Editorial
Implementation of Newborn Screening Programme for Inborn Errors of Metabolism in Hong Kong – Progress and Challenges
Hui

Original Articles
Clinical Utility of Second-tier Testing in Newborn Screening for Congenital Adrenal Hyperplasia: The Hong Kong Experience
Yeung, Chan, Mak

Ethical Issues of Dried Blood Spot Storage and Its Secondary Use After Newborn Screening Programme in Hong Kong
Ngan, Li

Evaluation of the 18-month "Pilot Study of Newborn Screening for Inborn Errors of Metabolism" in Hong Kong
The Task Force on the Pilot Study of Newborn Screening for Inborn Errors of Metabolism

Review Articles
20 Years After Discovery of the Causative Gene of Primary Carnitine Deficiency, How Much More Have We Known About the Disease?
Tang, Hui

Review of a Decade of International Experiences in Severe Combined Immunodeficiency Newborn Screening Using T-cell Receptor Excision Circle
Leung, Lee, Lau

Case Reports
The First Case of Homocystinuria Picked Up by Newborn Screening in Hong Kong: A Case Report
Ho, Hui

Clinical Course of Two Newborns Affected by Cobalamin C Deficiency Diagnosed in the Pre and Post Newborn Screening Era
Wong, Lin, Chow, But, Hui

Newborn Screening Pitfalls: A Missed Case of Salt-losing Type of Congenital Adrenal Hyperplasia
Wong, Wong, Belaramani

Classical Neonatal Propionic Acidemia: Diagnostic and Management Pitfalls
Lui, Fung, Belaramani

Abstracts of Articles in Chinese

MCQs

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Editorial

Implementation of Newborn Screening Programme for Inborn Errors of Metabolism in Hong Kong – Progress and Challenges

The successful implementation of the Newborn Screening Programme for Inborn Errors of Metabolism in Hong Kong is another major milestone towards delivery of high standard paediatric care to locally born Hong Kong children. Newborn screening (NBS) aims at the earliest possible recognition of diseases to prevent the most serious consequences by timely intervention. Individuals who are affected by these rare inborn errors of metabolism (IEM) can be diagnosed at the earliest instance where by with available treatment and with continued disease monitoring, these patients’ outcome and long term prognosis are deemed to be entirely different compared to their historic counterparts when the same diseases may be diagnosed only after catastrophic clinical presentation often leading to poor outcome or after symptomatic presentation when irreversible damages have already taken place. Expansion of Newborn screening with the use of tandem mass spectrometry (MS/MS) for the early detection and treatment of IEM conditions is considered one of the most notable advancements in public health in the 21st century.1

Delivery of services to IEM patients in Hong Kong has taken on a steady and gradual developmental process over the last 20 years. While a number of the necessary investigations for diagnosis and monitoring may not have been available locally, recognition of possible IEMs was not simple nor straightforward either due to the lack of clinical experiences 20 years ago. With collaborative and conjoint effort from clinicians, pathologists and patients support groups, the service model and the needs of this highly specialised group of patients become known to the society and the governmental funding bodies. Tang & Hui shared in their paper titled ‘20 years after discovery of the causative gene of primary carnitine deficiency, how much more have we known about the disease’ the journey of making the diagnosis of the first Primary carnitine deficiency (also known as Carnitine uptake deficiency) patient in Hong Kong at the time when very little was known about the disease.2,3 As it now turned out, Carnitine uptake deficiency is a potentially very treatable IEM condition with carnitine supplementation and is one of the most prevalent IEM in the Chinese population with over 1,000 cases diagnosed and treated all over China. Carnitine uptake deficiency is also one of the more frequent IEM conditions detected from our local screening programme.

Upon the announcement in the Chief Executive’s 2015 Policy Address, the Hong Kong Special Administrative Region’s Newborn Screening Programme for Inborn Errors of Metabolism was launched as a pilot programme at 2 government birthing hospitals in October of the same year. It was a dream realised for many generations locally, recognition of possible IEMs was not simple nor straightforward either due to the lack of clinical experiences 20 years ago. A comprehensive report was submitted to and reviewed by the government who then decided for full implementation of the programme in a stepwise fashion to involve babies born in all public birthing hospitals in Hong Kong. This full report prepared by The Task Force on the Pilot Study of Newborn Screening for Inborn Errors of Metabolism published in this issue as a formal documentation reviewed the course of events surrounding the pilot programme and discussed all the important clinical findings.4 A total of 9 IEM cases were confirmed from the pilot programme giving an incidence of 1 in 1,682 which is higher than the original estimation confirming that IEMs is indeed not rare in Hong Kong.

The case reports published in this issue help to highlight the value of newborn screening in the early diagnosis of asymptomatic conditions that will not manifest with symptoms until years later when irreversible damage especially on cognition may have occurred. The first published local case of Homocystinuria alerted us that conditions that have been considered extremely rare locally do occur and only through newborn screening may the true incidence of these rare and often difficult to diagnose IEM conditions be known. The report on the 2 cases of Cobalamin C deficiency, one diagnosed in the pre and the other in the post newborn screening era illustrates the entirely different clinical course between these 2 infants. Although
the baby with Propionic acidaemia was not picked up through newborn screening, with increasing awareness among the local paediatric community, this baby's condition was rapidly diagnosed and managed.

In the ideal world, a good screening programme is one that produces minimal false positives and no false negatives. Yet false positive and false negative results are part and parcel of any screening programme. There are multiple factors involved in causing false positive results in IEM screening programs. False positive results are frightening to families, causes extra workload for clinicians and are financially burdensome to the health care system. To improve diagnostic specificity without reducing diagnostic sensitivity, most NBS laboratories develop second-tier tests to measure additional metabolites that either strongly support the presumption of a true positive case or refute the notion that the patient has the disorder. Yeung et al shared their local experience of the clinical utility of a second-tier testing for Congenital adrenal hyperplasia (CAH) – a condition known to have high recall rate based on the measurement of the primary analyte 17-hydroxyprogesterone. The authors proposed that by adding second-tier steroid profiling with liquid chromatography-tandem mass spectrometry (LC-MS/MS), this can significantly reduce false positive rate and avoid unnecessary recalls in newborn screening for CAH. While second-tier testing is effective in reducing false positive recalls, the authors cautioned that false negatives can still occur and genuine cases of CAH can still be missed despite second-tier testing. This scenario was presented by Wong et al in their case report titled 'Newborn screening pitfalls: A missed case of salt-losing type of congenital adrenal hyperplasia'. The important lesson here is that a negative newborn screening result does not rule out the conditions completely. In the right clinical context, it remains important for clinicians to exercise their clinical judgement on investigating for conditions that have normal screening results and not be falsely reassured.

After completion of the screening procedures, residual dried blood spot (DBS) cards are usually stored for quality assurance purposes. Protocols vary among different laboratories on storage periods which can range between few months to indefinitely. In addition to quality assurance purpose, stored cards are a valuable resource as they represent a complete population and are highly useful for establishing reference intervals or carrier frequencies in the population – a rich resource for many potential biomedical research and public health epidemiological studies. However, with the sensitive information derived from stored DBS cards, secondary usage especially for research purposes need to be carefully planned and deliberated. Here is this issue, Ngan & Li discussed the ethical issues surrounding DBS cards storage and its secondary use after newborn screening. Issues on informed consent, privacy and confidentiality concern, returning research results and public transparency were discussed.

With advances in modern day technology on computerisation, automation and sensitivity of analytical instruments, newborn screening (NBS) tests have been rapidly proliferating in recent years. Furthermore, the demands for new tests have partly been driven by improvements in known (e.g. haematopoietic stem cell transplant, enzyme replacement therapy) as well as new innovative treatment options (e.g. chaperone therapy, read-through of premature stop codons, gene therapy). NBS tests for Lysosomal storage diseases, X-linked Adrenoleukodystrophy, Severe combined immunodeficiency and Spinal muscular atrophy are some examples that have been added to different countries' existing screening panel. In this issue, Leung et al reviewed a decade of international experiences on Severe combined immunodeficiency (SCID) newborn screening using T-cell receptor excision circle. SCID, asymptomatic at birth but often fatal in the first few years of life fulfils criteria for screening. Confirmation of diagnosis is relatively straightforward and with the availability of early haematopoietic stem cell transplantation, prognosis of affected patients is much improved through early detection from newborn screening. SCID was added to the disorders recommended for newborn screening in the USA in 2010. How far and how fast should Hong Kong move in terms of addition of these newer NBS tests. In addition to issues surrounding the disease itself and testing technology, financial and ethical justifications for the introduction of these new NBS tests would need to be properly addressed, discussed and planned among all the involved stakeholders prior to consideration for addition to the existing screening panels.

No doubt newborn screening saves lives. Towards the end of 2020, Hong Kong will celebrate its 5th year of implementation of Newborn Screening Programme for Inborn Errors of Metabolism when all babies born in the public birthing hospitals will be covered by the screening programme. While embracing the benefits that this programme brings to the affected IEM children in Hong Kong, there is continued improvement work on many different aspects of the screening programme. Quoting from Bridget Wilcken, the pioneer of newborn screening from Australia's review article on Fifty years of newborn screening, I would like to share her concluding statement – 'With care, the benefits of screening should burgeon – if only we can learn from the past experience and proceed at a good pace - not too fast, not too slowly.'

J Hui
Guest Editor

References

6. Ngan OMY, Li CK. Ethical issues of dried blood spot storage and its secondary use after newborn screening. Issues on informed consent, privacy and confidentiality concern, returning research results and public transparency were discussed.
Clinical Utility of Second-tier Testing in Newborn Screening for Congenital Adrenal Hyperplasia: The Hong Kong Experience

MCW Yeung, TCH Chan, CM Mak

Abstract

Objective: The present study aimed to evaluate the utility of steroid profiling using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as a second-tier test for congenital adrenal hyperplasia (CAH) newborn screening. Methods: The newborn screening results of 40,754 newborns who were screened for CAH from April 2016 to March 2019 were included in this study. First tier test involved measurement of 17-hydroxyprogesterone (17-OHP) in dried blood spot using dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA). Cases with positive first-tier screen were subjected to second-tier test utilizing LC-MS/MS to measure 17-OHP, androstenedione and cortisol in the same dried blood spot samples. Results: Of the 40,754 newborns screened, 422 (1.04%) were screen positive by first-tier test and required second-tier test. Among them, 4 (0.01%) were screen positive by second-tier test and were recalled for further workup. Two of whom were diagnosed with CAH. Also one neonate with negative screen was subsequently diagnosed with CAH during work up for fever and hyponatraemia and hyperkalaemia. The 2 true positive and 1 false negative cases are all salt-wasting 21-hydroxylase deficiency. The estimated incidence of classical CAH in the screened population was 1:13,585. Conclusions: Second-tier steroid profiling by LC-MS/MS can significantly reduce false positive rate and avoid unnecessary recalls in newborn screening for CAH. However, false negative screen still occurs and any patients with clinical features of CAH should receive diagnostic testing regardless of newborn screening results.

Key words Congenital adrenal hyperplasia; False positive; Newborn screening; Second-tier

Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders caused by defect in adrenal steroidogenesis. Among this group, 21-hydroxylase deficiency is the most common form and it accounts for around 95% of all CAH cases. In this article, CAH refers to 21 hydroxylase deficiency unless otherwise specified. 21-hydroxylase is responsible for converting 17-hydroxyprogesterone (17-OHP) to 11-deoxycortisol and progesterone to 11-deoxycorticosterone. A defect of this enzyme caused by mutations in the CYP21A2 gene can lead to varying degree of mineralocorticoid deficiency and hyperandrogenism, presenting clinically as salt wasting and virilising phenotype respectively. The incidence of CAH was reported to be 1 in 6,084 in Chinese. In view of the potentially fatal salt wasting crises in severe CAH and the morbidity of hyperandrogenism such as precocious puberty and shortened height in milder form of CAH, newborn screening for this disorder has been introduced in many countries.
Currently, first-tier screening methodology for CAH is based on measuring 17-OHP levels in dried blood spots by immunoassays, in particular dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA). This screening method, although being fast, simple and easily automated, suffers from poor specificity or high false positive rate due to several reasons: First, premature and low birth-weight neonates have elevated 17-OHP levels due to functional deficiency in steroidogenic enzymes and perinatal stress. Second, immunoassays are susceptible to cross-reactivity of antibodies with other steroids present in blood, which include steroid intermediates such as 17-hydroxyprenenolone and its monosulfate metabolite. Strategies to improve positive predictive value of CAH screening include improvement in analytical specificity of antibodies employed in immunoassay provided by assay manufacturers and adoption of gestational-age or birth weight adjusted cutoff values for 17-OHP, all with limited success. Another strategy is second-tier screening tests, which are tests performed on the same dried blood spot specimen used for first-tier screen. The most widely adopted second-tier test for CAH is steroid profiling by liquid chromatography-tandem mass spectrometry (LC-MS/MS). It was initially described by Lacey et al who simultaneously measured 17-OHP, androstenedione and cortisol by LC-MS/MS and subsequent report from their centre showed a nearly 90% reduction in false positive rate and an improvement of the positive predictive value from the reported 0.5 to 4.7%. Similar reports from other centers using this strategy further supported its clinical value. For this reason, our laboratory provided LC-MS/MS-based second-tier test when newborn screening for CAH was started in Hong Kong in April 2016. Drawing on data in the last 3 years, we aim to report our experience on first- and second-tier screening for CAH.

