comprised of 91 (61%) male and 57 (39%) female children. Mean length-for-age Z -score score was -2.71±0.51 in stunted children and -0.13±0.72 in non-stunted children. Regarding dietary diversity, about 58% mothers of case children consumed less than 5 food group on the previous day of the interview and this proportion was lower in control mothers (45%). Minimum dietary diversity score for mothers was 4.23±1.92 in cases and 4.89±1.80 in controls. Individual food groups like pulses, milk or milk like products, eggs and vitamin A rich fruits intake was higher in control mothers than cases. The risk of stunting was evident in both the unadjusted and adjusted analysis for women who consumed less than 5 food groups during 24 hr recall period. Children whose mothers consumed less than 5 food groups were 1.7 times more likely to be stunted than their counterparts (aOR=1.69, 95% CI=1.02-2.83, p-value 0.04). Other variables (maternal illiteracy, monthly family income of less than 11480 BDT, absence of bank account and unimproved sanitation) which were found associated during bivariate analysis, did not show any significant relationship with child stunting in logistic regression analysis.

Conclusion: As no single food contains all necessary nutrients, diversity in dietary sources is needed to ensure a balanced and healthy diet for mothers, and for improved nutritional status of children.
Effect of Environmental Enteric Dysfunction on Zinc (Zn) Absorption from Different Zn Doses in Micronutrient Powder: Findings from an Absorption Study in Peri-urban Slum of Dhaka, Bangladesh

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Background and Aims: Environmental enteric dysfunction (EED) alters gut integrity and is suspected to impair absorption of micronutrients, including zinc (Zn), due to abnormal small bowel mucosa. Interventions with micronutrient powders (MNP) containing 5 mg Zn have not yielded positive Zn sensitive outcomes. Zn requirements need to be better quantified in the context of EED to maximise the impact of preventive interventions. The aim of the study was to compare Zn absorption across a wide range of MNP Zn doses in young children at risk of EED.

Methods: Bangladeshi children aged 18-24 months old, living in a peri-urban slum, with and without EED (by urinary lactulose-mannitol excretion ratio test) were randomised to MNP with 0, 5, 10, or 15 mg (10 subjects/EED group/dose) of Zn. Outcomes by EED status included fractional Zn absorption (FAZ) from MNP meal plus from unfortified meals, measured with stable isotopes by urine dual isotope tracer ratio method; total dietary Zn intake (TDZ, mg/d) measured by duplicate diet collections; and total absorbed Zn (TAZ, mg/d) measured by FAZ x TDZ. TAZ data were applied to saturation response model (SRM), and to Estimated Physiologic Requirement (EPR, =0.74 mg/d).

Results: A total of 73 children aged between 18-24 months completed the study and comprised the analysable sample. Mean±SD age of the participants was 19±2 months and 50% were male. Approximately 47% of subjects had EED diagnosed as lactulose-mannitol ratio being ≥0.09. Mean excretion ratio for EED and non EED groups were 0.211±0.16 and 0.057±0.02 respectively (p<0.05). Mean FAZ for MNP dose 0 mg, 5 mg, 10 mg and 15 mg was 0.5±0.3, 0.5±0.3, 0.8±0.4, and 1.0±0.4, respectively (p<0.05), with no significant difference in TAZ when segregated by EED status. Absorption followed SRM pattern but the curves of both groups were lower at all MNP doses than the curve for healthy 9 month old breastfed U.S. infants (Krebs et al, AJCN, 2012). The latter group reached the EPR with <5 mg/d Zn intake, while for current study participants, only mean TAZ for 10 and 15 mg MNP doses exceeded the EPR.

Conclusion: Low Zn absorption was seen for both EED and non EED groups, suggesting lactulose-mannitol ratio was not able to identify EED. Final interpretation can be made based on findings of fecal biomarker analysis. Only children receiving 10 or 15 mg MNP were able to meet the EPR, suggesting increased Zn requirement of this population.

Impact of Nutritional Supplements on Cognitive Development of Children in Developing Countries: A Meta-analysis

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Background and Aims: Nutritional supplements may be important on cognition but the evidence is heterogeneous. This meta-analysis aimed (1) to determine whether nutritional supplements provided to pregnant women or young children could improve cognitive development of children in developing countries, and (2) to explore how supplementation characteristics could improve children's cognitive outcomes.

Methods: This meta-analysis examined nutritional supplementation studies in 9 electronic databases and 13 specialist websites. Experimental studies were included if they were published from 1992 to 2016, were conducted in developing countries, had nutritional supplementation for pregnant women or children aged ≤8, and reported effect sizes on cognitive outcomes. Interventions with confounded components, such as stimulation and parenting, were excluded.

Results: 67 interventions (48 studies) for 29814 children from 20 developing countries were evaluated. Childhood nutritional supplementation could improve children's cognitive development (d 0.08, 95% CI 0.03-0.13) and those with ≥5 nutrients was particularly beneficial (0.15, 0.08-0.22). Antenatal supplementation did not improve cognitive development (0.02, -0.01 to 0.06) except for those implemented in the first trimester (0.15, 0.03-0.28).

Conclusions: In conclusion, childhood nutritional supplementation was beneficial to cognitive development but could be optimised by providing multiple nutrients; antenatal supplementation should target pregnancy women in the first trimester for better cognitive benefits.
Outcome of a Standardised Inpatient Nutrition Replacement Protocol (NRP) for Adolescents with Eating Disorder (ED)

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Background and Aims: A highly conservative nutrition replacement approach has been adopted for adolescents with ED in Hong Kong for many years. This is because of the lack of international consensus on the optimal rate of nutrition replacement for this group of patients and the physicians' worry about refeeding syndrome, an uncommon complication of nutrition replacement but with high rate of mortality and morbidity. Increasing evidence in the medical literature has shown that replacing nutrition at a higher rate is both safe and effective and the risk of refeeding syndrome in inpatient NRP is more related to the degree of malnutrition, not the rate of nutrition replacement. Such reports included ED programs in North America and Australia. We performed this case control study to look into the preliminary outcome of our new NRP in local ED adolescents.

Method: This is a retrospective case-control study assessing the clinical outcome and safety (weight, electrolyte disturbance or signs of refeeding syndrome) of a new inpatient NRP for adolescents with eating disorder hospitalised in the Department of Paediatrics, Queen Elizabeth Hospital. The standardised inpatient NRP was started in May 2016, aiming at >0.5 kg/week weight gain (measured early morning after voiding according to protocol). Activity Responsibility stages, Nutrition Responsibility stages and house-rules were designed to standardise the treatment with respect to their activities on the ward, homemade food or standard nutrition replacement items if they could not finish the nutrition as prescribed.

Results: There was no significant change in the electrolytes level (i.e. serum sodium, potassium or phosphate) between the two groups and there was no sign of refeeding syndrome in any of the included adolescents. All 6 in the intervention group achieved the goal of 0.5 kg/week gain in weight on day 14 when compared to 5 of the 12 adolescents in the control group. The difference was statistically significant (Fisher's exact test; p value 0.038).

Conclusions: The case-control study provides preliminary evidence about the safety and improved weight outcome of our new NRP in adolescents with ED. A larger scale study would be able to provide more concrete information on the effectiveness and 1 year outcome of our new NRP.

The Interplay of Vitamin D Levels, Vitamin D Receptor Single Nucleotide Polymorphism and Diets on Serum Lipid Profiles in Chinese Han Adolescents

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Background and Aims: Abnormal serum lipids and glucose are closely related to the cardiovascular disease leading to high morbidity. Recent studies have shown that vitamin D and vitamin D receptor (VDR) FokI polymorphism as well as dietary patterns are involved in regulating serum lipids and glucose. But the existing conclusions are still controversial and debatable. The aim of this study is to investigate how dietary pattern of Chinese people, vitamin D and VDR polymorphism influence metabolism of serum lipid and glucose.

Methods: 590 volunteers, the eleventh grade students, participated in this study. According to the energy percentage of carbohydrate and fat, they were divided into two groups, high-carbohydrate (high-CHO) diet group and non-high-carbohydrate (non-high-CHO) diet group. Serum lipid, serum glucose and 25-hydroxyvitamin D [25(OH)D] were measured in laboratory. Based on the VDR FokI polymorphism, the participants were assigned into two groups, TT genotype and CC allele carriers. According to the serum level of 25(OH)D, the subjects were divided into three groups.

Results: There were quite a few differences in serum lipids and glucose between males and females under different vitamin D levels, dietary patterns and genotypes. To conclude below: (1) Considering dietary pattern, C allele carriers males with medium vitamin D in non-high-CHO diet group had higher HDL-C but lower TC/HDL-C and LDL-C/HDL-C than those in high-CHO diet group. As for females in non-high-CHO diet group, C allele carriers subjects with low vitamin D had lower TG than those in high-CHO diet group. (2) Considering FokI polymorphism, males with medium vitamin D in high-CHO diet group had higher HDL-C but lower TC/HDL-C and LDL-C/HDL-C than those...
in C allele carriers. As for TT genotype females in high-CHO diet group, subjects with low and medium vitamin D had higher TC than C allele carriers subjects. (3) Considering vitamin D, C allele carriers males in high-CHO diet group with medium vitamin D had lower HDL-C but higher TC/HDL-C and LDL-C/HDL-C than those with high vitamin D. As for C allele carriers females in high-CHO diet group, subjects with low vitamin D had higher TG than those with medium vitamin D.

**Conclusion:** In the youth China healthy Hans, serum lipid and glucose affected by serum level of vitamin D, VDR FokI polymorphism and dietary pattern, and these influences not only has sex dependent, but also interact with each other. This partly explains why the earlier results were inconsistent.

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**Prevalence of Vitamin D Insufficiency Among Children in Sunshine-abundant Southern China**


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**Background and Aims:** Vitamin D deficiency is related to numerous adverse health issues, and its public health consequences are enormous. Although inadequate vitamin D concentrations have been reported in different population around the world, limited data are available on vitamin D status among children in southern China regions with abundant sunshine. This study aimed to describe the 25-hydroxyvitamin D (25(OH)D) status among children in southern China and determine seasonal difference in serum 25(OH)D.

**Methods:** Children who visited the Guangdong Women and Children Hospital for health examination from January 2016 to May 2017 were included in this large cross-sectional study. Serum 25(OH)D concentrations were measured by electrochemiluminescence immunoassay. Vitamin D status was defined as deficiency (25(OH)D<50 nmol/L), insufficiency (50 nmol/L≤25(OH)D<75 nmol/L) and sufficiency (25(OH)D≥75 nmol/L).

**Results:** 16755 children aged 0-6 years were included. The serum 25(OH)D levels ranged from 10.5-307.4 nmol/L (mean±SD: 78.5±26.3 nmol/L). Overall, the prevalence of vitamin D deficiency, insufficiency and sufficiency were 10.8%, 59.0% and 30.3%, respectively. The concentrations of serum 25(OH)D varied with season, and serum 25(OH)D level in spring (71.8±4.9 nmol/L) was lower than that in other season. In some months (from January to April), we found a relative high vitamin D deficiency or insufficiency. The prevalence of vitamin D deficiency was higher in spring (17.1%) than that in summer (8.9%), autumn (5.6%) and winter (9.3%). The prevalence of vitamin D deficiency and insufficiency increased with age. Additionally, the logistic regression analysis revealed that vitamin D deficiency and insufficiency in children were significantly associated with age and season.

**Conclusions:** The deficiency and insufficiency of vitamin D is common among children in southern China despite in a sunshine-sufficient area. Age and season appear to be important for the variation in serum 25(OH)D levels, highlighting the consideration of these factors to achieve vitamin D adequacy.

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**Analysis of Energy Intake in Very-low-birth-weight Infants During NICU**

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**Background and Aims:** Very-low-birth-weight infants (VLBW, <1500 g) need a high energy intake to achieve intraterine growth rate. Our aim was to analysis the energy, protein, lipid intake in VLBWI during NICU, to find and improve the deficiency of clinical nutrition support.

**Methods:** This’s a retrospective survey. We collected the clinical data of the hospitalised VLBWI in Xin Hua Hospital in Shanghai between 1 October 2012 and 31 December 2016. The nutritional status and energy, protein, lipid intake were analysed.

**Results:** A total of 128 VLBWI accepted both enteral and parenteral nutrition (EN, PN) support was selected. The mean gestational age was 30.7±2.08 weeks and the mean birth weight was 1301.0±123.8 g. The duration of hospitalisation was 46.5±14.12 days, and the corrected gestational age was 37.4±2.24 weeks at the time of discharge. The mean time to achieve full enteral nutrition was 29.8±11.84 days. The proportion of growth restriction was 24.22% (31/128) at birth and increased to 71.88% (92/128). The mean energy intake was 104.5±12.06 kcal/kg/d. Further analysis revealed that the energy and protein intake in the 1st, 2ed weeks were not enough, accounted for 77.61%, 96.47% and 76.03%, 85.97.0% of the recommend amount respectively. The
energy and protein intake from 3rd to 9th weeks were enough. All the infants had stopped to receive PN support before the 10th week. However, the enteral energy and protein intake from 10th to 12th weeks were also inadequate, especially the protein intake in the 9th and 10th weeks accounted for only 86.93%, 84.80% of the recommended amount. The lipid intake is sufficient during NICU.

Conclusion: The incidence of extra-uterine growth restriction was high and the rate of weight gain was lower than intrauterine growth rate. The starting time of MEN and PN in most infants was late. There was a high rate of the interruption of enteral feeding. The intake of energy, protein was still inadequate in VLBWI, especially in the first two weeks. And too early to stop PN support might lead to insufficient supply.

Adult Outcome of Juvenile Idiopathic Arthritis: A Nationwide Population Retrospective Cohort Study in Taiwan
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Background: This population-based case-control study investigated the development of juvenile idiopathic arthritis (JIA) and the subsequent risks of developing rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), psoriatic arthritis, ankylosing spondylitis (AS), and Sjogren's disease in adulthood.

Methods: We analysed data from 107,433 children born between 1990 and 1997 to identify those with JIA. We then retrospectively followed them up until December 2013 to observe the occurrence of autoimmune diseases. There were 262 patients with JIA diagnosed between 2000 and 2012, and 107,171 controls. For those with JIA, the hazard ratios and percentage of developing adult autoimmune diseases were calculated. The hazard ratios were further stratified by age of JIA diagnosis (3-5 years, 6-10 years, and 10-15 years). Cox proportional regression models were used to compare the adjusted hazard ratios (aHR) of adult-onset autoimmune diseases among the study subgroups.

Results: The subsequent risks of RA, SLE, AS, psoriatic arthritis, and Sjogren's disease in adulthood were increased in the originally JIA group. The incidence rates (per 105 person months) were 83.56 (95% confidence interval [CI], 44.96-155.29), 16.61 (4.15-66.40), 58.39 (27.83-122.49), and 33.26 (12.48-88.61) for RA, SLE, AS, and psoriasis, respectively, in the JIA group. The adjusted hazard ratios (aHR) were 29.597 for any autoimmune disease (95% confidence interval [CI], 21.145-41.426), 129.518 for RA (70.185-239.012), 10.007 for SLE (3.189-31.402), 49.624 for AS (29.699-82.917), and 8.199 for psoriasis (2.618-25.675). The highest risk of adult-onset RA was in JIA patients who were first diagnosed at age 3-5 years old.

Conclusions: Children with JIA were at increased risk of developing RA, SLE, AS, psoriatic arthritis, and Sjogren's disease in adulthood. The increased risk was associated with the cumulative effect of concurrent rheumatologic diseases. Further study to investigate the role of JIA in the development of adult-onset rheumatic diseases is warranted.

Needle Length for Epinephrine Prefilled Syringes in Children and Adolescents
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Background: Intramuscular epinephrine is the first line drug in the treatment of anaphylaxis. This study was to identify the appropriateness of 1 inch needle length for epinephrine prefilled syringes in children.

Methods: Children aged 1 month to 18 years were enrolled. Skin to muscle depth (STMD) and skin to bone depth (STBD) were measured using an ultrasonography at the mid-anterolateral thigh. A 1 inch needle was considered as being appropriate if the STBD was more than 1 inch and the STMD was less than 1 inch.

Results: Seventy-five infants, 75 pre-school aged children, 75 school aged children and 147 adolescent were enrolled: 196 (52.7%) children were male. A 1 inch needle length was appropriate for 61% of the infants, for 88% of the preschool children, for 99% of the school aged children and for 95% of the adolescents. Thigh circumference ≥23 cm, BMI ≥16 kg/m² and BW ≥6 kg in infants provided the sensitivity of 74%-96% in predicting the appropriateness of 1 inch needle. In preschool group, thigh circumference ≥25 cm, BMI ≥13.5 kg/m² and BW ≥10 kg provided the sensitivity of 98.5-100% in the appropriateness of 1 inch
needle. Thigh circumference \( \geq 49 \) cm in adolescents provided the sensitivity of 75% in predicting that a 1 inch needle was too short.

**Conclusion:** One inch needle length may not be appropriated for intramuscular injection at thigh in all children. Thigh circumference, BMI and body weight are useful for predictor for using the 1 inch needle.

**Impaired Cellular Immunity Correlates with Severe Respiratory Syncytial Virus, Rotavirus and Varicella-zoster Virus Infection Among Patients with Primary Immunodeficiency**

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**Background and Aims:** Patients with primary immunodeficiency diseases (PID) are highly susceptible to various microorganisms. Respiratory syncytial virus (RSV) is one of the most common pathogens of lower respiratory tract infections in childhood, and is considered to be highly pathogenic in PID patients. In 2013, the use of palivizumab in children with immunocompromised conditions were approved in Japan. However, no population-based studies have been performed to clarify the actual severity of RSV infections in PID patients. Similarly, the reports on rotavirus (RV), varicella-zoster virus (VZV) and influenza virus (IV) infection among PID patients are limited. The objective of this study was to reveal the clinical burden of these four infections among PID patients in Japan.

**Methods:** We conducted a nationwide survey by sending questionnaires to 898 hospitals with paediatric departments throughout Japan.

**Results:** From 1996 to 2013, of the 2,886,836 persons from the NHIRD database, 1002 patients under 18 years old were identified that had HSP. Among them, 164 had more than 2 times of HSP. The recurrent rate of HSP was 16.4%. The incidence of recurrent HSP was 7.5 per 100 person-years. At the end of the 16-year cohort, 83.6% of patients who had first HSP remained free from secondary HSP. The average time interval between the first and second episode varied. Few studies have explored the incidence and risk factors of recurrent HSP.

**Methods:** This retrospective cohort study used a 16-year nationwide database to analyse the incidence of recurrent HSP. Patients with HSP were identified. Associated risk factors for the recurrence of HSP were explored. Kaplan-Meier and Cox regression model were performed for the analyses.

**Results:** The recurrent rate of Henoch-Scholein Purpura (HSP) was shown ranged from 2.7% to 30% in previous studies. The average interval between the first and second episode varied. Few studies have explored the incidence and risk factors of recurrent HSP.

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**Conclusion:** The annual incidence of recurrent HSP was
not high. However, children with underlying allergic rhinitis, presented with renal involvement and had steroid treatment more than 10 days should be notified the possibility of recurrence and should be followed up for at least 9 months.

Type I and III Interferon Production Is Impaired in Response to Oral Poliovirus But Not to H1N1 Influenza Virus in X-linked Agammaglobulinaemia

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Background and Aim: X-linked agammaglobulinaemia (XLA) is a primary immunodeficiency disease (PID) due to Bruton’s Tyrosine Kinase (BTK) mutation. Apart from being susceptible to bacterial infections, XLA patients are also susceptible to enteroviruses including poliovirus but not to other viral infections. To date the mechanism is unknown. However, recent studies have shown BTK plays major role in toll-like 3 receptors (TLR3) signaling, which is a critical component in innate antiviral immunity. We therefore hypothesise XLA patients have selective functional impairment in antiviral response to oral poliovirus (OPV) compared to influenza virus.

Methods: Peripheral blood mononuclear cells (PBMC) were obtained from 9 XLA patients aged 22-32 years old and 23 buffy coats from Hong Kong Red Cross donors. Monocyte-derived dendritic cells (mDC) were derived from PBMC. LFM-A13 was used as BTK inhibitor. Sabin type 1 OPV and H1N1 virus were used to stimulate mDC. RPMI was used as mock stimulation. Antiviral cytokine productions as well as expression of CD80, CD83, CD86 and MHC-II were analysed after 24 hours of stimulation.

Results: Production of IFN-α2, IFN-β and IFN-λ1 in patients mDC were significantly lower than those in healthy mDC in response to OPV stimulation (p=0.039, p=0.007 and p=0.0096 respectively). However, the production of IFN-α2, IFN-β and IFN-λ1 in patients mDC were similar to those in healthy mDC in response to H1N1 virus stimulation. Moreover, the selective production impairments in response to OPV but not H1N1 stimulation were reproduced when healthy mDC were treated with LFM-A13. The mean fluorescent intensities (MFI) of CD80, CD83, CD86 and MHC-II on patients mDC in response to OPV stimulation were similar to mock stimulation. In contrast, the MFI of CD83, CD86 and MHC-II in patients mDC were significantly higher in response to H1N1 stimulation than to mock stimulation.

Conclusion: Susceptibility to enterovirus infection in XLA patients may be due to selective impaired production of type I and III IFN only to enteroviruses but not other viruses. This impairment may be the result of impaired TLR3 signaling due to lack of functional BTK.

IDO Contributes to the Pulmonary Immunosuppression and the Alleviation of Pulmonary Fibrosis Induced by Human Mesenchymal Stem Cells in Humanised Mice

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Background and Aims: The contribution of indoleamine-pyrole 2,3-dioxygenase (IDO) is indicated in the human mesenchymal stem cells (MSC)-induced immunosuppression in vitro. However, the involvement of IDO in human MSC-induced immunosuppression and the attenuation of inflammation-related disease in vivo has not been investigated.

Methods: The inflammation is appeared at the early stage of pulmonary fibrosis and related to the pathogenesis of this disease. In this study, pulmonary fibrosis was induced by bleomycin in the human peripheral blood mononuclear cells (PBMC)-reconstituted humanised mice, which facilitated the direct investigation of human cell interaction in the animal model. The IDO production in human MSC was silenced by shRNA. Then, the IDO-silenced human MSC and control human MSC were injected respectively into humanised mice with bleomycin treatment. The weight loss, the lung function, and the fibrosis in the lung were monitored until day 21 post bleomycin injection. Meanwhile, the activation of human immune cells and the production of human cytokine/chemokine in the lungs of humanised mice were tested.

Results: In humanised mice, the administration of human MSC effectively rescue the weight change, the lung function and the fibrosis in the lung. Compared with the control human MSC, the IDO-silenced human MSC could not significantly induce pulmonary immunosuppression and the attenuation of pulmonary fibrosis.

Conclusions: IDO contributes to the pulmonary immunosuppression and the alleviation of pulmonary fibrosis induced by human MSC in humanised mice.
Development and Maturation of Polyfunctional Epstein-Barr Virus Antigen-specific CD4+ and CD8+ T Cell Responses in Children with Infectious Mononucleosis and Primary Asymptomatic Infection

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Background and Aims: Effective control of chronic viral infections was shown to require the generation of polyfunctional T cells (PFCs) which are capable of producing multiple cytokines and possess cytotoxic function. However, the development and maturation of PFC responses in Epstein-Barr virus (EBV) infection are not well understood. We carried out a longitudinal study to assess the development and maturation of T cell responses to EBV from the time of acute infection to recovery in a large cohort of children with infectious mononucleosis (IM) and primary asymptomatic (AS) infection.

Method: Evaluation of IFN-γ secreting CD8+ T cell responses upon stimulation of PBMC by HLA class I-specific peptides of EBV lytic and latent proteins was first performed by ELISPOT assay followed by assessment of CD4+ and CD8+ PFC responses upon stimulation of PBMC by a panel of overlapping peptides of EBV lytic and latent proteins using polychromatic flow cytometric analysis for the co-expression of IFN-γ, TNF-α, IL-2, perforin and CD107a. Cytotoxicity of T cells against autologous lymphoblastoid cell lines (LCLs) as well as viral loads in plasma and PBMC were determined.

Results: A trend of decrease in the magnitude of CD8+ T cell responses towards EBV lytic peptides in contrast to the increase towards latent peptides was demonstrated by the ELISPOT assay. Interestingly, both lytic and latent antigen-specific CD4+ and CD8+ T cells showed increased polyfunctionality (greater or equal to three functions) concurrent with enhanced cytotoxicity and sustained decrease in plasma and PBMC viral loads over time. Immunodominant EBV antigens (EBNA-3A, -3B and -3C) induced higher proportion of CD8+ PFCs than the subdominant ones. No significant difference in the pattern of development of EBV-specific PFC responses was found between the IM and AS patients.

Conclusion: Our data supported that the development and maturation of polyfunctional CD4+ and CD8+ T cell responses to both lytic and latent antigens are important in the long term control of EBV.

Genetic Basis of Chronic Granulomatous Disease in North India

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Background and Aims: Chronic granulomatous disease (CGD) is a genetic defect in the phagocyte function resulting for mutations in genes encoding for different components of the NADPH oxidase system. Impairment of NADPH oxidase complex results in defective generation of superoxides in phagocytic cells and defective killing of intracellular pathogens. Mutations in five different genes namely CYBB, NCF1, CYBA, NCF2 and NCF2 encoding for gp91phox, p47phox, p22phox, p67phox and p40phox respectively are responsible for the clinical phenotype. X-linked recessive disease due to mutations in the CYBB gene is the commonest form of CGD reported from the USA, UK and Europe. However, autosomal recessive forms of CGD due to defects in NCF1, NCF2 and CYBA genes are more common in geographical locales with high rates of consanguinity such as the Middle East and North Africa. There are very few reports on the genetic basis of CGD from India. We report the genetic basis of 32 patients from our cohort of 50 patients with CGD.

Methods: We analysed the molecular defects in our cohort of CGD patients, diagnosed and followed-up at the Pediatric Allergy and Immunology Unit, Advanced Pediatrics Centre, Postgraduate Institute of Medical Education and Research, Chandigarh. Fift y (50) cases of CGD were diagnosed over the past two decades. Diagnosis of CGD was made on the basis of nitroblue tetrazolium test, dihydrorhodamine test and NADPH oxidase component analysis by flow cyometry. Genetic mutation analysis was available for 32 patients. Mutational analysis was performed at our centre and centres in Japan and Hong Kong.

Results: Mutations in the CYBB gene were the commonest being detected in 17/32 patients (53%) in whom genetic analysis results were available. NCF1 gene mutations
accounted for the largest proportion of autosomal recessive form of CGD, being present in 12/32 patients (37%). Mutations in the NCF2 gene were present in 3 patients. All patients with NCF1 gene mutations had a GT deletion in Exon 2. Six of the 17 CYBB gene mutations were novel mutations. Prenatal diagnosis was performed in 6 families.

**Conclusions:** X-linked CGD with mutation in the CYBB gene was the commonest form of CGD (17 cases) in our cohort of 32 patients (53%) in whom genetic analysis results were available. However, AR CGD was also not uncommon present in 15 patients (47%). Novel mutations in the CYBB gene were also detected in our CGD cohort.

**The Plasticity of Innate Immune Responses to Lipopolysaccharide in Iron Dextran-overloaded Mice**

**Methods:** An experimental model of iron overload was generated by intraperitoneal injections of iron dextran (1 g/kg) administered once a week for 8 weeks in male C57BL/6 mouse strain. The serum levels of ferritin, ferrum, oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) as well as cytokines including TNF-α, IL-6, IL-10 were measured. Monocytes were identified and gated in a forward-scatter and side-scatter dot plot of PBMCs by flow cytometry. Two main types of monocytes were further defined by classical monocytes (Ly6ChighCCR2high) and non-classical monocytes (Ly6CloCCR2low). In *ex vivo* studies, cytokines secreted from PBMCs after LPS stimulation for 12 hrs were quantified.

**Results:** An increase in liver and spleen size with iron deposits were observed in iron overload mice. Compared to control, the group of iron overload had higher values of serum IL-6 (10.66±3.44 vs 2.52±0.47 pg/ml), ferrum (1096±112.9 vs 201.9±13.29 ug/dl), GOT (260.0±26.56 vs 70.71±12.58 U/l) and GPT (200.5±21.66 vs 30.43±3.37 U/l) but not ferritin level (10.63±0.50 vs 10.73±0.65 ng/dl). Two groups had the same population of Inflammatory, classical monocytes (0.53% vs 0.59%) of total monocytes but the group of Iron overload had higher tissue repairing, non-classical monocytes (0.22% vs 0.08%). At 12 hours after LPS exposure, the PBMCs of two groups produced the same level of IL-6 (236.3±13.42 vs. 227.9±17.62 pg/ml). However, the PBMCs of Iron overload mice secreted significantly higher pro-inflammatory cytokine, TNF-α (472.8±24.86 vs 118.9±15.87 pg/ml) and anti-inflammatory cytokine, IL-10 (744.0±1.69 vs 189.8±2.48 pg/ml).

**Conclusions:** Our results revealed the functional plasticity of monocytes during iron overload, wherein they simultaneously had pro-inflammatory and immunosuppressive phenotypes. These findings may explain the impaired innate immunity of thalassemic patients with chronic iron overload.

**Background and Aims:** The disturbance of iron homeostasis is associated with altered immune function. Transfusion-dependent thalassaemia patients have more complications in bacterial infections. However, the effect of iron overload on the function of immune system is unclear. In order to identify the differences in the innate immune responses to bacterial infection of iron overload, the present study assessed the subgroups of monocytes and responses of peripheral blood mononuclear cells (PBMCs) to lipopolysaccharide (LPS) in iron dextran-overloaded mice model.

**Methods:** An experimental model of iron overload was generated by intraperitoneal injections of iron dextran (1 g/kg) administered once a week for 8 weeks in male C57BL/6 mouse strain. The serum levels of ferritin, ferrum, oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) as well as cytokines including TNF-α, IL-6, IL-10 were measured. Monocytes were identified and gated in a forward-scatter and side-scatter dot plot of PBMCs by flow cytometry. Two main types of monocytes were further defined by classical monocytes (Ly6ChighCCR2high) and non-classical monocytes (Ly6CloCCR2low). In *ex vivo* studies, cytokines secreted from PBMCs after LPS stimulation for 12 hrs were quantified.

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**Conclusions:** Our results revealed the functional plasticity of monocytes during iron overload, wherein they simultaneously had pro-inflammatory and immunosuppressive phenotypes. These findings may explain the impaired innate immunity of thalassemic patients with chronic iron overload.
ovalbumin (OVA) of Balb/c mice intestinal sensitisation, food allergy research on intestinal barrier function in mice and the effects of regulatory T cells, so as to provide a new target for prevention and treatment of food allergy.

**Methods:** 30 Balb/c mice fed with no experimental protein, 18-22 g in body weight and half male and female, were randomly divided into two groups: experimental group and control group. The experimental group was given ovalbumin (OVA), the control group was given the same amount of normal saline, the model was made for thirty-first days, the eyeball was taken and the mice were killed. The content of intestinal secretory immunoglobulin A (sIgA) and serum total IgE and DAO were measured by ELISA. Meanwhile, the frequency of CD4+CD25+ regulatory T cells in their splenic cell suspension was analysed by flow cytometry, using different concentrations of short chain fatty acids and TSA (HDAC inhibitors), CD4+CD25+Foxp3+Treg cells were isolated and stained with intracellular factors, and the expression of IFN-γ, TNF-α and NF-kappa B was detected.

**Results:** compared with the control group, the serum total IgE and DAO content (A value) of the experimental group increased significantly, and the number of CD4+CD25+T cells in the splenic cell suspension decreased significantly. SCFAs may play an anti-inflammatory role in mouse PBMC by inhibiting the activity of NF-κB by inhibiting HDAC.

**Conclusion:** The food allergen ovalbumin on Balb/c mice intestinal sensitisation, food allergy research on intestinal barrier function in mice and the effects of regulatory T cells, to investigate the protective effect of short chain fatty acid and its receptor signal pathway.

**Immunosuppressive Properties of hTERT Mesenchymal Stem Cells (htMSC)-derived Extracellular Vesicles on Human Plasmacytoid Dendritic Cells (pDCs) and Its Therapeutic Potential in Autoimmune Diseases**

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**Background:** Mesenchymal stem cell (MSC)-based therapy has shown promises in treating inflammatory disorders and tissue damage by means of immunomodulation and promoting tissue regeneration. MSC exert its effect on target cells through cell-cell contact, secretion of soluble molecules and extracellular vesicles (EVs). In systemic lupus erythematosus, TLR7 and TLR9 activation in plasmacytoid dendritic cells (pDC) is recognised as one of the major pathogenetic mechanisms. We previously demonstrated that tumour necrosis factor-inducible gene-6 (TSG-6), a key anti-inflammatory protein secreted by activated MSC, could downregulate TLR-7 and TLR-9 activation in human pDC. Herein, we investigate the effect of MSC and MSC-derived EVs on regulating pro-inflammatory cytokines and interferon production in pDCs, and whether such effect is mediated by TSG-6.

**Methods:** hTERT-MSC (htMSC) were cultured in serum-deprived CDPF medium for 48 hours. EV were isolated by ultracentrifugation and characterised by transmission electron microscopy, Nanosight, and western-blot. Immunosuppressive effect of EV on TLR9-mediated cytokine production was determined in GEN2.2, a human pDC cell-line, following overnight, 4-hr or 30-min activation by CpG-A, and analysed by qPCR, ELISA and flow-cytometry.

**Results:** Upon activation of TLR9 signaling by CpG-A, IL-1β, TNF-α and IFN-α transcription was upregulated in GEN2.2. Such response was reduced when CpG-A-primed GEN2.2 were co-cultured with htMSC. Knockdown of TSG-6 in htMSC dampened its capacity to suppress IL-1β, TNF-α, IFN-α and IRF7 transcription in GEN2.2, suggesting the importance of TSG-6 in downregulating TLR9-mediated response. To find out whether MSC exert its immunosuppressive effect by means of EV, we isolated EVs from htTERT MSCs. We showed that htMSC-derived EV contained TSG-6 protein by western-blot. Coculture of EV with CpG-A-primed GEN2.2 resulted in downregulation of IFN-α transcription and protein expression, mediated via reduction in total and phospho-IRF7.

**Conclusion:** For the first time, we showed that MSC could downregulate TLR9 activation in human pDCs, and this was dependent on TSG-6. Furthermore, htMSC-derived EV contain TSG-6 and suppress IFN-α response in CpG-A primed pDCs through reducing total and phospho-IRF7. Our findings revealed mechanistic insights on the immunosuppressive properties of MSC, and their therapeutic potential in autoimmune disorders triggered by TLR9 activation shall be further explored.
Effect of Adipose-derived Stem Cell and Non-methylated CpG-ODN on Peripheral Blood CD4+CD25+ Regulatory T Cell in Young Mice of Food Allergy

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Background and Aims: To investigate the effects of adipose-derived stem cells (ADSC) and non-methylated CpG-oligodeoxynucleotides (CpG-ODN) on the expression of peripheral blood CD4CD25 regulatory T (Treg) cells in young mice with food allergy, as well as their immune intervention effects.

Methods: A total of 40 female BALB/c mice were randomly divided into control group, allergic group, ADSC treatment group, and CpG-ODN treatment group. A mouse model of food allergy was established by intraperitoneal injection and intragastric administration of ovalbumin (OVA) for sensitisation and challenge. The mice in the control group were treated with normal saline at the same dose; the mice in the ADSC treatment group were given intraperitoneal injection of ADSC (1*10^6cellsforeachmouse) before and after OVA challenge, and those in the CpG-ODN treatment group were given intraperitoneal injection of non-methylated CpG-ODN solution (40 ug for each mouse) at 1 hour before challenge by gavage. The allergic symptom scores determined for each group after model establishment. ELISA was used to measure the serum level of OVA-IgE. Flow cytometry was used to measure the percentage of peripheral blood CD4CD25 Treg cells. Haematoxylin and eosin staining was used for the pathological analysis of the jejunum.

Results: The allergic group had significantly higher symptom scores and serum level of OVA-IgE than the control group (p<0.05). There were no significant differences in the allergic symptom score and the serum level of OVA-IgE between the ADSC treatment group and CpG-ODN treatment group (p>0.05), but these two groups had significantly symptom scores and serum level of OVA-IgE than the allergic group and significantly higher allergic symptom scores and serum level of OVA-IgE than the control group (p<0.01). The allergic group had a significantly lower percentage of peripheral blood CD4CD25 Treg cells than the control group (p=0.05). The ADSC treatment group and the CpG-ODN treatment group had a significantly higher percentage of peripheral blood CD4CD25 Treg cells than the allergic group (p<0.05); there were no significant differences between these two groups or between them and the control group (p>0.05). Pathological results showed structural damage and edema in the jejunal villi, a large number of eosinophils, and lymphocyte infiltration in the allergic group, while the ADSC treatment group and the CpG-ODN treatment group had less structural damage and edema in the jejunal villi, a lower number of eosinophils, and less lymphocyte infiltration.

Conclusions: ADSC and non-methylated CpG-ODN have a certain effect in the treatment of food allergy and can increase the percentage of peripheral CD4CD25 Treg cells and reduce the level of OVA-IgE. They may be associated with the induction of immune tolerance.

B Regulatory Cells in Children Atopic Dermatitis

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Background: Immunoregulatory cells were identified to have suppressive role in immune responses. The B regulatory cells have been recently described for its ability to produce suppressive cytokines, to maintain Treg function, and to signal multiple immune cells. Atopic dermatitis (AD) is characterised by eczematous skin lesions showing inflammatory infiltrates of Th and memory T cells. It is believed the over sensitive inflammatory response is caused by a dysfunction of the regulatory immunity. The potential of using regulatory cells as therapeutic for autoimmune disease are currently under research.

Aim: To determine the regulatory cell profile in AD children, and correlate with AD symptoms and clinical parameters.

Methods: Chinese patients with AD were recruited from Paediatric clinics of The Prince of Wales Hospital, Hong Kong. Subjects were excluded with disease other than atopy. Use of corticosteroids, anti-histamine and other medication are recorded. Blood were collected from AD patients, and disease severity was assessed by physician. Blood were tested for serum IgE, lymphocyte count, and flow cytometry for B regulatory cells. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) statistical software for Windows, version 21. A probability value less than 0.05 will be considered significant. The study was approved by the CUHK-NTEC ethics committee.

Result: A total of 32 AD subjects (age: 9.5±4.6; 50% Male) were recruited. The IgE level ranged from 264-37296, with 62% classified as hyper-IgE (IgE >2000 IU/ml). The eosinophil percentage averaged at 7% with standard deviation of ±2.9%. Twelve out of 32 subjects were having...
active S. Aureus infection. The subjects were suffering from different severity of AD, with 6 mild; 14 moderate; 12 severe. B regulatory cells were detected from all samples, ranging from 3-12% (of all B cells). B regulatory cells % were significantly different between disease severity level \((p=0.05)\), with an increase in severe group. In addition, IL10 production in B regulatory cells were increased in severe group \((p=0.05)\).

**Conclusion:** In severe AD group, IL10 production and B regulatory cells were increased. The results were similar with B regulatory cell research of other autoimmune diseases. This suggested the B regulatory cell and IL10 production were not suppressed in severe AD patients, however the function of the cells and cytokines will need to be further investigated.

**Comparative Analysis of the Profiles of Short Chain Fatty Acids in Stool Samples of Healthy Infants and Infants with Eczema**

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**Backgrounds and Aims:** Short chain fatty acids (SCFAs) are metabolites produced by commensal bacteria. These major end products of anaerobic bacteria fermentation in the gut modulate host metabolism and regulate immune function. The aim of this study was to compare the profile of SCFAs in the first year of life between infants with eczema and healthy controls.

**Methods:** From a larger mother-offspring cohort, Growing Up in Singapore Towards healthy Outcomes (GUSTO), 34 children who developed eczema in the first 18 months of life and 40 non-eczema controls with similar demographic and clinical factors were selected. A total of 160 stool samples obtained over 3 times points (month 3, 6, 12) were analysed for the amount of short chain fatty acids (acetic-, propionic-, butyric-, isobutyric-, valeric-, isovaleric-, 2-methybutyric-, caproic- and 4 methylvaleric acids) by liquid chromatography tandem mass spectrometry. Longitudinal multivariate analysis was made and data was adjusted for possible confounders (mode of delivery, feeding pattern, antibiotics at labour and antibiotics in first year of life).

**Results:** Longitudinal analysis of individual SCFA levels showed higher butyric acid levels \((p<0.01)\) in controls compared to eczema. When the mode of delivery was taken into consideration, vaginally-delivered controls had higher butyric acid \((p<0.01)\) and 4-methyvaleric acid levels \((p<0.05)\) compared to those with eczema. For infants born by caesarean-section, controls had higher concentrations of isobutyric acid \((p<0.05)\) compared to those with eczema.

**Conclusions:** Specific SCFAs over the first year of life were lower in infants with eczema compared to non-eczema controls, suggesting an influence in eczema development. These differences are likely to result from the perturbation/impairment of the maturation of infant gut microbiome in infants with eczema.

**Thrombocytopenia in Cytomegalovirus Infection Can Lead To or Mislead From the Diagnosis of Wiskott-Aldrich Syndrome**

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**Introduction:** Cytomegalovirus (CMV) infection is frequently seen in the clinical setting of primary immune deficiency diseases (PIDs). Children with Wiskott-Aldrich syndrome (WAS) are predisposed to CMV infection. Thrombocytopenia is a dominant clinical finding in both congenital and postnatal CMV disease as well as WAS.
Thrombocytopenia may sometimes be falsely attributed to the CMV infection alone and thus the diagnosis of WAS can be missed. Here, we describe the case profile of 3 children who had CMV infection and WAS.

**Case 1**: A 4-month-old boy presented with blood stained loose stools. Family history was noncontributory. On examination, he was pale, had oral ulcers and hepatosplenomegaly. Severe anaemia with thrombocytopenia was noted. CMV IgM serology was positive and eye evaluation revealed active CMV retinitis. Colonic biopsy showed colitis with CMV inclusions. Postnatal active disseminated CMV disease was diagnosed and treatment initiated. However, thrombocytopenia persisted. Mean platelet volume was low and WASP protein expression was reduced. Mutation in Exon 1 of WAS gene confirmed the diagnosis of WAS.

**Case 2**: A 6-year-old boy presented with discharging sinus from right leg and headache. He had been unwell since the first year of life and was diagnosed to have severe atopic dermatitis. He had had blood mixed loose stools, multiple episodes pneumonia, and otitis media in past. He developed Staphylococcus aureus chronic osteomyelitis of right femur. Eye examination revealed elevated intraocular pressure, mild anterior uveitis, and unilateral acute glaucoma. Polymerase chain reaction from the aqueous was positive for CMV. CMV serology (IgM) was negative but CMV DNA PCR was positive. Review of old case records showed persistent thrombocytopenia which helped clinch the diagnosis of WAS.

**Case 3**: A 4-month-old boy presented with recurrent febrile episodes, progressive pallor and skin bleed. On examination, he had eczema, petechiae, and hepatosplenomegaly. He had severe anaemia and thrombocytopenia History of a death of male sibling 2 years back prompted the diagnosis of WAS. However, CMV IgM was found to be positive and CMV viral load was highly elevated. No active eye lesions were found. He received prolonged ganciclovir treatment and prophylaxis is being continued as he awaits transplant.

**Learning Objectives**: Proactively screening of children with CMV infection for WAS as well as children with WAS for CMV disease is necessary. Thrombocytopenia is a masquerader: can lead to or mislead the diagnosis.

Eye examination for CMV infection is necessary for children with WAS.

* This case has been published before J AAPOS. 2013 Dec;17(6):646-7. PMID:24210345

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**Association Between Breastfeeding in Infancy and Cognitive Function of Adolescents in Santiago, Chile**

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**Background and Aims**: The benefits of breastfeeding are well-established in literature, such as immunoprotection for infants, and reduced rates of depression and breast cancer for mothers. However, whether it relates to child intelligence is an ongoing scientific debate. Studies with robust designs have produced mixed results. Few have assessed whether benefits continue into adolescence. The aim of this study was to investigate the association between duration of breastmilk as the sole source of milk in infancy and cognitive function in adolescence.

**Methods**: Data were from 891 adolescents who completed an infancy iron deficiency anaemia preventive trial in Santiago, Chile. Date of the first bottle of formula was collected in infancy, and used to calculate the duration of breastmilk as the sole source of milk. Duration was categorised into 0-3 m, 3.1-6 m and >6 m. Cognitive function was assessed in adolescence using the Wechsler Intelligence Scale for Children-IV: Matrix Reasoning (WISC-MR) and Verbal Similarities (WISC-VS). Generalised linear modeling was used to assess differences in cognitive functioning scores by breastfeeding group. Models were adjusted for sex, birth weight, SES, maternal IQ, HOME score, maternal stress, age of WISC evaluation, randomisation group, and infancy iron deficiency anaemia.

**Results**: The sample was 50.2% female, 16.2±0.2 years of age at follow-up, and low-middle income. The average date of first bottle was 3.6 months (SD=3.1). 51.6% of participants were breastfed for at least 3 months. The average score for WISC-MR was 7.5 (SD=2.4), and WISC-VS was 8.4 (SD=2.1). Longer duration of breastmilk as the sole source of milk was associated with higher scores in WISC-MR (F=4.06, p=0.018). Those who breastfed for 0-3 m scored significantly lower on the WISC-MR at 16 y (M=7.31, SE=0.12) compared to those who breastfed for 3.1-6 m (M=7.83, SE= 0.15, p=0.004) and >6 m (M=7.72, SE=0.19, p=0.044). There was no difference in scores between the 3.1-6 m and >6 m groups. There were no significant associations between breastfeeding and WISC-VS score.
**Conclusion:** In a sample of healthy infants, at least 3 months of breastfeeding related to improved WISC-MR score in adolescence. Findings add to existing literature that breastmilk is a superior form of nutrition for infants, and its benefits extend to adolescence. Public policy advocacy campaigns to promote breastfeeding may benefit from this finding.

**Association of Household Tobacco Exposure in Hong Kong Young Children Under 2 Years of Age with Lower Family Socioeconomic Status and Medical Service Utilisation**

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**Background and Aims:** Household tobacco exposure in young children causes great disease and economic burden. Local prevalence of infant household tobacco exposure was previously reported to be 41.2%. Updated prevalence and identification of associated factors of household tobacco exposure in Hong Kong young children are important. This study aimed to examine the updated prevalence of household tobacco exposure in local young children under 2 years of age and to explore the associations between household tobacco exposure and family socioeconomic status, recent respiratory symptoms and medical service utilisation.

**Methods:** Analysis was performed on data obtained from a community-based cross-sectional pneumococcal carriage surveillance study of healthy children aged under 2 years across 4 main regions of Hong Kong. Information on demographics, household tobacco exposure, family socioeconomic status, children's recent respiratory symptoms and medical service utilisation was obtained by parent-reported questionnaires.

**Results:** A total of 1541 subjects (mean age: 11.2 months, male: 50.7%) recruited from June 2013 to June 2014 were included in the final analysis. The overall prevalence of household tobacco exposure was 31.5%, prevalence of prenatal and postnatal maternal smoking was 1.6% and 3.5% respectively. After adjustment for potential confounding factors, low household income (AOR=1.38, 95% CI: 1.08-1.76), overcrowding of household area (AOR=1.15, 95% CI: 1.11-1.25) and residing in New Territories West (AOR=1.65, 95% CI: 1.18-2.32) were independently and significantly associated with household tobacco exposure in young children. Practice of breastfeeding was significantly associated with lower odds of having household tobacco exposure (AOR=0.65, 95% CI: 0.50-0.84). For medical service utilisation, household tobacco exposure (AOR=1.33, 95% CI: 1.03-1.70) and postnatal maternal smoking exposure (AOR=2.30, 95% CI: 1.09-4.85) were significantly associated with doctor consultation in recent 3 months; postnatal maternal smoking exposure (AOR=2.70, 95% CI: 1.16-6.27) was significantly associated with hospitalisation in recent 3 months. However, household tobacco exposure was not significantly associated with recent respiratory symptoms in our cohort.

**Conclusions:** The prevalence of household tobacco exposure in young children under 2 years of age in our study was lower than previous local study. Lower family socioeconomic status was significantly associated with household tobacco exposure in young children. Household tobacco exposure in young children was associated with medical service utilisation. As home is the most significant source of environmental tobacco exposure for young children, efforts for reducing such exposure are essential especially in socially deprived population.

**A Sports Mentorship Program Improves Adolescent Mental Health and Physical Fitness: A Randomised Controlled trial**

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**Background and Aims:** To assess the effectiveness of a positive youth development (PYD)-based sports mentorship program on physical and mental well-being of adolescents recruited in a community setting.

**Methods:** This is a randomised controlled trial recruiting students from 12 secondary schools in Hong Kong, China. Participants were randomly assigned in a 1:1 ratio to an intervention or a control arm after stratification for school, from October 2013 to June 2014. Participants were not blinded to allocation due to the nature of intervention. Students in the intervention arm received an after-school PYD-based sports mentorship for 18 weeks. Each weekly session lasted for 90 minutes. Students in the control arm received exclusive access to a health education website.

**Results:** 664 students (mean age 12.3 [SD 0.76]; 386 females [58.1%]) completed baseline and post-intervention assessments. The intervention improved students' mental
well-being (Cohen's d 0.25, 95% confidence interval 0.10-0.40, P=0.001), self-efficacy (0.22, 0.07-0.37, P=0.01), resilience (0.19, 0.03-0.34, P=0.02), physical fitness (flexibility [0.28, 0.13-0.43, P=0.02], lower limb muscle strength [0.18, 0.03-0.33, P=0.03], dynamic balance [0.21, 0.06-0.37, P=0.01]), and physical activity level (0.39, 0.24-0.55, P<0.0001). The intervention did not significantly improve physical well-being (-0.01, -0.17-0.14, P=0.86), BMI z-score (-0.03, -0.18-0.12, P=0.69), body fat proportion (-0.15, -0.31-0.00, P=0.051), and social connectedness (-0.03, -0.18-0.12, P=0.72).

Conclusions: A PYD-based sports mentorship intervention improved healthy adolescents’ mental well-being, psychological assets, physical fitness, and PA level.

Accident & Emergency Department Attendances and Survival Patterns Among Children with Down Syndrome: A Population-based Cohort Study Follow-up From Birth

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Background and Aims: This study aims to, with the use of hospitalisation data, describe the survival patterns and attendance to Accident & Emergency Department (AED) among children with Down Syndrome (DS) in Hong Kong.

Methods: A population-based, retrospective cohort study was conducted on 1010 livebirths with DS delivered from 1995 to 2014, as identified from the territory-wide hospitalisation data. Kaplan-Meier product-limit method was adopted to estimate the survival probabilities of children with DS by selected demographic and clinical characteristics.

Results: Within the study period, the average live birth rate with DS in Hong Kong was 8.0 per 10,000 live births [95% confidence interval (CI): 6.8, 9.3]. Throughout the period, a total of 75 of 1010 livebirths with DS died, with the overall half-, 1-, and 5-year survival probabilities 95.8%, 94.4%, and 92.6%. Significant improvement in their survival has been observed, particularly among those born after 2000 compared to those born between 1995 and 1999 (p<0.05). Moreover, people with DS who were admitted to AED within their first half year of life had poorer survival rate in general.

Conclusions: The early life survival of children born with DS has improved incrementally across the last 2 decades. Further efforts are needed to educate the caregivers and health professionals to prevent the potential early onset of associated complications among children with DS, especially those with early AED visits.

A Scoping Review of Injury Outcome Indicators: Implications for Injury Surveillance

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Background and Aims: The aim of this review is to identify and scope injury outcome indicators for injury surveillance.

Methods: Literature search was conducted to identify existing injury outcome indicators using academic databases including Web of Science, PubMed, and ProQuest, and hand searching by Google Scholar. The method of this scoping review is an iterative process of search and analysis. After three rounds of review, researchers identified that saturation had occurred and stopped the searching process. Forty-seven search queries were generated to achieve a representative sample of the distribution of an evidence base across the topic area in this study. The searching process was outlined for identification of possible aspects of search terms which could contribute to more relevant results upon expansion of further literature search.

Results: Upon analysis of 142 articles, a total of 52 indicators were identified and were classified into four domains: in-hospital performance (17 indicators), functional/psychological outcomes (18 indicators), biological/physiological outcomes (9 indicators), and long-term impacts (7 indicators). There were injury severity definitions that were common across injury types. A synthesis of the findings and their significances in each domain were described.

Conclusions: Findings suggested that the list of injury outcome indicators in this review can serve as a set of indicators for different countries to extract their relevant set of indicators. A set of existing indicators relevant to a region is of particular importance for injury surveillance.
Effect of Socioeconomic Disparity on Childhood Injury in Hong Kong

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Background: Injury is one of the leading causes of childhood mortality and morbidity globally. In Hong Kong, injury has been consistently the 2nd leading cause of death among children of 0-14 years. Comprehensive injury data is needed in order to design specific intervention strategy and prioritise suitable resources to combat against the public health problem. Hong Kong, although small in size, is one of the most densely populated areas around the world and is divided into 18 districts. Each district has its own characteristics, including demographics, geographical features and housing attributes. The combination creates a unique environment with the district that pose varying injury risks across districts. The study aims to analysis on the difference in injury epidemiology among the 18 districts in Hong Kong and to explore the relationship between childhood injury and socioeconomic indicators.

Methods: A retrospective analysis of Hospital Authority's Accidents and Emergency Department (AED) visits of 0-19 year-old children due to injury between 2001 and 2012 was conducted through geo-spatial analysis and regression analysis of each injury sub-type. Sub-group analysis of age group and time sub-period were also conducted.

Results: There were a total of 742,552 episodes of AED visits due to injury during 2001-2012. 67% (n=495,207) were male. Rate of attendance of male was 5,839 per 100,000 population, which is 1.9 times more than that of female. Annual injury attendance rate was highest among 0-4 year-old children, at 6,799 per 100,000. There is an overall decreasing trend of children's AED attendance due to injury. Analysis by residential district revealed that Tai Po (6,500 per 100,000), North (5,290), Sai Kung (5,166) and Kwai Tsing (5,159) have the highest childhood injury risks. Regression analysis also showed that districts of higher socioeconomic indicators have lower risks of injury. Childhood injury's protective socioeconomic factors include smaller household size, higher household income media and higher employment rate.

Conclusion: Injury pattern varied in the 18 districts in Hong Kong due to the different characteristics in each district. There was also an inequity in injury risks due to socioeconomic disparities in Hong Kong. Further studies should be conducted to look into whether socioeconomic indicators affect the severity and outcome of injury among children, as well as how community level factors affect the risks of injury.

Instrument-based Visual Screening of 3-year-old Children in Japan

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Introduction: Amblyopia is a common and major cause of vision impairment for children, but the early diagnosis has been not easy because of the difficulty in examination of visual acuity in infant. Most of children have been examined by using picture eye chart performed by parents at home in Japan, but the accuracy of the results have been often suspected. Early diagnosis and treatment for amblyopia risk factors such as astigmatism, hyperopia, anisometropia, and myopia are known to lead better vision, but the treatment effectiveness is reduced after age five. Recently, instrument-based screening has developed and the amblyopia risk factors have been screened within seconds, one meter away from children. American academy of paediatrics showed the policy statement of visual system assessment in infants, children, and young adults by paediatricians in 2016, and the instrument-based screening were recommended. Here, we introduced instrument-based screening.

Method: We introduced the handheld photorefractor (Spot Vision Screener) at the 3-year-old health check-up of Tagawa city health centre. Children at the age of 3-year-old are screened by the instrument at the centre and conventional picture eye chart at their home.

Results: Over 150 children are screened, over 95% of them were tolerated. About 5-10% of them had amblyopia risk factors. Many of them are normal in the examination by picture eye chart.

Discussion: Early diagnosis of amblyopia risk factors was not achieved by conventional picture eye chart. This means
many children have been lost opportunity to prevent amblyopia. Although ocular history and ophthalmological examinations are important, the diagnosis of amblyopia risk factors is difficult. The age 3-year-old may be the best age for vision screening, because they can speak about their vision and they are also very sensitive to treatment of visual impairment. After 3-year-old health check, most Japanese children have no opportunities to examine vision until 6-year-old preschool health-check. Age 6-year-old seems to be late to prevent amblyopia, and the late treatment may lead to permanent vision impairment.

Conclusion: We conclude to avoid the treatment delay of amblyopia risk factors, instrument-based screening should be introduced for all 3-year-old children.

Assimilation of Reality of Health Index in National Health Promotion in Japan: As a Prospect of Flame-work Analysis and Reaction of External Causes

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Background and Aims: In Healthy Parent-Child 21 as a Japanese national plan of health promotion, the comprehensive success has been reported and on the other hands, the remarkable contribution with reality could not be impressed. So here we analysed the Assimilation of reality of health index in Healthy Parent-Child 21 and particularly of the index of child abuse.

Methods: (1) The time-dependent process of plural indexes in Healthy Parent-Child 21 in 2000, in 2004, in 2008 and in 2012 as the quantitate variables and three flame works such as the health and medical standards, citizen's action and some administrative groups and NGO etc's action and those structured by these plural indexes which could contribute the 5 step appraisal (being bad, not change, not enough improvement, improvement, impossible to be estimated). The logistic regression analysis was carried out as the grouped variables of 4 main topics in Healthy Parent-Child 21 such as adolescence health and education (topic A), safety of pregnancy and child birth and support of infertility (topic B), environmental preparation for child & maternal health and proper development of infant mind and child care support using SAS9.4 (EG7.1). The method of variable selection was the variable increase method and of optimisation was the Fishers Scoring method. (2) The health indexes of child abuse in Healthy Parent-Child 21 and other regional health indexes of child abuse were compared.

Results: (1) The odds ratio and 95% CI of topic A was 0.041 and 0.002-0.577 and of topic B was 0.193 and 0.032-0.984. (2) The difference and opposite trends between both indexes of child abuses were observed.

Conclusion: It was suggested that the contribution of some administrative groups was higher than citizen's action to the improvement of health indexes. Also in Japan, social capitalised citizen's action could be recognised most important in national health promotion. The difference and opposite trends between both indexes of child abuses were observed. It seemed that the results of our analysis had shown the in assimilation between national health promotion and reality of health-related matters.

Utility and Access of the Rotavirus Vaccine in Introducing Countries

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Background and Objectives: The third dose of the diphtheria, tetanus, pertussis (DTP) vaccine has been internationally recognised as a performance indicator providing insight into the utility and access of immunisation services. Since 2006, the World Health Organization (WHO) has encouraged countries to adopt the rotavirus vaccine to protect children against severe diarrhea and death caused by dehydration. The rotavirus vaccine schedule would follow that of DTP, meaning that the same service performance indicators could be used and applied to the Rotavirus vaccine.

Methods: Reported 2016 vaccine coverage of the first and last dose of the DTP and rotavirus vaccine series were collected from the WHO/UNICEF Joint Reporting Form (JRF) for the 84 rotavirus vaccine introducing countries. Eighteen countries were excluded from the analysis for either having missing, incomplete, or inconsistent vaccine coverage data. The final number of countries included in the analysis was 66.

Results: Low-income countries (LICs) have the highest reported rotavirus vaccine coverages (for the first and last dose within the series) compared to the other income groups. High income countries (HICs), on the other hand, reported the highest DTP coverage values but had the lowest reported rotavirus vaccine rates, which contributed to having the highest percent difference (15%) between the reportage coverage rates of the two vaccines. As outliers, São Tome Príncipe, Zimbabwe, Senegal reported higher Rotav1 coverage values than DTP1. A great majority (46/66, 70%)
of rotavirus vaccine introducing countries have good utilisation and access to the rotavirus vaccine while only 11% (7/66) scored poorly on these service indicators.

Conclusions: LICs and low-middle income countries (LMICs) have successfully integrated the rotavirus vaccine into their National Immunization Programs in comparison to HICs. There should be efforts geared towards improving rotavirus vaccine coverage in HICs. The seven poor performing countries (Greece, Guinea-Bissau, Haiti, Liberia, Marshall Islands, Micronesia, Venezuela) should be encouraged to decrease their immunisation service gaps, utility and access of rotavirus vaccination, to better protect infants against life-threatening rotavirus infections.

CYP2E1 Gene Polymorphisms Related to the Formation of Coronary Artery Lesions in Kawasaki Disease

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Background and Aims: Kawasaki disease (KD) is an acute febrile systemic vasculitis that disturbs coronary arteries. Patients' risks of adverse cardiovascular events and subclinical atherosclerosis have been found to significantly increase with polymorphisms of the human cytochrome P450. This current study aims to research the possible relationship between Cytochrome P450, Family 2, Subfamily E, and Polypeptide 1 (CYP2E1) polymorphisms with KD.

Methods: We selected six tag single-nucleotide polymorphisms (tSNPs) of the CYP2E1 gene for TaqMan allelic discrimination assay in 340 KD patients and performed analysis on the clinical phenotypes and coronary artery lesions (CAL). CAL associations of tSNPs were adjusted for age and gender in the logistic regression.

Results: This study consisted of 340 participants. A total of 35.6% of the KD cases experienced CAL formation. Of these, 12.6% did not respond to the initial intravenous immunoglobulin (IVIG) treatment. Furthermore, 150 patients with acute-phase KD were treated with high-dose acetylsalicylic acid (80 to 100 mg/kg/day). We examined six CYP2E1 gene polymorphisms and their potential association with KD in Taiwanese children. After genotyping six SNPs, we noticed that SNP rs2070676 and rs915906 of the CYP2E1 gene had a strongly association with the risk of CAL in the recessive model. For rs915906, the C/C genotype reflected a higher risk of CAL in KD patients (p=0.009). Regarding rs2070676, the G/G genotype was strongly associated with the risk of CAL formation (p=0.007). However, the SNPs of the CYP2E1 gene did not influence CAL formation in the participating KD patients either with or without high-dose acetylsalicylic acid. To determine the association between SNPs (rs915906 and rs2070676) of CYP2E1 and gene expression, we retrieved the tissue expression quantitative trait loci (eQTLs) data from the GTEx Portal (http://www.gtexportal.org/home/). The TT genotype of rs915906 had a higher expression of CYP2E1 when compared to the CC genotype in sun-exposed skin tissue (p=3.8e-14). Furthermore, the CC genotype of rs2070676 had a higher expression of CYP2E1 when compared to the GG genotype in esophagus tissue (p=4.9e-15).

Conclusions: This study is the first to find that the risk of CAL formation is associated with CYP2E1 gene polymorphisms in KD patients.

Increased Pituitary Volumes in Fontan Patients: The Other Portal Circulation

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Background and Aims: Current results of Fontan operation are acceptable as the final palliative surgery in patients with single ventricle physiology. There exist two portal systems in human being; the hepatic and pituitary portal systems, and these portal systems become super-portal systems after the completion of Fontan operation. Although the disturbance of the hepatic portal system has been shown to cause Fontan-associated liver disease or protein losing enteropathy, there is little information about the pituitary portal system in Fontan patients. The aim is to investigate pituitary volume measured by brain magnetic resonance imaging (MRI) and to compare pituitary volumes between Fontan patients and control subjects.

Methods: We performed brain MRI in 40 Fontan patients (26 males) and 74 age-matched control subjects (42 males). The median age at Fontan operation was 3.3 (1.6-5.7) years. Brain MRI was performed using a 1.5-T system at 9.3 (7-23) years of age in Fontan patients and at 10.4 (8.8-12.8) years in the control subjects, and T1-weighed images were
acquired. The pituitary volume was obtained by multiplying the high by depth by width by 0.52. We also measure the volume of the pons referred to the volume of the pituitary gland.

**Results:** The median the pituitary volume in Fontan patients was $472 \ (425-527) \ mm^3$, which was significantly larger than that in the control subjects $[267 \ (203-320) \ mm^3, p<0.001]$. However, the median pons volume was $7,286 \ (6,672-8,241) \ mm^3$, which was significantly smaller than that in the control subjects $[8,331 \ (7,531-9,148) \ mm^3], p<0.001]$. In Fontan patients, the larger pituitary volume was significantly related to an increase in central venous pressure, but there was no correlation with age, systemic saturation, cardiac index, and pulmonary arterial resistance.

**Conclusions:** The present study suggest that not only hepatic portal circulation but also the pituitary portal circulation is impaired in Fontan patients. Increased pituitary volumes in Fontan patients may surrogate congestion of the pituitary portal circulation which becomes super-portal circulation after Fontan operation.

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**Mechanism of Hyponatraemia in Kawasaki Disease: A role of Nonosmotic ADH Secretion and Salt Loss**

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**Background:** The mechanisms of hyponatraemia in Kawasaki disease (KD) have been reported to be hypotonic dehydration or syndrome of inappropriate secretion of antidiuretic hormone (SIADH). However, the precise mechanism of hyponatraemia remains elusive because assessment of volume status based on serial change in body weight is lacking.

**Methods:** Eighteen patients who were diagnosed with KD and hyponatraemia (serum sodium level less than $135 \ mEq/L$) were analysed. Hyponatraemia was diagnosed at febrile state before IVIG treatment in all subjects. Plasma arginine vasopressin (ADH), urine electrolytes, and serum cytokine levels were measured at diagnosis of hyponatraemia. Increase and decrease in body weight by $>3\%$ was defined as hypervolaemia and hypovolaemia, respectively. A diagnosis of SIADH was based on all the following criteria; (1) absence of hypovolaemia, (2) urine sodium level $>20 \ mEq/L$, (3) urine osmolality $>300 \ mOsm/kg$, and (4) detectable plasma ADH ($>0.8 \ pg/mL$).