Methods

From April 2016 to March 2019, a total of 40,754 newborns were screened for CAH in Hong Kong. Demographic data and screening and diagnostic information of screened neonates were collected and analysed. Dried blood spot samples were collected on whatman 903 filter paper between 24 and 72 hours of life. For neonates of less than 34 weeks gestation or birth weight less than 2,000 gram or neonates admitted to neonatal intensive care unit, a second dried blood spot sample will be collected on day 28 of life or at discharge whichever comes first. The first-tier test for CAH is based on 17-OHP measurements by automated, time-resolved fluoroimmunoassay (DELFIA, Perkin Elmer Life Sciences, Turku, Finland). For term neonates, a cutoff of 20 nmol/L was initially used to define elevated 17-OHP in first tier test and was later revised to 25 nmol/L in September 2018 after review of our reference data. For preterm neonates, cutoff is based on gestational age and age at sampling according to recommendations of the International Society for Neonatal Screening. Samples with elevated 17-OHP by this primary screen were submitted for second tier test as described by Lacey et al, in which LC-MS/MS is used to simultaneously measures 17-OHP, cortisol and androstenedione (4-AD). The ratio (17-OHP + 4-AD)/cortisol is then calculated. This ratio is based on the fact that the metabolic block at 21-hydroxylase causes reduced cortisol production and accumulation of precursors including 17-OHP, and shunting of precursors into androgen production, causing elevation in 4-AD. In sick neonates, whose elevation in 17-OHP is due to stress-related adrenal stimulation, cortisol is expected to be elevated, thus normalising the ratio. With the analytes 17-OHP, 4-AD and cortisol expressed in units of ng/mL serum, the cutoff for the ratio (17-OHP + 4-AD)/cortisol was set at 2 after review of our own population data and taking reference to cutoff used by other participants in the Newborn Screening Quality Assurance Program (NSQAP) CAH second tier proficiency testing program.

Results

During the 3-year study period, a total of 40,754 infants were screened by first-tier test for CAH. There were 1.04% of all screened infants (n=422) found to have elevated 17-OHP above cutoff. Their newborn screening dried blood spot samples were subjected to LC-MS/MS second-tier test and 4 were found to have elevated (17-OHP + 4-AD)/cortisol ratio and determined to be screened positive for CAH, giving the overall recall rate of 0.01%. We deduced that if second-tier test was not available and all elevated 17-OHP cases from first-tier immunoassay were recalled, the recall rate would be dramatically raised to 1.04%. But in reality the cutoff would need to be increased to avoid such high recall rate in newborn screening program for CAH without second-tier test.
51 preterm infants were screened positive by first-tier test and all of them were screened negative by second-tier test. Regarding the timing of sample collection, 82 dried blood spot samples were collected before 24 hours of life and 9 of them were screened positive by first-tier test and all 9 samples were normal on second-tier test. The higher screened positive rate (11%) on first-tier test in sample collected before 24 hours of life can be attributed to the fact that those are sick infants that required transfusion and neonatal intensive care unit admission and the dried blood spot collected before 24 hours of life was a pre-transfusion sample.

All screening-positive infants were assessed clinically by paediatricians and underwent further biochemical investigations, including serum 17-OHP, plasma electrolytes, short synacthen test, and urine steroid profiling. Consequently, among the 4 screened positive infants, 2 were diagnosed with CAH. First case (TP1) was a male full term neonate who was asymptomatic and was called back on day 7 of life for the abnormal newborn screening result. Physical examination showed normal blood pressure and normal external genitalia. Blood test on day 7 showed borderline low sodium of 134 mmol/L and mildly elevated potassium of 6.2 mmol/L. Second case (TP2) was a full term male neonate who was delivered by emergency cesarean section due to failed induction. He had transient tachypnea of the newborn and early onset neonatal jaundice due to glucose-6-phosphate dehydrogenase deficiency. His electrolyte was normal on day 2 of life but started to develop hyponatraemia (plasma sodium 136 mmol/L) and hyperkalaemia (plasma potassium 6.3 mmol/L) on day 5 of life, which was the time when the abnormal newborn screening result was available. Both positive cases had elevated plasma ACTH and renin and urine steroid profile of pattern compatible with 21-hydroxylase deficiency and both cases responded well to fludrocortisone and hydrocortisone. The clinical information and screening and subsequent follow up test results for these 2 true positive cases (TP1 and TP2) are summarised in Table 1. Regarding the 2 false positive cases, both had borderline 17-OHP results on first-tier test (21 nmol/L and 23 nmol/L) and also borderline elevated (17-OHP + 4AD)/cortisol ratio (2.2 and 2.4). Subsequent investigations showed both cases had normal plasma 17-OHP and normal urine or blood steroid profile.

There was one false negative case detected during this 3-year period. This false negative case (FN1) is a term female infant with borderline elevated 17-OHP of 25.4 nmol/L (cutoff >25 nmol/L). Subsequent second-tier LC-MS/MS test showed normal 17-OHP and normal (17-OHP + 4-AD)/cortisol ratio of 0.37 (see Table 1 case FN1 for summary). She presented at 1 month of age with fever and was noted to have clitoromegaly, hyponatremia and mild hyperkalaemia. Serum 17-OHP was 718 nmol/L (reference interval <8 nmol/L) at presentation and urine steroid profile detected significant elevation of metabolites of 17-OHP. She was treated with hydrocortisone and fludrocortisone with good response. With both true positive and false negative cases included, the overall incidence is estimated to be 1:13,585 for classical CAH.

**Discussion**

The purpose of this study was to explore the clinical utility of second-tier testing in newborn screening for CAH. Our data showed that the addition of LC-MS/MS-based second-tier steroid profile to immunoassay-based first-tier CAH newborn screening, the recall rate for CAH can be lowered from 1.04% to 0.01%, with an improvement in positive predictive value from 0.47% to 50%. This directly translates to less unnecessary hospitalisation and investigations of healthy infants, which can generate significant parental anxiety and potentially leads to

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Gestation (weeks)</th>
<th>Age of diagnosis</th>
<th>First tier 17-OHP (nmol/L)</th>
<th>Second tier 17-OHP (nmol/L)</th>
<th>Second tier (17-OHP + 4-AD)/cortisol ratio</th>
<th>CAH subtype</th>
<th>Serum 17-OHP at diagnosis (nmol/L)</th>
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</thead>
<tbody>
<tr>
<td>TP1</td>
<td>Male</td>
<td>40</td>
<td>7 days</td>
<td>127</td>
<td>89</td>
<td>2.0</td>
<td>SW</td>
<td>114</td>
</tr>
<tr>
<td>TP2</td>
<td>Male</td>
<td>38</td>
<td>5 days</td>
<td>207</td>
<td>120</td>
<td>4.1</td>
<td>SW</td>
<td>281</td>
</tr>
<tr>
<td>FN1</td>
<td>Female</td>
<td>41</td>
<td>1 month</td>
<td>25.4</td>
<td>6.8</td>
<td>0.37</td>
<td>SW</td>
<td>718</td>
</tr>
</tbody>
</table>

CAH: congenital adrenal hyperplasia; 17-OHP: 17-hydroxyprogesterone; 4-AD: androstenedione; SW: salt-wasting form of 21-droxylase deficiency
dysfunctional parent-child relationships. To further improve the positive predictive value of second tier test for CAH, inclusion of additional analytes in the LC-MS/MS assay has been suggested. Janzen et al improved on second-tier test method of Lacey et al, which is the method used by our laboratory, by adding 21-deoxycortisol and 11-deoxycortisol into the steroid panel. 21-deoxycortisol, being the product of 11β-hydroxylation of 17-OHP, is not elevated in preterm infants and is highly specific for 21-hydroxylase deficiency. In their study, the ratio of (21-deoxycortisol + 17-OHP)/cortisol is calculated and was shown to be significantly elevated in cases of 21-hydroxylase deficiency with no false positive result, resulting in a positive predictive value of 100%. Also, 21-deoxycortisol and 11-deoxycortisol allows differentiation of 21-hydroxylase deficiency and 11β-hydroxylase deficiency, hence this second-tier test can perform simultaneous confirmation and subtyping of CAH. Also, since this second tier test do not require additional equipment and the cost of acquiring the standards of these two analytes is limited, adding 21-deoxycortisol and 11-deoxycortisol into the second-tier steroid panel incurs minimal additional cost.

In this study, we have one cases of false negative case (FN1), who actually had mildly elevated 17-OHP on first-tier test but normal 17-OHP level and normal (17-OHP + 4-AD)/cortisol ratio by our LC-MS/MS second-tier test. This case is similar to the experience reported by the Minnesota newborn screening program. Using a two-tier newborn screening protocol, they reported 11 false negative cases with four of them actually had an abnormal first-tier screen which was subsequently overridden by a normal second-tier result. Also, the false negative rate of one-tier and two-tier protocols had no statistically significant difference. Therefore, it is clear that a normal second-tier steroid profile result should not be regarded as ruling out classic CAH totally and a high level of suspicion should be maintained in patients presenting with suspected CAH.

It is noteworthy that all three CAH cases (2 true positive and 1 false negative) are all salt wasting type of CAH. It is possible that there are other false negative cases that are not identified, especially milder forms of CAH including classical simple virilising and non-classical CAH. To minimise the risk of missing clinically important cases of CAH, some states in the United States mandated that a second screen be performed on all newborns at 8-14 days of age. A study comparing detection rates of first and second screen reported that majority of simple virilising and non-classical CAH cases were detected on the second screen that would have been missed by first screen. Also, the second screen allowed detection of an additional 6.5% of classical salt wasting cases. However, the practice of collecting a second specimen for all infants after discharge may not be practical in all newborn screen programs and its cost-effectiveness is still controversial.

Molecular genetic second-tier screening has been suggested as an alternative to biochemical second tier test in several small scale studies. The genotyping approach ranged from detection of a targeted panel of mutations using real-time PCR, or minisequencing to whole CYP21A2 gene sequencing with multiplex ligation-dependent probe amplification (MLPA). Molecular second-tier test has been well established in newborn screening programs for cystic fibrosis, however its application in newborn screening for CAH has several caveats: First, the functional CYP21A2 gene is in close proximity with its nonfunctional pseudogene CYP21P and they share 98% homology in exons and about 96% in introns. Hence, any PCR-based methods must utilise primers specific to sequence of CYP21A2 to avoid amplification of the pseudogene. Second, up to 30% of pathogenic variants causing CAH are gene deletions/gene conversions. Techniques to detect such copy number change, such as MLPA, needed to be incorporated, which complicates the genetic second tier testing protocols. Third, a single CYP21A2 allele may carry more than one pathogenic mutations. When two heterozygous mutations are detected, genotyping of the parents is needed to determine whether the mutations are in cis or in trans configuration. This approach is not possible in the setting of second tier newborn screen when the only sample available to screening laboratory is the dried blood spot card of the neonate. Overall, given the above technical difficulties, molecular genetic second screen for CAH is still considered to be tedious and costly compared with LC-MS/MS based approach. In 2018, the Endocrine Society stated that LC-MS/MS, in preference to all other methods (including genotyping), is the test of choice in second-tier screen for CAH.

**Conclusion**

In conclusion, this study suggests that LC-MS/MS based steroid panel as second-tier test for CAH reduces false positive rate and reduce unnecessary follow up testing,
healthcare expenditures and worry of parents. However, false negative result can occur in both first- and second-tier test and a negative newborn screening result does not rule out all possibility of CAH, even for severe classical salt wasting type. Therefore, any patient with clinical features of CAH should receive diagnostic testing.

**Conflict of Interest**

The authors have no conflicts of interest to disclose.

**References**

Ethical Issues of Dried Blood Spot Storage and Its Secondary Use After Newborn Screening Programme in Hong Kong

OMY Ngan, CK Li

Abstract

The advances in high-resolution tandem mass spectrometry led to a paradigm shift in expanded newborn screening, based on the dried blood spot (DBS). At the time of DBS collection, the quantity of blood collected is always more than sufficient for initial and repeated testing to validate screening results. Thus, the residual DBS for secondary use is almost always available. Current applications of residual DBS for various purposes, including quality assurance and test validation, new screening programme development and evaluation, biomedical research and public health epidemiological studies. However, because of the sensitive information that can be derived from the DBS, the storage and secondary use of residual DBS for research purposes raise several ethical considerations. This paper discusses some issues about the storage and secondary use of residual DBS in newborn settings, including informed consent, privacy and confidentiality concerns, the need for returning research results and public transparency.