**Results:** The volume status was hypervolaemic in 3 (17%), euvoalaemic in 14 (78%), and hypovolaemic in 1 (6%). The diagnoses were SIADH in 5 (28%) and hypotonic dehydration in 1 (6%). Plasma ADH levels were inappropriately high in 16 (89%). Contribution of decreased total exchangeable cations (salt loss) to occurrence of hyponatraemia $[5.0 \ (interquartile range, 2.5-6.5\%)]$ was significantly larger than contribution of increased total body water $[1.6 \ (-1.3-3.5) \%] \ (p=0.012)$. Fractional excretion of uric acid (FEUA) significantly correlated with increased total body water ($r=0.57, p=0.01$), and serum interleukin-6 levels significantly correlated with salt loss ($r=0.66, p=0.04$). Twelve patients (67%) other than SIADH or hypotonic dehydration were characterised by euvoalaemic or hypervolaemic hyponatraemia, salt loss, inappropriately high ADH levels, and a significant increase in total body water after diagnosis of hyponatraemia, which was not significantly different from SIADH patients.

**Conclusions:** Our study demonstrated that hyponatraemia in KD was euvoalaemic or hypervolaemic and might be explained by salt loss and water retention induced by nonosmotic secretion of ADH in the majority of patients. Physicians should avoid infusion of hypotonic solutions with low sodium concentrations. Restriction of infusion rate would also be recommended, especially when FEUA is high.

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**Ventricular Mechanics in Adolescents and Adults Late After Repair of Subarterial and Perimembranous Ventricular Septal Defects**

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**Background and Aims:** There have been concerns of ventricular dysfunction late after surgical repair of non-subarterial ventricular septal defects (VSD). We assessed and compared right (RV) and left ventricular (LV) mechanics in adolescents and adults after surgical closure of subarterial and perimembranous defects.

**Methods:** A total of 75 subjects were studied: 29 patients after subarterial VSD repair (group I), 17 patients after perimembranous VSD repair (group II) and 29 age-matched controls (group III). RV and LV mechanics were assessed using tissue Doppler and speckle tracking echocardiography,
while RV outflow systolic function was quantified by systolic excursion and fractional shortening (FS).

**Results:** Compared with group III, groups I and II had significantly reduced tricuspid annular systolic and diastolic velocities, isovolumic myocardial acceleration, RV global longitudinal systolic and diastolic deformation parameters, and RV outflow systolic excursion (all p<0.05). Group I, but not II, had reduced RV outflow FS (p=0.008) and the lowest global LV longitudinal systolic strain (p=0.008) and systolic strain rate (p=0.014). In group I, postoperative aortic regurgitation was associated with lower LV longitudinal systolic strain (p=0.009) and early diastolic strain rate (p=0.048). As a group, RV outflow excursion (p<0.001) and FS (p=0.001) were correlated with LV global systolic strain.

**Conclusion:** Adolescents and adults late after repair of subarterial and perimembranous VSDs show impairment of RV systolic and diastolic myocardial deformation. The RV outflow function and LV systolic deformation appear to be worse after repair of subarterial defects.

**Clinical and Genetic Profile of Congenital Long QT Syndrome in Hong Kong: 18-year Experience in Paediatrics**

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**Background and Aim:** To report the clinical characteristics and management of all patients diagnosed with congenital long QT syndrome (LQTS) in a Hong Kong single tertiary paediatric cardiology centre.

**Methods:** All paediatric patients diagnosed at our centre with LQTS from January 1998 to December 2016 were included. LQTS was established if corrected QT interval was greater or equal to 480 ms in repeated 12-lead ECG, a Schwartz score >=3.5 or a presence of pathogenic mutation.

**Results:** Fifty-nine patients (33 boys) fulfilled the inclusion criteria, with a mean age of 8.90 ± 5.74 years at diagnosis. The mean follow-up duration was 5.33 ± 4.65 years. 84.7% individuals were probands. Around 1/5 (20.3%) of our LQTS patients had malignant presentation of VF/VT episodes. 23 (39.0%) and 7 (11.9%) patients first presented with syncope and convulsion respectively. Seven patients (11.9%) had cardiac arrest on presentation. Fetal bradycardia and neonatal 2:1 atrioventricular block were also the mode of presentation in 2 patients. The mean corrected QT interval in our cohort was 504 +/- 47 ms. 42.4% individuals had a positive family history. 38 patients (64.4%) confirmed to have pathogenic mutation for LQTS (LQT1-16.9%; LQT2-18.6%; LQT3-11.9%; LQT5-5.1%; LQT8-10.2%; LQT16-1.7%). No deaths were reported but 5 patients had subsequent cardiac arrest despite on treatment. Pacemaker was implanted in 4 patients, while implantable cardioverter-defibrillator was implanted in 14 patients. Two patients underwent left cardiac sympathectomy in addition to ICD therapy. 72.3% individuals were put on medical therapy, with metoprolol being the preferred initial choice of beta-blocker therapy in our cohort. For LQTS patients with initial presentation of dizziness, syncope or convulsion, nearly 70% became asymptomatic on medical treatment and lifestyle modifications. All our asymptomatic LQTS patients remain event-free throughout our follow-up period.

**Conclusions:** 18-year experience in management of paediatric LQTS in a tertiary cardiology centre is described, with a significant proportion of probands. Syncope and convulsion were the main forms of presentation. Our cohort demonstrated relative good survival.

**The Number of Lymphocytes Can Be a IVIG Treatment Failure Predictor in Kawasaki Disease As Well As Neutrophil-lymphocyte Ratio, Platelet Lymphocyte Ratio**

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**Background and Aims:** Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are used as systemic inflammatory markers and prognosis of adverse cardiovascular events. Recently there have been reports that NLR and PLR in the acute stage of Kawasaki Disease (KD) may be a predictor of refractoriness against intravenous immunoglobulin (IVIG) treatment in Kawasaki disease. To validate whether NLR and PLR could be predictors of IVIG treatment in the acute phase of KD.

**Methods:** A retrospective study was performed with 398 patients with KD that we experienced in our department between January 2009 and December 2016. And we divided them into responders and non-responders according to the initial IVIG responsiveness. NLR, PLR and the absolute number of differential blood count were calculated. Furthermore, those values were evaluated when they were used with RAISE (Randomized controlled trial to Assess Immunoglobulin plus Steroid Efficacy for Kawasaki disease) score.
Results: Both NLR and PLR of non-responders were significantly higher than those of responders (P<0.01). The sensitivity and the specificity were 70.3% and 63.9% respectively by using a cut-off of NLR ≥3.12. The sensitivity and the specificity were 70.4% and 50.6% respectively by using a cut-off of PLR ≥100. In addition, IVIG non-responder group showed significantly less number of the absolute lymphocyte count than responder group (p<0.01). We determined the cut-off level of absolute lymphocyte count as 2600/ml. Then, the sensitivity and the specificity were 61.1% and 68.2% respectively. The positive predictive value of the refractory prediction combined NLR and RAISE score ≥5 was 33.3% [95% CI 23.9-44.4] and the odds ratio was 3.1 [1.1-8.0]. The positive predictive value of the refractory prediction combined PLR and RAISE score ≥5 was 35.1% [95% CI 25.2-46.5] and the odds ratio was 3.8 [1.3-9.7]. The negative predictive value of lymphocyte alone was 92.1% [95% CI 88.1-94.8].

Conclusion: We demonstrated that NLR, PLR and the number of lymphocyte play an important role in predicting IVIG-resistance in Kawasaki disease. NLR and PLR are values which can be calculated easily. However, we can see the number of lymphocytes more quickly. It is crucial to pay attention to lymphocyte count for predicting IVIG-resistance. Further investigation is necessary to improve the accuracy of NLR, PLR and the number of lymphocyte.

Selection of Endotracheal Tube Cuff for Children with Congenital Heart Disease Based on an Ultrasound-based Linear Regression Formula

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Background and Aims: The use of empirical age-based formula to determine the endotracheal tube (ETT) size has been reformed after the introduction of ultrasound technology. However, it remains to be discovered whether an ultrasonography-based formula used to determine the subglottic transverse diameter for the selection of an appropriate ETT cuff for children with normal heart anatomy is useful for children with congenital heart disease (CHD).

Methods: A regression formula for predicting the subglottic diameter (SGD_formula) was established after assessing 60 children ≥8 years without CHD. The formula was validated on a group of 60 children with CHD. We selected the ETT cuff based on the SGD determined by ultrasound (SGD Ultra). Subsequently, the fit of the ETT cuff in 60 children who underwent cardiosurgery was examined via air leak test. The maximum allowed difference between the SGD predicted using the formula and the ETT cuff size that fit was 0.2 mm. The agreement among and accuracy of SGD Ultra, SGD formula and the ETT used in children with CHD was analysed.

Results: For children without CHD, we adopted a linear regression formula, given by SGD_formula(mm)=0.4*age+5.3. For children with CHD, we adopted an allometric formula, given by SGD_formula(mm)=5.4*age^0.18. A stronger agreement existed between SGD Ultra and ETT compared to that between SGD formula and ETT, and bias was 0.21 mm (95% confidence band, -0.59 to 1.01 mm) and -0.00 cm (-0.79 to 0.84 mm), respectively. Furthermore, for the CHD group, the ultrasound-based method yielded a 78% (47/60) success rate, while the formula-based method permitted an appropriate selection of ETT in only 32% (19/60) of subjects (P=0.00).

Conclusion: Our analysis shows that the allometric equation is ideal for children with CHD, and the linear regression equation is best suited for children without CHD. SGD Ultra was more accurately able to predict the ETT size for children awaiting cardiovascular surgery, while the linear regression equation was better for predicting the relationship between age and SGD.

Waist-to-height Ratio Remains an Optimal and Easier Index for Indicating Obesity-related Cardiovascular Risk in Children and Adolescents

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Background and Aims: Which obesity measures, including body mass index (BMI), waist circumference (WC) and waist to height ratio (WHtR), better indicate cardiovascular risk remains controversial for paediatric population since cardiovascular disease rarely occur in this population. Recently tri-ponderal mass index (TMI) is reported better correlated with body adiposity than BMI. The current study aims to evaluate the accuracy of body adiposity indexes based on BMI, TMI, WC, WHtR and percentage of body fat (PBF) as indicators for cardiovascular risk in children and adolescents.

Methods: Eligible subjects were recruited from 4 schools in Shanghai and 6 schools in Chongqing by random cluster sampling. Height, weight, WC, blood pressure, fasting blood glucose (FBG) and lipid profiles (elevated triglyceride, total cholesterol, low density lipoprotein cholesterol, decreased
high density lipoprotein cholesterol) were examined by standard protocols. PBF were measured by dual energy X-ray absorptiometry and standardised into Z scores using American recommendation. BMI and WHtR were computed. BMI and WC were standardised into Z scores to adjust effect of age by gender. Central obesity was defined by age-specific cutoff recommended by China Children's Obesity Working Group. Two outcomes were defined. Subjects with any 3 or more the following abnormalities, elevated FBG, lipid profiles, blood pressure, or central obesity, were defined as CVD3 (outcome 1). Subjects with at least 2 above abnormalities were defined as CVD2 (outcome 2). Pearson correlation coefficients were calculated between WHtR, TMI, BMI, WC and PBF. Receiver operation curves (ROC) were performed to assess and compare the performance of WHtR, TMI, BMI, WC and PBF in predicting two CVD outcomes, respectively.

**Results:** A total of 1863 subjects aged 7 to 18 years with complete data were included in this analysis. WHtR and TMI were very weakly correlated with age (r=-0.05 and 0.05, Ps<0.001). WHtR, TMI, BMI and WC were highly correlated to PBF (r=0.71, 0.67, 0.68 and 0.72, Ps<0.001). To predict CVD3, AUCs of WHtR, TMI, BMI, WC, PBF were 0.83 (95% CI: 0.79-0.87), 0.82 (95% CI: 0.78-0.86), 0.83 (95% CI: 0.79-0.87), 0.83 (95% CI: 0.79-0.88), 0.81 (95% CI: 0.77-0.85), respectively. To predict CVD2, AUCs were 0.80 (95%CI: 0.77-0.83), 0.79 (95%CI: 0.77-0.82), 0.82 (95%CI: 0.80-0.84), 0.82 (95% CI: 0.80-0.85), 0.78 (95% CI: 0.75-0.81), respectively.

**Conclusion:** Considering performances in indicating CVD risk and simplicity in application, we believe that WHtR remains an optimal index to evaluate obesity related CVD risk in children and adolescents in public health.

**Urine Biomarkers for Monitoring Renal Function in Premature Infants**

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**Background and Aims:** Premature infants are at high risk for acute kidney injury (AKI) from various causes. The serum creatinine level has limitations in evaluating the renal function of premature infants because neonatal serum creatinine reflects maternal levels at early postnatal period. Furthermore, it is difficult to repeat invasive blood sampling to monitor serum creatinine levels for them. The purpose of this study is to evaluate whether urine biomarkers can be used to monitor development of AKI in premature infants.

**Methods:** A prospective cohort study was conducted in premature infants born less than gestational age (GA) 37 weeks and admitted to the neonatal intensive care units of Kangnam Sacred Heart Hospital and Inha University Hospital. Urine biomarkers and serum creatinine were measured on postnatal day 1, 3, 5, 7, 10, and 14. Urine biomarkers include neutrophil-gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), liver fatty acid binding protein (L-FABP), cystatin-C (CysC), osteopontin (OPN), and epidermal growth factor (EGF). AKI was classified using modified Acute Kidney Injury Network (AKIN) definition.

**Results:** A total of 83 infants were recruited. (male:female 1:4.1) Mean GA and birth weight was 30.5±3.0 weeks and 1514±549 g, respectively. AKIs occurred in 17 (20.5%) infants at mean age of 7.4±2.6 days. When classified using the modified AKIN definition, 9 (12.0%) infants were classified as the stage 1, 5 (6.0%) and 2 (2.4%) infants were classified as the stage 2 and 3, respectively. One (1.2%) infants died because of sepsis and AKI. Because AKI did not occur in infants born after GA 32 weeks, we compared demographic factors and urine biomarker between AKI and non-AKI groups under GA 32 weeks. There are no differences in baseline characteristics except GA in multivariate logistic regression analysis. Urine levels of NGAL, CysC, KIM-1, L-FABP and OPN were higher in AKI group than in non-AKI group. Urine levels of IL-8 were higher in AKI group than in non-AKI group at around the onset of AKI. Conversely, urine levels of EGF were lower in AKI group than in non-AKI group before the onset of AKI.

**Conclusion:** In this study on premature infants, though there was no demographic risk factor for AKI except GA, several urine biomarkers were significantly different between AKI and non-AKI groups. Urine biomarkers could be useful to monitor renal function and predict AKI development in premature infants.
records of patients below 18 years old who had received CRRT in either NICU or PICU of Queen Elizabeth Hospital from January 1998 to May 2017.

Results: Altogether 76 patients received 81 episodes of CRRT during the period. 55.6% were male and 25.9% were neonatal patients. The median (interquartile range) age was 6.0 (11.6) years for paediatric patients and 3.0 (12.5) days for neonatal patients. 76.5% of CRRT were performed for AKI-related indications including abnormal renal function, acidosis or electrolytes imbalance (91.9%), volume overload (56.5%) and removal of toxic substances (4.8%), whereas 23.5% of CRRT were performed for non-renal indications including metabolic disease (57.9%), sepsis (15.8%), medication intoxication (15.8%) and tumour lysis syndrome (10.5%). The overall mortality was 33.3% and the duration of ICU stay was 17 (30) days. 81.5% of survivors managed to stop CRRT but 18.5% remained dialysis-dependent.

Besides, 54.5% of those who were off CRRT had impaired renal function. Multivariate analysis identified PRISM III score (Odds ratio [95% confidence interval] 1.14 [1.03-1.25]) and pre-CRRT fluid overload (OR: 1.39 [1.10-1.77]) as independent predictors for mortality. Comparison of variables between survivors with and without renal recovery revealed that diagnosis of primary renal diseases (p<0.001), PRISM III score (p=0.047), pre-CRRT urine output (p=0.001) and baseline estimated glomerular filtration rate (p<0.001) were significant determinants of renal outcome.

Conclusion: Children required CRRT carried high mortality. 63% of survivors had impaired renal function or remained dialysis-dependent. PRISM III score and pre-CRRT fluid overload were independent predictors for mortality. Survivors with primary renal disease, lower PRISM III score and poorer baseline renal function carried worse renal prognosis.

Chronic Intermittent Hypoxia Exposure Induces Kidney Injury in Growing Rats

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Objective: To examine the effect of chronic intermittent hypoxia (CIH) on the morphological changes in the kidney of growing rats and to explore the mechanisms underlying the CIH-induced renal damage.

Methods: 40 Sprague-Dawley rats were randomly divided into two groups: 2 and 4 weeks CIH groups (2IH, 4IH), and in the control group 2 and 4 weeks air stimulated groups (2C, 4C), with 10 rats in each group. Pathological changes of renal tissue were observed by HE staining, PAS staining and Masson staining. Real-time PCR method was used to detect the mRNA expression of HIF-1α, Cu/ZnSOD and MnSOD in renal tissue.

Results: (1) Intermittent hypoxia (IH) caused morphological damage in the kidney. Hypertrophy of epithelial cells in the kidney tubules and dilation in the glomeruli were observed under light microscope in HE and PAS stain, especially in 4IH group. Besides, 54.5% of those who were off CRRT had impaired renal function. Multivariate analysis identified PRISM III score (Odds ratio [95% confidence interval] 1.14 [1.03-1.25]) and pre-CRRT fluid overload (OR: 1.39 [1.10-1.77]) as independent predictors for mortality. Comparison of variables between survivors with and without renal recovery revealed that diagnosis of primary renal diseases (p<0.001), PRISM III score (p=0.047), pre-CRRT urine output (p=0.001) and baseline estimated glomerular filtration rate (p<0.001) were significant determinants of renal outcome.

Conclusion: Oxidative stress played a critical role in renal damage by up-regulating HIF-1α transcription and down-regulating Cu/ZnSOD and MnSOD transcription after chronic intermittent hypoxia exposure in growing rats.

Inhibition of MicroRNA-155 Alleviates Lipopolysaccharide-induced Kidney Injury in Mice

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Background and Aims: Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Accumulated evidences suggest that microRNAs (miRNAs) are related with inflammation-associated diseases. The aim of this study is to investigate whether miR-155 is involved in lipopolysaccharide (LPS)-induced kidney injury, and to explore the underlying mechanisms.

Methods: Mice were intraperitoneally injected with LPS to construct endotoxemia mice model, and miR-155 inhibitor was injected via tail vein to suppress the expression of miR-155 in kidney.

Results: The results indicated that the expression of miR-155 was markedly increased in renal tissues of LPS-treated
mice. And miR-155 inhibitor protected mice from LPS-induced kidney injury associated with the lower levels of TNF-α and IL-6 in renal tissues. Furthermore, inhibition of miR-155 increased the expression of suppressor of cytokine signaling 1 (SOCS1), a target gene of miR-155 and a negative regulator of Janus activated kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway. Consistently, inhibition of miR-155 suppressed the expression of JAK2, STAT3 and phosphorylated STAT3 (p-STAT3).

**Conclusions:** All these results indicated that inhibition of miR-155 protects mice from LPS-induced kidney injury possibly through regulating SOCS1-JAK2/STAT signaling pathway, which suggested that miR-155 might be an important and potential target in developing therapy for preventing sepsis-associated kidney injury.

**Clinical and Pathological Correlation in Diagnosis of Paediatric Invasive Pulmonary Aspergillosis**

**Background and Aims:** Invasive pulmonary aspergillosis (IPA) has been one of the major causes of mortality in immunocompromised patients. Therefore, early diagnosis and appropriate treatment could improve survival outcome. The gold standard in diagnosis IPA is histopathological examination of lung tissue; however, post-procedural bleeding limits the feasibility of lung biopsy.

The European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and The National Institute of Allergy and Infectious Disease Mycoses Study Group (EORTC/MSG) defined definitions for IPA as proven, probable, and possible IPA. To our knowledge, there is limited data about validity of these definitions comparing to histopathological diagnosis of IPA, especially in paediatric population. The onbjective in this study was to validate the EORTC/MSG 2008 definition of IPA, comparing to the gold standard of histopathological result, in paediatric population.

**Methods:** Histopathological examination of lung tissue of 1 month to 18 years old patients, with respiratory tract infection at the time obtaining biopsy/autopsy, between January 2006 to December 2016, were identified. Retrospective chart review for clinical characteristic; including underlying disease, immune status, diagnostic tests, treatment and outcome was done. IPA diagnosis was classified according to EORTC/MSG 2008 definition. Data was analysed using SPSS 18 software.

**Results:** During the 10-year period, there were 256 histopathology of lung tissue, 58 of which suspected pulmonary infection. Fourteen (24%) was proven IPA by histopathology. Seven (50%), 7 (50%) and none were classified as probable, possible, and no IPA, respectively, by using EORTC/MSG 2008 definition. While, histopathological negative for IPA showed 14 (32%), 14 (32%) and 16(36%) were classified as probable, possible, and no IPA, respectively. When compare probable/possible IPA to no IPA, we found that EORTC/MSG 2008 definition had 100% sensitivity, 36% specificity, 33% positive predictive value, and 100% negative predictive value in diagnosis of IPA.

**Conclusion:** Our study show the EORTC/MSG 2008 consensus definitions have a 100% sensitivity but low specificity for diagnosis of IPA.

**The accuracy of Dengue NS1 Antigen Test for the Early Diagnosis of Dengue Infection: A Systematic Review and Meta Analysis**

**Background:** Dengue Fever is a major international public health concern. There has been a dramatic global increase in the incidence of Dengue Fever (DF), DHF and DSS. There is thus, an urgent need for an affordable, time saving and convenient diagnostic test for the early diagnosis of Dengue. Dengue NS1 antigen was introduced before for the early diagnosis of Dengue. Several studies have been conducted to evaluate the accuracy of this tool.

**Aims:** General: To determine the accuracy of Dengue NS1 Antigen test for the early diagnosis of dengue infection by systematic review and meta analysis. Specific: To determine the Sensitivity and Specificity of Dengue NS1 antigen based on all literatures reviewed. To determine the PPV and NPV of Dengue NS1 antigen based on all literatures reviewed. To determine the likelihood ratio of Dengue NS1 antigen test based on all literatures reviewed. To determine the overall accuracy of Dengue NS1 antigen test based on all literatures reviewed.

**Methods:** Inclusion Criteria: Cross Sectional Studies using Dengue NS1 Antigen as an index test for the early diagnosis of Dengue infection. Search Strategy: Electronic Searches were done using the library of the local references, Cochrane central Register of Controlled Trials, Pubmed, Elsevior, Google Scholar, NEJM and the reference lists of
identified studies. Data Extraction and Statistical Analysis: Two authors independently assessed the studies and extracted data from the studies. Data synthesis was carried out using Review Manager version 5.3.

**Result:** 5 studies were included with a total of 1,976 serum samples. Results showed; Sensitivity: 63.5% (Mean), 59% (Median), 62% (Mode); Specificity: 98.6% (Mean), 97% (Median), 100% (Mode); PPV: 98.6% (Mean), 97% (Median), 100% (Mode); NPV: 48.4% (Mean), 55.9% (Median); LR+: 27.4 (Mean), 43.5 (Median); LR-: 0.36 (Mean), 0.37 (Median), 0.57 (Mode); and for overall accuracy the mean was 73.1%.

**Conclusion:** This meta-analysis implies that the Dengue NS1 antigen test can be used as a valid method and deserve inclusion in the diagnostic evaluation of early Dengue infection.

**Cadherin-related Family Member 3 Expression and Their Regulation in the Human Respiratory Explant Cultures**

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**Background:** Cadherin-related family member 3 (CDHR3) is identified as a susceptible gene for early childhood asthma with severe exacerbations in genome-wide association study published in 2014. In early 2015, the same transmembrane protein is found to be the cellular receptor for the human rhinovirus C (RV-C). Intriguingly, the HRV-C infection is the leading cause of childhood wheezing illnesses and asthma exacerbation. The rs6967330 in CDHR3 is one of the top SNPs identified and the tyrosine at position 529 is found to be more cell surface expression and yielded ten-fold more RV-C progeny viruses. This finding infers that factors that could induce an overexpression of CDHR3 at the cell surface might lead to a greater susceptibility of RV-C, therefore, a higher chance of wheezing or asthma exacerbation.

**Aims:** To examine the expression of CDHR3 in human respiratory epithelial cells upon the exposure to wheezing and asthma exacerbation associated risk factors, and its effect in alternating RV-C susceptibility.

**School-based Surveillance for Childhood Influenza in Hong Kong, 2014-15**

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**Background and Aims:** Influenza imposes substantial healthcare burden in terms of hospitalisation and mortality in children, which can be prevented by vaccination. Influenza vaccination coverage varies widely among childhood populations worldwide, which has significant impact on herd immunity and usefulness of influenza vaccine. However, there is limited real-life data on influenza vaccine effectiveness (VE) in children. This study aimed to investigate clinical spectrum of influenza infection and VE in preventing influenza in Hong Kong children.

**Methods:** This prospective cohort study recruited children aged 2-12 years from 15 kindergartens and primary schools. Parents completed a questionnaire on subjects’ health status and history of influenza vaccination. Flocked nasopharyngeal swabs (NPSs) were collected at biweekly
school visits during influenza seasons in 2014-15, and illness visits were arranged for children with influenza-like illness (ILI). Influenza A and B were detected and typed by polymerase chain reaction, and influenza immunity measured by haemagglutination inhibition (HAI).

**Results:** 623 children provided a total of 2,633 NPS samples. Two samples were obtained from 607 (97.4%) of subjects. Thirty-six (11.2%) subjects had influenza A or B in 2014 whereas all 19 (6.3%) subjects had influenza A in 2015. Seropositivity rates for A (H1N1) pdm09, A/H3N2, A/H3N2_Switzerland, B/Victoria-lineage and B/Yamagata-lineage were 92%, 91%, 68%, 49% and 85%, respectively. Ninety-nine subjects reported ILI and nine illness visits were arranged. Seasonal influenza vaccination was protective against ILI but not laboratory-confirmed influenza by surveillance. Influenza VE for ILI varied between 42.1 (10.5-63.1) % and 51.9 (24.5-70.1) % depending on the year of vaccination. Subgroup analyses showed higher VE for both ILI (70.9% vs 34.6%) and mild laboratory-confirmed influenza (44.0% vs -6.2%) in school-age children than preschoolers who were vaccinated within 12 months. HAI titres and seropositivity did not differ in subjects with and without ILI. Logistic regression confirmed protective effect of influenza vaccination against ILI. There was no reported transmission of influenza within subjects’ classes and household.

**Conclusions:** Mildly symptomatic influenza is common in children during influenza seasons. Seasonal influenza vaccination is effective against ILI but not mild influenza identified by surveillance. HAI titres do not appear to indicate protective immunity for childhood influenza.

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**Effects of Different Immune and Non-immune Factors on CDHR3 Expression in Airway Epithelial Cells and Ex vivo Bronchus Culture**

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**Background and Aims:** Human rhinovirus C (HRV-C) infection was reported to be a major risk factor for asthma exacerbations and wheezing illnesses in children. This respiratory virus has not been widely studied because it was not culturable in standard cell culture until the recent identification of cadherin-related family member 3 (CDHR3) as its cellular receptor on airway epithelial cells. We hypothesised that cellular distribution of CDHR3 in the human airways was associated with host susceptibility to HRV-C infection. This study aimed to investigate changes in CDHR3 expression of the human respiratory epithelial cells upon exposure to asthma-related stimuli.

**Methods:** Both human alveolar type II epithelial cells (A549) and primary human nasopharyngeal epithelial cells were subjected to challenges with dexamethasone, lipopolysaccharide (LPS), and cigarette smoke medium (CSM). CDHR3 expression levels on these respiratory epithelial cells were determined at gene and protein levels using quantitative PCR and western blot. The localisation of CDHR3 was detected by immunofluorescence staining. The effects of these stimuli on the susceptibility of respiratory epithelial cells to HRV-C infection were evaluated by the subsequent inoculation with HRV-C isolate. The replication kinetics of HRV-C was assessed by titrating the culture supernatant using CDHR3-expressing H1-HeLa cells.

**Results:** A 549 cells incubated with dexamethasone expressed 4-fold higher CDHR3 than control cells at 24 hours post treatment, while LPS induced 8-fold increase in CDHR3 at 48 hours post-treatment. Stimulation with 0.625% CSM upregulated CDHR3 expression by 5-fold and 150-fold at 24 and 48 hours following treatment, respectively. The effects of CSM on the human primary nasopharyngeal epithelial cell culture were consistent to those observed in A549 cells, although primary nasopharyngeal epithelial cells were less responsive to dexamethasone and LPS treatments. CDHR3 expression only increased 2-fold at 48 hours after these treatments.

**Conclusions:** The exposure of respiratory epithelial cells to asthma-related stimuli such as LPS and CSM can enhance CDHR3 expression. Interestingly, the incubation of these respiratory epithelial cells with dexamethasone, a corticosteroid useful for suppressing asthmatic airway inflammation, can also alter the expression of HRV-C receptor.

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HIV Coreceptor Tropism in Treatment-naïve and Treatment-experienced HIV-1 Infected Children and Adolescents

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Background and Aims: HIV-1 enters host cells by interaction with the envelope glycoprotein gp120 with CD4 molecule and coreceptor. The chemokine receptor 5 (CCR5) and chemokine receptor 4 (CXCR4) are usually the main coreceptors. Initially, the CCR5 antagonist, maraviroc, was approved for treatment-experienced adults infected with CCR5-using strains. However, CCR5 antagonists could be considered in the future as alternative drugs in HIV-1-infected children and adolescents who have virologic failure after standard treatment. Coreceptor tropism testing should be performed prior to initiation of therapy with CCR5 antagonists. HIV-1 coreceptor tropism varies among different HIV-1 subtypes. The majority of HIV-1 subtype in Thailand is CRF01_AE. To our knowledge, data on coreceptor tropism of HIV-1 CRF01_AE in children and adolescents are limited. We aim to evaluate the prevalence of coreceptor tropism in HIV-1-infected children and adolescents in Thailand.

Methods: HIV-1 infected patients, aged <20 years, who failed the standard ARV’s and had viral HIV-1 RNA >1000 copies/ml or who were treatment-naïve were enrolled from September 2015 to February 2017. Plasma samples were collected for determining the HIV-1 coreceptor tropism by using genotypic testing methods. Coreceptor tropism was predicted base on V3 sequences using GENO2PHENO version 2.5 with a false positive rate of 5%. Results: Fifty-two HIV-1 infected participants, aged 14.9 years (IQR 8.9-16.8) were recruited. The median CD4 cell count was 396 (IQR 72-630.25) cell/uL. HIV-1 RNA viral load was done in 47 (90.38%) patients and the median was 4.6 (IQR 3.8-5.3) log10 copies/ml. Thirty-nine patients (75%) had experience virological failure of the standard ART and the remainder were antiretroviral-naïve. The disease spectrum ranges from asymptomatic to severe respiratory complications and in rare instances, subsequent death. It belongs to the family Picornaviridae and the genus Enterovirus, a member of D species. In the US, there is a National Enterovirus Surveillance System since the 1960s, however, in Hong Kong, the surveillance system of such is lacking. In addition, the whole genome data of EV-D68 is limited. With the effort of screening through 6,800 nasopharyngeal aspirate (NPA) sample from 2010-2014 by a local group, a 0.44% (n=30) positive rate was found in two regional hospitals on Hong Kong Island.

Aim: Our study aims to extend the EV-D68 surveillance in the in-patient settings in the two major hospitals in the East New Territories with a catchment of 1.5M population
and 11.7% are under 17 years old. This will allow a better understanding of the molecular epidemiology of EV-D68 strains in Hong Kong.

**Methods:** NPA samples collected in September 2014 to December 2015 from in-patient under 17 years old were retrospectively examined. Samples which were PCR screened to be enterovirus positive were further genotyped by PCR of the VP4/VP2 region followed by Sanger sequencing. Nucleotide sequences with published EV-D68 sequences were aligned using Clustal Omega and phylogenetic trees were generated by MEGA software using the neighbor-joining method with a reliability with 1,000 bootstrap replications.

**Results and Discussion:** Within the 10,529 NPA collected from hospitalised children, 23.3% (n=2,457) of them were positive with an enterovirus in the regular diagnostic testing. 1,033 (42%) underwent genotyping and fifteen EV-D68 were identified followed by full genome sequencing. The EV-D68 viruses detected in the winter of 2015 showed high similarity with the published strains from Southern China, Taiwan and Japan during a similar study period.

**Molecular Detection and the Whole Genome Sequencing of Echovirus Serotypes Identified in Hong Kong Hospitalised Children**

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**Background:** Enteric cytopathic human orphan virus (Echovirus) is single-stranded positive sense RNA virus which was first isolated from the feces of asymptomatic children in the context of epidemiological studies of polioviruses in the 1950s. It can cause mild and self-limited disease but sporadically, it can cause a severe central nervous system infection, such as aseptic meningitis, encephalitis, paralysis. So far, there were no complete genome data of echovirus strains from Hong Kong’s isolates and in general, genomic data of such is limited. Echovirus has a genome of approximately 7.5 Kb. It belongs to the species Enterovirus B, genus Enterovirus of the Picornaviridae family, echoviruses include 33 serotypes and the genome consists of a 5’ untranslated region (UTR), structural polypeptide P1, nonstructural polypeptides P2 and P3, and a 3’ UTR.

**Aim:** By conducting the *Enterovirus* surveillance in the nasopharyngeal aspirate (NPA) obtained from hospitalised population below 17 years old, we would like to improve the understanding of the molecular epidemiology and evolution of echovirus serotypes in Hong Kong.

**Methods:** Retrospective NPA samples collected from September 2014 to December 2015 in the two major hospitals in the East New Territories from in-patient settings were screened. Genotyping of echovirus was carried out by using the PCR of the VP4/VP2 region followed by Sanger sequencing. When echovirus was screened positive, whole genome sequencing was performed and nucleotide sequences with published echovirus sequences were aligned using Clustal Omega and phylogenetic trees were generated by MEGA software using the neighbour-joining method with a reliability with 1,000 bootstrap replications.

**Results and Conclusions:** Within the 10,529 NPA collected from hospitalised children, 23.3% (n=2,457) of them were positive with an enterovirus in the regular diagnostic testing. One thousand and thirty-three (42%) underwent genotyping and ten echoviruses were identified followed by full genome sequencing. Six different echovirus serotypes, E6 (n=1), E9 (n=3), E16 (n=1), E18 (n=2), E25 (n=2), E3 (n=1), were detected using VP4/VP2 region specific PCR, whole genome sequencing was performed to get their complete genome data. This could indicate a wide range of echovirus serotypes circulating in Hong Kong. Our study contributed the first Hong Kong echovirus whole genome data to this research arena all over the world. Data from a variety of geographic areas promote the investigation on associations between variants and clinical symptoms.

**Serum Inflammatory Cytokine Levels Correlate with CD4+ T Cells in Hand-foot-mouth Disease**

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**Background and Aims:** To investigate the correlation between the expression of proinflammatory and anti-inflammatory cytokines associated with CD4+ T cells and severity of disease and prognosis in severe hand, foot, and mouth disease to find out predict factors for further immunotherapy.

**Methods:** From January 2014 to January 2016, the serum of 433 cases of children with HFMD and divided into five groups: death group of 30 cases of death, survival group of 58 cases, severe group of 167 cases, mild group of 118 cases, subclinical infection group of 60 cases and 50 cases of
normal control group were collected, IFN-α, IFN-β, IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, TNF-α, IL-1β, GM-CSF, TGF-β were measured by quantitative cytokines antibody microarray.

**Results:** Serum IFN-γ, IFN-α, IFN-β in children with death group significantly increased compared with severe group, the mild group, the recessive infection group increased compared with normal control group (p<0.05). Proinflammatory cytokine TNF-α, TNF-β, IL-6, IL-1, IL-2, IL-12 levels increased significantly both in death group and survival group compared with severe group, the mild group and normal control group (p<0.05). IL-18 and IL-8 levels significantly increased of the death group (p<0.05), IL-1β, IL-6, IL-8 levels higher in subclinical infection group than NC group (p<0.05). In the death group, anti-inflammatory cytokines of IL-10, TGF-β levels were high than survival group, severe group, mild group, the recessive infection group and normal control group (p<0.05). IL-4 levels increased in mild, recessive infection group and normal control group (p<0.05), but there was no significant difference between death group, survival group and severe group. The levels of GM-CSF, IL-21 of death group increased significantly compared with the severe group, the mild group, the recessive infection group and normal control group (p<0.05).

**Conclusion:** The proinflammatory cytokine and anti-inflammation cytokine levels are increased by disease severity, but the death group had higher IL-10 which reveal that compensatory antiinflammation syndrome exist in severe hand, foot, and mouth disease.

**Development and Application of Enterovirus 71 Vaccine in China**

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**Background and Aims:** Enterovirus 71 (EV71)-associated hand, foot and mouth disease (HFMD) causes significant morbidity and mortality, leads to some severe neurological complications, and poses a serious burden on the health of children and the public. Due to the lack of effective drug treatment, vaccine is the main method to control disease. This article summarises EV71 vaccine development progress and application status in China.

**Methods:** Through document retrieval, all literatures related to EV71 vaccine research and evaluation, clinical trial and application since 2001 were reviewed and summarised.

**Results:** A total of 50 related literature were retrieved in the database. Varieties of EV71 vaccine are in research and development. At present, EV71 vaccine mainly includes inactivated whole vaccine, live attenuated vaccine, recombinant VP1 vaccine, VP1-based DNA vaccine, synthetic peptide vaccine and virus-like particle vaccine. Three inactivated vaccine in China have been completed phase III clinical trials. Among them, the vaccine developed by Chinese Academy of Medical Sciences and Sinovac Biotechnology Co., Ltd. have been approved on December 2015.

The vaccine developed by Beijing Vigoo biological is undergoing a permit review process. Three vaccines have good safety, efficacy and immune response in each clinical trials. The rate of prevent infection EV71-associated HFMD infection is 90%-97.3%, severe case protection is 100%. After 1 year two-pin base immunisation, inoculation of 1-pin boosted immunisation could cause long-term protection of the disease.

**Conclusions:** EV71 vaccine in China is in continuous progress, expect to collect larger sample size and longer follow-up monitoring data include safety, efficiency, EV71 genotype and gene recombination etc. Prevention of other enteroviruses such as Coxsackievirus A16 and Echovirus are still challenges of vaccine applications. The development of multivalent broad-spectrum high-performance enterovirus vaccine, and how to combine with the pentavalent vaccine and other immunisation need further efforts.

**Kinetic Measurements of Human Neutrophil Lipocalin Can Monitor Antibiotic Efficacy in Patients with Local Bacterial Infections**

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**Background and Aims:** Human neutrophil lipocalin (HNL) is a protein released from neutrophil granules and is regarded as a useful marker for discriminating acute bacterial and viral infections. The aim of this study was to explore its potential to monitor the effect of antibiotic treatment in patients with local bacterial infections.

**Methods:** Sera HNL were quantified in 40 healthy individuals and 105 patients confirmed with acute infections (30 with sepsis; 45 with local bacterial infections; 30 with viral infections). HNL levels were measured in all cases before antibiotic treatment, as well as 48 h and 72 h post antibiotics treatment for cases with local bacterial infections. Levels of serum CRP were measured at the same time for comparison.
Results: Prior to antibiotic treatment, serum HNL levels were significantly higher in patients with local bacterial infections than those with viral infections (p<0.001). After treatment for 48 h and 72 h, HNL levels declined rapidly as the infections went under control.

Conclusions: In summary, serum HNL may serve as a highly sensitive and specific early diagnostic marker for acute bacterial infections. Kinetic detection of HNL may monitor the efficacy of antibiotic treatment in patients with local bacterial infections.

Blocking Integrin CD11b Inhibits LPS-induced HMGB1 Release and Translocation During Sepsis

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Background and Aims: High mobility group box 1 (HMGB1), a chromatin-binding nuclear protein, plays a critical role in the generation and development of sepsis by acting as a key "late-phase" inflammatory mediator. Due to its unique secretion pattern and severe pro-inflammatory effect, HMGB1 has been recognised as an alarm in the regulation of immune response to infection and the pathophysiological process of sepsis and other autoimmune disease. Here, we investigate the role of integrin CD11b in HMGB1-mediated sepsis and LPS-induced HMGB1 release.

Methods: Firstly, the exact role of CD11b in sepsis was investigated in mouse CLP-induced sepsis model in vivo. The detailed molecular mechanisms of CD11b in LPS-induced HMGB1 release was forward to evaluate by Western blot, flow cytometry, immunocytochemical analysis and CoIP analysis in vitro.

Results: In CLP-induced septic model, antagonism of CD11b by using blocking antibody or pharmacological CD11b inhibitor could protect mice from septic death and inhibit the level of circulating HMGB1 but not TNF-α. Consistent with this, compared to wild-type mice, CD11b knockout (CD11b−/−) mice exhibited improved survival rate with decreased HMGB1 level in serum. Further study showed that pharmacological antagonism and genetic knockdown/knockout of CD11b could hamper LPS-induced HMGB1 cytoplasmic translocation and active release from macrophages. To clarify the underlying mechanism of effect of CD11b-mediated HMGB1 inhibition, immunofluorescence microscopy and co-immunoprecipitation were carried out. As observed, CD11b knockdown blocked HMGB1 nucleocytoplasmic translocation both by hampering interaction with a nuclear export factor CRM1 and inhibiting phosphorylated modification of HMGB1 by cPKC.

Conclusions: Taken together, all results give us the direct evidence of antagonism of integrin CD11b could exert protection against sepsis and inhibit LPS-induced HMGB1 cytoplasmic translocation and active release by hampering interaction between HMGB1 with CRM1 and PKC. Our studies will help us to clarify that targets for CD11b could be an alternative therapeutic target of HMGB1-mediated endotoxemia and sepsis.

RETA: An R Package for Whole Exome and Targeted Region Sequencing Data Analysis

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Background and Aims: Whole exome and targeted region sequencing play a major role in diagnoses of Mendelian diseases. However, currently analysis of these data involves using a number of different complex tools and understanding of the analysis results is no easy job. So here we aim to creating a user-friendly integrative analysis tool, and using R programming, we have developed an easy-to-use package, RETA, to provide a one-stop analysis for whole exome and targeted region sequencing data.

Methods: RETA presents analysis results as an interactive report with many visualisation features. The report is divided into six sections: general QC, in-depth QC, candidate gene QC, structural variants, inheritance mode analysis and detailed figures for CNV and coverage analysis. General QC shows overall summary of the targeted regions and sequencing reads. In-depth QC includes IBD relationship check, consanguinity check and summary of sequencing coverage for targeted regions. Candidate gene QC reports low coverage or quality regions within the focused genes specified by the user. Structural variants section is for CNV analysis currently. Inheritance analysis provides a list of high quality variants that are consistent with the inheritance mode for e.g. autosomal dominant or recessive for families. Finally, the detailed figures for CNV context and low coverage regions are shown in the last section and users may click gene names and jump back and forth conveniently.

Results: The final results were presented in a well-organised interactive HTML file.
Conclusions: RETA should help researchers and medical professionals to analyse the huge amount clinical sequencing data with ease.

Impact of Clinical Geneticist’s Analysis on the Diagnostic Process of Whole Exome Sequencing: Experience from 104 Families
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Background and Aims: To evaluate the role of clinical geneticists in the diagnostic process and subsequent clinical utility of whole exome sequencing results.

Methods: 104 prospectively recruited patients with undetermined diagnoses had Whole exome sequencing (WES) performed by two laboratories, Genome Diagnostics Nijmegen (Nijmegen, Netherlands) and Ambry Genetics (Aliso Viejo, CA). Among them, 93% were children below the age of 18, and families were predominantly Chinese (94%). The WES result of each patient was comprehensively reviewed with incorporation of clinical and molecular data, review of literature and databases, segregation analysis, subsequent clinical investigations, functional studies, expert review and exome reanalysis. The subsequent changes in diagnosis and management was evaluated.

Results: Among the 104 patients, singleton WES was performed in 81 patients and trio-based WES in the remaining 23 families. Review by the clinical geneticist changed variant classification in 18 patients (17%) and variants were either promoted (n=10), demoted (n=5), or additional variants (n=3) were identified. For example, clinical review and discussion prompted reanalysis for variants in the FGD1 gene and a diagnosis of Aarskog-Scott syndrome (OMIM #305400) was identified. Overall the diagnostic yield was 40%, some of which involved recently discovered disease genes (e.g. PURA, DDX3X, WAC, PPP1CB, KMT2B). Recommendation in clinical management was made in 77% of the diagnosed patients after WES.

Conclusions: Comprehensive review of WES reports by the clinical geneticist can have substantial impact on the diagnostic yield of exome reports. Clinical geneticists have a unique advantage, as they have direct contact with the families, prior knowledge of the clinical background, and possess the skills in dysmorphic assessment and gestalt analysis which gives insights missed by the use of phenotypic keywords. We demonstrate that even when the clinical and exome teams are not co-located geographically, a good collaboration is still achievable by enhanced cross talk between institutions.

Acknowledgement: The study is supported by (i) SK Yee Medical Foundation and (ii) the Society for the Relief of Disabled Children.

Blood cell type-specific Genome-Wide DNA Methylation Analysis of Chinese Patients with Early-onset Systemic Lupus Erythematosus Identifies Loss of DNA Methylation in Genes Related to the Type I Interferon Pathway
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Background and Aims: Around 20% of Systemic Lupus Erythematosus (SLE) is diagnosed in children under 18. These children usually present severer disease than adult-onset patients. Since whole blood comprised of different immune cells, we aimed to identify the cell type-specific DNA methylation signatures of CD4+ T cells, CD8+ T cells, B cells, neutrophils and whole blood in individuals with SLE patients who presented before 18-year of age.

Methods: We compared the DNA methylation profiles of different blood cells for 16 Chinese SLE patients to that of 13 healthy controls using the Illumina HumanMethylationEPIC BeadChip. Data pre-processing was performed to remove cross-reactive probes, probes with SNPs at the target site, and probes from sex chromosomes. For each specific region, Wilcoxon rank-sum test was used for group comparisons, and false discovery rate was used for multiple testing corrections. Differentially methylated CpG sites were defined as CpG sites with an adjusted p-value <0.05 and a mean methylation change >0.1.

Results: 775280 probes remained after data preprocessing and principal component analysis showed that samples clustered according to specific cell types rather than disease manifestation. Global changes of DNA methylation were not observed among different cell types. The number of differentially methylated CpG sites ranged from 46 to 160 in comparisons of different cell types, with more CpG sites showing hypomethylation than hypermethylation. Gene ontology analysis was performed for each cell type and revealed that in all the cell types examined, hypomethylated
genes identified were overrepresented in the type I interferon pathway.

**Conclusions:** Our results suggest that the DNA methylation changes in different immune cells of SLE patients target the same biological pathway. As type I interferon has long been believed to be involved in the pathogenesis of SLE, our findings support the importance of an epigenetic mechanism in the dysregulation of type I interferon in SLE pathogenesis.

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**Genetic Epidemiology of Novel Genetic Mutations Identified by Next Generation Sequencing in the Ciprofloxacin-resistant Nontyphoid Salmonella Isolates in Taiwan**

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**Background and Aims:** The ciprofloxacin resistance rate in non-typhoid Salmonella (NTS) has increased to 8% in non-specific serotypes of Salmonella. Our previous study using the next generation sequencing (NGS) identified 2 novel (parC g.1307delA and parE g.1031G>T) and 2 reported (gyrA g.248C>T and parC g.170C>G) genetic mutations in one NTS isolate from a paediatric patient in TMU-SHH. Thus, we conducted this study to investigate the incidences of these four mutational targets in the NTS clinical isolates from different areas in Taiwan.

**Methods:** A total of 39 ciprofloxacin-resistant NTS isolates were used in this study, including 34 NTS isolates from northern, middle, southern, and eastern Taiwan in the Taiwan Surveillance of Antibiotic Resistance (TSAR) from NHRI during 2010-2016 and 5 NTS isolates from TMU-SHH during 2012-2016, including the isolates with all 4 mutations as positive control. The 39 NTS isolates were cultured in LB broth at 225 rpm at 37°C for 18 hours. The bacterial genomic DNAs were purified for the mismatch amplification mutation assay (MAMA) PCR using the primers specific to the 4 genetic loci, and the amplified fragments were visualised using 1.3% agarose gel. Finally, the incidences of the individual 4 genetic mutations in the 39 isolates were obtained and expressed in percentage (%).

**Results:** We demonstrated that 11 among the 39 NTS isolates (28.2%) have at least one of the four genetic mutations. Single mutation was detected in 9 NTS isolates. Double mutations were present in the other two NTS isolates. Quadruple mutations were confirmed in the NTS isolate as positive control. The known mutation gyrA g.248C>T occurred in 7 NTS isolates (17.9%), and the known mutation parC g.170C>G was found in 6 NTS isolates (15.4%). The novel mutation parE g.1031G>T was present in 3 NTS isolates (7.7 %), including the positive control, the other one coexistant with gyrA g.248C>T, and another one in single mutation (1/39, 2.6%). The deletional frameshift mutation parC g.1307delA was not identified in the additional 38 NTS isolates. gyrA g.248C>T was the most commonly seen but not the predominant genetic mutation. The novel genetic mutation parE g.1031G>T was found alone in one NTS isolate without coexistence of the other 3 genetic mutations related to ciprofloxacin resistance.

**Conclusions:** In this study, no single genetic mutation predominates in ciprofloxacin-resistant NTS isolates. For the first time, our NGS-identified novel genetic mutation parE g.1031G>T was detected in the ciprofloxacin-resistant NTS isolate without presence of the other genetic mutation. Further studies are warranted for validating the genetic epidemiology of all the genetic mutations related to ciprofloxacin resistance in NTS.

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**The Development of a Web-based Tool Generating Graphical Plots of Functional Domain with Reported Pathogenic Variants, Population Variants and Amino Acid Conservation as Evidence for ACMG Variant Classification**

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**Background and Aims:** Despite American College of Medical Genetics and Genomics (ACMG) developed standards and guidance for the interpretation of sequence variants, there are lots of tedious and manual works to classify variants by using and interpreting typical types of variant evidence, e.g. population, computational and functional data. One of the classification rules is to locate whether the variants in a mutational hot spot and well-established functional domain. We aim to develop a publicly available tool to easily classify the variants by interpreting the graphical representations of mutation and functional data.
and facilitate easy clinical management by the graphical plots.

Methods: A web-based tool is to be developed by using HTML5 web technologies to link up with database of population data (gnomAD), protein sequence and functional information (UniProt), evolutionary conservation of amino/nucleic acid positions in a protein (ConSurf) and variant database (Clinvar). A series of graphical plots of functional domain with reported pathogenic variants, population variants and amino acid conservation for a region of a gene against the amino acid residue position will be generated based on the input of the gene and the variant position.

Results: Take an example of a variant p.Arg76 in SRY gene. After inputting SRY gene on the website, 3 graphical plots of SRY gene were generated and shown on the website. The first one is a plot of 204 residue sequence of SRY with a black horizontal line that HMG box shown by a box and above the line are shown positions of mutations of different syndrome represented by different symbols. The second one is shown with the ConSurf grade, ranging from 1 (minimum) to 9 (maximum), plotted by amino acid position. The third one is with the gnomAD missense variants plotted by position (x-axis) against allele frequency (y-axis). Based on the above information, it provides strong evidence to support variant, p.Arg76, as PM1.

Conclusion: The tool provides an easy and convenient way to generate strong evidence of a series of graphical plots for the classification and interpretation of the variants based on ACMG’s PM1 rule.

Acknowledgement: This work was supported by the Edward & Yolanda Wong Research Fund.

Application of Whole Exome Sequencing in Diagnosing Movement Disorders in Hong Kong

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Background: Movement disorders (MD) are neurologic syndromes involving a variable combination of impaired voluntary movements, dysfunction of posture, abnormal voluntary movements and normal appearing movements at inappropriate or unintended times. It has been a challenge to make a molecular diagnosis for paediatric patients with MD because of their genetic and clinical heterogeneity. The aim of our study is to utilise whole-exome sequencing (WES) as an alternative or additional diagnostic tool for children with MD in Hong Kong.

Methods: This is an ongoing project with a target cohort of 100 patients. Paediatric patients up to 18-year-old with unexplained MD were recruited. They were either having active follow-up in clinics, or newly referred from other hospitals. WES and analysis were performed using in-house diagnostic pipeline in our department. First-tier screening was performed on a list of MD-associated genes, and second-tier analysis was open to the entire human exome. Validation of target mutations and segregation analysis were performed by Sanger sequencing.

Results: Out of the 15 patients recruited, we identified two pathogenic mutations in three patients (20%) (TGM6: p.L517W and ATP1A3:p.E818K); one patient with a likely pathogenic mutation (POLG:p.E944K); and one patient with a variant of uncertain clinical significance (KCND3: p.N639K). The patient with TGM6 mutation had a phenotype of hereditary spastic paraplegia (HSP) with distal amyotrophy and mild intellectual disability. The two patients with ATP1A3 mutation are siblings with CAPOS syndrome (Cerebellar ataxia, Areflexia, Pes cavus, Optic atrophy and Sensorineural hearing loss), and their mother with the same phenotype is being tested.

Discussion: In particular to TGM6 mutation, we had conflicting interpretation towards the previously reported TGM6:p.L517W. Despite the high allelic frequency in East-Asians (i.e. 0.0015), the mutation was revealed in multiple patients with suspected HSP, dystonia, spinocerebellar ataxia, or acute myeloid leukaemia, with a functional characterisation supporting pathogenicity. We postulated that TGM6 is a pleiotropic gene causing multiple phenotypes. As there are asymptomatic individuals with TGM6 mutations (e.g. p.L517W), other genetic modifiers or environmental factors may contribute to the disease aetiology.

Conclusions: The preliminary yield of WES in unexplained MD was 20%. Further analysis is on-going. However, due to the limitations in technology and current knowledge in genetics, careful judgement and interpretation on the pathogenicity of variants are necessary when performing WES in clinical settings.

Acknowledgements: We would like to thank The Society for the Relief of Disabled Children and The Edward and Yolanda Wong Fund for the support.
Mutations in PI3K-AKT-mTOR Signaling Pathway Are the Major Cause of Macrocephaly with Developmental Delay/Autism

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Introduction: Macrocephaly is a common dysmorphic feature in children with developmental delay/autism. PTEN was the first gene identified in patients with developmental delay and macrocephaly. Since then, other genes in the PI3K-AKT-mTOR signaling pathway have also been reported in patients with macrocephaly and developmental delay/autism. In this study, we aim to characterise the mutation spectrum of patients with macrocephaly (head circumference ≥+2 SD) and developmental delay/autism.

Methods: Whole-exome sequencing was performed for 21 patients with macrocephaly and developmental delay/autism, with the source of DNA either from blood, buccal mucosa or saliva. Germline mutations were validated by sanger sequencing, whereas somatic mutations were validated by droplet digital PCR.

Results: A total of 11 pathogenic mutations were identified in PTEN (n=5), PIK3CA (n=3), MTOR (n=1) and PPP2R5D (n=2) in ten patients, with one patient harboring biallelic PTEN mutations. Besides germline mutations, somatic mutations of PIK3CA were identified in two of the ten patients, which could be easily missed by testing on blood DNA. While nine mutations were de novo mutations, two mutations were inherited maternally but both of the parents did not have remarkable clinical history. MRI findings showed that polymicrogyria and periventricular white matter lesions were common in these patients.

Conclusion: Mutations in PI3K-AKT-mTOR signaling pathway are a major cause of macrocephaly with developmental delay/autism, which can be found in nearly half of the patients tested. Genetic testing is recommended for this group of patient because mutations in the PI3K pathway potentially increase the risk of cancer. Clinically it is hard to distinguish patients with germline mutations and somatic mutations, therefore the use of buccal or saliva DNA is important to identify somatic mosaicsisms and to maximise diagnostic yield. We propose an umbrella term "mTOR pathway-related macrocephaly spectrum" to encompass patients with macrocephaly and developmental delay/autism who are associated with germline or somatic mutations of mTOR signaling pathway.

Comparing MEFV Variants in Chinese and Moroccan Patients with Familial Mediterranean Fever

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Background: Familial Mediterranean fever (FMF) is defined as an autosomal recessive disease characterised by recurrent attacks of fever with serosal inflammation.

Aims: To compare the frequency and the spectrum of MEFV variations in Chinese and Moroccan patients clinically suspected of FMF.

Methods: Thirty-six males and 16 females who had symptoms of FMF were analysed for their genomic sequences of all MEFV exons by PCR direct sequencing.

Results: MEFV variations associated with FMF were detected in 16 out of 23 Chinese patients. Thirteen out of 29 patients referred from Morocco were found to have MEFV variations. E148Q and R202Q were the predominant variations in our Chinese and Moroccan patients respectively. E148Q was found in 8 Chinese patients (50%) and 2 Moroccan patients (15%) whereas R202Q was identified in 9 Moroccan patients (69%) and 2 Chinese patients (13%). Of the remaining 6 Chinese patients, 3 patients were carriers of the complex allele L110P-E148Q, 1 patient was a carrier of G304R and 1 patient each was a carrier of E148Q-R202Q or L110P-E148Q-I641F. Two novel variations were found in Moroccan patients, V620D and R133P which were unreported in HGMD professional and INFEVERS.

Conclusions: The identification of MEFV variations could facilitate FMF diagnosis. Genetic analysis revealed that the frequency of the variation was different across ethnic groups.

Acknowledgement: Hong Kong Society for the Relief of Disabled Children.
**ARHGAP18 Is a Novel Gene Under Positive Natural Selection Influences HbF Levels in β-thalassaemia**

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**Background and Aims:** Foetal haemoglobin (HbF) plays a dominant role in ameliorating the morbidity and mortality of β-thalassaemia. Better understand loci and genes involved HbF expression is beneficial for treatment for β-thalassaemia major. However, many genes associated with HbF expression remain largely unknown.

**Methods:** In this study, we firstly explored large-scale data sets and examined the human genome for evidence of positive natural selection to screen out single nucleotide polymorphisms (SNPs). A genetic analysis of HbF levels was conducted in a Chinese cohort with β-thalassemia to confirm the result of bioinformatics assay. A total of 1,151 subjects with β-thalassaemia were recruited.

**Results:** The results showed that the SNP rs11759328 in the ARHGAP18 gene was significantly associated with HbF levels (P=4.6x10^-4). Secondly, determining that ARHGAP18 was highly expressed in the human K562 cell line, we used lentiviral-mediated small interfering RNA to knock down ARHGAP18 expression, then assessed cell proliferation and apoptosis using cell proliferation assays and flow cytometry, respectively. The downregulation of ARHGAP18 expression in K562 cells significantly increased the HBG1/2 expression and apoptosis, but proliferation was not significantly changed in vitro.

**Conclusions:** Our data suggest that the ARHGAP18 gene, which was located by the SNP rs11759328 with positive selection, plays a potential role in regulating HbF expression in β-thalassaemia, may be a promising therapeutic target for β-thalassaemia. Knockout studies of ARHGAP18 warrant further investigation into its aetiology in HbF.

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**Clinical and Molecular Studies in 203 Chinese Patients with Mitochondrial Disorders**

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**Background and Aims:** Because of the complexity and heterogeneity of the phenotypes and genotypes, the diagnosis of mitochondrial disorders is difficult. We aimed to establish a comprehensive analysis method for the etiologic diagnosis and prenatal diagnosis of mitochondrial disorders.

**Methods:** 203 patients were enrolled in this study. PCR-RFLP and NGS were used to detect mtDNA and nDNA sequence. Pathogenic study was performed to confirm the pathogenicity of suspected mutations. Amniocytes mutation analysis was performed for families with definite gene diagnosis.

**Results:** Candidate pathogenic mutations were identified in 152 patients among 203 cases. The detection rate was 74.88%. Seventy-nine cases (51.97%) had mtDNA variations, while 73 cases (48.03%) had nDNA variations. The most common mtDNA mutation was m.3243A>G (44.30%). Two novel mtDNA mutations were identified, then pathogenicity was confirmed. The most common nDNA mutant gene was SURF1 (16.44%). Ninety-two novel mutations in nDNA were identified. m.3243A>G is the most common genotype for mtDNA associated mitochondrial diseases, while the most common mutated nDNA is SURF1 and PDHA1. The most common phenotype of either mtDNA or nDNA associated mitochondrial disorders is Leigh or Leigh-like syndrome. Seventeen fetuses got precise prenatal diagnosis.

**Conclusion:** The m.3243A>G is the most common mtDNA mutation. Two novel mtDNA mutations and 92 novel nDNA mutations were found, expanding mutation spectrum. The pathogenicity of part NDUFS3, AIFM1 and SERAC1 mutations was confirmed. Leigh or Leigh-like syndrome is the most common phenotype of mitochondrial disease. Prenatal diagnosis for 17 fetuses were carried out successfully. NGS greatly improved the etiologic diagnosis rate of mitochondrial disease.
Systematical Analysis of Eleven Chinese Children with Fibrodysplasia Ossificans Progressiva for Potential Treatment
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Background: Fibrodysplasia ossificans progressiva (FOP) is a rare severely disabling heritable connective tissue disease characterised by congenital malformations of the great toes and progressive heterotopic ossifications. The onset of FOP is presented in early lifetime, and its course is inevitably progressive. Although clinical and animal model studies on pathogenesis of FOP implicated that most patients with FOP were caused by the same recurrent mutation c.617G>A, p.R206H in the ACVR1 (activin A receptor type 1) gene, there is no effective treatment so far. Because of the rarity of FOP, clinicians, especially paediatricians lack the awareness of FOP flare-ups and many patients were misdiagnosed. Therefore, the purpose of the present study is to provide useful information for the early and precise diagnosis, the early prevention and the potential treatment of FOP.

Results: The clinical manifestations, radiographic features and follow-up records of the eleven FOP children were collected. All the eleven cases had no family history, and experienced the onset of FOP prior to six years of age and diagnosed with FOP before ten. All the cases had malformations of great toes, and 10 of them with episodic soft tissue swellings. We found that by the time when they were diagnosed with FOP, respectively, six patients under the age of 6 had not yet developed heterotopic ossifications, while the other five patients above the age of 6 presented with heterotopic ossifications. Sanger sequencing showed that the heterozygous missense mutation c.617G>A, p.R206H in the ACVR1 gene were identified in all the patients.

Conclusions: Eleven cases in the present study presented with classic FOP characteristics were heterozygous for the canonical mutation c.617G>A, p.R206H in the ACVR1 gene. Therefore, patient with congenital malformations of the great toes should be sequenced for the ACVR1 gene and paediatricians need to gain awareness of the possibility of FOP flare-ups. For the patient with the canonical mutation, we proposed a time window, about seven months to 1.7 years, for clinicians and paediatricians to give potential effective treatment to prevent heterotopic ossifications for FOP patients, since that usually happens after the first flare-up of soft tissue swelling during one and a half year.

Search for Congenital Radioulnar Synostosis Causative Gene and Modelling in Mouse
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Background and Aims: Congenital radio-ulnar synostosis (RUS, MIM:179300) is a rare disease characterised by congenital synostosis of the radius and ulna, which results in limited rotational movement of the forearm. The upper limb bud arises embryologically from the unsegmented body wall at 25-28 days, and the radius and ulna are initially connected and share a common perichondrium. Failure of segmentation during embryo development has been hypothesised as the cause of RUS. Further, an autosomal dominant inheritance pattern has been indicated based on RUS case reports. However, the causative genes and underlying mechanisms of isolated RUS are still unknown. In this study, we performed whole genome sequencing (WGS) to search for candidate causative gene for RUS in a rare dizygotic-twin family, which comprises a girl with bilateral RUS, her normal twin-sister and healthy parents.

Methods: Genomic DNA were extracted and subjected WGS using HiSeq X10 (Illumina, San Diego, CA) sequencing system. Crispr/Cas9 technology was used to establish a novel knock-in mouse line harboring the exactly same mutation found in patient.

Results: WGS results revealed a de novo missense mutation in SLC04A1 gene, which locates adjacent to/in the protein trans-membrane region and may affects the transport of thyroid hormone (TH), rostaglandin and taurocholate. TH is well known for regulating development, growth and metabolism. SLC04A1 mutation in fetus may affect the transport of TH from maternal blood, or local cellular transportation of TH during embryogenesis, which in turn affects bone development and segmentation. As this hypothesis could only be firmly confirmed in an appropriate animal model, we are establishing a new knock-in mouse model, which carries exactly the same patient-specific missense point mutation, to facilitate evaluation of the causative roles of the candidate SLC04A1 mutation in RUS.

Conclusions: We identified a novel SLC04A1 missense mutation in a rare RUS twin family case using WGS. To facilitate evaluation of the causative role of this candidate mutation in RUS, a new knock-in mouse model carrying this patient-specific mutation is under developing.
Identification and Characterisation of Heart-Specific Enhancers in Zebrafish Genome

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Background and Aims: Enhancers are cis-acting DNA elements which are critical for precise patterns of gene expression during embryonic development. Indeed, one challenge toward understanding the function of genome is to identify the regulatory elements embedded in DNA sequences. Also, little information about how spatiotemporal patterns of gene expression driven by these elements is available. Here we apply a combined computational and experimental approach to discover heart-specific enhancers within zebrafish genome. After identification, we then uncover critical motifs within these enhancers from common features of transcription factor binding sequences. The aim of this research is to provide new insight into the gene regulatory network of heart development.