Key words

Bioethics; Dried blood spot; Expanded newborn screening; Hong Kong; Informed consent

Introduction

Newborn screening is regarded as one of the most successful public health programmes, with clinical benefits that include providing information for family planning, enabling early diagnosis and treatment, and preventing childhood morbidity and mortality. The advances in high-resolution tandem mass spectrometry led to a paradigm shift in the development of expanded newborn screening, based on the dried blood spot (DBS). DBS is a form of sampling, where a few drops of blood are obtained from the heel through a prick and blotted on filter paper. Samples are then analysed via tandem mass spectrometry to determine the concentrations and activity of certain compounds, which allow detection of inborn errors of metabolism (IEM) – rare metabolic diseases caused by the accumulation of toxic metabolites or lack of essential metabolites in the metabolic pathway. The biospecimen in dried samples can be conserved for long periods at ambient temperature or under refrigeration, and have a minimal risk of bacterial contamination or haemolysis. The DBS collection procedures are also inexpensive, reliable for testing, and could be stored and transported easily. It offers an excellent opportunity to explore ground to routinely store and use the residual DBS collected in the newborn screening programme for research purposes. However, because of the sensitive information that can be derived from the DBS, its storage and secondary use raise several ethical considerations.
Advantages of Storing Residual DBS in the Newborn Settings

Current applications of residual DBS for various purposes include quality assurance and test validation, new screening programme development and evaluation, biomedical research and public health epidemiological studies.4

At the individual level, residual DBS has a continued value to the newborn when re-testing or additional testing is required to validate the test results. In particular, newborn infants with IEM often have variable and non-specific clinical presentations and can become acutely ill with severe complications within a short period. These diseases are rare but treatable, where therapeutic options, such as special modified diets, or bone marrow transplantation, may possibly restore normal metabolism with good prognosis. It is recommended that these therapeutic interventions should be implemented prior to the onset of signs. With doctors’ poor understanding of uncommon diseases, newborn infants with mild or atypical signs often diagnosed late and thus unable to receive early treatment. Residual DBS could be an effective alternative to identify affected infants at the earliest instance, often before they develop signs and facilitate early treatment to ensure the best possible outcome. For example, studies showed that residual DBS allows an early diagnosis of rare IEM, such as Gaucher disease and lysosomal storage disorder.5,6 The residual biospecimens can also be used for forensic identification purposes in the case of a missing or deceased child after an unexplained death.7

At the institutional level, residual DBS enables quality assurance and control. A newborn screening programme is not a one-time screening but involves multistage procedures, such as confirmation of diagnosis, management, and long-term follow-up. Regular audit and continuous quality improvement of the programme are essential to assess laboratory analytical processes in detecting, reducing, and correcting deficiencies prior to the release of patient results.

At the community, residual DBS foster biomedical research and public health programme development. In the 1990s, many developing countries used residual DBS as a surveillance tool for HIV epidemiology research to monitor the use of antiviral treatment among patients, estimate maternal-infant HIV transmission rates at birth, and perform drug resistance genotyping.8,9 It is particularly useful to identify HIV-exposed infants so that timely primary paediatric medical care could be provided.10

Residual DBS is also an invaluable source for developing a new screening method that requires large sample sizes especially to study rare diseases, characterise genetic variation frequencies, and establish a baseline measure for longitudinal comparisons.

Ethical Consideration about the Storage and Secondary Use of Residual DBS

Research use of residual DBS has a high societal value at the population level, although the immediate benefit for individual biospecimen donor may not be self-evident. There is some discussion advocating formalising use of residual DBS in a more systematic way as a biobank for future population research,11 but this practice does not come without disputes.

Informed Consent

The most frequently discussed concern is informed consent, which is the clinical practice of adhering to the principles of respecting autonomy. Autonomy broadly refers to individual freedom to make an informed choice voluntarily based on self-value and informedness without an undue coercive or deceptive circumstance.12 In health care practice, the consent procedures usually involve providing specific information about the nature of the research, as well as anticipated risk and benefits. Healthcare providers or researchers has an ethical responsibility not expose biospecimen donors to any uninformed research risk.

The need for informed consent is indisputable, and there are broadly two arguments against waiving informed consent. Firstly, the consent procedures enable individuals to learn what the research entails and what the risks and benefits they may face may be. There is some scholarly debate arguing that residual DBS is considered part of routine care so that consent is unnecessary for any related secondary use.13 It is, however, essential to note that autonomy entails the notion that biospecimen donors should not be treated as merely means to an end. Consent procedures provide an opportunity allowing parents to express approval or disapproval in storing children's DBS, while also allowing professionals to build up a rapport with them. Secondly, the DBS contains genetic information that can potentially readily identify the donor. Consent procedures allow parents to protect their children's privacy
by restricting the use of residual DBS in some research projects that they may consider to be sensitive or against their moral view.

Approaches for research with residual DBS, or broadly human biospecimens, employ different types of consent procedures and there are three types of consent majorly discussed in the literature, namely – specific, blanket, or broad – consent.14

The first type is known as specific consent, where explicit details specific to research are mentioned prior to study enrolment. Parents must be recontacted to obtain new permission for each future study. Residual DBS research, however, rarely takes place in a clearly defined scope such that explicit information often cannot be provided at the study recruitment. Also, it is hard to determine what is an acceptable scope for clarity in specific consent.15 Adopting the specific consent model is logistically limited in research for such biospecimens, and it may make informed consent too routine, leading to a reflexive denial when subjects are being asked to give consent too often.16 This approach may cause researchers to spend excessive amounts of time in these practical procedures rather than spending time in conducting research analysis.

Another type is known as blanket consent, which involves a one-time consent to future research without any limitation or oversight.17,18 This framework is favourable when the future research uses cannot be known at the time of consent and when samples are de-identified that there is no risk or concern about tracing identity to the residual biospecimen donor. It is, however, weak in terms of providing sufficient informedness to research participants.

The third type is known as a generic or broad consent that contains non-specific information with some specified procedural oversight, including governmental regulation, institutional committee review, and researcher integrity and privacy protection training.17 In this approach, parents have a higher degree of autonomy than the blanket consent as they are allowed to give a propositional agreement in giving out residual DBS on the types of research they would like to join.19 The flexibility account in the broad consent with some governance to some degree is regarded as ethically acceptable for biospecimen research, although it raises questions as to whether a generic consent with nonspecific information suffices because of the complexities of identifying future research with DBS, and ever-changing nature of genetic advancements.

Empirical research studied the parental understanding of residual DBS from newborn screening and found that parents of young children lacked knowledge towards the programme and were not familiar with the handling of biospecimens.20 Some were not even aware of the implemented policy of residual storage and thought that all samples were discarded immediately after clinical use.21 Parents showed a high level of trust and support towards the storage of DBS, and were willing to give out child's DBS if permission was obtained. In this way, they felt respected and given room to express consent or dissent to research prior to the enrolment.22,23 Specifically, they preferred being asked to give out consent for researchers to obtain and retain samples for research (an opt-in consent approach) over being assumed and stored, unless parents contact the researchers to have their child's sample removed (an opt-out consent approach).24,25 Healthcare providers or researcher involved in newborn screening also equally found consent procedure crucial to prevent adverse consequences from newborn screening.26 Small numbers of Hong Kong healthcare providers working in paediatrics, pathology or obstetrics, however, did not believe that parental consent should be mandatory for baby blood sampling.27 A possible explanation is that they see drawing blood is normalised as a part of regular clinical care that consent is regarded as not crucial in such a routine setting.

Privacy and Confidentiality Concern

The storage and secondary use of residual DBS card can lead to privacy and confidentiality concerns when the phenotype or personally identifiable information (e.g., name, ID card number, and date of birth) is not delinked from the collected biospecimen. The solution to alleviate these concerns is to guarantee anonymity grounded in two positions. First, it encourages altruism.28 Secondly, it protects biospecimen donors against potential bias or disadvantageous social consequences, such as exclusion from insurance.29 This approach, however, may limit the implications of the storage and secondary use of residual biospecimens. At the individual level, the donor of DBS may no longer directly benefit from the research, since the identity cannot be traced and results returned to parents or their physicians. At the population level, anonymity limits the research utility in transforming residual DBS into broader biobanks research, since DNA analysis gives meaningful implication only when interpreting with health care information. Empirical research studied how parents made a trade-off decision between anonymity and return
of research findings. In the vignette, parents were put in a situation to choose between anonymity for greater privacy protection, and a chance to receive important infant health information if retaining identifiers with the samples. Parents, who are knowledgeable about NBS, lower-income, and received lower education put infant health information at a higher priority than privacy. In other words, parents regarded the importance of benefiting a child's health as more imperative than privacy protections.

Parental attitude towards retaining the child's residual DBS for research purposes was also partly influenced by the question as to who or which entities manage the data and what purposes what these data serve. Parents found confident with research participation selectively when academic institutions lead a project, and are less favourable to give out samples of a child to non-academic institutions, such as commercial enterprises, insurance companies and pharmaceutical companies, with the concern of potential misuse of information. Examples of types of research uses that were considered unacceptable include, commercially-oriented research targeting profitable diseases by pharmaceutical and biotechnology companies, research lead to potential discrimination by workplaces, schools, insurance companies, or governmental agencies. Another research studied factors influencing parental decision to enrol in residual DBS research and the vast majority respondents prioritised consent as the most critical factors, distantly followed by privacy and research identity. Parents generally preferred their consent to be sought for every use of the child's DBS, child's identity not linked to the DBS, and research projects conducted by university researchers.

An alternative way not to completely obviate anonymity is to store personal identifier separately from the residual samples and label a unique study identifier to each sample. In this way, it not only enables the adoption of the double-blinded standard that permits unbiased laboratory analyses but also offers the feasibility to retrace participants for returning research findings. This approach requires additional considerations in data management and handling, such as restricting limited research investigators access right to the sensitive information.

Return of Research Findings

There has been active ethical debate about whether, when, and how residual DBS research findings should be returned to parents or their physicians, even if there are of no direct medical benefit (e.g., some IEM conditions are incurable, and there is no treatment plan upon returning the results). The underlying arguments supporting the return of research findings are autonomy, beneficence, reciprocity, and empowerment. Some physicians and researchers believe that they are obliged to warn research participants upon finding abnormalities and believe early warning can enable parents to take appropriate actions after receiving relevant test results. The counterargument against returning results concerns the potential violation of the original altruistic intent to help research benefiting for the greater public good instead of wishing for anything in return. Healthcare providers worry that clinically unactionable information might not lead to meaningful clinical implication but rather emotional burden, such as anxiety, and fear of discrimination by parents. They may feel that there is an obligation to avoid harm to parents by not disclosing the information, even though there is a possibility to deprive their right to know.

The discussion about what incidental research findings should be released or not is not new to the paediatric community, and consensus remains elusive. Some ethicists recommended that the information disclosure should be grounded upon the standard of best medical interests, where results that are accurate, has clinical significance to health and has available clinical interventions should be returned. However, for conditions with adult-onset or may have relevance only to the health of other family members is more controversial, since there are also some familial implications in pregnancy planning and concerns about the vulnerability of the child who will grow up with risk information. This approach is consistent with the purpose of newborn screening for early disease detection reducing child morbidity or mortality.

There is now a shift of consensus that physicians and research investigators should be prepared to return findings discovered in the course of the research and meeting an actionability threshold, but they have no ethical obligation to search for such results actively. However, the returning of results should be packaged with a referral for appropriate clinical follow-up. The workload and expertise in genetic counselling of the results at the time of releasing to participants can be significant. How to return the results and who should be responsible for returning of results should be well-planned. The research protocols requesting the use of residual samples should include a clear action plan in returning results.
Public Transparency

The storage and secondary use of residual DBS without parental consent and public transparency have culminated in lawsuits in the United States and Canada and raised international controversy. In 2009, the Texas State health office retained residual DBS from the mandated disease screening and then used them for research purposes without parental consent. Five families filing against the Texas State Department of Health claimed that uninformed storage and use of NBS for research purposes violated their constitutional protection with unlawful search and seizures and also failed to protect their liberty and privacy interests. The Texas Department of Health later admitted that they had provided 800 de-identified samples without parental consent to the United States Armed Forces for forensics purposes between 2003 and 2007. The lawsuit was settled and led to the destruction of over five million biospecimens and influenced succeeding research practice that parental consent must be sought before retaining residual DBS for storage and secondary use.

Another litigation in British Columbia, Canada took place in 2011. A lawsuit was filed in the Supreme Court of British Columbia against the Provincial Health Services Authority alleging the torts of fraudulent and negligent misrepresentation. The plaintiffs alleged that the Authority knowingly or negligently misrepresented the purpose of the collection and storage of the newborn blood samples and subsequently used the samples beyond indicated purposes without explicit parental consent. The court later ruled that the Authority making the unconsented samples available to outside parties for the purpose of medical research was unlawful and tortious that constituted a breach of privacy and fiduciary duty to the plaintiffs. The lawsuit was settled and led to a recent policy revision, where parents now have the right to choose from dissenting storing their children's DBS after the completion of the tests.