Methods: Comparative genomic analyses are effective and efficient tools to identify conserved non-coding elements (CNEs) which may act as potential candidates for enhancers. Based on literature and gene expression database, group of heart-specific/enriched genes were chosen as candidates for CNEs selection. Using website "ECRbrowser", we screened CNEs between zebrafish genome and human genome. Then, a Tol2-based enhancer trap method was used to test enhancers in zebrafish. After identifying heart-specific enhancers, we later conducted de novo motif prediction algorithms to uncover top-ranked 6-nucleotide motifs that are significantly enriched in these enhancers. Finally, mutation motifs within selected heart-specific enhancers were carried out to determine whether these predicted motifs are critical for heart-specific enhancer activity.

Results: In this study, we chose a set of 83 CNEs near 32 heart-specific/enriched genes. Subsequently, we tested their ability to drive reporter gene GFP expression using a transient transgenic method. We found that 12% of tested CNEs exhibited heart-specific enhancer activity. Application of de novo motif prediction algorithms on a set of ten heart-specific enhancers revealed three top-ranked 6-nucleotide motifs that were significantly enriched in these enhancers. Experimental analyses of these motifs in zebrafish demonstrated that they are functionally critical for heart-specific enhancer activity.

Conclusions: Taking advantage of this combined computational and experimental method, we successfully discovered heart-specific enhancers within zebrafish genome. This efficient and practical approach can be adopted to other tissues of interests for enhancer trap screening. Moreover, characterisation analyses and experimental validation revealed functional motifs that are important for gene-specific expression. Our results provide important resources for further analyses of regulatory network of heart development and function.

Identification and Clinical Implications of Novel MYO15A Mutations in a Non-consanguineous Chinese Family By Targeted Exome Sequencing

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Introduction: Autosomal recessive nonsyndromic hearing loss (ARNSHL) is a genetically heterogeneous sensorineural disorder, generally manifested with prelingual hearing loss and absence of other clinical manifestations. The aim of this study is to identify the pathogenic gene in a four-generation consanguineous Chinese family with ARNSHL.

Case Report: Two novel frame-shift mutations, c.5964+3G>A and c.7395+1G>A, in the myoxin XVa gene (MYO15A) was identified by exome sequencing and Sanger sequencing. The compound heterozygous MYO15A c.5964+3G>A and c.7395+1G>A variants co-segregated with the phenotypes in the ARNSHL family and was absent in one hundred normal controls. The variant was predicted to interfere with the formation of the Myosin XVa-whirlin-Eps8 complex at the tip of stereocilia, which is indispensable for stereocilia elongation.

Learning Points: Our data suggest that the compound heterozygous MYO15A c.5964+3G>A and c.7395+1G>A variants might be the pathogenic mutation, and exome sequencing is a powerful molecular diagnostic strategy for ARNSHL, an extremely heterogeneous disorder. Our findings extend the mutation spectrum of the MYO15A gene and have important implications for genetic counseling for the family.
The Generation of Embryonic Stem Cells with Erythrocytic Expression of Marker Genes

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Blood diseases, including sickle-cell anaemia and thalassaemia that are characterised by aberrant versions of haemoglobin gene, are common in children. How do these diseases proceed, and how to treat these diseases are some of the most important questions to ask. The embryonic stem cells (ESCs) with specific marker genes (such as GFP) may be useful to study these issues. Herein, we aimed to establish the murine ES cell lines with erythrocytic expression of GFP.

First of all, HG mice (A HS23-GFP transgenic mice line with erythroid-specific GFP expression) were produced by pronuclei microinjection. The GFP expression was driven by a human β-globin gene promoter, and the transgene was detected at generation F10 in metaphase chromosome with one integration site by FISH method. ESCs were derived using these HG mice blastocysts. Pluripotent tests of the ESCs were performed both in vivo and in vitro through studying genic expression at the mRNA level, and the formation of embryonic bodies and teratoma. Chimeric mice of HG ESCs were generated. Pluripotent genes such as Sox2, Oct3/4 and Nanog were expressed. Embryo bodies were formed in vitro. Moreover, erythrocytes derived from HG ESCs had special GFP expression in the blood smear of the offspring of HG ESC chimera. HG ESCs also had the capacity of germline transmitted. Thus this dynamic cellular model system would be useful for the study of ESCs differentiation into haemopoietic stem cells, and specifically to evaluate ES cellular quality of differentiation into erythroid cells. This would also have implications of the safety and reliability of ESCs clinical applications.

Association Between Dietary Intake, Psychosocial Status, Physical Activity and Constipation in School Children: The Results from Toyama Birth Cohort Study

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Background and Aims: Childhood constipation is one of the major causes of clinic visit and affects quality of life of patients. Although psychosocial status (PS) have been thought as a potential risk factor, an epidemiological study in large population have been rarely conducted. Our aim was to clarify the association between PS, lifestyle factors and child constipation.

Methods: Children were from Toyama Birth Cohort Study in Japan. A total 7,478 children aged 9-10 years were analysed by questionnaire. 'Less frequent than once every two days' was defined as constipation. We also surveyed children's lifestyle, food frequency and PS. PS included the frequency of irritation, feeling of school refusal and talk with their parents. Food frequency was divided into three: more than once a day, 3 to 5 days/week, or less frequent. Multivariate logistic regression analyses were performed to explore the association. This research has been approved by an ethical committee.

Results: Of all, 276 children (3.7%) had constipation. Girls were more likely to have constipation (2.6% in boy and 4.8% in girl). In multivariate analysis, constipation was significantly associated with girl (Odds Ratio (OR)=1.92), physical inactivity (OR=1.48), obesity (OR=0.52), less frequent intake of milk (OR=1.30), fruits (OR=1.88), and vegetable (OR=1.56). In addition, frequent irritation (OR=1.58), feeling of school refusal (OR=1.81) and insufficient talk with parents (OR=1.42) were associated with constipation. In stratified analysis by sex, OR of physical inactivity became higher (=2.73) in boys, while ORs of psychosocial status became higher in girls (OR of irritation=1.89 and insufficient talk with parents=1.61).

Conclusions: Our epidemiological study showed that psychosocial status was as strongly associated with childhood constipation as conventional risk factors, such as fiber intake and physical activity. It is be beneficial for parents and health practitioners to be aware that caring psychosocial status of children can reduce their constipation.
Exclusive Enteral Nutrition Versus Infliximab in Inducing Therapy of Paediatric Crohn's Disease

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Background and Aims: Infliximab, a monoclonal antibody targeting tumour necrosis factor-alpha (TNF-α), is one of the primary treatment strategies for active Crohn disease (CD), while exclusive enteral nutritional (EEN) therapy have been shown advantages in inducing remission, improving growth and MH in paediatric CD patients. However, acute infusion reactions (AIR), infections and risk of malignancy are the main concern of patients who are receiving infliximab as induction remission therapy. In the meanwhile, patients' poor compliance may lead to EEN treatment failure. Thus, the balance between efficacy, risk of side effects and patients' compliance is an important consideration in choosing therapeutic regimens. Since rare studies showed comparative effectiveness of those approaches, we prospectively compared the efficacies, growth improvements, and adverse effects of the two regimens in children with newly diagnosed CD.

Methods: In a prospective study of children initiating EEN or infliximab therapy for CD, we compared clinical outcomes using the paediatric Crohn's disease activity index (PCDAI), growth improvement as evaluated by height for age (HFA) z score and body mass index for age (BMIFA) z score, endoscopic mucosal healing and adverse effects. Data were measured at baseline and after 8 weeks of therapy.

Results: We enrolled 26 children with CD, of whom 13 were treated with infliximab, 13 with EEN. Clinical response (PCDAI reduction ≥15 or final PCDAI ≤10 was achieved by 83.3% in EEN group and 90.9% in IFX group. BMIFA z scores were significantly increased in both 2 groups (p<0.05). No significant differences were observed in PCDAI, HFA or BMI recovery between two groups. Adverse effects were detected in 30.7% on infliximab and 0% on EEN. Mucosal healing was achieved in 71.4% cases in EEN group versus 85.7% in IFX group.

Conclusion: EEN provided similar improvement as IFX in clinical symptoms, mucosal healing and BMI, but not height, in a short term. EEN therapy had less adverse effects when compared with IFX. To our knowledge, this is the first study comparing the efficacy of nutritional status between EEN and IFX.

Obericholic Acid Protects Against Lipopolysaccharide-induced Liver Injury and Inflammation

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Background and Aims: Liver injury occurs frequently due to cholestasis during sepsis. The farnesoid X receptor (FXR) is a ligand-activated transcription factor that plays important roles in regulating bile acid homeostasis. The aim of the present study was to investigate the effect of obeticholic acid (OCA), a novel synthetic FXR agonist, on lipopolysaccharide (LPS)-induced acute liver injury.

Methods: A total of 72 male C57BL/6J mice were randomly divided into three groups (n=24 per group) according to the treatment with 0.9% NaCl (control group) or 10 mg/kg LPS only (LPS group) or 10 mg/kg LPS plus oral OCA (LPS + OCA). In LPS + OCA group, mice were gavaged with OCA (5 mg/kg) once a day for 5 days before LPS administration and 2 days after LPS administration. The serum and livers were collected at 12h, 24h, 48h and 72h after LPS administration for further analysis. Serum levels of total bile acid (TBA), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured at 12h, 24h, 48h and 72h after LPS administration in each group. Histological examinations were performed on livers at 48h after LPS administration in each group, and liver sections were stained with haematoxylin & eosin (H&E). Furthermore, the mRNA levels of FXR, bile salt export pump (BSEP), Interleukin-6 (IL-6), IL-1β and Tumour Necrosis Factor-α (TNF-α) in livers were analysed by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Results: The mRNA levels of FXR and BSEP in livers of mice significantly increased at 48h, 72h after LPS administration. As expected, OCA stimulated the expression of FXR and BSEP in livers of mice in LPS + OCA group. Interestingly, OCA protected mice from LPS-induced hepatocyte oedema, necrosis, inflammatory cells infiltration, ductal proliferation and expansion. Consistently, LPS significantly induced the higher serum levels of TBA, ALT, and AST, which were significantly suppressed in mice with FXR agonist treatment. Furthermore, the mRNA levels of IL-1β, TNF-α and IL-6 decreased in livers of mice in FXR agonist group compared with LPS group.

Conclusions: OCA induces the expression of FXR and BSEP in livers of mice and protects mice from LPS-induced liver injury, which contributed by suppressing the expression of IL-1β, TNF-α and IL-6 in livers.
**Antibiotic Selection and Drug Sensitivity Analysis of Cholangitis After Hepatico-portoenterostomy for Biliary Atresia**

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**Background and Aims:** It is the key point to choose appropriate antibiotics for the children with congenital biliary atresia Kasai postoperative cholangitis, as the developing of antibiotic resistant and the changing of the bacteria spectrums. To explore the antibiotic selection of cholangitis in children who underwent hepatico-portoenterostomy for congenital biliary atresia.

**Methods:** The clinical data of 300 children with congenital biliary atresia Kasai postoperative cholangitis had been collected to analyse pathogenic bacteria and antibiotics sensitivity according to clinical types of the cholangitis in our hospital from 2006 to 2016.

**Results:** (1) In 300 cases of children with occasional cholangitis accounts for 202 cases, 98 cases of frequent cholangitis; early cholangitis in 166 cases, 134 cases of late cholangitis. (2) The main pathogens of cholangitis followed by *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus*, *Acinetobacter baumannii*, *Enterobacter cloacae* and *Candida albicans*. (3) The sensitivity rates of *Escherichia coli* and *Pseudomonas aeruginosa* to cefoperazone sulbactam were 75% and 78%, to piperacillin tazobactam were 82% and 84%, and to meropenem were 93% and 76%. The sensitivity rates of *Enterococcus* to vancomycin or linezolid were 100%.

**Conclusion:** Cefoperazone sulbactam and piperacillin tazobactam can be used to the first choice of antibiotics for biliary atresia Kasai postoperative cholangitis. And meropenem should be used to replace them when treatment effect was poor. Late cholangitis and frequent cholangitis should be alert to *Enterococcus*, *Acinetobacter baumannii* and other pathogens.

**Detection and Analysis of Faecal Intestinal Microflora in Children with Henoch-Schonlein Purpura**

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**Background and Aims:** Henoch-Schonlein purpura (HSP) is the most common systemic vasculitis in children, which mainly involves the skin, joints, and gastrointestinal and renal small blood vessels. More and more studies have found that intestinal microflora play a very important role in autoimmune and allergic diseases. HSP is also an immune-mediated disease, the aetiology and pathogenesis of which is not yet fully understood. This study aimed to detect and evaluate the intestinal microflora in children with HSP and to explore the relationship between HSP and intestinal microflora.

**Methods:** Faecal samples were collected from children without HSP and children with HSP at the active stage and remission period. A 16SrDNA high-throughput sequencing technique was used to detect the intestinal microflora.

**Results:** The abundance of intestinal microflora (based on OTUs) in children without HSP was significantly higher than that in children with HSP at both the active stage and convalescent phase. The average abundance of the Bacteroidetes phylum in children with HSP at the active stage increased along with *Dysgonomonas*, *Parabacteroides*, *Prevotella* and unclassified Bacteroidetes at the genus level. The average abundance of the Firmicutes phylum decreased significantly, accompanied by a significant decrease of *Megamonas*, *Acetivibrio*, *Anaerostipes*, *Butyricicoccus*, *Clostridium XI*, *Clostridium sensu stricto*, *Coprococcus*, *Dorea*, *Faecalibacterium*, *Lachnospira*, *Lachnospiracea incertae sedis*, *Roseburia*, and unclassified Lachnospiraceae at the genus level. The average abundance of Proteobacteria phylum significantly increased, accompanied by significant increases in *Comamonas*, *Escherichia/Shigella*, *Halomonas*, *Succinivibrion*, and *Sutterella* at the genus level. In addition, the average abundance of *Eggerthella*, which belongs to actinomycetes, was significantly higher in patients at the acute phase than that in the children without HSP. The intestinal microflora of children in the convalescent group seemed not to shift back to normal. The abundance of intestinal microflora increased in the children with HSP at the acute phase and was the highest in children at the convalescent phase, while the abundance of intestinal microflora decreased at the acute phase and was the lowest during the remission period.

**Conclusion:** There is a dysregulation of intestinal microflora in children with HSP at the active stage and recovery stage. The relationship between such disorders and the pathogenesis, clinical and prognosis of HSP is worthy of further study.
A Comparative Study on Outcomes of Anus Retention Enema with Total Colonic Enema After Transendoscopic Enteral Tubing for Inflammatory Bowel Disease
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Background and Aims: Salicylates are classic and traditional first-line therapy for inflammatory bowel disease. The usual administration is oral and/or anal retention enema. Transendoscopic enteral tubing (TET) is a technology which can fix implanted tube in the intestinal tract of deep under the endoscope assisted. In vitro side of tube communicates with the outside world along the intestinal tract, usually including endoscopic colon Catheterisation and endoscopic naso-jejunal Catheterisation, which both can be used to keep a long time. The former is new technology that emerged in 2015. The purpose of this study is to investigate and compare efficacy and safety of Anus retention enema with total colonic enema by TET for IBD in children.

Methods: 23 cases of hospitalised children diagnosed IBD in our hospital from January 2014 to February 2017 were researched. All cases were treated with oral mesalazine. Among them, 15 cases were treated with anal retention enema, 8 cases were treated with total colonic enema by TET (mesalazine enema-Shaerfu enema, dose as follows: bedtime dosing, 1 g/time, 1 time/day, a total of 4-8 weeks). We followed up the patients every month, reviewed endoscopy and inflammation index, and observed the differences about adverse reactions and clinical curative effect of the two groups.

Results: The clinical symptoms of the two groups were improved, and the inflammatory indexes were obviously decreased compared with that before treatment. There was no significant difference between the two groups (p>0.05); for the group treated with total colonic enema by TET, the colonic mucosa repaired faster after 1 month treatment, and repaired completely after more than 2 months treatment, and efficiency is significantly higher, compared with that of the group treated with anal retention enema. The difference was statistically significant (p<0.05). There was no significant difference between the two groups in the incidence of adverse reactions (gastrointestinal discomfort, skin rash, liver and kidney damage) (p>0.05). And the group treated with total colonic enema by TET had obvious anal foreign body feeling, discomfort, but did not affect defecation, can adapt after long time.

Conclusions: Total colonic mesalazine enema by TET in the treatment of IBD, greatly improved the clinical efficacy, and didn't increase adverse reactions, and was safe and effective combined with oral mesalazine. But this method has very high technical requirements for colonoscopy. Although the clinical effect is good, it needs multi-center large-sample clinic trial to observe its curative effect and the technical popularisation should be carried on.

Antibiotics Resistance of Helicobacter pylori in Children with Upper Gastrointestinal Symptoms in Hangzhou, China
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Background and Aims: The decreasing eradication rate of Helicobacter pylori is mainly because of the progressive increase of its resistance to antibiotics. Studies on antimicrobial susceptibility of H. pylori in children is limited. This study aimed to investigate the resistance rates and patterns of H. pylori strains isolated from children.

Methods: Gastric mucosa biopsy samples obtained from children who had undergone upper gastrointestinal endoscopy were cultured for H. pylori and susceptibility to six antibiotics (clarithromycin, amoxicillin, gentamicin, furazolidone, metronidazole and levofloxacin) was tested from 2012 to 2014.

Results: A total of 545 H. pylori strains were isolated from 1390 children recruited. The total resistance rates of H. pylori to clarithromycin, metronidazole and levofloxacin were 20.6%, 68.8%, and 9.0%, respectively. No resistance to amoxicillin, gentamicin and furazolidone was detected. 56.1% strains were single resistance, 19.6% were resistant to more than one antibiotic, 16.7% for double resistance, and 2.9% for triple resistance in 413 strains against any antibiotic. And the H. pylori resistance rate increased significantly from 2012 to 2014. There was no significantly difference in the resistance rates to clarithromycin, metronidazole and levofloxacin between different gender, age groups, and patients with peptic ulcer diseases or non-ulcer diseases.

Conclusions: Antibiotic resistance was observed in H. pylori strains isolated from children in Hangzhou and it increased significantly during the three years. Our data strongly support current guidelines which recommend antibiotic susceptibility tests prior to eradication therapy.
Early Nasogastric Versus Nasojejunal Tube Feeding in Paediatric Acute Pancreatitis: A Randomised Controlled Trial

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**Background and Aims:** Nasojejunal tube feeding is a standard of care in patients with predicted acute pancreatitis (AP) and several recent trials suggested that nasogastric tube feeding (NGT) is as safe and efficient as nasojejunal tube feeding in these patients. However, the efficacy and safety of NGT in paediatric AP has not been investigated yet. The aim of this study was to investigate whether NGT presents any benefit to patients with mild to moderate AP in children.

**Methods:** A total of 49 consecutive paediatric patients with AP were randomised to receive either NG or NJ feeding via a fine bore feeding tube within 72 hours of hospital admission. The primary outcome was tolerance of enteral nutrition support. Complications (tube-associated, infections, feeding-associated) were monitored and comparisons made of both total hospital and intensive-care stays, duration of tube feeding, occurrence of any complications.

**Results:** A total of 49 children with acute pancreatitis were recruited into this study and were randomised to NG group (25) or NJ group (14). There were no significant difference of age, gender, paediatric acute pancreatitis scores, CT Severity Index (CTSI) and gastrointestinal symptoms or abdominal pain between the two groups. Eighty-eight percent (22/25) of NG group and 96% (23/24) of NJ group can tolerate with tube feeding (p>0.05). The duration of hospital stay was 14.5 ± 4.7 d for NG group and 16 ± 6.3 d for NJ group. For duration of tube feeding were 12.5 ± 7.4 d and 16 ± 4.4 d separately. One children of NJ group has tube-associated complication. Eight patients of NJ group and 9 of NG group have feeding-associated complications such as diarrhea, vomit and abdominal pain. None of all patients has complication of any infection. Clinical differences between the two groups were not significant. Overall mortality was 0.

**Conclusion:** The simpler, cheaper, and more easily used NG feeding is as good as NJ feeding in paediatric patients with AP. This appears to be a useful and practical therapeutic approach to enteral feeding in the early management of patients with AP.

Clinical Application of Treatment of Large Colorectal Polyps by Purse String Suture with Nylon Loop and Titanium Clips in Combination with Colonoscopy-assisted High-frequency Electrocision

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**Objective and Aims:** Endoscopic high-frequency electrocision has been widely used in the treatment of colorectal polyps in children. But for big polyps with wide base, electrocision is likely to cause complications, such as intestinal perforation and intestinal bleeding. The aim of this study was to assess the effect and safety of high-frequency electric snare used for removal of big polyps with wide base (Diameter >2.5 cm) under endoscopy with the assistance of purse string suture with titanium clips and nylon loop.

**Methods:** 27 cases of hospitalised children diagnosed big colorectal polyps with wide base from January 2015 to December 2016 were researched and their clinical data were retrospectively analysed.

**Results:** 27 cases of big polyps in children include that P-J syndrome was 11 cases, familial polyposis was 9 cases, inflammatory polyps was 6 cases, lymphoma was 1 case. Among the distribution of these 33 polyps, 26 of them were located in colon and 7 in rectum. Fourteen polyps were ligatured by nylon loop firstly and then were cut by high-frequency electric snare, and finally clipped by titanium folders. Bleeding occurred during operation in 1 case, and delayed haemorrhage after operation had been happened in 3 cases (after intravenous haemostasis treatment, 1 case stopped bleeding; 2 cases used endoscopy again to clip bleeding site with titanium clip, no more bleeding); delayed intestinal perforation after operation had been happened in 1 case, and the perforation was successfully cured by anti-infection treatment, no diet, and applying titanium folder under endoscopy for emergency treatment. Nineteen colorectal polyps were cut by high-frequency electric snare, and the stump was treated by purse string suture with titanium clips and nylon snare. No intraoperative and postoperative bleeding occurred. No perforation occurred. No other complications occurred.

**Conclusion:** The application of high-frequency electrocision with the assistance of titanium folder and nylon loop can effectively prevent the bleeding and perforation during cutting big colorectal polyps with wide base or after the operation. Compared the two methods, purse string suture is more effective to prevent and cure complications, so it is worth popularising in clinic.
Disease Spectrum in Children with Inherited Intrahepatic Cholestasis in China
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Objective: Genetic causes account for a substantial proportion of paediatric intrahepatic cholestasis. Several genetic defects have been reported in China, but the disease spectrum is still largely unknown. The aims of this study are to explore the disease spectrum of paediatric inherited intrahepatic cholestasis that is still largely unknown in China.

Study Design: Between January 2012 and June 2016, 877 paediatric patients with intrahepatic cholestasis were evaluated by Sanger sequencing, panel sequencing, or whole exome sequencing. Clinical data and sequencing results were retrospectively collected by reviewing the medical records.

Results: A total of 295 (33.6%) received a molecular diagnosis. There were 18 distinct genetic disorders diagnosed. The top 7 resulted from mutations in SLC25A13 (41.4%), JAG1 (25.4%), ABCB11 (9.2%), ATP8B1 (6.1%), ABCB4 (4.1%), ABCC2 (4.1%), and CYP27A1 (2.7%). Patients with disease onset at younger age were more likely to receive a genetic diagnosis. The majority (85.4%) of diagnosed cases developed cholestasis before 4 months of age, and Citrin deficiency was the most common cause. But ABCB4 deficiency became the most common cause of patients with disease onset after one year old. Among the 295 diagnosed patients, 245 distinct mutations were identified in disease genes, including 61 novels. Recurrent mutations were detected in SLC25A13, ATP8B1, CYP27A1, and AKR1D1; and together accounted for 47.0% of the total mutant alleles.

Conclusion: SLC25A13 was the most common disease one of paediatric inherited intrahepatic cholestasis in China, followed by JAG1, ABCB11, ATP8B1, ABCB4, ABCC2, and CYP27A1.

Activation of the Renin-angiotensin System Promotes Colitis Development
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Background and Aims: The renin-angiotensin system (RAS) plays pathogenic roles in renal and cardiovascular disorders, but whether it is involved in colitis is unclear. The study was designed to explore the role of the RAS in the pathogenesis of colitis.

Methods: RenTgMK transgenic mice that overexpress active renin from the liver, and wild-type mice chronically infused with Ang II or treated with AT1 receptor blocker (ARB), were studied using 2, 4, 6-trinitrobenzenesulfonic acid (TNBS)-induced colitis model. Intestinal mucosal biopsies from IBD patients who were on ARB therapy were also analysed.

Results: RenTgMK mice developed much more severe colitis compared with wild-type controls, where >50% RenTgMK mice died and all wild-type mice recovered. RenTgMK mice exhibited more robust mucosal T₉17 and T₉1/T₉17 immune responses and more severe colonic epithelial cell apoptosis compared with wild-type controls. Treatment with aliskiren (a renin inhibitor), but not hydralazine (a smooth muscle relaxant), ameliorated colitis in RenTgMK mice, although both drugs normalised blood pressure. Chronic infusion of Ang II into wild-type mice mimicked the severe colitic phenotype of RenTgMK mice, and treatment with losartan (an ARB) ameliorated colitis in wild-type mice, confirming a colitogenic role for the endogenous RAS. In human biopsies, pro-inflammatory cytokines were suppressed in IBD patients who were on ARB therapy compared with patients not on ARB therapy.

Conclusions: These observations demonstrate that activation of the RAS promotes colitis by a blood pressure independent manner. Ang II appears to drive colonic mucosal inflammation by promoting intestinal epithelial cell apoptosis and mucosal T₉17 response in colitis development.

The Effect of Anti-Gr-1 Antibody in Rhesus Rotavirus Induced Mouse Biliary Atresia Model
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Background and Aims: Biliary atresia (BA) is a common obstructive jaundice disease in paediatric patients with poor prognosis and high mortality. The aetiology of the BA is not fully understood, but the virus infection, autoimmune dysregulation and genetic background might all get involved. The inflammation was a typical phenomenon in BA, but the immune cells involved and their interaction were not totally clear. The aim the study is to investigate the function of Gr-1⁺ cells in the mouse BA model.
Methods: Rhesus rotavirus inoculated into newborn mice was well established as a mouse BA model. In this study, the virus was injected into the mice at day 5 instead of 24 hours after birth. The anti-Gr-1 antibody was injected 4 hours before virus injection and repeated every 3 days for another 3 times. Isotype control antibody was used in the control group. The morphological changes of the mouse were monitored and the survival curve and body weight were recorded. The liver samples were collected and the Haematoxilin and Eosin and Sirius Red staining were used for histology and fibrosis evaluation.

Results: The results showed the similar observation with previous studies that in isotype control groups, no obvious jaundice was observed and the body weight was comparable to that of normal mice. However, in anti-Gr-1 antibody group, the oily hair was developed at day 10-12 and reduced body weight and jaundice were observed, some of them was dying at day 15-17 day after birth. The development of the BA syndrome was similar to that of virus injection at 24 hours after birth than 5 days time different suggested the similar immune response. Furthermore, the chronic BA was obtained by which, in the liver tissue section the collagen deposition was found indicated the liver fibrosis was in progress.

Conclusions: Our data indicated the Gr-1+ cells play an important role in prevention of virus infection induced postnatal biliary atresia in mouse, the detail virus-Gr-1+ cells interaction is still need to further examine for understanding the mechanism in disease process.

Diagnosis and Treatment of Functional Constipation Caused by Cow Milk Protein Allergy in Infants

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Background and Aims: Functional constipation (FC) account for more than 90% of children with constipation, which often lead to abdominal pain and pain during defecation, and bring children with anxiety and unease. In addition, because of the long period of illness, parents also appear anxious and the quality of family life was affected. Cow Milk protein allergy (CMPA) is an immunological reaction of the body to one or more milk proteins, and its clinical manifestations are diverse, which mainly related to the digestive system, respiratory system, skin and so on. There are few reports about the relationship between CMPA and FA at home. The purpose of this study was to analyse the clinical features of infants with functional constipation (FC) associated with CMPA and explore its ways of diagnosis and therapy.

Methods: The detail clinical information of the infants (<1 year old) diagnosed FC relating to CMPA in our hospital from January 2015 to May 2017 were analysed retrospectively. All patients completed routine blood test, serum IgE, allergen IgG examination, anal digital examination, thyroid function, barium enema, abdominal B ultrasound, anorectal manometry, and so on.

Results: 67 cases of milk protein allergy manifested functional constipation as the main manifestation, accounted for 9.1%(67/736) of all milk protein allergy. Twenty-eight (41.8%) cases were males, and 39 (58.2%) cases were females. The onset age was 17 days to 11 months, and the average age was 4.4 months. The course of disease was 1-8 months, and the average course of disease was 3.5 months. His mother and/or father had an allergic history in 21 cases (31.3%). The proportion of eosinophils increased in 57 cases (85%), and serum IgE elevated in 8 cases (11.9%). Twenty-one cases (31.3%) were positive for allergen IgG test, especially milk, eggs, cod and so on. DRE, thyroid function, abdominal ultrasound, barium enema, anorectal manometry examination showed no abnormality in all cases. Diet therapy: mothers avoid allergic food for breastfeeding children, and free amino acid formula was used for formula fed children. After 2-4 weeks treatment 45 cases (67.1%) of children with constipation has been improved.

Conclusion: CMPA may be one of the causes of FC in infants. Avoiding diet in the treatment of some milk protein allergy related functional constipation in infants was effective.

Clinical Analysis of Gastroscopic Results of 525 Cases of Paediatric Abdominal Pain

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Xi'an Children's Hospital, Xi'an, China

Background and Aims: Abdominal pain is one of the main causes of digestive diseases in children, with the characteristics of multiple, and easy recurrence. The invention of gastroscopy provides convenience for people to explore the cause of abdominal pain. The purpose of this study was to observe the microscopic manifestations and analyse the causes of abdominal pain in children, and to provide guidelines for the diagnosis and treatment of abdominal pain in children.

Methods: 525 patients with upper abdominal pain who were examined by gastroscope were collected from Xi'an
Children's Hospital between March 2015 and May 2016. The patients were examined by using electronic gastroscope Olympus GIF-Q260 and electronic nose gastroscope GIF-XP290N. Meanwhile, results were recorded in details, including medical history, family history, other signs, biochemical tests results and abdominal imaging results. For children with long history or serious abdominal pain, further endoscopic mucosal biopsy and 13C-UBT test for *helicobacter pylori* were done to clear aetiology of abdominal pain.

**Results:** Gastroscopy results showed 36 cases among 525 (6.9%) cases were normal, the other 489 cases (93.1%) were abnormal, and the upper digestive tract diseases were common. Endoscopic biopsy was performed in 122 cases, and the biopsy rate was 23.2%. According endoscopic and biopsy results, Simple chronic superficial gastritis was 284 cases (58.1%), peptic ulcer was 47 cases (9.6%), superficial gastritis complicated with reflux esophagitis, or bile reflux was 61 cases (12.5%), superficial gastritis with duodenitis was 36 cases (7.36%), gastric volvulus was 3 cases (0.6%), erosion gastritis was 18 cases (3.68%), chemical corrosive gastritis (0.6%), eosinophilic gastroenteritis was 3 cases (0.6%), allergic purpura was 34 cases (6.95%). Among them, 385 cases received 13C-UBT test to check *Helicobacter pylori*, the positive rate was 53%.

**Conclusion:** The causes of abdominal pain in children are complicated and the clinical manifestations are varied. Gastroscopy is an important method for the definite aetiology of abdominal pain in children, and the improvement of the rate of biopsy is beneficial to the diagnosis of the disease.

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**Pharmacokinetics of Intravenous Levetiracetam in Thai Children**

S. Horsawan, N. Jenjarattithigarn, C. Sukasem,
C. Khongkhatithum, A. Visudtibhan, L. Thampratankul
Ramathibodi, Bangkok, Thailand

**Background and Aims:** Levetiracetam (LEV) is a new generation antiepileptic drug approved for partial onset and generalised seizures in adults and children aged over one month. Intravenous LEV, approved by the United States' FDA in 2006, is an alternative when oral [ad1] [D2] is not feasible. Currently, LEV is frequently used in children with hepatic and/or cardiovascular problems and concerns over drug interaction. The recommended dosage varies from 20 to 50 mg/kg. Very limited information from pharmacokinetic studies of LEV infusions exists, particularly regarding children. This study is to determine pharmacokinetics (Pk) of intravenous LEV in Thai children.

**Method:** This was a prospective study conducted in Thai children with clinical indication for IV LEV therapy aged between 1 month and 18 years old. Patients with glomerular filtration rate (GFR) <50 ml/min/1.73 m², severe cirrhosis and [ad3] [D4] history of LEV allergy were excluded. Data collection included demographic data, underlying disease, seizure type and aetiology, response and adverse effects, as well as GFR (revised Schwartz formula). All received LEV infusions of 30 mg/kg in 15 minutes. Plasma LEV levels, analysed by LC-MS/MS, were measured at 0, 30, and 60 minutes, and 4, 8 and 12 hours after the loading dose. Pharmacokinetic parameters were determined using Kinetica 2.0 (Thermo Fisher Scientific, MA, USA) with non-compartmental model.[ad5] [D6] [D7]

**Results:** 14 patients (50% male, mean age 115.6 ± 14.9 months) were enrolled. Four patients received concomitant treatment with enzyme inducers, while another 4 patients received enzyme inhibitors. Notably, 3 patients had significantly prolonged half-life and delayed clearance when compared with the others. From these, two patients had sepsis and acute kidney injury, while the other had steroid resistant nephrotic syndrome. Their results were excluded from analysis. Pk of IV LEV in 11 patients showed a half-life of 5.12 ± 1.68 hr, a clearance of 9.00 ± 5.65 L/hr, and a volume of distribution of 59.86 ± 34.56 L. No significant differences in gender, age group, or concomitant medication were found.

**Conclusions:** Pharmacokinetics of IV levetiracetam in these children revealed short half-life and rapid clearance. Patients with kidney problems had significantly prolonged half-life and slower clearance.

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**Impact of Sleep Duration on Neurocognitive Outcomes in Hong Kong Preschoolers**

F. Chen, P. Chan, X. Yu, A. Li, S. C. L. Chau, H. S. H. S. Lam
Department of Pediatrics, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong

**Background and Aims:** Sleep is important for infants and children in terms of growth and development. Other than sleep quality, sleep duration has been found to be associated with behavioural problems, early school failure and neurocognitive dysfunction. There is evidence to suggest that Hong Kong children tend to sleep less when compared to their Western counterparts. Our aim was to compare neurocognitive outcomes of short and median sleep duration in local preschool Chinese children, and to provide local data.

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**Background and Aims:** Sleep is important for infants and children in terms of growth and development. Other than sleep quality, sleep duration has been found to be associated with behavioural problems, early school failure and neurocognitive dysfunction. There is evidence to suggest that Hong Kong children tend to sleep less when compared to their Western counterparts. Our aim was to compare neurocognitive outcomes of short and median sleep duration in local preschool Chinese children, and to provide local data.
Methods: Based on our former community-based study (n=3041) in Hong Kong, we calculated the percentiles of sleep duration for 4- and 5-year old children. Children with the shortest sleep duration (≤10th percentile) and children with average sleep duration (25th-75th percentile) and their parents were invited to record a 14-day sleep dairy, and to undergo neurocognitive assessments: Child Behaviour Checklist (CBCL), the Connor’s Kiddie Continuous Performance Test (K-CPT, attention test), and Beery’s Visual Motor Integration (VMI, a test for perceptual and fine motor abilities). SPSS v.22 was used to analyse the difference in neurocognitive performance.

Results: The 10th and 25th-75th of sleep duration for 4 and 5-year-old children were calculated respectively, and in total 199 children were included. Mean total sleep duration was 575.45 min (n=80) for the short sleep group and 621.40 min (n=119) for the median group. Majority of the test results were within normal range. For the short sleepers, higher scores were noted in items of internalising problems (p=0.043), sleep problems (p=0.008), anxious/depressive (p=0.013) in CBCL, and lower score in the item of preservations in K-CPT (p=0.025), showing better performance in median sleepers. No significant difference was detected in the VMI tests.

Conclusions: Most of the neurocognitive performance results were within normal range. Significant differences were observed in CBCL and CPT subtests between short and median sleepers, indicating that short sleep duration may be associated with cognitive and behavioural problems.

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Screen Use and Its Relationship with Emotional and Behavioural Difficulties in Pre-school Children with Neurodevelopmental Disorders: Do Screen Content and Context Matter?

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Introduction: Use of screen media has been linked to poorer developmental outcomes in typically developing children. Despite increasing use of screen media in children, little is known about screen use in children with neurodevelopmental disorders (NDD), a population already at a higher risk for poorer developmental, emotional and behavioural outcomes. The present study examined the relationship between screen use and emotional and behavioural difficulties (EBD) in Singaporean pre-school children with NDD. Specifically, we explored whether the content (type of programmes) and context (amount of co-viewing with others) of screen use moderates this relationship.

Methodology: Parents of 367 children with NDD below the age of six years seeking services at the Department of Child Development, KK Children’s and Women’s Hospital, participated in this cross-sectional study. Children’s screen use patterns and EB difficulties were assessed using parent-report questionnaires.

Results: The average daily screen time for children in this study was 3.98 hours. The average age of First Screen Exposure (FSE) was 1.74 years, with 52% exposed to screens at less than 18 months of age. Child video programmes (CVP) was the screen content ranked as being ‘used most often’, followed by interactive educational applications (EA), and mobile games (MG). Use of CVP ‘more often’ was significantly negatively associated with use of EA ‘more often’. Earlier age of FSE was associated with more EBD, with a small effect size. Average daily screen time and screen context (time spent on solitary viewing of screens) was not associated with EB difficulties. Using CVP during screen time was found to be a significant moderator between age of FSE and EBD. Other screen
Discussion/Conclusion: Screen time of children with NDD in our study exceeded APA recommendations, but was similar to previous Singapore studies in younger children. Children who are introduced to screens at an earlier age had more EBD at recruitment. Mixed results in the moderating effects of screen content and screen context are in line with previous studies, but could also be due to limitations in measurement of screen content and context. Recommendations for future studies are discussed.

Rasch Validation of the Chinese Parent-Child Interaction Scale (CPCIS)

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Background: Proper parent-child interaction is crucial for child development, but an assessment tool in Chinese is currently lacking. This study aimed to develop and validate a parent-reported parent-child interaction scale for Chinese preschool children.

Method: The Chinese Parent-Child Interaction Scale (CPCIS) was designed by an expert panel based on the literature and clinical observations in the Chinese context. The initial CPCIS had 14 parent-child interactive activity items. Psychometric properties of the CPCIS were examined using the Rasch model and confirmatory factor analysis (CFA). Convergent validity was investigated by the associations between CPCIS and family income, maternal education level, and children's school readiness.

Results: The study recruited 567 Chinese parent-child pairs from diverse socioeconomic backgrounds, who completed the CPCIS. Six out of the 14 items in the initial CPCIS were dropped due to suboptimal fit values. The refined 8-item CPCIS was shown to be valid and reliable by Rasch models and CFA. The person separation reliability and Cronbach's α of the CPCIS were 0.81 and 0.82, respectively. The CPCIS scores were positively associated with family socioeconomic status ($\eta^2=0.05, p<0.001$), maternal education level ($\eta^2=0.08, p<0.001$), and children's school readiness ($\eta^2=0.01, p<0.01$).

Conclusions: CPCIS is an easily administered, valid, and reliable tool for the assessment of parent-child interactions in Chinese families.
Application of Whole Exome Sequencing to Identify Genetic Causes of Syndromic Craniosynostosis

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Background and Aims: Syndromic craniosynostoses are a group of multiple conditions with high heterogeneity. To identify and analyse causative genetic variants in 9 unrelated family pedigrees mainly manifested as syndromic craniosynostosis without pre-existing clinical diagnoses.

Methods: We reviewed the relevant medical information of the study subjects. Whole exome sequencing was performed in the probands and relevant variants were verified with Sanger sequencing and parental background. Bioinformatics analysis was used to evaluate the potential pathogenicity through evolutionary conservation alignment, multi-predication, and variants classification according to the criteria recommended by the American College of Medical Genetics and Genomics.

Results: We shared the strategies of interpreting the genetic results and the results revealed 9 variants in four different genes: TWIST1, FGFR2, IFT122 and SMC1A. Five (5) of the 9 variants have been identified previously, while 4 variants including three missense mutations (c.628C>T, c.3385C>T in IFT122 gene, c.3581A>G in SMC1A gene) and a frameshift mutation (c.434dupA in TWIST1 gene) were novel or extremely rare and have not been previously reported.

Conclusions: Our study not only expanded genotype-phenotype correlations, but also confirmed the underlying causative variations of syndromic craniosynostoses, and emphasized the importance of genetic testing applied in patients with syndromic craniosynostoses.

EEG Power Spectra as Early Biomarkers of Autism Spectrum Disorder

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Objective: To identify electrophysiological biomarkers of autism spectrum disorders (ASD) and to investigate their diagnostic value of ASD.

Methods: We recruited toddlers aged 18-30 months who diagnosed with ASD and age-matched toddlers with typical development (n=20, respectively) in our study. EEG absolute and relative powers of 17 frequency bands were measured when subjects were awake. Then, we selected the power spectra which were different between toddlers with ASD and typical toddlers, and performed receiver-operator-characteristic (ROC) area-under-the-curve (AUC) analyses using clinical diagnosis as reference to evaluate their application values for early diagnosis of ASD, and to compare the sensitivity and specificity of the EEG power candidates using optimal cutoffs. Finally, we used the EEG power indexes of statistical significance to establish a model for early diagnosis of ASD. Meanwhile, we performed linear regression analysis and correlation analysis to explore the probable relationship between EEG powers and severity of clinical symptoms/developmental outcomes.

Results: (1) Compared with those of typical toddlers, the beta relative powers of central (C3, Cz), frontal (F3, Fz, F4), left temporal (F7, T3) and left parietal (P3) areas were significantly higher in toddlers with ASD. (2) After performing ROC-AUC analyses, beta relative powers of 3 electrodes (F3, Fz, F4) of frontal area were confirmed with statistical significance. The AUCs were higher than 0.7 (p<0.05). Of these, the AUC of the F4 beta relative power was the best (AUC=0.827, p=0.006). Logistic regression model was established with the 3 beta relative powers, but only the F4 beta relative power entered the equation. The regression equation is logitP=-2.454+0.517*F4 beta RP. (3) The beta relative powers of left frontal (F3) and left anterior temporal (F7) lobe were positively correlated with the developmental quotients (DQs) of hand-eye coordination and performance subscales, and the general quotient (GQ), respectively (r>0.3, p<0.05). No other correlations were founded between EEG relative powers and clinical outcomes.

Conclusions: (1) There are some differences in awake EEG power between toddlers with ASD and typical toddlers, mainly manifesting as higher beta relative powers of some areas in toddlers with ASD, especially in left brain areas. (2) The beta relative powers of frontal areas (F3, Fz,
F4) can be the biomarkers for early diagnosis of ASD, and the beta relative power of right frontal area (F4) might become an independent index for the early diagnosis of ASD. (3) The beta relative powers of left frontal (F3) and left anterior temporal (F7) lobes might be used for predicting cognitive ability of ASD toddlers.

**Altered Functional and Structural Brain Connectivity in ASD Individuals with SHANK3 Defect**

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**Background and Aims:** SHANK3 is a postsynaptic scaffolding protein, whose molecular variations is thought to be responsible for 22q13 deletion syndrome (Phelan-McDermid Syndrome) and autism spectrum disorders (ASD). However, it remains unclear how SHANK3 defect are related to abnormal brain development in ASD. Our study aims to assess the GM and WM development of SHANK3 defect children ascertained for ASD and explore the relationship with clinical phenotypes.

**Methods:** MLPA and Sanger sequencing were carried out to confirm the SHANK3 deficiency of 8 Chinese children with ASD (SHANK3 group), followed by systematic and comprehensive evaluations. Then we recruited 24 ASD children without SHANK3 deficiency (ASD group) and 25 typically developing controls (TD group). ADOS scale was applied to examine the severity of autism and Griffith scale was used to assess the development level of SHANK3 group and ASD group. In addition, MRI scans of the three groups were analysed using voxel-based morphometry (VBM) and Diffusion tensor imaging (DTI). Normalised modulated GM maps were statistically analysed using the general linear model. The integrity of WM fiber was evaluated using fractional anisotropy (FA).

**Results:** The sample was characterised by high rates (100%) of ASD, developmental delay, hypotonia, several dysmorphologies and perception abreaction. SHANK3 defect children displayed severer developmental delay in language, performance and other items compared with ASD group (p<0.0005). As to the MRI performance, VBM showed that SHANK3 group showed significant gray matter volume decrease in the left middle frontal gyrus, right postcentral and left dorso-lateral superior frontal gyrus, compared with TD and ASD group (p<0.001). What's more, SHANK3 group had significantly less gray matter in the left cerebellar crus, left triangle inferior frontal gyrus, left inferior parietal and left fusiform gyrus than ASD group (p<0.001). Additionally, as to the DTI result, corpus callosum (body, splenium and genu) tracts in SHANK3 group displayed significantly lower FA than ASD and TD group (p<0.001). Other tracts, such as middle cerebellar peduncle, bilateral superior and anterior corona radiate and bilateral superior longitudinal fasciculus, also had significant abnormalities (p<0.001).

**Conclusions:** These results imply that SHANK3 defect associated with ASD may be rooted in neural anatomy, and autism symptoms in individuals with SHANK3 defect and ASD might have, at least partially, different underlying etiologies. Moreover, an obvious phenomenon of imbalance between left and right side in SHANK3 defect children was existed, which may be associated with the different functions of each brain region and the diversity of clinical phenotypes.

**Aberrant Expression of Histone Homocysteinylation: Implications for Neural Tube Defects**

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**Background and Aims:** Neural tube defects (NTDs) are serious congenital malformations. A superphysical maternal homocysteine (Hcy) level increases the risk of NTDs, but the mechanism behind this remains elusive. Previous studies have shown that cellular one-carbon metabolism (associated with NTDs) can function to directly affect histone modification and consequently plays a critical role in early embryogenesis in particular. Therefore, we hypothesised that cellular Hcy, inter-metabolites within the one-carbon metabolism, that modifies histones and aberrant histone homocysteinylation due to the disturbance of one-carbon metabolism are involved in the failure of neural tube closure (NTC).

**Methods:** Mass spectrometry was used to identify the new histone homocysteinylation in human fetal brain and neural stem cell; Immunoblot was used to validate the histone homocysteinylation; Hey and HTL treatment were used to find if Hey and HTL can regulate the level of histone homocysteinylation in neural stem cell; ChiP-seq and RNA-seq were used to find the genes regulated by histone homocysteinylation; Human NTDs and normal controls were used to test out hypothesis.

**Results:** In total, 39 histone homocysteinylation sites were identified in human embryonic brain tissue by QE-HF
mass spectrometry. Then, the conservatism and extent of histone Hcy and H3K79Hcy were evaluated using specific anti-KHcy antibody and anti-H3K79Hcy antibody. Higher expression levels of histone KHcy and H3K79Hcy were detected, while the cellular Hcy levels were increased. ChiP-seq analysis revealed that histone H3K79Hcy bound to neural development-related genes participating in developmentally controlled processes, namely, nervous system development, generation of neurons, and neurogenesis. Combining with data from RNA-seq, our results showed that H3K79Hcy regulated the expression of selected NTC-related genes including Cecr2, Smarca4, and Dnmt3b. Lastly, in human NTDs, the decreased expression of Cecr2, Smarca4, and Dnmt3b was also detected in brain tissues with high levels of Hcy and H3K79Hcy.

**Conclusions:** Collectively, our results suggest that high levels of Hcy may contribute to the onset of NTDs through the upregulation of histone H3K79hcy, leading to a decreased level of expression of selected NTC-related genes.

**Clinical Practice of CMA in NDD in China**

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**Background and Aims:** Neurodevelopmental disorders (NDDs), including intellectual disability, global developmental delay, and autism, affect more than 15% of children. NDDs are a condition of great concern for public health and society which have the complex genetic understanding in its aetiology. Chromosomal microarray (CMA) is currently the first-line diagnostic genetic test for patients with idiopathic neuropsychiatric diseases in many countries. Large-scale whole-genome copy number variation (CNV) studies have established the importance of de novo CNV in NDD, CNV study in DD/ID and/or ASD has not been well investigated in non-Caucasian population.

**Methods:** To evaluate the pathogenic responsibility and clinical impact of chromosomal microarray (CMA) on the paediatric patients in China. We performed CytoScan™ HD system, Affymetrix array on 364 children with intellectual disability (ID)/developmental delay (DD), autism spectrum disorders (ASD) in the department of developmental behavioural paediatrics of Shanghai Children's Medical Center and Xinhua hospital affiliated to Shanghai Jiaotong University, School of Medicine from July 2014 to August 2016. The medical records of patients were reviewed, focusing on the pathogenic/likely pathogenic CMA findings and their clinical management.

**Results:** 56 patients were reported to have pathogenic/likely pathogenic results. This gives a detection rate of 24.4% for DD/ID and 8.33% for ASD with clinically significance. The significant findings have prompted clinical actions in 48 patients (86%). A total of eight recurrent CNVs that spanned in different chromosome were identified. The 22q13.33 deletion is more common in ASD patients of our study cohort. Our study clearly demonstrates a dosage effect of 17q11.1-q12 copy number on the various clinical findings, and suggests the presence of dosage sensitive genes and level of somatic mosaicism within the rearranged interval.

**Conclusion:** Our effort helped to collect the information of the de novo mutation in Chinese children with DD/ID and/or ASD. Our data shows that CMA provides immediate clinical utility for patients. Our study provided further evidence of an increased diagnostic yield of CMA and supported its use as a first line diagnostic tool for Chinese individuals with DD/ID, ASD. We advocate using diagnostic yield of clinically actionable results to evaluate CMA as it provides information of both clinical validity and clinical utility. The same framework can be applied to other genomic testing strategies enabled by next-generation sequencing.

**Collaborative Project of Medical Service and School Health System to Care for School Age Children's Mental Health in Shanghai**

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**Objective:** The present study aimed to build a mode of collaboration with medical service and school health system to care for school age children's mental health in Shanghai.

**Methods:** The sample of this study consisted of 4884 children aged 6-9 years (8.10±1.19) from 12 primary schools in four districts in Shanghai. The Strengths and Difficulties Questionnaire (SDQ) was administered to screen. Screening positive children were interviewed with Mini International Neuropsychiatric Interview for Children and Adolescent (MINI-KID) by trained teachers from school health system. Developmental and behavioural paediatricians then further interviewed children with positive results of MINI-KID to made diagnosis. Eight-week individualised intervention for
children with mental issues, mainly Attention Deficit Hyperactivity Disorder (ADHD) was done in schools with the monitoring of developmental and behavioural paediatricians and therapists. The intervention includes group play therapy, behaviour therapy, language and neuro-motor therapy and medication. The Chinese version of the Swanson Nolan and Pelham, Version IV (SNAP-IV) Scale, Self-esteem Scale, SDQ and neuro-motor scale were reassessed at the end of the intervention and 3 months later.

**Results:** The positive rate of SDQ screening is 9.38\% (458/4884), in which about 80\% shows positive in domains of conduct problem, partnership problem and hyperactive and attention problem. MINI-KID positive is 18.85\% (69/366, 92 children didn't attend interview). All 69 children show ADHD pattern and 12 comorbid anxiety, 7 comorbid ODD, 5 comorbid depression, 4 comorbid conduct disorder and 2 comorbid tics. Fifty-eight from 69 MINI-KID positive children accepted interview of developmental and behavioural paediatricians and 46 were diagnosed as ADHD. The detection rate of ADHD by school health system is 79.31\%. Eight-week individualised intervention significantly improved ADHD children’s symptoms of inattention and hyperactivity, neuro-motor ability, and self-esteem (p<0.05). The same pattern of improvement showed 3 months later.

**Conclusion:** The mode of collaboration with medical service and school health system to care for school age children's mental health in Shanghai is feasible and effective. ADHD is the main aetiology for primary school children with behavioural and mental health problems.

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**A Randomised Controlled Study and Valuation of Children with Cerebral Palsy by Mind Acupuncture**

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**Objective:** To investigate the effects of clearing the Governor Vessel and refreshing the mind needling in neural development and remediation of children with cerebral palsy.

**Methods:** 200 cases of children with cerebral palsy were randomly divided into the treatment group (n=100) and the control group (n=100). The treatment group was given the combined therapy of acupuncture and rehabilitation training, and the chosen acupoints were 13 points of the Governor Vessel, Shenshu (BL 23), Taixi (KI 3), Yanglingquan (GB 34), Zusanli (ST 36) and Sanyinjiao (SP 6), and points of refreshing the mind were also selected, which included puncturing Shenting (GV 24) toward Qianding (GV 21), puncturing Qianding (GV 21) toward Baihui (GV 20), puncturing Baihui (GV 20) toward Naohu (GV 17) and Sishencong (Ex-HN 1). The control group was only treated with rehabilitation training. A contrastive analysis of the therapeutic effect of acupuncture combined with rehabilitation training and pure rehabilitation training was made after a treatment course of 3 months. The Gross Motor Function Measure (GMFM) and Beijing Gesell Developmental Scale were adopted to assess the neural development and rehabilitation outcomes of the two groups. In addition, skull CT/MRI was adopted to evaluate the plerosis of injured cerebral nerve after treatment.

**Results:** The total effective rate in treatment group was 87\%, significantly higher than the 55\% in the control group. The children's development quotient (DQ) tested by Gesell Developmental Scale and scores tested by GMFM in the treatment group was obviously higher than the control group (p<0.01). The improving and curing rates presented by skull CT/MRI in the treatment group were higher than the control group (p<0.01).

**Conclusions:** Clearing the Governor Vessel and refreshing the mind Needling could accelerate the recovery of injured brain nerve and the reconstruction of brain function. The acupuncture therapy could ameliorate both the motor development and cognitive development. On the other hand, the forward curative effect of acupuncture combined with rehabilitation training was significantly better than the pure rehabilitation training.
of BCRI early behavioural intervention, and provide positive affects to the clinical outcome for children with ASD.

**Methods:** 130 children diagnosed with ASD between the ages of 18 and 30 months were randomly assigned to the early BCRI group and CI group (community intervention). Both BCRI group and CI group participate in a two-day ASD seminar to receive basic knowledge about behavioural intervention in Phase-one. In Phase-two, BCRI group participate in a 24-halfday workshop to gain hands-on experience and one-on-one training from master trainers, then these family take another 11 months to implement BCRI intervention to their children in home settings, and CI group receives community service. Psycho-educational Profile-3rd Edition (PEP-3) assessment indexes were collected, and single and multiple Wilcoxon signed rank test were performed for statistical analysis.

**Result:** 85 participants (53 in BCRI group, 32 in CI group) completed primary endpoint at one year after enrollment. Statistically significant post-intervention improvements were found in BCRI group, which included Cognitive Verbal/Proverbial (CVP), Expressive Language (EL), and Receptive Language (RL) subsets in the Communication domain (p<0.05). By Comparison of pre-and post-intervention between BCRI and CI group, significant improvements were reported in combined score of Communication domain (CVP, EL, RL) plus Fine motor (FM) subset (p<0.05), and in combined score of Visual Motor Imitation (VMI) subset and FM subset (p<0.05).

**Conclusions:** It's the initial large sample randomised controlled study for family-based early behavioural intervention for ASD. We suggest that BCRI Intervention model have positive effects for children with ASD, to develop early communication skills as well as visual motor Imitation and fine motor skills. BCRI model emphasize the initial appliance of behaviour management and problem-solving strategies, which following by structural teaching infrastructure with appropriate level of education. BCRI also emphasize to emerge ‘relationship elements’ as part of social interaction intervention throughout every step of BCRI model, to Improve social skills of children with ASD. The study indicated that BCRI model is an effective early behavioural intervention method for child with ASD, and the feasibility to implement in middle-income countries.

**Association Between Maternal Gestational Diabetes Mellitus and Offspring’s Neurodevelopment Delay at One Year of Age: A Prospective Cohort Study**

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**Objective:** The incidence of gestational diabetes mellitus (GDM) is rising rapidly in China. Effects of maternal GDM on offspring's neurodevelopment remain unclear. We aimed to examine whether children whose mothers were diagnosed with GDM had increased risk of neurodevelopment delay in their first years of life.

**Methods:** We used data from 5107 mother-child pairs recruited from the Born in Guangzhou Cohort Study between February 1, 2012 and April 30, 2015. Maternal GDM status was assessed by 75-g oral glucose-tolerance test (OGTT) at 22 and 28 week’s gestation. Five dimensions of child's neurodevelopment, including adaptive behaviour, gross motor, fine motor, language and social behaviour, was evaluated at 12 months of age using the Gesell Development Scale. Neurodevelopment delay for each dimension was defined as a score of <85 respectively. Log-binomial regression models were used to examine the association between maternal GDM status and children's neurodevelopment outcomes, and risk ratios (RR) and 95% confidence intervals (CI) were calculated after adjustment for child's sex, birth weight, mothers' maternal age, pre-pregnancy body mass index (BMI), second-hand smoking status, maternal education, income, parity and feeding practice. Multiple linear regression was applied to explore the relationship between fasting glucose level and neurodevelopment scores.

**Results:** Maternal GDM was statistically associated with lower risk of fine motor development delay (RR=0.59, 95% CI: 0.35-0.97, p=0.04), but not other neurodevelopment dimension (p values >0.05). Fasting glucose levels was positively related to the scores of gross motor (regression coefficient $\beta=0.81$, p=0.03), fine motor ($\beta=0.80$, p=0.01) and social behaviour ($\beta=1.64$, p=0.001).

**Conclusions:** We did not observed evident harmful effects of maternal GDM on children's neurodevelopment at 12 month of age. In contrast, higher fasting glucose level appeared to be associated with improved development of gross motor, fine motor and social behaviour. Further research is needed to confirm our findings.
Regional Brain Chemical Alterations Between Verbal and Non-verbal Autistic Children: A 1H-MRS Study
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Background: Language deficit is one of the most difficult to treat autistic features; around 25% autistic patients remain non-verbal; the pathophysiology of non-verbal is unclear.

Objectives: In vivo investigated cellular neurochemicals with proton magnetic resonance spectroscopy imaging (1H-MRS) in the brain regions associated with language in children with autism spectrum disorder (ASD).

Methods: The autistic children aged between 2-14 years old were recruited. The concentrations of cerebral N-acetyl-aspartate (NAA), creatine (Cr), choline (Cho), were determined by 3 T 1H-MRS examinations in 91 non-verbal (NV) and 75 verbal (V) autistic children at the inferior frontal cortex (IFC), superior temporal cortex, cerebellum, and hippocampus respectively. The binary multivariate logistic regression analysis was adopted.

Results: The higher level of NAA/Cr ratio at the right IFC was associated with increased risk of non-verbal (2.51 [1.17-5.38]; p=0.018), whereas the lower levels of Cr (0.60[0.41-0.88]; p=0.01) and Cho (0.73 [0.56-0.97]; p=0.027) at the right IFC, Cho/Cr ratio (0.46 [0.23-0.90]; p=0.023; 70/91 [NV] versus 54/75 [V]) at the left cerebellum, and NAA (0.70 [0.52-0.93]; p=0.015; 53/91 [NV] versus 37/75 [V]) at the right hippocampus were related to decrease risk of non-verbal, after adjusting the confounding factors including age, gender, gestational age, birth weight, developmental quotient/intellectual quotient, severity of autism, maternal education and paternal education.

Conclusions: Among autistic children, some regional chemical alterations were associated with the risk of non-verbal feature.

Experimental Study of Lentiviral Vector-mediated Ephrinb2 Gene Transfection Rat Bone Marrow Mesenchymal Stem Cells
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Background and Aims: To culture and identify rat bone marrow-derived mesenchymal stem cells, to detect the over-expression and morphological changes after ephrinb2 gene transfected to BMSCs and study the effect of EphB4/ephrinB2 on rat bone marrow mesenchymal stem cells (BMSCs) in vitro.

Methods: The simple adherent method was adopted in isolating and culturing experiments of bone marrow mesenchymal stem cells (BMSCs). The inverted microscope was used to observe cells. Expression of BMSCs marker antigen was examined by flow cytometry. Lentivirus carrying ephrinb2 infected BMSCs (MOI=10) and (MOI=100), using qPCR and Western blot to detect ephrinb2 after transfection, detect the mRNA and protein's expression. Morphological changes of BMSCs after differentiation was observed. In 28 days the cell differentiation was determined by microtubule-associated protein 2 (MAP2), CD133 and Nestin immunofluorescent staining. For migration assay. Transwell assay was used to detect the ability of cell migration. The migration rate was assessed to study the role of Ephb4/Ephrinb2 pathway in the stem cells migration by transwell chambers. The expression of Grb4, Jnk and C-jun protein in EphB4/EphrinB2 reverse signal pathway were detected by Western blot.

Results: Cell bodies of major BMSCs were unified into polygonal or fusiform, germinated into a spiral shape. The BMSCs were successfully isolated, since flow cytometry results showed that rat BMSCs CD90 and CD29 positive, CD34 and CD45 negative. We confirmed exogenous ephrinb2 expression in ephrinb2-BMSCs by qPCR and Western blot. 3 days after ephrinb2 gene transfection, BMSCs cell body began to shrink, refraction enhanced cell protrusions, and differentiate into the typical neuron-like cell, RT-PCR and Western blot detection of Nestin positive expression. In 15 days, expression levels of (MAP2), CD133 and Nestin in the low and high concentration transfected group were significantly higher than those in the negative control group (p<0.05). Transwell assay showed the number of transmembrane cells in the low and high concentration transfected group were obviously higher compared to non-transfected group and negative control group (p<0.05). Western blot analysis
showed that the expression of Grb4, Jnk and C-jun protein of were increased.

**Conclusion:** The simple adherent method was a feasible way to isolate, culture and purify BMSCs successfully. It layed foundation for later experimental procedures of this research. Lentiviral-mediated ephrinb2 can efficiently infect BMSCs, and differentiate into neuron-like cells. EphB4/ EphrinB2 pathway can play the role of migration in bone marrow mesenchymal stem cells.

**A New Interpretation of Overnight Pulse Oximetry to Diagnose Moderate to Severe Childhood Obstructive Sleep Apnoea**

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**Background and Aims:** Pulse oximetry (PO) has been used relatively most often as an alternative test to polysomnography (PSG), which was expensive and not widely available. Three clusters of desaturation on POTG (POTG) were found to have 97% positive predictive value. Since 2012, the American Academy of Sleep Medicine has changed PSG scoring rules. It was unknown whether POTG is still useful or there is any other interpretation method. We aimed to determine the accuracy of 3 desaturation clusters to diagnose moderate to severe OSA as compared to PSG and to find a new simple interpretation of PO to diagnose moderate to severe OSA, which was considered to be suitable candidates for adenotonsillectomy.

**Methods:** We recruited consecutive snoring children with adenotonsillar hypertrophy, aged 1-15 years, referred for PSG at Ramathibodi Hospital from January 2013-August 2014. They were monitored with Masimo® pulse oximeter while performing PSG. Apnoea-hypopnoea index (AHI) ≥5 per hour of sleep was defined as moderate to severe OSA. POTG were created by Masimo® Trend-Com Graph software program and number of desaturation clusters were determined. Other parameters of SpO₂ data including minimum, maximum, mean, median, skewness, kurtosis and standard deviation (SD) were calculated automatically from the software. All of them were analysed to identify the most useful diagnostic one.

**Results:** Among 166 children, 103 (62%) were male. Mean age was 6.2±2.7 years, range 2-15 years. Ninety-one (54%) were diagnosed with moderate to severe OSA by PSG. Three clusters of desaturation on POTG provided a positive predictive value of 92.3%, an accuracy of 62%, a sensitivity of 28%, a specificity of 97.5% and an area under ROC curve (AUC) of 0.629. Among all parameters, SD of SpO₂ was found to be the most promising one. The SD of SpO₂ ≥1.3 provided a positive predictive value of 93.9%, an accuracy of 62.7%, a sensitivity of 34%, a specificity of 97.3% and an area under ROC curve (AUC) of 0.646 for diagnosis of moderate to severe OSA patients.

**Conclusions:** SD of overnight PO can be used as an initial test for snoring children. It yields good diagnostic performance compared with determination of desaturation cluster numbers on POTG but the calculation of SD of SpO₂ is much easier, less time consuming and least likely to generate interpersonal disagreement on interpretation.

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**Functional and Ultrastructural Analysis of Respiratory Cilia in Healthy Chinese Children in Hong Kong**

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**Background and Aims:** Respiratory cilia beat in a coordinated manner with a specific frequency and pattern. Ineffective movement of cilia impairs clearance of mucus and debris from the airways that may cause sinusitis and recurrent chest infections, leading to bronchiectasis in primary ciliary dyskinesia (PCD). Normal reference range of ciliary beat frequency (CBF) and structure and is available in Western population but lacking in the Chinese population for the early diagnosis of PCD. Our study aims to establish the normal reference range for respiratory CBF and determine the ciliary ultrastructure in a healthy Chinese paediatric and adolescent population.