Hong Kong Context

Since 1984, two metabolic conditions, namely congenital hypothyroidism and Glucose-6-Phosphate Dehydrogenase deficiency, are screened under the governmental-subsidised neonatal screening pathway using umbilical cord blood samples under Clinical Genetic Service, Department of Health. A limitation of using umbilical cord blood samples in newborn screening is that it is not suitable for detection of IEM. In 2008, a local case reported the sudden death of a 14-year-old child resulting from glutaric acidaemia type II, confirmed by a post-mortem genetic diagnosis. In 2010, a 2-year-old boy died of aspiration pneumonia, which was suspected to be related to methylmalonic academia. The two cases of child mortality due to a metabolic disease could have been prevented if earlier diagnoses were made, leading to reconsideration in expanding the screening panel for IEM, which is not yet covered in the universal newborn screening in Hong Kong. The universal congenital hearing impairment screening programme was also introduced and offered in 2007.

In 2012, a first territory-wide prospective study on expanded newborn screening for IEM in two public hospitals was conducted by the University of Hong Kong. The team specifically implemented a 10-step model in delivering quality health service, including parental education, informed consent procedure, DBS collection and handling, laboratory assay, test result reporting, post-test counselling and follow-up, as well as treatment monitoring. Among all 2,440 newborn babies were recruited for newborn screening from October 2012 to August 2014. There were six (0.25%) false-positive cases confirmed by subsequent laboratory findings. No true-positive cases were found.

Starting from July 2013, the Centre of Inborn Errors of Metabolism at The Chinese University of Hong Kong started a private expanded newborn metabolic screening programme. During the period of July 2013 and July 2016, out of a total of 30,488 local newborn babies screened, there were 35 cases with mildly abnormal results and four with markedly abnormal results that were highly suggestive of IEM. Followed by further metabolic investigations, six neonates were subsequently confirmed to have IEM and one had a deficiency in maternal carnitine uptake, which had an incidence of 1 per 4355 births. The false-positive rate was 0.105% (32/30,448). One false-negative result was also identified. In particular, this study incidentally diagnosed a mother with carnitine uptake defect through the abnormal result for her baby in the newborn screening. The mother reported history of good past health during the pregnancy period, and her cardiac function was normal at the time of diagnosis. Later, she was referred to a cardiologist for follow-up.

The feasibility to implement expanded newborn screening for IEM was positively demonstrated, and the government decided to introduce a full-territory wide implementation of the programme in phases to involve all babies born in public hospitals.
framework, all residual DBS cards will be stored in the laboratory for a minimum of two years according to laboratory accreditation guideline. Once reaching the end of the retention period, blood samples will then be destroyed and disposed of. With informed consent from parents, the laboratory stores the cards longer for medical research after removing all identifying information. With the current annual delivery rate, it is estimated that over 40,000 dried blood spots would be collected yearly. As DBS cards represent a big wealth of data, its proper utilisation in clinical and research settings needs a thorough discussion among different stakeholders.

To date, there is a limited policy consideration about the long-term storage of these biospecimens in Hong Kong, despite its important value for research benefiting the children, pregnant women, and the general public. Policy aspect of managing residual DBS storage and its secondary uses are multifaceted.

First is about biospecimen retention period. In countries where universal newborn screening is currently implemented, the policy regarding the storage practices of newborn bloodspots varies. While some states in the United States destroy the cards after completing the laboratory quality check,46 other such as Denmark47 and Canada48 retain the leftover DBS collected in a routine standard of clinical care and stored for a lengthy period (10 years or more) or indefinitely for research purposes. What is an acceptable timeframe in the local context should be openly discussed, since the decision is likely to be influenced by many factors, for example, public knowledge and awareness, civic involvement, as well as scientific infrastructure.

Second is about the biospecimen data research uses, handling, sharing and management. Hospital Authority is not a research-orientated organisation, and so partnership with academic and not-for-profit organisations is essential to enhance utility for research. In response to incidental findings not only related to the newborn but also immediate family members, do physicians or researchers have duty of care? What research purposes, other than for clinical service, do residual samples serve or not serve? How should biospecimens be managed and shared among qualified investigators and institutions? These questions remain unanswered and require further exploration among stakeholders.

Third relates to the parallel development of healthcare infrastructure and regulatory oversight aligned with the residual DBS programme. Without compatible support in the clinical settings, including trained medical expertise to act upon abnormal results or access to healthcare services, the limited supporting healthcare services impede the benefit of the biospecimen storage programme. An oversight process for quality assurance and research are equally essential to ensure public transparency and public trust, such as launching an open website. Hospital Authority and the government play vital roles in taking up leadership in developing a regulatory framework.

Conclusion

We discussed some major ethical issues associated with the storage and secondary use of residual DBS in newborn settings (see Table 1). The discussion highlighted the essence of the informed consent model, privacy and confidentiality framework, returning research results protocol, transparency and public accountability in running a local-wide DBS storage programme and transforming it to a sizeable biobank-like database in supporting biomedical research. Stakeholders’ opinion is

<table>
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<tr>
<th>Table 1</th>
<th>Some ethical consideration of dried blood spot (DBS) card storage and its secondary use after the expanded newborn screening programme</th>
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<tbody>
<tr>
<td>What are the pros and cons of each informed consent model?</td>
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<tr>
<td>What types of research should be allowed to use the residual DBS?</td>
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<tr>
<td>How long should the residual DBS be stored?</td>
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<tr>
<td>Who has access to the residual DBS? How shall it be shared and managed?</td>
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<tr>
<td>Who has the ownership of the residual DBS?</td>
<td></td>
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<tr>
<td>Do researchers have the duty to return parents the research findings from DBS? What results should be returned or not returned?</td>
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<tr>
<td>What measures should be taken to enhance the transparency and public accountability in newborn screening programmes?</td>
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</table>
context-dependent and varies by countries, primarily affected by data sharing and protection policy, consent regulation, and governmental oversight. When the period of residual DBS storage prolongs and formalise as a biobank, the discussion should involve researchers, paediatricians, ethicists, policymakers, and other stakeholders to inform public policy. Normative analysis, such as empirical research among different stakeholders (e.g., parents and ethics committee) is also helpful to inform the development of acceptable research guidelines governing residual DBS in the local context.

Declaration of Interest

The authors declare that there is no conflict of interest.

References

37. Beleno v. Tex. Dep't of State Health Servs: No. 5:09-cv-00188-FB (W.D.Tex., San Antonio Division filed Mar. 12, 2009)
38. LD (Guardian ad litem of) vs. Provincial Health Services Authority of British Columbia British Columbia Supreme Court, BC, Canada.
48. Morrison A, Dowler J. Newborn Screening for Disorders and Abnormalities in Canada [Environmental Scan issue 26]. Ottawa: Canadian Agency for Drugs and Technologies in Health; 2011.
Evaluation of the 18-month "Pilot Study of Newborn Screening for Inborn Errors of Metabolism" in Hong Kong

The Task Force on the Pilot Study of Newborn Screening for Inborn Errors of Metabolism

Abstract

Introduction: After the release of the Chief Executive's 2015 Policy Address in Hong Kong, a pilot study was planned and implemented to study the feasibility of trying out in the public healthcare system a screening programme for newborn babies for inborn errors of metabolism (IEM). After six months of preparation, the "Pilot Study of Newborn Screening for Inborn Errors of Metabolism" was launched in October 2015 in two public birthing hospitals (Queen Elizabeth Hospital and Queen Mary Hospital). It lasted for 18 months in two phases: Phase I from October 2015 to March 2016 (covering 21 IEM diseases) and Phase II from April 2016 to March 2017 (covering totally 24 IEM diseases). Aim: This paper is to review the course of events and discuss about the clinical findings of the Pilot Study. Results and conclusion: The Pilot Study had been operated smoothly, in aspects of parental education, specimen collection, preparation and dispatch of specimens. There were effective communication and cooperation among different parties involved in baby recall, arrangement of further investigations and clinical management. 15,138 out of 15,361 (98.5%) eligible babies had parental written consents to join the Pilot Study and 9 IEM cases were confirmed (incidence of the Pilot Study was 1 in 1,682 (Confidence interval (CI): one in 909 to one in 3,333)). Two mothers were incidentally picked up with IEM of carnitine uptake deficiency (CUD) and classic phenylketonuria respectively, and two false negative cases of Citrullinaemia type II (CIT type II) were notified. Incidence was increased to 1 in 1,376 if the two false negative cases were also included and it is higher than those in other countries or regions. Collectively, IEM cannot be claimed to be rare in Hong Kong.

Key words Hong Kong; Inborn errors of metabolism; Newborn screening; Pilot study

Introduction

Screening is defined as the "systematic application of a test or enquiry to identify individuals at sufficient risk of a specific disorder to warrant further investigation or direct preventive action, amongst persons who have not sought medical attention on account of symptoms of that disorder."¹ Worldwide newborn screening programmes were started in 1960s. They can improve infants' health, through early identification and timely intervention of particular disorders which can be life-threatening or cause long-term disabilities to babies.²

With advancement of technology, tandem mass spectrometry (MS/MS) is the most commonly used technology for the newborn screening (NBS) of inborn errors of metabolism (IEM) because it is more effective than conventional screening method. It replaces the conventional testing method of one analysis of one metabolite for one specific disease with one analysis of many metabolites for different diseases.³ Expanded newborn screening for IEM exists in most western European

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countries, Australia, New Zealand as well as neighbouring Asian countries – China, Japan, Singapore and other places like Taiwan. Internationally, the screening programmes for IEM has been implemented at various pace for different panels of disorders, and even at various states of the same country. The differences are mainly due to various factors, such as the prevalence of specific disorders and medical practice.

In Hong Kong, the territory-wide Neonatal Screening Programme (NSP) was started in 1984 under the Clinical Genetic Service (CGS) of the Department of Health (DH) of the Hong Kong Special Administrative Region (HKSAR). It has a public health endeavor providing free-of-charge service to screen two relatively prevalent disorders, congenital hypothyroidism (CH) and glucose-6-phosphate dehydrogenase (G6PD) deficiency, among babies who are born in the eight public hospitals with obstetric services. The Workgroup on Expansion of Neonatal Screening Programme in Hong Kong (“Workgroup”) was set up in July 2013 to review the information and relevant evidence for the expansion of newborn screening (NBS) programme to cover IEM. It comprised of representatives from the Food and Health Bureau (FHB) of HKSAR, the DH, the Hospital Authority (HA) and included disciplines of Clinical Genetics, Paediatrics, Obstetrics, Pathology and Public Health. After the announcement of the Policy Address 2015, the Workgroup set up a Task Force (TF) to plan and prepare for the implementation of the Pilot Study to assess the feasibility of trying out the screening programme for newborn babies for IEM in the public healthcare system in our locality.

The pilot study was titled "Pilot Study of Newborn Screening for Inborn Errors of Metabolism" (Pilot Study). In this paper, we review the course of events and discuss about the findings of the Pilot Study.

**Methods**

The Pilot Study was conducted for a period of two years, including the first six months for preparatory works. The protocol was produced by the TF members for the implementation of the Pilot Study, which was launched in October 2015. Two public hospitals under the HA, namely, Queen Elizabeth Hospital (QEH) and Queen Mary Hospital (QMH) and the Newborn Screening Laboratory (NSL) of Princess Margaret Hospital (PMH) participated in the Pilot Study.

Apart from the existing healthcare professionals (HCPs) in QEH, QMH and NSL, additional medical staff of Advanced Practice Nurses (APN), registered nurses, laboratory technicians, phlebotomists and clerical staff were recruited for increased workload in the Pilot Study. They were equipped with education and training materials, including training sessions and competency assessments, resource books and training kit, before the launching of the Pilot Study.

Dried blood spots (DBS) from newborn babies for laboratory testing was taken for 18 months from October 2015 to March 2017. Courier services were arranged to collect the DBS from different hospitals and send to NSL. Such LC-MS/MS, Fluorometric-based auto-analyser as essential equipment in NSL were purchased for specimen processing, analytics and reporting. The NSL operated 5 days (From Monday to Friday) per week.

Among many different IEM, a total 24 (Table 1) were selected to be included in the Pilot Study. The four criteria that were agreed upon and adopted for selection of IEM included: 1) screening capability – availability of accurate, reliable screening, diagnostic testing and laboratory capability; 2) clinical significance – seriousness and number of cases encountered in our locality; 3) availability of treatment - efficacy and/or effectiveness of the treatment; and 4) favourable outcome after early treatment – adequacy of the understanding of the natural history of the condition and its long-term outcome with early treatment.