**Methods:** Nasal epithelial cells were obtained from 162 children (age range 2-17 years) by brushing inferior turbinate. CBF The movement of cilium was captured and examined using a high speed camera. CBF of each ciliated strip was counted and a maximum of 10 ciliated edges were analysed per subject. CBF was determined by the number of frames required to complete 10 cycles. Ultrastructure – Nasal brushings were processed by standard
techniques. Ciliary ultrastructures were examined using transmission electron microscopy. Data analysis – Age were first sub grouped into 2-6, 7-12 and 13-17. The mean for CBF was compared among each age group using ANOVA. Standard deviation, 5th and 95th percentiles, and 95% confidence intervals of whole age group were calculated if there is no significant observed.

Results: Nasal brush samples obtained from 141 healthy children (70 male) were included for CBF evaluation following the inclusion criteria. The mean CBF for children was 10.4Hz (SD 2.2, 95% CI 10.0 to 10.8). There was also no significant difference in mean CBF between the individual age groups (unpaired t-test, p>0.5). For ciliary beat pattern (CBP), circulating beating cilia was found on ciliated strips from a child. Normal CBP was observed in the other samples. One hundred and twenty five samples were sufficient for ultrastructural analysis. Dynein arm defects were not found in the cilia. The mean outer and inner arm counts were 8.5 and 7.8 for children. Microtubular defects were found in 9.2% of cilia counts from children. Other ciliary ultrastructural defects were found in less than 3% of cilia for individual age groups.

Conclusions: Our study showed that the normal reference range of CBF for children was 10.4Hz (SD 2.2, 95% CI 10.0 to 10.8). A further study will be followed to investigate the microtubular defects of cilia.

Long-term Changes in Spirometric and Airway Inflammatory Parameters in Asthmatic Children
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Background and Aims: Asthma is caused by complex interactions between many predisposition genes and early-life and environmental factors. A proportion of asthmatic children had decreased lung function with time. On the other hand, there is limited longitudinal data as well as the genetic influences on lung function growth of asthmatic children. This study characterised the pattern of and explored determinants for changes in spirometric indices among Chinese asthmatic children.

Methods: 186 Chinese asthmatic children aged 6-12 years were recruited from paediatric allergy clinic of our university-affiliated teaching hospital. These patients were prospectively followed by the same paediatrician for five years. Pre-bronchodilator spirometry was recorded at baseline and then annually. Genomic DNA from these patients was genotyped for single-nucleotide polymorphisms (SNPs) on major asthma loci by TaqMan genotyping assays. Generalised estimating equation was used to analyse longitudinal changes in these lung function outcomes.

Results: The mean (SD) age of patients at baseline was 9.7 (1.9) years, and 117 (63%) of them were male. Twenty-nine percent had passive smoking and 54% ever received inhaled corticosteroid (ICS) treatment during follow-up. Adjusting for age and presence of upper respiratory infection within 2 weeks before visits, we found significant decline in FVC of 1% per year, and significant increase in FEV1/FVC and FEF25-75 of 1.5% and 3.7% per year respectively. Male patients had 4.8% higher FEV1 and 6.9% higher FEV1/FVC than females in any single year. However, there was no significant gender disparity in longitudinal changes for FEV1 and FEV1/FVC. Patients treated with ICS had 4.0% lower FEV1 and 3.1% lower FEV1/FVC than those without ICS, but the former group had increased FEV1, and less rapid FVC decline over time. Among asthmatics, there was no association between lung function growth and passive smoking. Rs1342326 of IL33 was associated with FEV1, FVC, FEV1/FVC and FEF25-75. Rs2305480 of GSDMB was associated with FEV1/FVC.

Conclusions: Chinese schoolchildren with asthma have significant annual decrease in FVC and increase in FEV1/FVC and FEF25-75. Boys with asthma had higher FEV1 than females. ICS-treated asthmatics have lower baseline lung function but improved lung function growth. IL33 may be a candidate gene for longitudinal changes in several spirometric indices among Chinese children with asthma.

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Case-control Study for Disease Phenotypes Associated with Respiratory Rhinovirus Infection in Hong Kong Children
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Background and Aims: Human rhinovirus (HRV) is the major pathogen for a range of acute respiratory infections and wheezing illnesses in children. Among the genogroups A, B and C, HRV-A and HRV-C were more prevalent and clinically important than HRV-B. Our published results
suggested that HRV-C was associated with childhood asthma exacerbation. However, the clinical manifestations and associations with asthma and different lower respiratory tract infection for different HRV species remain unclear. This study aimed to investigate the epidemiology and disease spectrum of HRV detected in Hong Kong children between 2014 autumn to 2015 spring.

Methods: This retrospective study obtained archived nasopharyngeal aspirate (NPA) samples from patients aged below 18 years who were hospitalised for acute respiratory illnesses in a university-affiliated hospital during the periods September-November 2014 and January-April 2015. Their clinical information was retrieved from computerised record. HRV was detected by RT-PCR, and isolates were sequenced to determine the genogroups and serotypes.

Results: 90 patients whose NPA was positive for HRV and 160 patients being negative for an extended panel of respiratory viruses by multiplex PCR method were identified. Mean age of these groups was 3.6 years and 3.5 years respectively. HRV infection was significantly associated with asthma exacerbation (OR 16.54, 95% CI 7.11-38.48), wheezing illnesses (OR 8.90, 95% CI 4.74-16.71) and lower respiratory tract infection (OR 3.53, 95% CI 2.15-5.78). Among patients with HRV and asthma exacerbations, HRV-C was more commonly found than HRV-A (75% vs 25%; OR 2.50, 95% CI 0.66-9.47). Similar proportions of HRV-A and HRV-C were found in patients with upper respiratory tract infection. We observed a trend towards changes in HRV from HRV-C in 2014 autumn to HRV-A in 2015 spring (HRV-A: 20.9% vs 34.0%). The predominant HRV serotypes in these two periods changed from HRV-C-8713-MY-10 to HRV-A30/HRV-C15.

Conclusions: HRV is a strong risk factor for asthma exacerbations and wheezing illnesses in Hong Kong children. Although these disease associations were mainly accounted for by HRV-C, the predominant HRV species isolated from our hospitalised children changed from HRV-C in 2014 autumn to HRV-A in 2015 spring. Prospective studies with larger sample size are needed to confirm our observations.

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Balloon Dilatation in Management of Central Airway Stenosis in Children

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Background and Aims: To access the clinical efficacy and safety of balloon dilatation of central airway stenosis in children.

Methods: Retrospective analysis 49 cases of central airway stenosis in children, who were classified into 2 groups, balloon dilatation and non-balloon dilatation, to assess the aetiology of central airway stenosis and evaluate the optimal indications, efficacy and safety of bronchoscopic interventional therapy.

Results: Among the 49 cases of central airway stenosis diagnosed by bronchoscopy, 12 cases (24.5%) were associated with severe pneumonia, followed by primary pulmonary tuberculosis with or without tracheobronchial tuberculosis (22.4%, 11/49), stenosis after tracheal intubation (22.4%, 11/49), congenital tracheal stenosis (22.4%, 11/49), stenosis by compression (4.1%, 2), traumatic stenosis (4.1%, 2). According to the pathology of the stenosis, these cases were divided into two types: 30 (61%) cases were muscular stenosis and 19 (39%) cases were non-muscular stenosis. The response rate was 82% (14/17). The location of stenosis in 49 patients included: 14 cases (28.6%) in upper trachea, 9 cases (18.4%) in lower tracheal stenosis, 15 cases (30.6%) in left main bronchus, 7 cases (14.3%) in right main bronchus and 2 cases (4.0%) in right middle bronchus. Group of balloon dilatation had apparent hypoxia (p<0.05). The symptoms of cough, wheezing and shortness of breath were relieved after using balloon dilatation. Expansion of the airway before and after treatment the average diameter stenosis was (2.5±1.3), (4.7±0.8) mm (p<0.01). Due to Intraoperative transient hypoxemia. Most patients got improved after more oxygen inhalation. Two cases (4.1%) had postoperative laryngeal wheezing and shortness of breath.

Conclusions: Children of central airway stenosis with severe hypoxia was effective using Bronchoscopic balloon dilatation, compared with dyspnoea, hypoxia and other serious clinical signs caused by central airway stenosis, and bronchoscopic balloon dilatation was safe and feasible.
Clinical Applications of Impulse Oscillometry in Asthma Management After Exacerbation in Preschool Children

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Background and Aims: Determination of the values of specific physiologic tests has not been well studied in long-term asthma management in preschool children. We sought to determine the utility of impulse oscillometry in long-term management in preschool children after asthma exacerbation.

Methods: 40 outpatients, aged 3 to 5 years old, with mild-to-moderate asthma exacerbation from Shengjing Hospital of China Medical University were enrolled. The impulse oscillometry was performed immediately after enrollment (T₀). And then during 24 weeks of therapy with inhaled corticosteroid, which were adjusted according to GINA report, impulse oscillometry was performed at 4 (T₁), 12 (T₂) and 24 (T₃) weeks separately for every children. The differences of resistance at 5Hz (R₅), resistance at 20Hz (R₂₀), R₅-R₂₀, resonant frequency (Fres) and low frequency integrated reactance from 5Hz to Fres (AX), among four visits (R₂₀), R₅-R₂₀, resonant frequency (Fres) and low frequency integrated reactance from 5Hz to Fres (AX), among four visits were measured by repeated-measures analysis.

Results: For the 40 children, 26 were boys, the average age was 3.68±0.58 years old, with the weight was 17.74±3.17 kg and the height was 103.95±6.49 cm. R₅ was 1.27±0.33, 1.12±0.26, 1.01±0.26 and 0.89±0.24 kPa/L/s at T₀, T₁, T₂ and T₃ separately and the differences were significant when compared in pairs. R₂₀ was 0.77±0.19, 0.67±0.16, 0.66±0.16 and 0.59±0.15 kPa/L/s separately, and the differences were significant except between T₀ and T₁. The Fres was 25.38±6.91, 22.70±3.19, 21.41±2.40 and 20.13±2.69 Hz separately, and the differences were significant. The AX was 4.29±1.91, 3.23±1.33, 2.48±1.28 and 1.81±0.90 kPa/L/s separately, and the differences were significant.

Conclusions: In preschool children, with the management of asthma after exacerbation, lung function assessed by impulse oscillometry improved in different degrees. R₅, Fres and AX may reflect the ongoing improvements. Assessment of respiratory mechanics over time with oscillometry might offer useful insights into the response of asthmatic preschool children to therapy. Further studies should focus on longer term of management and the relationship between impulse oscillometry and airway inflammations.

Attenuation of Acute Lung Injury by Upregulating SP-B Expression via Pulmonary Epithelial Cell Specific Knockdown of NAMPT

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Rationale: Our preliminary study found that pulmonary epithelial cell specific knockdown of Nampt gene could attenuate LPS induced acute lung injury (ALI) in mice. Since pulmonary epithelial cell expressed SP-B plays a significant role in normal lung physiology and its downregulation was implicated in the pathogenesis of ALI, we intended to investigate whether therapeutic effect of epithelial cell specific knockdown of Nampt on ALI is in part due to its upregulation of SP-B expression to shed some light on the underlying molecular mechanisms in order to further develop a new NAMPT based therapeutic modality to ALI.

Methods: Four groups of C57BL/6J male mice, 8-12 weeks old [(1) wild type, (2) lung epithelial cell specific Nampt knockdown (NamptPE+/-) mice prepared in our lab by crossing Nampt gene exon2 floxed mice with mice expressing Cre in lung Clara cells, (3) wild type mice + intratracheally delivered Ad- SPC-Nampt-antisense scFv for 72 h, (4) wild type mice + intratracheally delivered Ad-SPC-Nampt-scFv, an adenovirus based and SPC promoter driven anti-Nampt single chain variable fragment antibody, for 72 h] were intratracheally administered with LPS (2 mg/kg) or PBS for 24 h before their bronchoalveolar lavage (BAL) and lung tissues were harvested for various assays.

In vitro, A549 cells and H441 cells had been transfected with pCAGGS vector only, pCAGGS-NAMPT cDNA, pCAGGS NAMPT H247E, scRNA or NAMPTsiRNA or treated with pharmacological inhibitors for 48 h plus additional 6 h with or without LPS or TNFa treatment before their SP-B mRNA or protein levels were quantified.

Results: NamptPE+/- mice or mice receiving the Ad-SPC-Nampt-scFv treatment exhibited attenuated ALI (lung injury score: 4.10±1.11 vs 7.20±0.89, n=6 per group, p<0.01 or 5.71±1.23 vs 8.50±1.12, n=5 per group, p<0.01, respectively) and significantly increased their BAL SP-B expressions over their controls. Down regulation ofNAMPT expression by either NAMPT siRNA or FK866, a NAMPT enzymatic inhibitor, increased the expression of SP-B at a basal level as well as rescued the TNF-α or LPS mediated inhibition of SP-B expression while overexpression of NAMPT inhibited SP-B expression in human A549 cells or H441 cells. NAMPTH247E like NAMPT wild type similarly...
inhibited SP-B expression. The JNK inhibitor abolishes NAMPT's effect on SPB expression.

Conclusion: Lung epithelial cell-specific knockdown of Nampt gene expression significantly attenuated LPS induced ALI in part via its upregulation of SP-B expression through its nonenzymatic functions and JNK pathway.

Association of T Lymphocyte Immune Imbalance and IL-10 Gene Polymorphism with the Risk of Obstructive Sleep Apnoea in Children with Obesity

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Background and Aims: To determine the role of T lymphocyte immune imbalance and interleukin (IL)-10 gene polymorphism in the development of obstructive sleep apnoea (OSA) in obese children.

Methods: One hundred obese children at high-risk and low-risk for OSA based upon a sleep questionnaire were selected. Peripheral blood T lymphocyte subsets were measured by flow cytometry, and plasma IFN-γ, IL-4 and IL-10 cytokines were detected by ELISA. The relationships between OSA and the above variables were analysed. IL-10 gene polymorphisms were analysed by DNA sequencing.

Results: Ninety subjects completed all the tests. Forty-two patients were diagnosed as OSA by PSG. Compared with non-OSA children, the levels of CD4+ T cells, IFN-γ, IL-4 and IL-10 cytokines were increased (p<0.05) whereas the numbers of CD4+ CD25+ Treg and NKT cells, and the levels of IL-10 were reduced (p<0.05). Multiple linear regression analysis showed that IL-10 level was negatively associated with OAHI (OR: 0.352, 95% CI: 0.286-0.540; p<0.05). In multivariate analysis, IL-10 also had a strong negative association with OSA after adjustment for confounding factors from Models 1 to 3. Correlative analysis showed that IL-10 levels had a positive association with CD4+CD25+Treg (r=0.628, p<0.01). Furthermore, the IL-10/A-1082G gene polymorphism correlated with OSA.

Conclusions: T lymphocyte immune imbalance was associated with OSA and IL-10 may play an important protective role in the pathogenesis of OSA in obese children.

Protective Effect of Nasal Mucosa on PM_{2.5}-Induced Lung Injury in Mice

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Objective: The purpose of this study was to explore whether the nasal mucosa can protect the PM_{2.5}-induced lung injury in mice.

Methods: The study was carried out in BALB/C mice. 40 mice were randomly divided into four groups, including normal control group (NC group), nebulised by normal saline group (NS group), nebulised by PM_{2.5} suspension group (PS group) and nebulised by PM_{2.5} suspension with blocked nose group (PB group). Mice in NS, PS and PB groups were treated by nebulisation, lasting for 40 minutes once a day, for total 14 times with an interval of 24 hours. All animals were sacrificed after the last nebulisation within 24 hours, then bronchoalveolar lavage fluid (BALF) was collected at the same time. The levels of acidic phosphatase (ACP), alkaline phosphatase (AKP), lactate dehydrogenase (LDH) and the levels of IL-6, TNF-α, IL-1β in BALF were examined. Pathological of lung and nasal mucosa were also observed meanwhile. The data were analysed by SPSS 20.0.

Results: (1) After nebulisation, the increase of mice weight in PS group was lower than that of NC group (p=0.041), while that of PB group was the least among all the groups (p<0.05). (2) Lung and nasal mucosa histopathology: lung histopathology demonstrated that inflammatory changes in different degrees were observed in some animals, along with lymphocytic infiltration, lung inflation, wall thickening, pulmonary interstitial hyperplasia and increased oedema and lung consolidation. Qualitative scores were used to compare pathological manifestations conveniently. The scores of PB, PS, NS, NC were 11.90±1.79, 9.90±2.07, 7.20±1.55, 5.70±1.16 respectively. The scores of pathological injury of PB group were higher than those of the other three groups (p<0.05). The nasal mucosa of mice had no obvious manifestation of inflammation. (3) Comparison of the levels of enzymes and cytokines in BALF: The levels of ACP and LDH in PB group and PS group were higher than those in NC and PS groups respectively (p<0.05). And furthermore, the level of LDH in PB group was higher than that in PS group (p<0.05). (4) Comparison of the levels of inflammatory cytokines in BALF: The levels of IL-6 and IL-1β in PB group and PS group were higher than those of NC group and NS group (p<0.05). In addition, the level of IL-6 in PB group was higher than PS group.
Conclusion: (1) PM$_{2.5}$ inhalation can be involved in the lung inflammatory response. (2) Nasal breathing may alleviate PM$_{2.5}$-induced lung injuries than mouth breathing.

Silencing of Pin1 Suppresses Oxidative Stress-Induced Apoptosis of A549 Cells

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Background: The peptidyl prolyl cis/trans isomerase (Pin1) has attracted considerable interest as an inhibitor of tumour cell targets. Pin1 is important in the oxidative stress pathway. We hypothesise that inhibition of Pin1 expression might dampen hyperoxic lung injury. Moreover, the mechanism responsible for this process is poorly understood.

Objective: We aimed to explore the role of Pin1 in the oxidative stress-signaling pathway and apoptosis in hyperoxia-exposed A549 cells.

Methods: The gene sequences were cloned into the pLenR-GPH-shRNA lentiviral vector, which was selected by Genebank searches. The pLenR-GPH-shRNA and lentiviral vector packaging plasmid mix were cotransfected into 293T cells to package lentiviral particles. Culture virus supernatant was harvested, and then the virus titer was determined by serial dilution assay. A549 cells were transduced with the constructed lentiviral vectors, and real-time polymerase chain reaction (qPCR) and Western blot were used to evaluate Pin1 expression. The study is divided into a control group, a hyperoxia group, an A549-Pin1shRNA hyperoxia group, and a negative lentivirus group. Cell apoptosis was detected by flow cytometry (FC) after 24 hrs, the expression of XIAP (X-linked inhibitor of apoptosis protein) and Caspase-9 were detected by immunohistochemistry. The production of ROS (reactive oxygen species), and cellular mitochondria membrane potential ($\Delta\Psi_m$) were determined by fluorescence microscopy.

Results: We established an A549-Pin1shRNA and inhibited its expression. Both qPCR and Western blot demonstrated downregulation of Pin1 expression in A549 cells. In the A549-Pin1shRNA hyperoxia group, we found dampened oxidative stress.

Conclusion: Pin1 mediates the oxidative stress-induced signal pathway in hyperoxia-exposed A549 cells.

Genetic Variation of Fusion Protein of Respiratory Syncytial Virus from Children with Community-Acquired Pneumonia in China

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Background: Respiratory Syncytial Virus (RSV) is mostly reported as an important pathogen of respiratory tract infection, especially acute lower respiratory tract infection (ALRTI), in infants. The RSV fusion protein is an important transmembrane glycoprotein associated with virus infection and immunity, which can be useful as a vaccine candidate. To clarify the genetic characteristics and antigenic sites variation in F protein of the prevalent genotypes in our country, comprehensive analysis was performed with 550 RSV positive samples from hospitalised children with community-acquired pneumonia (CAP) from 9 provinces between November 2014 and December 2016.

Methods: The complete F protein gene and partial G gene of RSV was amplified by RT-PCR and sequenced. The phylogenetic analysis were performed by MEGA5.03 program. The identity matrices and genetic sites variation were determined with Bioedit software.

Results: Complete F protein genes and partial G protein genes were gained from 258 samples, which contained 189 RSV A and 69 RSV B. Phylogenetic analyses based on partial G gene revealed that ON1 and BA were the main subgenotypes in China, which accounted for 93.2% and 97.8% of RSV A and B, respectively. The F protein gene sequence is highly conserved, while all the complete F gene sequences could be divided into two groups which corresponded to the grouping based on the G genes. Pairwise nucleotide (amino acid) sequences identities were 98.7%~100% (98.4%~100%) among 189 subtype A, 98.8%~100% (98.4%~100%) among 69 subtype B, and 81.3%~82.4% (88.6%~90.7%) between groups A and B, respectively. There were 11 and 9 significant amino acid changes at group A and B corresponded to their prototype, respectively; however, 46 amino acid differences were found between the two groups. Compare to the prototype Long strain, variations at antigenic site $\varnothing$ were observed in amino residues 67(N→T), 200(D→N), 201 (K→N) and 209 (K→Q). There were 3 variations at antigenic site I in amino residues 380 (N→S), 384 (V→I/T), 389 (P→S). Nine amino residues mutation were found on the antigenic p27. No more mutation was found on the antigenic sites IV except the 276 (N→S) on palivisumab binding site of antigenic sites II. Mutations were also observed in the human histocompatibility
leukocyte antigen (HLA)-restricted CTL epitopes.

**Conclusions:** The nucleotide and amino acid of RSV F protein, especially, in the antigenic sites area, were highly conserved except limited genetic variations. These results revealed that F protein remains the potential candidate for the development of vaccine and drug.

**Loss of Thy-1 from Myofibroblasts in Progressive Pulmonary Fibrosis and Reversibility of Myofibroblast Phenotype with Soluble Thy-1**

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**Background:** Previous studies have demonstrated Thy-1 expression increases in aging lungs, and is silenced in fibroblastic foci associated with idiopathic pulmonary fibrosis (IPF). Repeat-dose intratracheal bleomycin (Bleo.) administration in mice results in progressive fibrosis, unlike the self-resolving fibrosis seen in the more widely used single-dose Bleo. model. In this study, we utilised a repetitive lung injury mouse model to determine whether progressive, non-resolving fibrosis is associated with Thy-1 loss in lung fibroblasts, and to what extent soluble Thy-1 (sThy-1) reverses myofibroblast differentiation in vitro.

**Methods:** Progressive lung fibrosis was induced in Col-GFP mice (6-8 weeks old) by 1 unit/kg Bleo. or saline (control) instilled intratracheally (IT) every 12 days for four doses. Single dose injection mouse model were instilled IT with 4 unit/kg Bleo. Thy-1 expression and fibrotic tissue remodelling were evaluated at 4 weeks and 8 weeks after final instillation by qPCR and immunofluorescence staining. Normal human fibroblasts were treated with TGFβ1 to monitor myofibroblast differentiation, the ability of a sThy-1 fusion protein (sThy-1-Fc) or mutated constructs of soluble human Thy-1-Fc (sThy-1 RLE) inhibit myofibroblast differentiation were determined by measuring expression of fibrotic genes both in transcriptional and protein levels.

**Results:** Using a repetitive lung injury mouse model and a traditional single dose model of intratracheal injection of Bleo., we show that Col-1A1, Col-III gene expression and collagen I deposition are reduced at 8 weeks after single dose Bleo. injection. No significant change in Thy1 gene expression was observed at 4 or 8 weeks post single dose administration. However, repetitive Bleo. instillation induced persistent fibrosis as assessed by Col-1A1, Col-III gene expression and immunofluorescence staining to detect collagen I and αSMA levels. Furthermore, Thy-1 gene expression significantly decreased at 8 weeks. Histological examination with IF confirmed that Thy-1 noticeably was down regulated in GFP-expressing myofibroblastic regions at 4 weeks, and that no significant recovery occurred at 8 weeks. By administrating sThy-1, ACTA2 and Col-1A1 gene and protein expression were significantly decreased after 1000 ng/mL sThy-1-Fc treatment when compared with sThy-1 RLE and controls.

**Conclusion:** Single-dose Bleo.-induced lung injury promotes reversible lung fibrosis associated with decreased myofibroblasts and recovery of Thy-1 expression, while repetitive lung injury induces progressive, non-resolving lung fibrosis associated with sustained silencing of Thy-1 expression in myofibroblasts. sThy-1 partially reverses differentiation of senescent human lung myofibroblasts, and the effect of sThy-1 requires the RLD integrin-binding motif (Supported by NIH1 R01 HL111169-01A1).

**Effect and Mechanism of PM2.5 on Airway Inflammation in Asthmatic Mice**

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**Background and Aims:** To explore the effect of fine particulate matter (PM2.5) on airway inflammation in normal and asthmatic mice as well as the regulation of PM2.5 on the expression of 11βHSD1 and 11βHSD2 in lung, and to investigate the preliminary mechanisms of PM2.5 for promoting inflammation.

**Methods:** 42 BALB/c mice were randomly divided into six groups (n=7): normal control group (NC), PM2.5 low-dose group (NP1) (3.5 mg/kg), PM2.5 high-dose group (NP2) (10 mg/kg), asthmatic group (AC), asthma + PM2.5 low-dose group (AP1), asthma + PM2.5 high-dose group (AP2). Mice model of allergic asthma and PM2.5 exposure was established respectively. All animals were sacrificed for leukocyte count, HE staining and BALF ELISA kit detection. Real-time quantitative PCR and Western Blot method were applied to observe effect of PM2.5 on 11βHSD1 and 11βHSD2 mRNA and protein expression.

**Results:** (1) BALF lymphocyte count and proportion, eosinophil count and proportion of NP2, AC, AP1, AP2 groups are higher than the control group and the difference was significant. The number of eosinophils in AP2 group was higher than that in AC group and AP1 group (p<0.01). (2) The levels of IL-4 and IL-5 in BALF of AP1 group and
AP2 group were higher than those in normal group (p<0.05). The concentration of IFN-γ in BALF of AP1 and AP2 groups was lower than that of normal group (p<0.01). The concentration of IL-4 and IL-5 in AP2 group were significantly higher than those in AP group. The concentration of IFN-γ in AP2 group was lower than that of AC group and AP1 group (p<0.05). (3) The expression of 11βHSD1 mRNA in NP2 group was higher than that in NP1 group. The expression level of 11βHSD1 mRNA in AP2 group was lower than that in normal group (p<0.01). The expression of 11βHSD2 mRNA in NP2 group and AP2 group was higher than that in normal group (p<0.05). (4) Western blot showed that the expression of 11βHSD1 and 11βHSD2 in NP1 group, NP2 group and AC group was higher than that in normal control group. Compared with the AP1 group and normal group, the expression of 11βHSD2 in AP2 group was increased (p<0.05).

**Conclusions:** PM2.5 promote the immune response of Th2 cells and leading to the airway Th1/Th2 immune imbalance. PM2.5 can upregulate the 11βHSD1 level in lung tissue of normal mice. High dose PM2.5 down regulate the expression of 11βHSD1 in asthmatic mice. PM2.5 can upregulate the level of 11βHSD2 in lung tissue of normal and asthmatic mice.

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**Epigallocatechin Gallate Ameliorates Airway Inflammation by Inducing Regulatory T Cells in an Asthmatic Mouse Model**

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**Objective:** Epigallocatechin gallate (EGCG) is a polyphenol that is found in green tea that has been shown to ameliorate airway inflammation in an ovalbumin-sensitised asthmatic mouse model. The purpose of this study was to investigate whether the immunomodulatory and anti-inflammatory effects of EGCG by enhancing the regulatory T cell (Treg) in this model.

**Methods:** Female BALB/c mice were sensitised and challenged with ovalbumin by intraperitoneal injection. EGCG was administrered to asthmatic mice intraperitoneally one hour before each OVA challenge. Airway hyperresponsiveness (AHR) was measured, and lung inflammatory infiltrates were assessed by haematoxylin and eosin (HE) staining. Serum OVA-specific IgE level and Interleukin-10 (IL-10) levels in the bronchoalveolar lavage fluid (BALF), serum, and splenocyte culture supernatants were measured by ELISA. Flow cytometry was used to assess the effects of EGCG on the number of CD4⁺CD25⁺Foxp3⁺Treg cells in the splenocytes and real-time PCR was used to measure the expression of Forkhead box P3 (Foxp3) mRNA in the lung tissue.

**Results:** The results showed that administration of EGCG significantly decreased AHR and OVA specific IgE in the serum, increased IL-10 levels in the BALF, serum, and splenocyte culture supernatant, and the number of CD4⁺CD25⁺Foxp3⁺Treg cells in the splenocytes and asthmatic mice. Administration of EGCG also ameliorated airway inflammation and eosinophil infiltration in asthmatic mice. These results suggested that EGCG likely ameliorated OVA-induced airway inflammation by increasing the production of IL-10, the number of CD4⁺CD25⁺Foxp3⁺Treg cells and expression of Foxp3 mRNA in the lung tissue.

**Conclusions:** EGCG could be an effective agent for treating asthma by inducing regulatory T cells to ameliorate airway inflammation.
Going to Study at School or University in the UK?
– New UK Meningococcal Vaccination Policies: What This Means for You

BC MILLAR, JE MOORE

The incidence of meningococcal disease (MD) in Hong Kong is relatively low. Over the past five years, the number of cases has decreased significantly since the mid 1980s, where currently, there is approximately five cases per annum, equating to an incidence of 0.04 cases/100,000 population. Comparable data from mainland China has shown that the incidence of disease in Hong Kong is similar to neighboring provinces, with a rate of 0.003-0.05 cases/100,000 population. As a result, there is currently no provision for routine meningococcal vaccination within the Child Immunisation Programme, however there is provision for vaccination in certain groups, including travellers to Saudi Arabia and sub-Saharan Africa and vaccination during outbreak situations. Currently, the incidence of MD in the UK is approximately 25-fold higher (1 case/100,000 population).

Worldwide, there are six main virulent serogroups which cause the majority of clinical cases, including MenA, B, C, X, W135 and Y. Most of the meningococcal cases in recent years in Hong Kong have been due to serogroup B. Presently, there are four licensed meningococcal vaccines in Hong Kong, three of which cover serogroups A, C, W135 & Y (MENACTRA [SANOFI-AVENTIS HONG KONG Ltd; HK-60659], MENCEVAX ACWY [PFIZER CORPORATION HONG KONG Ltd; HK-48475] & NIMENRIX [PFIZER CORPORATION HONG KONG Ltd; HK-62095]) and one which covers serogroups A & C (MENINGOCOCCAL A+C POLYSACCHARIDE [SANOFI-AVENTIS HONG KONG Ltd; HK-36398]). At present, there is no licensed vaccine against serogroup B in Hong Kong, although such vaccines do exist and are licensed elsewhere.

The UK remains a popular choice with Hong Kong parents, as a country to send their children for higher education. In academic year 2014/2015, there were 29,705 students from Hong Kong, who were studying wholly in the UK, comprising of 25,035 undergraduate and 4,760 postgraduates. In the same year, 16,215 Hong Kong students entered a UK Higher Educational institution for the first time. Over the period 2012/2013 to 2014/2015, there has been a 24.1% increase in first-time Hong Kong students in the UK. Overall, to put this into context, there are more Hong Kong students taking up university places each year in the UK, than any other individual EU country, such as France or Germany. Reasons for this popularity has been suggested to include confidence in the academic freedom and rigorous standards of the British education system. Additionally, there has been an increase in children aged 14-15 years, travelling to the UK for completion of their secondary education, many of whom are boarders at private secondary schools.

Hong Kong students arriving in the UK for the first time do so, without having a protective meningococcal childhood immunisation programme in place locally, to a country with a higher incidence of disease and a highly elaborate active national immunisation programme. So what does this mean for the health and wellbeing of the Hong Kong student arriving in the UK?

As of 2014/2015, Public Health England reported that serogroup B accounted for the majority of capsular-associated cases (n=418; 57.7%), followed by serogroups
W135 (24.3%), Y (12.9%), C (3.9%) and others (1.2%). Of concern has been the recent increase of serogroup W135 in the UK, due to the expansion of a single endemic hypervirulent strain belonging to the sequence type 11 clonal complex. In response to this, the UK Department of Health has introduced a new national meningococcal vaccination policy, targeting vaccination of adolescents with the MenACWY glycoconjugate vaccine.