In consideration of technical readiness, the Pilot Study initially covered 21 IEM diseases in Phase I during the first six months (October 2015 to March 2016) of its implementation for all babies born in QEH and QMH of ≥34 weeks of gestation and birth weight >2000 g with DBS taken in postnatal ward (namely Babies of Category A). In Phase II from April 2016 to the end of the Pilot Study in March 2017, three additional diseases were included in the programme (Table 1) and all newborn babies were recruited. Besides babies of Category A, babies born with preterm <34 weeks of gestation; or birth weight <2000 g; or being admitted to Neonatal Intensive Care Unit (NICU) (namely Babies of Category B) were also included in Phase II (Table 2). Other differences between Phase II and Phase I of the Pilot Study were the number of dry blood spot (DBS) required for screening and the timing of DBS taking among babies classified under different categories according to different clinical circumstances (Table 2).

The workflow of the Pilot Study started with parental education. Video and education pamphlet were distributed
Table 1  24 inborn errors of metabolism included in the Pilot Study

<table>
<thead>
<tr>
<th>Disorders of Organic Acids (7) (Phase I &amp; II)</th>
<th>有機酸障礙 (七項) (第一及第二階段)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple carboxylase deficiency</td>
<td>多發性羧化酶缺乏症</td>
</tr>
<tr>
<td>Glutaric acidemia type I</td>
<td>戊二酸血症 I 型</td>
</tr>
<tr>
<td>Methylmalonic acidemia</td>
<td>甲基丙二酸血症</td>
</tr>
<tr>
<td>Propionic acidemia</td>
<td>丙酸血症</td>
</tr>
<tr>
<td>Isovaleric acidemia</td>
<td>異戊酸血症</td>
</tr>
<tr>
<td>3-hydroxy-3-methylglutaryl-CoA lyase deficiency</td>
<td>白胺酸代謝異常症</td>
</tr>
<tr>
<td>Beta-ketothiolase deficiency</td>
<td>貝塔酮硫解酶缺乏症</td>
</tr>
<tr>
<td>Disorders of Amino Acids (8) (Phase I &amp; II)</td>
<td>氨基酸障礙 (八項) (第一及第二階段)</td>
</tr>
<tr>
<td>Classical phenylketonuria</td>
<td>苯丙酮尿症</td>
</tr>
<tr>
<td>6-pyruvyl-tetrahydropterin synthase deficiency</td>
<td>六 - 丙酮酰 - 四蝶噪合酸酶缺乏症</td>
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<tr>
<td>Argininosuccinic acidemia</td>
<td>精氨酸酸血症</td>
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<tr>
<td>Maple syrup urine disease</td>
<td>槟糖尿病</td>
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<tr>
<td>Citrullinaemia type I</td>
<td>瓜氨酸血症 I 型</td>
</tr>
<tr>
<td>Citrullinaemia type II</td>
<td>瓜氨酸血症 II 型</td>
</tr>
<tr>
<td>Tyrosinaemia type I</td>
<td>酪氨酸血症 I 型</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>高氨氨酸尿症</td>
</tr>
<tr>
<td>Disorders of Fatty Acid Oxidation (6) (Phase I &amp; II)</td>
<td>脂肪酸氧化障礙 (六項) (第一及第二階段)</td>
</tr>
<tr>
<td>Carnitine uptake deficiency</td>
<td>卡尼丁吸收障礙</td>
</tr>
<tr>
<td>Carnitine-acylcarnitine translocase deficiency</td>
<td>卡尼丁穿透障礙</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase II deficiency</td>
<td>卡尼丁結合酵素 II 缺乏症</td>
</tr>
<tr>
<td>Medium-chain acyl-CoA dehydrogenase deficiency</td>
<td>中鏈類輔酶 A 去氫酶缺乏症</td>
</tr>
<tr>
<td>Very long-chain acyl-CoA dehydrogenase deficiency</td>
<td>極長類輔酶 A 去氫酶缺乏症</td>
</tr>
<tr>
<td>Glutaric acidemia type II</td>
<td>戊二酸血症 III 型</td>
</tr>
<tr>
<td>Others (3) (Phase II)</td>
<td>其他 (三項) (第二階段)</td>
</tr>
<tr>
<td>Congenital adrenal hyperplasia</td>
<td>先天性腎上腺增生症</td>
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<tr>
<td>Biotinidase deficiency</td>
<td>生物素缺乏</td>
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<tr>
<td>Classic galactosaemia</td>
<td>半乳糖血症</td>
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</table>

Table 2  Number of DBS specimen and timing of DBS taking for screening

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Babies of Category A</th>
<th>Babies of Category B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Criteria</td>
<td>≥34 weeks + 0 day of gestation and birth weight &gt;2000 g with DBS taken in postnatal ward (Phase I &amp; II)</td>
<td>Preterm &lt;34 weeks + 0 day of gestation; or birth weight &lt;2000 g; or being admitted to NICU (Phase II)</td>
</tr>
<tr>
<td>No. of DBS required</td>
<td>Single</td>
<td>Serial of three</td>
</tr>
<tr>
<td>Time of DBS taking</td>
<td>Within 24 to 72 hours of life and before discharge</td>
<td>1st specimen should be collected on admission regardless of the age and before any treatment except respiratory support</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2nd specimen should be taken at 48 to 72 hours of life</td>
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<tr>
<td></td>
<td></td>
<td>3rd specimen should be taken at discharge or on day 28 of life whichever comes first</td>
</tr>
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</table>

DBS: dried blood spots; NICU: Neonatal Intensive Care Unit
during both the antenatal and postnatal periods to help parents understand IEM and the Pilot Study. Parents decided whether they would voluntarily join the Pilot Study or not. Consent was obtained from parents of eligible babies before DBS were collected by heel pricking. The number of DBS collected would depend upon which category the baby belonged to (Category A or B). Collected DBS were dispatched to NSL by pre-arranged courier services. Laboratory results were issued as one of the following three categories viz. normal results, invalid specimens and positive results (with two sub-categories – uncertain result and abnormal result). Normal results would not be notified with no further action necessary. For invalid specimens, resampling was required upon notification of individual birthing units. Babies with positive results would be recalled for clinical assessment with repeat DBS testing +/- further diagnostic and confirmatory testing by the respective paediatric department which would be responsible for the subsequent on-going treatment and monitoring of these babies. In the Pilot Study, residual DBS were stored for six months in the screening laboratory and were discarded afterwards. Positive DBS specimens would be kept for quality assurance purpose.

Results

15,138 out of 15,361 (98.5%) eligible newborn babies were included in the Pilot Study. All DBS were sent together with completed particulars of babies and mothers to the NSL within three working days of specimen collection. 99.5% specimens were collected within the required time according to the babies’ category. More than 99.8% of DBS were received by the NSL as valid and optimal for testing.

Among the total of 15,138 babies screened, 53 showed positive results. Nine (four boys and five girls) were subsequently confirmed to have various IEM conditions (Table 3). The collective incidence of IEM of the Pilot Study was calculated to be 1 in 1,682 (9 confirmed cases from 15,138 babies screened (CI: one in 909 to one in 3,333).

Eight of the nine patients were in stable clinical condition when the screening result became available. They were treated with appropriate dietary advice +/- medication as indicated for their specific conditions. Confirmatory genetic testing with counselling were given to the parents. Only the baby who was subsequently confirmed to have Methylmalonic acidaemia presented symptomatically prior to the availability of the screening result. Baby presented early with shortness of breath and poor feeding on Day 3 of life (Sunday) before the availability of the screening result on the following working day (Monday). The baby required intensive care unit support initially but her condition gradually stabilised with medical treatment.

Incidentally, two babies (one male and one female) with abnormal screening results were confirmed not to be affected with IEM. Their screened false positive results were explained by their mothers who were, afterwards, diagnosed to have carnitine uptake deficiency (CUD) and classic phenylketonuria respectively.

Although there was no established mechanism to report false-negative cases (i.e. babies having normal screening results but finally diagnosed to have IEM) in the Pilot

<table>
<thead>
<tr>
<th>Sex</th>
<th>Ethnicity</th>
<th>Diagnosis</th>
<th>Any symptom when being picked up by screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>Chinese</td>
<td>Carnitine uptake deficiency (CUD)</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>F</td>
<td>Nepalese</td>
<td>Phenylketonuria (not classic PKU) (Mild PKU)</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>M</td>
<td>Indian</td>
<td>Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)</td>
<td>Asymptomatic</td>
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<tr>
<td>M</td>
<td>Chinese</td>
<td>Citrullinaemia type II (CIT type II)</td>
<td>Asymptomatic</td>
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<tr>
<td>F</td>
<td>Chinese</td>
<td>Citrullinaemia type II (CIT type II)</td>
<td>Asymptomatic</td>
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<tr>
<td>F</td>
<td>Chinese</td>
<td>Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD)</td>
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<td>F</td>
<td>Chinese</td>
<td>Methylmalonic acidaemia (MMA)</td>
<td>Symptomatic</td>
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<tr>
<td>M</td>
<td>Chinese</td>
<td>Carnitine uptake deficiency (CUD)</td>
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<td>M</td>
<td>Chinese</td>
<td>Carnitine uptake deficiency (CUD)</td>
<td>Asymptomatic</td>
</tr>
</tbody>
</table>
Study, we were informed of two babies (one male and one female) with normal screening results who were subsequently confirmed to have Citrullinaemia type II (CIT type II) after their symptomatic presentation with prolonged jaundice at one month of age.

Discussion

The purpose of conducting the Pilot Study was to look into the feasibility of trying out in the public healthcare system a screening programme for newborn babies for IEM. Being the first of its kind to be conducted in a large scale among babies born in the government birthing units, careful design and effort would need to go into the protocol design to ensure seamless collaboration among the various stakeholders who are involved in the screening programme. Upon the smooth implementation of the pilot study, this same protocol was then adopted for subsequent territory-wide extension after some modifications. With the changing needs and advancement of screening technology, the scope of IEM for screening would continue to be monitored and reviewed on a regular basis.

Effective parental education was crucial for success of any newborn screening programme. This might be reflected by the high uptake rate 98.5% (15,138 out of 15,361 eligible babies participated) of the pilot study. Great appreciation and credit went to the frontline Health care professionals to achieve an overall 99.5% of specimen collection within the required time. More than 99.8% of the specimens were received in the NSL as valid and optimal for screening test. The handling of babies of Category B who needed serial DBS testing created some confusion and stress to the frontline HCPs initially. This was successfully tackled by streamlining the specimen collection arrangement.

With the nine IEM cases confirmed in the Pilot Study, the collective incidence of IEM conditions detected during the pilot study period was one in 1,682 (9/15,138). This incidence was higher than the previously estimated local collective incidence of one in 4,122 to one in 7,580. This collective incidence is higher than those reported worldwide, such as 1 in 5,800 in Mainland China, 1 in 5,882 in Taiwan, 1 in 2,000 in Korea, 1 in 9,330 in Japan, 1 in 3,600 in India, 1 in 6,000 in Australia which excluded hyperphenylalaninemia, 1 in 2,400 in German, 1 in 4,000 in America. Understandably, incidence figures vary dependent on different factors, such as the panels of IEM disorders that were screened for, the proportion of various ethnic group in the community. Also our pilot study only lasted for a relatively short period of eighteen months. Thus more prolonged data collection and further in-depth study of local epidemiology of IEM cases should be undertaken to reflect the true local incidence figures.

The baby with MMA presented clinically before the screening result became available. It is well known that MMA patients can present acutely even before the screening result becomes available. The issue of specimen transportation and laboratory working hours needs further deliberation to balance between efficient utilisation of facilities and rapid result turnaround time. As the demand increases, the number of laboratory working days should be reviewed and adjusted with additional resources. Also, the schedule of courier service from different hospitals to the NSL should be well planned and monitored.

Two mothers were incidentally found to have IEM conditions - Carnitine uptake deficiency (CUD) and Classic phenylketonuria (PKU). The mother in the first case (CUD) was stable and her baby was asymptomatic since birth. The baby affected by maternal PKU suffered microcephaly, congenital heart defects and developmental delay. The diagnosis of maternal PKU was made only after reviewing the IEM screening result of the newborn. Therefore, the implementation of universal newborn screening may also help to detect undiagnosed IEM in mother in future.

There were two false-negative cases of Citrullinaemia type II (CIT type II) during the pilot study period. Both babies presented with prolonged jaundice. It is well known that Citrullinaemia type II (CIT type II/Citrin deficiency) can be easily missed if newborn screening is performed early during the first week of life. It is important for frontline staff to educate parents on the possibility of false negative results during the education and consent process before blood taking. Paediatricians also need to be alerted to the relative higher false negative rate of certain screened IEM, in particular Citrullinaemia type II in our locality. Paediatricians should proceed to investigate for this possibility in babies presenting with prolonged neonatal jaundice even with a normal newborn screening result. Setting up a notification mechanism for false-negative cases would need to be considered for the future territory-wide implementation of the IEM NBS programme to capture the genuine incidence figures.

Conclusion

The Pilot Study has operated smoothly, especially in
terms of parental education, specimen collection, preparation and dispatch. There were effective communication and cooperation among different parties involved in baby recall, arrangement of further investigations and clinical management. The health education effectively helped parents to make informed decision before joining the Pilot Study and led to the overall encouraging parental consent rate. With the concerted effort from different disciplines, nine IEM cases were identified and treated. Effectiveness in reducing morbidity and mortality due to IEM was well demonstrated in this pilot study. With a collective incidence of 1 in 1,682, IEM should not be considered as rare in Hong Kong.

Acknowledgement

The members of the Task Force on the Pilot Study of Newborn Screening for Inborn Errors of Metabolism would like to take this opportunity to acknowledge all parties involving in the preparation, implementation, operation and monitoring of the Pilot Study, including Obstetricians and Nursing colleagues in Departments of O&G in QEH and QMH for antenatal education, consent seeking and handling specimens; Chemical Pathologists and colleagues in Newborn Screening Laboratory in PMH to perform testing; Paediatricians and nursing colleagues in Departments of Paediatrics in QEH and QMH for managing babies; Healthcare colleagues in Maternal and Child Health Centres of the Department of Health to contribute their expert advice; Health Administrators and IT experts from Headquarter of the Hospital Authority for their support and significant contributions in the Pilot Study.

Declaration of Interest

The authors declare that there is no conflict of interest.

References

### Appendix

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20 Years After Discovery of the Causative Gene of Primary Carnitine Deficiency, How Much More Have We Known About the Disease?

NLS Tang, J Hui

Abstract

We review the development of molecular diagnosis of primary carnitine deficiency, also known as carnitine uptake defect. Knowledge of mutations of the causative gene, SLC22A5 (previously called OCTN2), greatly enhanced our understanding of the physiology, pathogenesis and therapy. DNA diagnosis and newborn screening are very useful in early diagnosis and early therapy which leads to favourable prognosis. With the latest incidence data, primary carnitine deficiency turns out as one of the most common inherited metabolic disease in Chinese (the incidence as high as 1 in 10,000) and up to a thousand patients have been diagnosed and treated in the Greater China region during the last 20 years.

Key words

Carnitine; Carnitine uptake defect; Fatty acid oxidation defects; Primary carnitine deficiency; SLC22A5

Introduction

In 1996, a family with recurrent unexplained infant deaths were investigated with all available pathology and genetic methods possible at that time. After a thorough examination, the diagnosis of primary carnitine deficiency was made for the first time in this locality.1 As it was the first case, there was little insight how prevalent this disease could be in Chinese. There was also no prior experience in greater China region. After 20 years, this disease now turns out to be one of the most prevalent inherited metabolic disease (IMD) in Chinese and up to 1,000 cases have been diagnosed and treated all over China. This article reviews and highlights special features about primary carnitine deficiency which is also commonly known as carnitine uptake defect (CUD).

Early reports of "Primary carnitine deficiency" were in fact mis-diagnosed cases of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency and the carnitine deficiency was secondary to blockage of fatty acid β-oxidation. It was not until the development of an in-vitro carnitine uptake assay on cultured fibroblast, a clear-cut definitive diagnosis of CUD could be made.2 However, in-vitro functional assay has many limitations and the most obvious one is the unavailability of living fibroblast as fibroblast culture from skin biopsy is not a routine investigation that is readily available. Furthermore, failure to setup a culture sometimes happens which makes it less reliable as a diagnostic test. To overcome these troubles, we and two other teams identified the causative gene which was called OCTN2 historically, now known as SLC22A5, around the same time in 1999.3-5 The discovery allows making use of a simple DNA mutation test to make a definitive diagnosis. A battery of early mutations reported by the three papers were archived in the OMIM database (#MIM:603377, https://www.omim.org/entry/603377). As we were the only one of few laboratories performing
Primary Carnitine Deficiency / Carnitine Uptake Defect

Physiological Role of the Plasma Membrane Carnitine Transporter

Intracellular carnitine concentration is much higher than extracellular concentration which is achieved by this plasma membrane sodium carnitine co-transporter with ATPase activity (Figure 1). This transporter belongs to a large family of active organic ion transporters which share similar protein structure and common molecular evolution. Other member transporters play important role in transport and absorption of drugs and other endogenous organic molecules.

Carnitine is an essential compound in fatty acid oxidation pathway. Fatty acid is a key energy source for cardiac muscle and skeletal muscle. Therefore, carnitine transporter is highly expressed in muscle tissues to maintain a high carnitine concentration in the cytoplasm. Kidney tubular epithelial cells also have a high expression of the carnitine transporter. As carnitine has a low molecular weight, it is passively filtered across glomeruli. Over 90% of filtered carnitine are reabsorbed presumably in the proximal tubule by this active transporter (Figure 1). If carnitine transporter is defective, extensive renal wastage of carnitine causes a very low plasma carnitine concentration and subsequently carnitine deficiency.

In addition to support fatty acid metabolism of muscular tissue itself, fatty acid oxidation in liver produces the emergency energy currency, ketone bodies. Central nervous system is dependent on ketone bodies as its primary energy after depletion of body carbohydrate stores and during hypoglycaemia. This phenomenon happens in patients with various fatty acid oxidation defects and manifests as hypoketotic hypoglycaemia, which is a common hallmark of this group of IMDs. In fact, CUD may be in Fujian around 1 in 10,000 newborns.9 On the other hand, incidence was lower in Northern China, like Shandong.11

The high disease incidence in this locality is due to common mutations. R254X is common in Southern China. It is also found in Xuzhou and Shanghai but it is less frequent in Beijing and Vietnam7,8,12 Interestingly, the same nonsense mutation was found as far as in Western Asia countries including Lebanon, Turkey and Saudi Arabia (Figure 2). There are 2 alternate possibilities to account for the geographical distribution of R254X mutation. Firstly, these mutations might arise separately in history through independent mutation events happened to different ancestors. Alternatively, the mutation happened to a single ancestor and descended through generations and spread to new locations with people movement or migration. It is called a founder mutation. It is possible to differentiate the two scenarios by looking at the neighbour segments of the chromosome carrying the mutation. If the mutation arose attacks during infancy particularly among those patients who had not been treated with carnitine. This was also the case of the referral from Macau who was a 10-year-old proband with dilated cardiomyopathy.6

Regional Disease Prevalence and Geographic Distribution of Common Mutations, R254X and S467C

Founder Mutation R254X

In 2002, we reported for the first time a common mutation, R254X which was found in referral cases from Macau, Taipei, and Kaohsiung.6 R254X is a mutation changing the codon 254 from Arginine to a stop codon which causes a loss of function of the transporter. This mutation also had an unusually high carrier frequency of 1 in 125 in local Chinese. This carrier frequency predicts a disease incidence of 1 in 62,500 and suggested carnitine uptake defect (CUD) was a common IMD in Hong Kong.

We do not have good statistics of the disease incidence in Hong Kong as territory wide newborn screening NBS commenced only recently. A high disease incidence of CUD (~1 in 40,000) was confirmed in Taiwan where NBS for fatty acid oxidation defect (FAOD) had been started in 2002. In fact, CUD was the most prevalent FAOD in Taiwan. Similarly high incidence has been recently reported in Chinese provinces south of the Yangtze River (Figure 2), including Guangdong, Fujian, Hunan, Shanghai and Xuzhou in Zhejiang.7-10 In fact, the highest incidence of CUD may be in Fujian at around 1 in 10,000 newborns.9 On the other hand, incidence was lower in Northern China, like Shandong.11
only once to a single common ancestor supporting the scenario of a founder mutation, the neighbour segments of the chromosome of nowadays patients carrying the R254X mutation should be descended from this common ancestor and thus identical among nowadays patients. This is called haplotype analysis. We showed that R254X among Southern Chinese indeed shared the same haplotype presumably that of the common ancestor and so this is a founder mutation.

Shortly after our publication, very much to our surprise, R254X was also found in 3 different countries as far as in Western Asia and Middle East.\textsuperscript{13-16} The research team in Lebanon followed our protocol and showed that R254X in Lebanon patients had the same haplotype as ours. It suggests that R254X in Lebanese patients shared the same ancestor as Chinese patients. On the other hand, R254X in Saudi Arabia patients had a different haplotype (our unpublished data) and it arose independently (marked as triangle in Figure 2). Haplotype of R254X in Turkish patients was not examined so its origin remained as a question. At first sight, it was incomprehensible how Chinese and Lebanese could share a mutation descended from one common ancestor. Historically, both Lebanon and Turkey formed the western end of Silk Road through which heavy trade traffic happened up to the 14th Century. Marco Polo was the prototype historical figure who

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{(A) The primary function of the plasma membrane carnitine transporter. It is an active sodium-carnitine co-transporter which pumps carnitine into cytoplasm against a concentration gradient. Mutations of its encoding gene \textit{SLC22A5} (previously called \textit{OCTN2}) result in impairment of carnitine uptake and significant reduction of intracellular carnitine level. (B) This plasma membrane carnitine transporter is crucial for uptake of carnitine into heart and skeletal muscle which are dependent on fatty acid oxidation for energy supply. It is also required in reabsorption of carnitine from tubular fluid back to the circulation in renal tubules.}
\end{figure}
travelled Silk Road between 1271-1295. After his departure from Venice, he landed at the city Arce which is next to the current border of Lebanon. On his return, he took a route through Turkey (Wikipedia).

By analysis of the length of shared segments of nowadays mutation chromosomes that were descended from the putative common ancestor, an estimation of age of the founder mutation can be performed. The principle is based on probability of recombination is related to (1) length on the chromosome and (2) number of generation (number of meiosis). The longer the shared segment of common haplotype the shorter the history (passed fewer generations) of the mutation. The shared haplotype segment of Chinese and Lebanese R254X extended over 6 million base pairs next to the \textit{SLC22A5} gene on chromosome 5. Age calculation suggested that the mutation arose 620 years ago (confident interval: 375 to 1075 years ago).\textsuperscript{17} This coincided with Macro Polo's historical period when the Silk Road traffic was also most active. Of course, this hypothesis is pending for further confirmation, until then it remains speculative.

\textit{Maternal Carnitine Uptake Defect (mCUD) and S467C}

While we performed mutation analysis for NBS screen-positive CUD neonates referred from Taiwan screening centres, there were occasional newborns with whom only one mutation was found. As CUD is an autosomal recessive disease, affected patients are expected to have two mutations, one on each of two chromosomes. In a putative affected case, missing one mutation represent a major diagnostic dilemma. Steps are needed to differentiate among several scenarios, and importantly each of them leads to a different diagnosis and management. These scenarios may represent (a) false negative mutation analysis, (b) wrong diagnosis, or (c) mosaicism and other rare genetic aberrations.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{R254X mutation is particularly prevalent in the Southern China region. Molecular evolution analysis indicates that the mutation was originated from a single founder in the past. Interestingly this founder mutation was also found in Lebanese patients. It is uncertain R254X reported in Turkey is the same founder mutation while the mutation in Saudi Arabia arose as a separated origin (not from the same founder).}
\end{figure}
Colleagues from the referring centre found that several mothers of these cases had unusually low serum carnitine concentration which were comparable to that of CUD patients. Mutation analysis of maternal samples of these cases readily confirmed that the mothers had two mutations in SLC22A5 gene. The results indicated that the real patients were in fact the mothers and those children picked up by NBS were just obligate carriers.

When we first looked at the list of mutations in these maternal CUD cases, it took only a quick glance to spot an eye-catching pattern. All except one case were carriers of the same mutation, S467C.15 This is change of Serine to Cysteine at codon 467. In fact, S467C was among the first batch of mutations ever diagnosed in CUD.19 Among the Japanese patients, this mutation was prevalent in Akita region where carrier frequency was as high as 1 in 100. As it is a common mutation in Japanese, functional study had been performed to evaluate the biochemical function of mutated carnitine transporter. In-vitro study confirmed that S467C had a substantial residual function.20 Examples in other diseases indicate that mutants with residual function may lead to a milder phenotype. S467C may serve as an example to illustrate this point.