Following the initial introduction of the MenACWY vaccine in August 2015, which targeted university entrants in the UK, we undertook pilot surveillance at two UK universities, through the completion of a voluntary questionnaire following short presentations on meningitis awareness and vaccination. Responses were analysed to ascertain levels of disease and vaccine awareness in recently arrived new university entrants to the UK from Hong Kong. Students were aged 19-23 years (n=22), with a mean age of 20.3 years, of which 96% of students lived in communal university-controlled accommodation. Survey response rate amongst Hong Kong students was extremely high (90.9%). Regarding meningitis disease awareness, 36% of respondents did not know what meningitis was, nor did they know the signs and symptoms. 18% of students believed that if vaccinated, they could not contract meningitis. Regarding vaccination awareness, 96% of students were not aware of the UK MenACWY vaccination programme for new university entrants and as such had not receive the vaccine prior to entering university. Initially, 68% of students wished to receive the vaccine, however, after attending the presentations, 91% of students intended to obtain the vaccine. A final vaccination uptake rate of 96% was recorded in this student cohort. When this surveillance was undertaken, none of the Hong Kong students had registered with a UK General Practitioner (GP). However, following the presentations, all students subsequently registered with a local GP. In addition, all students indicated that presentations on disease and vaccine awareness from healthcare professionals were useful in informing newly arrived students, followed by information posters.

Given the contrasting incidence of meningococcal disease and vaccination policies in Hong Kong and the UK, it is of utmost importance that Hong Kong students arriving in the UK to commence further and higher education at UK academic institutions are made fully aware about this disease, as well as available vaccine provision. In the UK, there are considerable resources available to arriving students, to help them learn more about the signs and symptoms of meningococcal infection. In particular, Meningitis Now and the Meningitis Research Foundation (MRF) are UK-based patient charities, with extensive and elaborate resources to help with this (www.meningitisnow.org/how-we-help/campaigns/uni/ & www.meningitis.org).

Current UK vaccination policy regarding meningococcal immunisation is set out in the UK Department of Health’s "Green Book", which is available freely online at www.gov.uk/government/publications/mentingococcal-the-green-book-chapter-22.

Current provision of meningococcal vaccination in the UK for newly arriving students from Hong Kong, allows that "Children and young adults aged 10 years to less than 25 years (including students up to 25 years attending university for the first time) may also be eligible, or will shortly become eligible, for the teenage MenACWY conjugate vaccine. Those in this group who have never received a MenC-containing vaccine should be offered a single dose of the MenACWY conjugate vaccine. No further vaccination is then required".

In contrast to the USA where MenACWY vaccination is mandatory, prior to admission of new undergraduate students to many universities, meningococcal vaccination in the UK for new students is voluntary. It is, however, highly recommended that foreign students register with a local GP and avail of the freely available healthcare provision, including the various vaccination programmes.

Additionally, the 4CMenB (Bexsero®, GSK Ltd) vaccine is licensed in the UK. Currently, this is available to babies <1 year old, as part of the UK National Routine Childhood Immunisation Programme. This vaccine is available to young adults privately at a cost at certain pharmacies and private clinics.

Meningococcal infection still remains a relatively rare disease in the UK. It is hoped that this commentary will help to guide Hong Kong General Practitioners, students and their families in preparations for study in the UK.

Declaration of Interests

None

References


Lingual Thyroglossal Duct Cyst: A Case Report and Review of the Literature

Dear Editor,

We would like to present a rare case of lingual thyroglossal duct cyst (TGDC) in a 3-year-old male. His chief complaint was a recurrent mass in sublingual area. Other symptoms were drooling, swallowing difficulty and pain. His problems appeared 15 months ago. The case had been misdiagnosed as a sublingual abscess, so incision and drainage has been carried out. However, 3 months later, the swelling recurred and he was referred to us. A soft, fluctuant and tender mass was completely involved the sublingual area. There was a fistula in the midline of submental region. Thyroid function tests were normal. CT revealed a hypodense lesion with a rim enhancement (Figure 1). Also, the presence of thyroid gland in the normal position was confirmed by CT. Intra and extra-oral approaches were carried out under general anesthesia. A 5 cm x 4 cm sized cyst was completely excised intra-externally (Figure 2). Externally the fistula tract and the central portion of hyoid were resected through Sistrunk procedure. The tongue musculature had been displaced by the mass, without any involvement. So, there was no tongue deformity or speech

morbidity postoperatively. TGDC was confirmed histopathologically. After 24 months the patient showed no recurrence.

TGDC is the most common congenital cyst in the neck which is usually located below hyoid (85%). Only 1-2% of cysts occurs around the tongue.1 The main differential diagnoses of lingual TGDCs are ranula, mucocele, abscess, dermoid and epidermoid cysts.2 Ultrasonography and CT scan are useful in diagnosis. Definitive diagnosis is histopathological.

In this case the mass was settled under the tongue which is very rare in TGDCs. Although Sistrunk procedure is the gold standard for management of all cases of TGDCs,3 practically it is difficult in the cysts involving the sublingual space.4 Also, a wide anterior neck dissection has been suggested for recurrent thyroglossal duct cysts.5

Lingual TGDC is a rare presentation of a common congenital malformation and should be considered if a young patient suffers from a sublingual mass.

Declaration of Interest

None declared.
References


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Our proband is a 5-month-old female who was referred from Maternal & Child Health Centre for suspected hypotonia with decreased limb movement since birth. She was born to a non-consanguineous couple via vaginal delivery at 40 weeks of gestation after an uneventful pregnancy, weighing 3.27 kg at birth. The family history was unremarkable and there was no history of neurometabolic diseases. Mother also had a history of first trimester spontaneous miscarriage, the cause of which was unknown.

Parents reported paucity of limb movement since birth. There was not much anti-gravity movement observed and the child did not withdraw with painful stimulation. No seize-like activity or cyanosis was noted. Growth was satisfactory despite some choking, with body weight at 25-50th centile, height at 90th centile and head circumference at 3-10th centile at 5 months of age. Clinical photographs can be seen in Figure 1.

Physical exam at 5 months of age showed a responsive non-dysmorphic baby with generalized hypotonia with frog like posture, positive scar sign and heel ear test and complete head lag. She also had tongue fasciculation and generalized areflexia. Minimal horizontal movement over lower limbs and some passive grasping over fingers were noted. She was not distressed in room air but there was some paradoxical breathing. Examination of other systems was unremarkable.

Basic blood tests were unremarkable and chest X-ray showed signs of atelectasis/aspiration likely related to weak respiratory muscle. She was referred to clinical genetics for further evaluation.

![Clinical photographs of our patient at 6 months old](image)

The clinical quiz was prepared by:

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Answer to "Clinical Quiz" on Pages 151-152

N.B. The Editors invite contributions of illustrative clinical cases or materials to this section of the journal.
Abstracts of Articles in Chinese

住院：檢測兒童發育遲緩的好時機

本研究在兒童住院期間應用發育監測工具，目的為測定其發育遲緩的風險。對年齡為2-42月的113名住院兒童的發育進行評估，包括兒童發育監測指南（GMCD）中的表現性語言、接受性語言、精細運動、大運動、社會情感和聯絡功能、遊戲、自我幫助好技能方面。發育遲緩兒童有49名（42.4%），表現對發育遲緩有所關注的母親當中，孩子發育遲緩達72.9%（p<0.001）。在沒有定期產前回診（p<0.001）、低養育水平（p<0.001）、以往有流產史（p<0.013）的母親中，孩子出生體重低於2500克（p<0.002）、和父母近親血緣（p<0.007），孩子的發育遜緩明顯多見。住院期間是一個良好機會來找出發育延緩高風險兒童，並轉介進一步評估和早期干預。

關鍵詞：兒童，發育，住院

PICU病童父母的焦慮：土耳其一兒童深切治療部的經驗
B Akyildiz, G Zararsiz. Parental Anxiety During PICU Admission: A Single Centre Experience from Turkey. HK J Paediatr (new series) 2018;23:8-12

目的：本研究旨在評估入住兒童深切治療部（PICU）病童父母的焦慮程度，並發現住院的影響因素。方法：共有170名重病兒童及其父母納入本研究。從病歷記錄中採錄病人的人口資料、兒童死亡風險評分（PRISM III）、兒童內臟器官功能全達評估（PELOD）、入院原因、併發症、機械通氣操作、住院長短和病童存活率。採用貝克焦慮量表（BAI）評估父母的焦慮情況。另外，記錄父母的性別、年齡、入院時間、入院地點、教育水平、和家庭收入。結果：總共170名兒童及其父母納入本研究。92例病人有慢性疾病史。PICU住院兒童的家庭成員經歷中等程度的焦慮。BAI統計母親的焦慮程度比父親高（p=0.009）。根據兒童的病史，在急性病期父母雙方的BAI中位數並沒有統計學意義（p=0.52）。在另一方面，慢性病兒童的母親BAI高於父親（p=0.03）。作者發現2個變數對PICU相關的父母焦慮有顯著增加，包括入院時間（OR 1.05, 95% CI 1.00-1.10）和機械通氣操作（OR 5.93, 95% CI 2.66-10.53）。結論：急性病重兒童的父母均常出現焦慮，慢性病重兒童的母親需要更多的情緒支援，在PICU入院時需建立良好的病人—醫護關係。在此期間，確定父母的需求和壓力來源，有利於改善父母的長短期心理狀態。

關鍵詞：焦慮，兒科，兒童重症監護室，父母
羅馬尼亞兒童出生後第一年的餵養情況


背景：幼兒早期營養習慣的影響對日後發育和營養狀態極為重要。嬰兒營養的許多方面都基於社區的傳統和流行觀念。本研究目的為：評估輔食餵養影響因素與羅馬尼亞社會經濟環境的相關性。方法：在羅馬尼亞首都布加勒斯特喬治亞歷山大急診兒童醫院急診部，對 1 歲幼童體檢時進行評估。對父母當面提問答卷方式採集資料，關於幼童出生後首年營養行為和社會人口統計方面的多方面結果。結果：共 382 對父母完成答卷，回應率為 85.29%。生後第一天的母乳餵養嬰兒達 68.1%，然而純母乳餵養 4-6 個月的只有 41.6%。關於餵哺時斷奶的時點，85.6% 在 4-6 月時開始添加輔食，8.9% 提前斷奶，5.5% 在 7 個月大後斷奶。多變數分析顯示農村地區、低收入家庭和低教育水平母親，成為不恰當餵食添加行為的風險因素(p<0.05, CI 95%)。結論：底層人群易於出現輔食餵養錯誤，他們代表著需要物質支援和接受資訊的目標人群。對父母和保健人員進行正確的綜合宣傳政策，可改善羅馬尼亞母乳餵養率和輔食餵養行為。

關鍵詞：輔食餵養、母乳餵養、飲食習慣、營養

陳健良醫師

新生兒中維生素 D 缺乏與下呼吸道感染


目的：文獻報告維生素 D 在免疫系統功能和調節中的關鍵和複雜作用。關於輕型維生素 D 缺乏的後果瞭解不多。關於非佝僂病兒童中亞臨床維生素 D 矮小症與急性下呼吸道感染的關係已有報道。本研究對患有下呼吸道感染的非佝僂病新生兒中的血漿 25- 羧維生素 D 濃度進行測定。方法：本病例對照研究在一所大學轉介醫院進行。40 名診斷為下呼吸道感染的住院新生兒納入為病例組。40 名無呼吸道症狀的門診健康新生兒作為對照組。用化學發光免疫分析法檢測血漿 25- 羧維生素 D 濃度。結果：在患有肺炎的新生兒中，維生素 D 平均濃度為 9.6±6.8 ng/ml，對照組為 14.7±9.3ng/ml (p=0.02)。病例組中確認維生素 D 缺乏新生兒 37 例 (92.5%)，對照組有 26 例 (65%) (p=0.005)。病例組中 36 名 (90%) 母親有維生素 D 缺乏，對照組為 23 名 (57.5%) (p=0.002)。病例組中 26 例 (65%) 維生素 D 濃度低於 10 ng/ml，11 例 (27.5%) 新生兒為 10-20 ng/ml，3 例 (7.5%) 高於 20 ng/ml。結論：本研究顯示如何有效改善本地人群維生素 D 缺乏是常見疾病，患有急性呼吸道感染的新生兒有低維生素 D 濃度。因此，後續研究需要確定如何有效提高維生素 D 的水平和優化其功能。

關鍵詞：下呼吸道感染、新生兒、維生素 D
從外週血塗片診斷感染


外週血塗片檢查很可能是臨床診斷中沒有充分應用的一項實驗室檢查。在此報告的三個病例說明：檢查血塗片可提供直接確定感染病原體的重要線索。第一例為一男性新生兒，出生時血小板減少，發現有多形性非典型淋巴細胞增多。可疑傷寒期巨細胞病毒感染，血漿檢測到病毒DNA後確定診斷。第二例為一名7個月大女孩，因呼吸窘迫和肺炎徵入院。淋巴細胞增多，伴發細胞核捲曲的非典型細胞，強烈提示百日咳，從鼻咽拭子監測到百日咳桿菌DNA確定此診斷。第三例病人是一名16歲男孩，發熱和腹瀉病史3個月，檢出非典型淋巴細胞增多，伴發大量大型顆粒的淋巴細胞指向感染性單核細胞增多的診斷。血漿中檢出EB病毒DNA，證實這一診斷。經過恰當治療，所有病人的初發症狀和體徵已經消退。外週血塗片檢查能成為有力的輔助方法，指導診斷性檢查，並在診斷性治療中更準確地應用抗生素。

關鍵詞：全血細胞計數、巨細胞病毒、感染性單核細胞、外周血塗片、百日咳

高血壓可成為急性髒性白血病嬰兒中彌散性毛黴菌感染的臨床表現

FL Huang, CF Lin, PY Chen, TK Chang. Hypertension as the Presentation of Disseminated Rhizopus in an Infant with Acute Myeloid Leukaemia. HK J Paediatr (new series) 2018;23:29-33

毛黴菌病對免疫缺陷病人會造成病變，病童可出現血管病變，為一致命性感染。作者報告一例急性髒性白血病嬰兒，患有腹腔包塊，並通過多腔頭CT和組織活檢，診斷出毛黴菌感染相關的左腎動脈、腸系膜上動脈和脾動脈栓塞導致進展性高血壓。血漿雖然經過積極治療處理，包括手術和脣腫體內二性黴素B用藥，但仍因白血病不斷惡化而夭折。以作者所知，尚未有文獻報告嬰兒因毛黴菌感染而同時出現三支動脈阻塞。作者結論：白血病兒童伴中性粒細胞缺乏時，毛黴菌病是一種致命性感染，毛黴菌感染可與多發動脈阻塞有關。所以，病童需要進行早期診斷，並予以恰當的手術及治療。

關鍵詞：高血壓、阻塞、白血病、毛黴菌
兒科急診部的一例罕見自發性腸穿孔

JJ Chen, CH Lien. A Rare Case of Spontaneous Intestinal Perforation at a Paediatric Emergency Department. HK J Paediatr (new series) 2018;23:34-36

摘要：腹痛是兒科急診部的常見病例。腸穿孔為罕見病，通常由其他腸道疾病引發，或是存在其他危險因素的病人中出現。作者報告1例身體完全健康的自發性腸穿孔病人。本例提醒我們，無潛在疾病或其他危險因素的身體健康者，也可出現自發性腸穿孔。

關鍵詞：腹痛、腸穿孔、腹膜炎、氣腹

陳健良醫師

患顯性耳聾—帶指甲發育不良綜合徵的華裔家庭


顯性耳聾—指甲發育不良綜合徵（DDOD syndrome; MIM 124480）是一罕見疾病，表現為先天性感覺神經性聽力喪失和指甲發育不良或缺失。作者在此報告一個家庭中二位成員患有先天性聽力喪失和指甲發育不良或缺失，通過常染色體顯性遺傳。遺傳分析顯示父母二人均帶有ATP6V1B2基因突變（c.1516C>T（p.Arg506X））。本家庭與以往文獻報告中的描述表型相類似。本例報告有助證明：ATP6V1B基因突變可導致DDOD綜合徵，該家庭情況跟DDOD 疾病貎融合，將為該病的診斷提供有用的資料。

關鍵詞：顯性耳聾—指甲發育不良綜合徵、聽力喪失、突變
MCQs

Instruction:
1. Please use pencil to shade the box for the best and correct answer (only one answer for each question).
2. Send back the answer sheet (see loose leaf page) to the Hong Kong College of Paediatricians. One point will be awarded to each article if ≥3 of the 5 answers are correct. The total score of the 4 articles will be 4 CME points.

(A) Hospitalisation: A Good Opportunity to Detect Developmental Difficulty in Children

1. Developmental conditions occur in approximately what percentage of children in the United States?
   a. 1-5%
   b. 5-10%
   c. 10-20%
   d. 30%
   e. 50%

2. At what ages should children be screened for development with a relevant instrument according to The American Academy of Pediatrics even if the parents or caregivers have no concerns?
   a. 9, 18, 24 or 30 months
   b. 6, 12, 24 or 36 months
   c. 12, 24 months
   d. 12, 18, 24 months
   e. 2, 9, 18 or 30 months

3. Which of the following is incorrect about the characteristics of Guide for Monitoring Child Development (GMCD)?
   a. It is used in children aged under 42 months
   b. It is administered with an open ended, 10-minute interview with the primary caregiver.
   c. The parents are first asked whether they have any concerns.
   d. It only has one component, which is developmental monitoring.
   e. It has a large multinational study on international standardisation.

4. Which of the following developmental milestones are assessed with GMCD?
   i. Expressive language,
   ii. Receptive language,
   iii. Fine motor functions,
   iv. Gross motor functions,
   v. Social-emotional and relational functions,
   vi. Play, and
   vii. Self-help skills are assessed with GMCD.
   a. (i), (ii), (iii), and (iv)
   b. (i), (ii), (v), and (vi)
   c. (iii), (iv), (vi), and (vii)
   d. (v), (vi), and (vii)
   e. All

5. Which of the following is incorrect?
   a. There is a higher incidence of developmental disabilities in hospitalised children
   b. Parents' concerns are less effective in directing the primary caregiver to the early detection of behavioural and developmental problems
   c. A child may be confronted by many risk factors within a certain period or during development. This situation is called double jeopardy.
   d. The hospitalisation period provides an opportunity to determine the presence of developmental problems and appropriate referral, especially in LAMI countries
   e. Children from LAMI countries have a higher risk of developmental difficulties and other medical problems.
1. Which is a scoring system used to identify organ failure in the paediatric intensive care unit?
   a. SOFA
   b. PRISM
   c. PELOD
   d. APACHE II
   e. SAPS II

2. Which is a scoring system used to identify risk of mortality in the paediatric intensive care unit?
   a. PRISM
   b. PELOD
   c. APACHE II
   d. SNAP II
   e. FLACC

3. Which of the following shows the anxiety in parent?
   a. 36-item short form survey
   b. The self-esteem rating scale
   c. Maslach burnout inventory
   d. Beck depression inventory
   e. Beck anxiety inventory

4. How many parameters does the Beck anxiety scale consist of?
   a. 19
   b. 20
   c. 21
   d. 22
   e. 23

5. Which of the following scores indicate a severe anxiety level in Beck anxiety inventory?
   a. 36 and above
   b. 0-21
   c. 22-35
   d. 0-19
   e. 34 and above

1. Delaying introduction of solid food is undesirable because it promotes suboptimal acquisitions of:
   a. zinc
   b. protein
   c. iron
   d. vitamins B and D
   e. All of the above

2. Timely introduction of solid foods promotes:
   a. good health
   b. adequate nutritional status
   c. balanced growth for babies and toddlers
   d. resistance to infections
   e. a, b, c

3. The families need support to optimise the infant's nutritional well-being. Which of the following influence that in our study:
   a. Low income families
   b. Mother's education level
   c. Living in rural area
   d. Birth weight
   e. a, b, c

4. According to this study, the introduction of allergenic food is:
   a. Late (after 7 months)
   b. Early
   c. Not investigated
   d. Between 4 and months
   e. None of the above

5. Breastfeeding for more than 4 months is associated with:
   a. Delay timing of complementary feeding
   b. Early weaning practices
   c. Not investigated
   d. Vaginal delivery
   e. None of the above
**Vitamin D Deficiency and Lower Respiratory Tract Infections in Newborn Infants**

1. Vitamin D plays a role in:
   a. Regulation of calcium and phosphorous haemostasis.
   b. Prevention of anaemia.
   c. Sodium metabolism.
   d. Potassium metabolism.
   e. RBC integrity

2. What is the most abundant vitamin D metabolite?
   a. 25 hydroxy D3
   b. 1,25 dihydroxy vitamin D
   c. 24,24 dihydroxy vitamin D
   d. Vitamin D2
   e. 1 hydroxy D3

3. Vitamin D deficiency causes:
   a. Impaired bone mineralisation.
   b. Increased risk of cancers.
   c. Type 1 and 2 diabetes.
   d. Respiratory infections
   e. All of the above.

4. How long of sunlight exposure during summer will it need for the body to produce adequate vitamin D?
   a. 1-5 minutes
   b. 10 minutes
   c. 5-15 minutes
   d. Less than 10 minutes
   e. 20-30 minutes

5. Vitamin D concentration in human milk is:
   a. Higher than its levels in regular formula.
   b. Equal to its levels in regular formula.
   c. Lower than its levels in regular formula.
   d. Not predictable.
   e. Enough to meet infants needs

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**Answers of October issue 2017**

(A) 1. c; 2. e; 3. d; 4. c; 5. c  
(B) 1. d; 2. d; 3. d; 4. d; 5. d  
(C) 1. c; 2. e; 3. b; 4. a; 5. e  
(D) 1. e; 2. c; 3. e; 4. a; 5. e
Exim Assessment for Fellowship of the College will be organized twice yearly and the next Exit Assessment will be held on 21 June 2018. The deadline for application will be on 3 April 2018.

Application forms are available on the College website at http://www.paediatrician.org.hk/index.php?option=com_content&view=article&id=47&Itemid=48 and applications should be returned to the Hong Kong College of Paediatricians, Room 801, Hong Kong Academy of Medicine Jockey Club Building, 99 Wong Chuk Hang Road, Hong Kong. For enquiries, please call the Secretariat at 28718773.

The completed application form should be submitted with:

(i) Certified true copies of certificates
   a) Annual Practicing Certificate (current year)
   b) Primary medical qualification
   c) Certificate of an overseas higher qualification recognized by the Hong Kong Academy of Medicine (Appendix)
   d) The letter from the Honorary Secretary stating the date of commencement of the candidate's Higher Training (for Hong Kong trainees only)
   e) Certificate of Intermediate Examination OR Membership Certificate issued by the Hong Kong College of Paediatricians (for Hong Kong trainees only)
   f) Child Protection Course Certificate (for all trainees who started their basic paediatric training on or after 1st July 2009)

(ii) 2 photographs certified by one of the proposers

(iii) 2 dissertations with one from each of the following 2 categories (For details, please refer to the Revised Guidelines October 2004 available on the College website at http://www.paediatrician.org.hk/index.php?option=com_content&view=article&id=47&Itemid=48)
   a) Research project/study
   b) Case report and review of literature of a clinical problem related to the case.

   However, full research study can replace the case report and review of literature. Research protocols are not acceptable as submission for the purpose of the Exit Assessment.

   For candidates re-sitting the Exit Assessment, the previously submitted dissertations with revision can be used.

   Dissertations should not be on the same disease condition.

Please submit 4 hard copies of each dissertation. Please also submit a soft copy of each dissertation in MS Word format to enquiry@paediatrician.org.hk

From 2015, dissertations submitted for Exit Assessments will be considered for the Best Dissertation prize awarded every year by the College. Case reports will not be considered for the prize.

The Scientific & Research Subcommittee's decision about the Best Dissertation Prize will not retrospectively affect candidates' scores in the Dissertation Section of the Exit Assessment.
Announcement

(iv) Log sheets of higher training and assessment by Trainers and Supervisors for local trainees.

(v) Exit Assessment Form A-Record of Higher Training in Paediatrics duly completed.

(vi) Exit Assessment Form B-Checklist for Dissertations duly completed.

(vii) For overseas applicants, documentation of retrospective accreditation of paediatric training undertaken by the applicant by the Accreditation Committee of the Hong Kong College of Paediatricians must be submitted.

(viii) A cheque of $6,300 for the Exit Assessment made payable to the "Hong Kong College of Paediatricians".

(ix) Candidates who withdraw their applications before the application deadline will be entitled to a full refund of the assessment fee. Candidates who withdraw after the application deadline will not receive any refund. A 50% assessment fee will be refunded to candidates who withdraw from the Assessment after the application deadline due to bereavements of a near relative or illness. A medical certificate must be submitted as supporting evidence for claims due to medical illness.

An acknowledgement will be issued upon receiving an application with assessment fee but only applications with the complete submissions of documents required will be processed.

Appendix

Overseas Higher Qualifications Recognized by Hong Kong Academy of Medicine for Paediatrics:

<table>
<thead>
<tr>
<th>QUALIFICATION</th>
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<tbody>
<tr>
<td>1. Diplomate of the American Board of Paediatrics</td>
<td>DAB Paed</td>
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<tr>
<td>2. Fellow of the Royal Australasian College of Physicians</td>
<td>FRACP</td>
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<tr>
<td>3. Fellow of the Royal College of Physicians (Canada)</td>
<td>FRCP (Canada)</td>
</tr>
<tr>
<td>4. Fellow of the Royal College of Physicians (Edinburgh)</td>
<td>FRCP (Edin)</td>
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<tr>
<td>5. Fellow of the Royal College of Physicians (Glasgow)</td>
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<tr>
<td>6. Fellow of the Royal College of Physicians (Ireland)</td>
<td>FRCP (Ire)</td>
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<tr>
<td>7. Fellow of the Royal College of Physicians (London)</td>
<td>FRCP (London)</td>
</tr>
<tr>
<td>8. Member of the Royal College of Physicians (Edinburgh)</td>
<td>MRCP (Edin)</td>
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<tr>
<td>9. Member of the Royal College of Physicians (Glasgow)</td>
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<tr>
<td>10. Member of the Royal College of Physicians (Ireland)</td>
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<tr>
<td>11. Member of the Royal College of Physicians (London)</td>
<td>MRCP (London)</td>
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<tr>
<td>12. Member of the Royal College of Physicians (UK)</td>
<td>MRCP (UK)</td>
</tr>
<tr>
<td>13. Fellow of the Royal College of Paediatrics and Child Health</td>
<td>FRCPCH</td>
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<tr>
<td>14. Member of the Royal College of Paediatrics and Child Health</td>
<td>MRCPCH</td>
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What is the diagnosis?

Clinical suspicion for spinal muscular atrophy (SMA) was raised in view of her severe hypotonia and muscle weakness. Multiplex Ligation-dependent Probe Amplification (MLPA) for gene dosage of survival motor neuron 1 (SMN1) gene and survival motor neuron 2 (SMN2) gene was performed. Homozygous deletion of exon 7 and exon 8 in SMN1 gene with two copies of SMN2 gene was detected in the patient (Figure 2A), confirming the diagnosis of spinal muscular atrophy. Parents are both heterozygous SMN1 deletion carrier (Figure 2B & C).

Figure 2  Multiplex Ligation-dependent Probe Amplification (MLPA) for gene dosage of survival motor neuron 1 (SMN1) gene and survival motor neuron 2 (SMN2) gene. (A) Result of the affected child showed homozygous deletion of exon 7 and exon 8 of SMN1 gene (ratio ~0 for both exon 7 and exon 8 probes). There are 2 copies of exon 7 and exon 8 of SMN2 gene (ratio ~1 for both exon 7 and exon 8). (B) & (C) Results of the mother and father showed heterozygous deletion of exon 7 and exon 8 of SMN1 gene (ratio ~0.5 for both exon 7 and exon 8 probes).
What is the genetic anomaly associated with SMA?

Spinal muscular atrophy (SMA) is an autosomal recessive inherited neurological condition with a spectrum of clinical severity. It is caused by homozygous deletions or mutations in the survival motor neuron 1 (SMN1) gene on chromosome 5q13.2, resulting in deficiency of the SMN1 protein.1,2 The SMN protein is critical to survival of neurons in the spinal cord, the absence of which results in enhanced neuronal death. The SMN2 gene, often called the SMA "backup gene", produces some functional SMN protein which can partially compensate for the loss of SMN1 protein. Thus, its number plays a role in determining the clinical severity of the disease. The presence of three or more copies of SMN2 is associated with a milder phenotype. In our patient, only two copies of SMN2 gene were detected.

What is SMA?

SMA is characterised by progressive hypotonia and muscular weakness due to degeneration of the anterior horn cells in the spinal cord. In some, the motor neurons of cranial nerves are also involved, but sensation and cognition are intact. Depending on the age of onset and clinical course, SMA is classified as type 0 to type 4, with type 0 (prenatal onset) and type 1 (infantile onset) being more severe and type 2-4 being less severe with later onset.1 Our patient is likely to have SMA type 1 in view of genetic workup showing two copies of the SMN2 gene, as well as infantile onset of symptoms.

Apart from symmetrical proximal muscle weakness, SMA patients face other physical challenges including progressive respiratory failure, feeding problem, joint contractures and scoliosis.3 Those with milder types of SMA may be ambulatory initially but become wheelchair dependent with time. On the other hand, SMA type 1 patients may die from respiratory related complications by 2 years of age.

What is the treatment approach?

A team-based approach with multi-disciplinary care tailored for each family should be provided to SMA patients to optimise their quality of life. Apart from supportive care, intrathecal Nusinersen, a novel disease modifying drug, was approved to treat SMA patients in 2016.4 Nusinersen is an antisense oligonucleotide that increases the expression of the survival motor neuron protein, thus resulting in improvement in motor function in SMA patients. In view of the high cost and uncertain long term effects, individualised treatment decision has to be made before commencement of such therapy.

References

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Manuscripts are accepted on the condition that they are submitted solely to the HKJP and have not been published elsewhere previously and are not under consideration by another journal. A complete report following presentation or publication elsewhere previously and are not under consideration by another journal. A complete report following presentation or publication of preliminary findings elsewhere can be considered.

Categories of articles include the following:

Original Articles The text should not usually exceed 5,000 words excluding references; the number of tables, figures, or both should normally be not more than six, and references not more than 50.

Review Articles Reviews are usually invited systematic critical assessments of literature.

Case Reports Length should not exceed 1,500 words; the number of tables or figures used should not be more than two, and references should not be more than 10. Limit the number of authors to 4.

Commentaries Commentary on current topics is welcome. Length should not exceed 1,200 words; no tables or figures allowed, and references should not be more than 20.

Letters to the Editor Letters discussing a recent article in the HKJP are welcome. Original letters that do not refer to an HKJP article may also be considered. Letters should not exceed 500 words and have no more than five references. Published letters may be edited.

Manuscript Preparation

1. Use Arabic numerals for numbers above nine, for designators (e.g. case 5, day 2, etc.) and for units of measure; numbers should be spelled out if below 10, at the beginning and end of sentences, and for fractions below one.

2. Manuscripts should be submitted as a Word document in British English in the following format: Typed double-spaced, page size 22 cm. x 29 cm. (8 1/2 in. x 11 in.), page margins 2.54 cm (1 in), font size 12 pt.

3. Do not use abbreviations in the title or abstract and limit their use in the text. Standard abbreviations may be used and should be defined on first mention in the text unless it is a standard unit of measurement.

4. SI units should be used or included in parentheses.

Ethical Consideration

For original clinical study, authors must state that the protocol for the research project has been approved by the Ethics Committee of the institution within which the work was undertaken. All investigations on human subjects must include a statement that informed consents have been obtained. Patient anonymity must be preserved. Photographs and video clippings need to be prepared to prevent human subjects being recognized unless prior written permission has been obtained. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

The manuscript should usually be arranged as follows:

Title page This page should include the full names, and affiliations of all authors. A short title of no more than 40 characters should also be given. Up to three academic degrees for each author are allowed. If an author’s affiliation has changed since the work was done, list the new affiliations as well. Limit the number of authors to 4 for case reports and clinical quiz.

Abstract and key words The abstract should be no more than 150 words summarising the purpose, methods, findings and conclusions. Authors should provide no more than five key words to assist with cross-indexing of the paper. Key words should be taken from Index Medicus.

Introduction

Methods

Results

Discussion

References

Number references in the order they appear in the text. References should follow the Vancouver style and should appear in the text, tables and legends as Arabic numerals in superscript. Journal titles should be abbreviated in accordance with Index Medicus. List all authors and/or editors up to six; if more than six, list the first three and "et al".

Examples of References:

Articles in Journals


Illustrations (Figures)

Each illustration must be submitted as a separate figure file. The file name should be the same as the figure number. Preferred formats for digital artwork submission include Encapsulated PostScript (EPS), Portable Document Format (PDF), and Tagged Image Format (TIFF). Letters, numbers and symbols should be clear and of sufficient size to retain legibility when reduced. Photographs of persons must be retouched to make the subject unidentifiable, or be accompanied by written permission from the subject to use the photograph.

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Supplementary Video Clips

Video clips can be submitted with your manuscript in MP4 file format with H.264 codec. The size of the video should not exceed 5 MB. Patient anonymity must be preserved unless prior written permission has been obtained. If accepted, the video will appear online on the Journal's website, http://www.hkjpaed.org.

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