S467C was first reported as mutations found in Akita region of Japan where is well known for a high incidence of CUD. Asymptomatic adults were screened for serum carnitine levels and those with low carnitine were analysed for mutations and carriers of S467C were found. Although residual transporter function, almost up to 10% of normal update capacity, was demonstrated, its particular role in mCUD was not aware at that time.19,20 Pregnancy leads to major changes in physiological function and adaptation, like increased cardiac output, renal filtration etc. Many of them demand for additional calories/energy supply, and thus represent major stress to cellular function and also fatty acid oxidation. It is unexpected to have a CUD patient (the mother in this case) to go through all the biochemical challenges of the 40-week pregnancy without any clinical presentation. This could be related to such residual function of S467C which allows the necessary fatty acid oxidation to proceed. As one mutant with residual function would be sufficient to support the cell function in recessive disease, it does not matter which mutant is found on the other chromosome. So the most common genotype of mCUD was (S467C; R254X). Another allele F17L was also more frequently found among mCUD than clinical presented cases, and it may represent another mild mutant contributing to mCUD.9,18,21

**Excellent Treatment Response But Good Compliance is the Key**

High dose supplement therapy with carnitine is the key treatment for patients. Cederbaum et al reported favourable prognosis after carnitine supplement of the first three ever-diagnosed CUD patients.22 As some of their patients diagnosed after symptomatic attacks or cardiomyopathy, such already established pathology were not reversible. Subsequent to the discovery of OCTN2 gene (SLC22A5), molecular analysis has become a routine test to establish definitive diagnosis when patients present with symptoms (clinical symptomatic patients). Latest implementation of NBS allows the treatment to start even earlier. As early diagnosis and treatment prevent development of metabolic crisis and cardiomyopathy, most NBS-diagnosed patients are expected to live a normal life span free of symptoms.

However, good prognosis is dependent on good compliance to carnitine supplement, irrespective of age. CUD patients are at risk of sudden death if carnitine supplement is abruptly stopped. It might take as short as a few day after cessation of carnitine, cardiac arrhythmia and cardiac arrest might come suddenly leading to fatal outcome.23,24 On the other hand, it is uncertain if asymptomatic carrier (with one mutation) should be treated with carnitine. Some reports suggested improve cardiac function indicator after carnitine supplement25 but many clinicians are not convinced that they should be treated.

**Newborns Are Now Screened for CUD But...**

Many colleagues and us have been advocates for implementations of NBS in Hong Kong for many years. The Hong Kong government started NBS in 2015 for a battery of inherited metabolic diseases including CUD and other fatty acid oxidation defects. Given the population allelic frequencies of common mutations, the incidence of CUD in Hong Kong is estimated to be ~1 in 60,000.26 This estimate matches with early results from our NBS data published in this issue and reported recently.27,28

However, NBS is not 100% sensitive. Early report indicated that sensitivity of NBS for CUD was not perfect.29 So, there is a small proportion of clinical CUD cases that might be missed by NBS and will present at the time of metabolic derangement or cardiomyopathy. Paediatricians need to be alerted of such possibility and carry out appropriate investigations to make the diagnosis when face with such scenario. In fact, this is a very difficult situation. For example, doctors and parents would not readily challenge a negative NBS report. Therefore, clinical
vigilance and readily available investigation algorithms are helpful. A diagnostic algorithm for hypoglycaemia which is the most common presentation of CUD, is shown here as a quick reference (Figure 3).

Hypoglycaemia is a common presentation of many differential diagnoses. From the perspective of IMD, hypoglycaemia could be broadly categorised by presence of urine reducing substances and ketone bodies. These tests are very important bedside dipstick test with good utility in early triage of patients into various diagnostic groups which might need different management. Of course, bedside dipstick urine test results need to be confirmed by subsequent definitive assays in laboratory. It is also very important to collect pre-treatment specimen or any specimen as early as possible for laboratory investigation as treatment might alleviate or alter the biochemical derangement and thus the laboratory results. Hypoketotic hypoglycaemia is the typical feature for the group of IMDs belonging to fatty acid oxidation defect, and CUD is one of them. The diagnosis of CUD may preliminary be made with results of serum free carnitine, acylcarnitine profile, and urine metabolic screening while the definitive diagnosis is made after DNA mutation analysis. Carnitine treatment should be given if clinical suspicious is high or as soon as a preliminary diagnosis is available because it may be lifesaving and CUD patients typically have good response if treated early in the course of biochemical derangement.

**Declaration of Interest**

The authors declare that there is no conflict of interest.

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**Figure 3** Diagnostic algorithm of hypoglycaemia. CUD and other fatty acid oxidation defects are characterised by hypoketotic hypoglycaemia.
References


17. Gandolfi LC, Bahlo M, Speed TP. Dating Rare Mutations from Small Samples with Dense Marker Data. Genetics 2014;197:1315-27.


Abstract

Severe Combined Immunodeficiency Disorders (SCIDs) are a diverse group of monogenic inborn errors of immunity (IEIs) characterised by severe lack of T lymphocyte number and function, with or without affecting B and NK lymphocyte numbers. Severe infections lead to early infant death. SCID infants are seemingly well prior to onset of infections, causing them to miss the golden window for haematopoietic stem cell transplant (HSCT) prior to the age of newborn screening. T cell receptor excision circle is a clinical marker for naive T cell number and can be used to screen for SCID with high sensitivity. International experiences showed that no classical SCIDs have been missed by screening programs so far. Due to extremely favourable outcomes (90% long term survival) for SCID patients picked up by screening and savings incurred by early HSCT, screening for SCID with TREC assay is great value for money. Hong Kong should join the global trend of screening for SCID and save the lives of SCID children.

Key words

Newborn screening; Primary immunodeficiencies; Severe combined immunodeficiency; T cell receptor excision circle

Severe Combined Immunodeficiency Disorder

Severe Combined Immunodeficiency Disorders (SCIDs) are a genotypically and phenotypically diverse group of monogenic diseases in which adaptive immunity is profoundly impaired due to a severe lack of T lymphocyte number and function, with or without low B and NK lymphocyte numbers. SCIDs belong to a large class of disorders known as primary immunodeficiency disorders (PIDs), or in a more recent term, inborn errors of immunity (IEIs), which are mostly due to defects of a single gene in the immune system. Collectively, PIDs are not 'rare' and have an estimated prevalence of 1 in 1200; yet SCID per se occurs at around 1 in 50000 - 1 in 65000 in populations with newborn screening and low consanguinity.

SCID infants have grim prognosis due to severe recurrent infections. Serious infections with both common and opportunistic pathogens, such as Candidiasis, and at times by live vaccines such as Bacillus Calmette-Guérin vaccine, form the classic SCID triad together with chronic diarrhoea and failure to thrive. They are often seemingly well prior to onset of infections at about 2 months, due to the protective effect of residual transplacental maternal IgG in the first months of life. In some patients, transplacental maternal T cells cause graft-versus-host disease (GVHD) due to impaired infant immunity. Atypical, or leaky SCID are caused by hypomorphic mutations, and therefore have a less severe clinical picture and may present after 1 year in life.

In the laboratory, SCID infants usually have absolute lymphocyte counts lower than 3×10⁹/L. Apparently normal lymphocyte count may be due to maternal engraftment, therefore lymphocyte subset analysis demonstrating low

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amount or proportion of CD45RA+ (naive) T cells is also preferred. Primary Immunodeficiency Treatment Consortium diagnostic criteria for typical SCID require less than 300 autologous T cells/mm³ in peripheral blood sample with less than 10% response to PHA when compared against control or demonstration of maternal engraftment. Atypical or leaky SCID, such as Omenn syndrome, is characterised by less severe T lymphopenia. Presence and absence of B cells and NK cells distinguish the type of SCID.

With improving technology and increased use of exome sequencing for SCID patients, in this decade, more than 90% of SCID patients receive a genetic diagnosis; and among the more than 30 causes of SCID, the most common causes of SCID are IL2RG, RAG1/2, and ADA. SCID mostly exhibit autosomal recessive inheritance pattern, except for X-linked IL2RG SCID and moesin deficiency and autosomal dominant BCL11B SCID, RAC2 SCID and complete DiGeorge syndrome. Populations with high consanguinity have a lower proportion of IL2RG SCID. Phenotypic variations may help clinicians identify the candidate gene for sequencing in some cases (Table 1).

Lymphopenia in SCID mostly originates from abnormal development of T, B and/or NK cells. Impaired lymphocyte signalling pathways, such as cytokine signalling (IL2RG, JAK3, IL7R, BCL11B), T cell receptor signalling pathway (CD3D, CD3E, CD3Z, CD45, CORO1A, LAT, ZAP70), major histocompatibility complex class II antigen presentation pathway (RFXANK, CIITA, RFXAP, RFX5) or others (ORAI1, MSN and PGM3) present intrinsic blocks in lymphocyte development. Genetic defects impairing thymus development, the site for T cell maturation, can cause SCID (FOXN1, chromosome 22q11.2 deletion). Inborn errors of metabolism may also affect lymphocytes severely due to metabolic stress or the role of metabolites in immune system signalling, such as inside the purine salvage pathway, adenosine deaminase (ADA) deficiency and purine nucleoside phosphorylase (PNP) deficiency, and outside, adenylate kinase 2 (AK2) which creates cellular ATP supply, are well known to cause SCID. SCID may also arise from mistakes in V(D)J recombination which confers antigen specificity. Null mutations in RAG1/2 which initiates the process by inducing DNA strand breaks and non-homologous end-joining pathway (NHEJ) effectors, including NHEJ1 (Cernunnos), DCLRE1C (Artemis), LIG4 and PRKDC, which repair the breaks, cause arrest in T and B cell development. Genomic instability due to TTC7A, RMRP, RPP25 and SMARCAL1 mutations increase DNA breaks and may cause early death of lymphocytes. As a unique cause of SCID, TPP2 is involved in extra-lysosomal peptide degradation; murine models suggest TPP2 deficiency promotes premature stress-induced senescence in and causes apoptosis of T and B cells, leading to severe immunodeficiency and autoimmunity.

Treatment considerations in SCID infants vary according to their genotype. In general, when a diagnosis of SCID is suspected in an infant presenting with acute infection, they should be put in protective isolation and given aggressive antimicrobial treatment. While Pneumocystis jirovecii prophylaxis (septrin) and antifungal prophylaxis (itraconazole and fluconazole) must be given, acyclovir is reserved for those with history or risk of herpes infection. Live vaccines such as Bacillus Calmette–Guerin vaccine and rotavirus vaccine, unirradiated blood products, and breastfeeding by CMV IgG positive mothers must be avoided. Infants screened for SCID and have received BCG vaccine may begin antimycobacterial treatment. For cure, allogenic haematopoietic stem cell transplant (HSCT) is well-established, with different conditioning regimens for different types of SCID and donors. Younger age (<3.5 months) and infection-free status at transplant have been shown to improve survival significantly in large cohort studies infants who receive transplant earlier than 3.5 months of age have a 94% 5-year survival, compared to 66% for those later than in one cohort. Gene therapy has been shown to be as effective and safe as HSCT in ADA-SCID (Strimvelis®), and is showing great promise for X-SCID and perhaps other forms of SCID.

T Cell Receptor Excision Circle

T Cell Receptor Gene Recombination

The dried blood spot (DBS) high throughput quantitative polymerase chain reaction (qPCR) screening strategy used worldwide nowadays for SCID was first proposed by Chan and Puck in 2005. The invention stems from our knowledge of V(D)J recombination of T cell receptors (TCR). T cell receptors are the cell surface antigen receptors of T cells, and are heterodimers consisting of either α and β, or γ and δ chains, with the α and β combination forming the major TCR type found in thymus and blood. The extracellular portion of the chains fold into the antigen-binding variable region of the receptor, which confers the specificity of the lymphocyte, and the rest constitute the constant region. The diverse repertoire of TCR is created...
### Table 1  Genetics and phenotype variations in severe combined immunodeficiencies

<table>
<thead>
<tr>
<th>Gene</th>
<th>Lymphocyte</th>
<th>Special features</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2RG^</td>
<td>T-B+NK-</td>
<td>The most common SCID, X-linked recessive</td>
<td>~30%</td>
</tr>
<tr>
<td>RAG1/2</td>
<td>T-B-NK+</td>
<td>Autoimmune haemolytic anaemia; Omenn syndrome.9</td>
<td>~20%</td>
</tr>
<tr>
<td>ADA^</td>
<td>T-B-NK-</td>
<td>Low IQ, sensorineural hearing loss, non-infectious pulmonary disease, radiologic skeletal abnormalities, renal sclerosis,10</td>
<td>~10%</td>
</tr>
<tr>
<td>IL7R^</td>
<td>T-B+NK+</td>
<td>Isolated T cell deficiency</td>
<td>~10%</td>
</tr>
<tr>
<td>22q11.2*</td>
<td>T-/+/B+NK+</td>
<td>Chromosome 22q11.2 deletion syndrome (DiGeorge syndrome): conotruncal cardiac anomalies, hypocalcaemia, thymic hypoplasia/aplasia11</td>
<td>~5%</td>
</tr>
<tr>
<td>JAK3^</td>
<td>T-B+NK-</td>
<td>Identical to IL2RG SCID, but autosomal recessive</td>
<td>~5%</td>
</tr>
<tr>
<td>DCLRE1C / Artemis^</td>
<td>T-B-NK+</td>
<td>Sensitivity to ionising radiation12</td>
<td>~5%</td>
</tr>
<tr>
<td>RMRP*</td>
<td>T-B+/NK+</td>
<td>Cartilage hair hypoplasia13</td>
<td>~5%</td>
</tr>
<tr>
<td>CD3D, E&amp;Z^</td>
<td>T-B+NK+</td>
<td>Isolated T cell deficiency</td>
<td>Rare (&lt;5%)</td>
</tr>
<tr>
<td>CD45 / PTPRC</td>
<td>T-B+NK+</td>
<td>Isolated T cell deficiency; can be caused by uniparental disomy14</td>
<td>Rare</td>
</tr>
<tr>
<td>CORO1A</td>
<td>T-B+NK+</td>
<td>Detectable thymus on chest X-ray, Epstein-Barr virus induced lymphoproliferation,15 attention-deficit and hyperactivity disorder16</td>
<td>Rare</td>
</tr>
<tr>
<td>LAT</td>
<td>T-B+NK+</td>
<td>Serious autoimmunity, e.g. autoimmune haemolytic anaemia and immune-mediated thrombocytopenia17</td>
<td>Rare</td>
</tr>
<tr>
<td>ZAP70*</td>
<td>T-B+NK+</td>
<td>Ulcerative colitis, cytopenia, eczema18</td>
<td>Rare</td>
</tr>
<tr>
<td>FOXL1*</td>
<td>T-B+NK+</td>
<td>'Nude SCID': alopecia universalis and nail dystrophy; CNS defect, Omenn syndrome. Present in carriers as nail disease.19</td>
<td>Rare</td>
</tr>
<tr>
<td>BCL11B*</td>
<td>T-B+NK+</td>
<td>Craniofacial abnormalities, agenesis of corpus callosum, umbilical hernia, erythematous psoriasiform dermatitis7</td>
<td>Rare</td>
</tr>
<tr>
<td>SMARCAL1*</td>
<td>T-B+NK+</td>
<td>Schimke immunoosseous dysplasia—spondyloepiphyseal dysplasia, renal insufficiency, cerebral ischaemia, pancytopenia,20,21</td>
<td>Rare</td>
</tr>
<tr>
<td>RFXANK, CIITA, RFX5, RFXAP*</td>
<td>T-B+/NK+</td>
<td>Muscular hypotonia, anhidrotic ectodermal dysplasia24</td>
<td>Rare</td>
</tr>
<tr>
<td>OAR1*</td>
<td>T+B+NK+</td>
<td>Muscular hypotonia, anhidrotic ectodermal dysplasia24</td>
<td>Rare</td>
</tr>
<tr>
<td>PNP*</td>
<td>T-B+/NK+</td>
<td>Hypotonia, delayed development, undetectable uric acid in plasma, brain atrophy25</td>
<td>Rare</td>
</tr>
<tr>
<td>PGM3*</td>
<td>T-B-NK+</td>
<td>Facial dysmorphism, short limbs, atrial septal defect, intestinal malrotation, horseshoe kidney, bilateral exaggerated lesser trochanter26</td>
<td>Rare</td>
</tr>
<tr>
<td>LIG4</td>
<td>T-B-NK+</td>
<td>Characteristic bird-like face, microcephaly, growth and developmental delay, pancytopenia, dermal anomalies, radiosensitivity27</td>
<td>Rare</td>
</tr>
<tr>
<td>NHEJ1 / Cernunnos</td>
<td>T-B-NK+</td>
<td>Microcephaly, growth retardation, sensitivity to ionising radiation28</td>
<td>Rare</td>
</tr>
<tr>
<td>PRKDC / DNAPKcs</td>
<td>T-B-NK+</td>
<td>Granuloma, organ-specific autoimmunity29</td>
<td>Rare</td>
</tr>
<tr>
<td>TTC7A*</td>
<td>T-B-NK+/-</td>
<td>Multiple intestinal atresias,30 very early onset inflammatory bowel disease31</td>
<td>Rare</td>
</tr>
<tr>
<td>RAC2</td>
<td>T-B-NK-</td>
<td>Gain-of-function mutation: myeloid dysfunction8</td>
<td>Rare</td>
</tr>
<tr>
<td>AK2</td>
<td>T-B-NK-</td>
<td>Agranulocytosis, sensorineural deafness32</td>
<td>Rare</td>
</tr>
<tr>
<td>MSN*</td>
<td>T-B-NK-</td>
<td>Neutropenia, monocytopenia6</td>
<td>Rare</td>
</tr>
<tr>
<td>TPP2*</td>
<td>T-B-NK-</td>
<td>Refractory multilineage cytopenia; neurodevelopmental delay33,34</td>
<td>Rare</td>
</tr>
<tr>
<td>RPP25*</td>
<td>Unclear</td>
<td>To be reported</td>
<td>Rare</td>
</tr>
<tr>
<td>12p duplication*</td>
<td>Unclear</td>
<td>Pallister-Killian syndrome: hypotonia, intellectual disability, hearing and vision impairment, facial anomalies35</td>
<td>Rare</td>
</tr>
</tbody>
</table>

^Mainly manifests as typical SCID^9

*Yet to be listed as a cause of severe combined immunodeficiency but may be included in other categories in the International Union of Immunological Societies inborn errors of immunity classification updated in November 2019^36
Figure 1  Immunophenotypes of major types of severe combined immunodeficiencies.

Figure 2  Pathogenesis of SCID.

Figure 3  V(D)J recombination at the TCRA/D gene cluster on chromosome 14.
by recombination in the individual segments of the TCR
genes – V stands for variable, D for diversity and J for
joining, as shown in Figure 3. Each TCR gene contains
several alternative versions of these gene segments for
diversity. To initiate the process, the recombination-
activating genes (RAG1/2) complex recognises the
recombination signal sequences that flank the gene
segments and creates double-strand DNA breaks which are
repaired by nonhomologous end joining (NHEJ), forming
a signal joint T-cell receptor excision circle (sjTREC) and
a coding joint T-cell receptor excision circle (cjTREC).
Quantification of the signal joint TREC formed at the
TCRA/D gene cluster on chromosome 14 in blood is
therefore an indicator of naive T cells that have completed
TCR VDJ recombination.

**Measuring TREC**

DNA contained in the dried blood spots is isolated; and
TREC and a control gene fragment, e.g. beta-actin or RNase
P, are quantified by qPCR. The average healthy newborn
would have approximately 250 copies of TREC per
microliter of blood. SCID infants, who have very low naive
T cells, can be identified by very low or zero TREC count
regardless of exact genetic aetiology. In the USA,
Wisconsin state began the world’s first SCID TREC DBS
NBS pilot in 2008, and many countries have since then
started their own pilots using in-house developed assay or
commercial product. To date, only one commercial product,
the PerkinElmer EnLite™ Neonatal TREC kit, has been
approved by the US Food and Drug Administration for
SCID newborn screening.

Alternative screening methodologies have since then
also been developed for SCID. Vidal-Folch et al identified
the room for improvement in the qPCR method and
proposed the use of multiplex droplet digital PCR (ddPCR)
instead in 2017, which would allow the measurement of
absolute TREC amount without normalisation, repetition
nor standardisation. Initial results from a small group of
subjects have been encouraging as expected, yet the
feasibility and cost-effectiveness of the technology being
implemented for large-scale population screening should
be further investigated. To increase coverage of the qPCR

### Table 2: Findings of published SCID TREC screening pilot services worldwide

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay choice</td>
<td>EnLite™</td>
<td>Mixed</td>
<td>Mixed</td>
<td>EnLite™</td>
<td>EnLite™</td>
<td>EnLite™</td>
<td>In-house</td>
<td>In-house</td>
</tr>
<tr>
<td>Screened</td>
<td>3252156</td>
<td>3030083</td>
<td>920398</td>
<td>190517</td>
<td>177277</td>
<td>130903</td>
<td>58834</td>
<td>5160</td>
</tr>
<tr>
<td>Low TREC</td>
<td>562 (0.02%)</td>
<td>1265 (0.04%)</td>
<td>173 (0.02%)</td>
<td>165 (0.09%)</td>
<td>46 (0.03%)</td>
<td>30 (0.02%)</td>
<td>16 (0.03%)</td>
<td>5 (0.1%)</td>
</tr>
<tr>
<td>TCL</td>
<td>213</td>
<td>463</td>
<td>136</td>
<td>62</td>
<td>35</td>
<td>21</td>
<td>N/A</td>
<td>5</td>
</tr>
<tr>
<td>Total SCID</td>
<td>50</td>
<td>52</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(incidence)</td>
<td>(1 in 65000)</td>
<td>(1 in 58000)</td>
<td>(1 in 130000)</td>
<td>(1 in 32000)</td>
<td>(1 in 22000)</td>
<td>(1 in 131000)</td>
<td>(1 in 59000)</td>
<td></td>
</tr>
<tr>
<td>Typical SCID</td>
<td>39</td>
<td>42</td>
<td>7</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>Leaky SCID</td>
<td>11</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>SCID missed</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SCID survival</td>
<td>0.94</td>
<td>0.87</td>
<td>1</td>
<td>0.67</td>
<td>0.88</td>
<td>1</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Non-SCID TCL</td>
<td>163</td>
<td>411*</td>
<td>125</td>
<td>56</td>
<td>27</td>
<td>20</td>
<td>N/A</td>
<td>4</td>
</tr>
<tr>
<td>Syndromes</td>
<td>72</td>
<td>136</td>
<td>34</td>
<td>7</td>
<td>9</td>
<td>6</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>Preterm</td>
<td>33</td>
<td>29</td>
<td>59</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>N/A</td>
<td>2</td>
</tr>
<tr>
<td>Secondary</td>
<td>25</td>
<td>117</td>
<td>24</td>
<td>15</td>
<td>4</td>
<td>2</td>
<td>N/A</td>
<td>2</td>
</tr>
<tr>
<td>Idiopathic*</td>
<td>33</td>
<td>12</td>
<td>8</td>
<td>27</td>
<td>5</td>
<td>10</td>
<td>N/A</td>
<td>0</td>
</tr>
</tbody>
</table>

*Includes newborn screening programmes from 11 states
*California switched from in-house assay to EnLite™ neonatal TREC kit in 2015
*Information for 117 infants with non-SCID T cell lymphopenia not available
*Idiopathic T cell lymphopenia includes both persistent and transient T cell lymphopenia of unknown causes
to X-linked agammaglobulinemia (XLA), a B cell defect in which mutated Bruton tyrosine kinase leads to near absence of CD19+ B cells, Borte et al developed a triplex qPCR for detection of TREC, kappa-deleting recombination excision circle (KREC), the equivalent of TREC in B cells, and a control gene.52 Alternatively, TREC assay can be multiplexed to \textit{SMN1} assay, allowing the detection of patients with spinal muscular atrophy (SMA) as well.53 The practice of including certain conditions in the national screening programmes yet varies among regions, but it is believed that more conditions will be added to newborn screening for the benefit of more rare disease patients at a lower added cost as both XLA and SMA can be screened by the same methodology as SCID.

### Potential New Methods

Apart from PCR, new screening methodologies are being developed to screen for more primary immunodeficiencies. Collins et al investigated the application of peptide immunoaffinity enrichment coupled with selection reaction monitoring mass spectrometry (immuno-SRM) to assay for Wiskott-Aldrich syndrome protein (WASP), BTK and CD3E proteins on dried blood spots as markers for Wiskott-Aldrich syndrome, an immunodeficiency with neutropenia or thrombocytopenia, XLA and SCID.54 In the future, with discovery of novel disease markers, lowered price of cutting-edge laboratory assays and new testing methods, more and more primary immunodeficiency patients can benefit from population screening.

### International SCID Screening Experience

Ever since the first SCID TREC NBS pilot started in Wisconsin in 2008, numerous feasibility studies and pilot services have been rolled out around the world, screening millions of neonates. Beginning from December 2018, all newborns in the United States are screened for SCID. The biggest and most representative study so far published is perhaps the California TREC screening service, which has screened 3.3 million infants in the period of 6.5 years between 2010 and 2017.55 Experiences of population screening with TREC have also been published by programs in Catalonia, Seville (Spain), France, Israel, Sweden, Taiwan and other states in the US.56-64 These pilot studies offer a large amount of useful data and new insights about T cell lymphopenia, demonstrating the effectiveness, feasibility and vital importance of SCID screening in all situations.

### Screening Protocol

While in-house assays are occasionally used by population screening programs worldwide, the PerkinElmer EnLite\textsuperscript{TM} Neonatal TREC kit is widely used, including in California (after 2015), Catalonia, France, Israel, Taiwan (Taipei Institute of Pathology) et cetera. Multiple pre-service evaluative studies using the PerkinElmer EnLite\textsuperscript{TM} Neonatal TREC kit on anonymised dried blood spots have also been published, including those by Australian (Victoria), Dutch, and Saudi scientists.65-67 Taiwan's Chinese Foundation of Health is unique in using the Roche LightMix\textsuperscript{®} Modular TREC assay in their service.62

There are various screening algorithms used by these studies as decided locally by the relevant authorities. Prior to screening pilot service, a retrospective study using already collected dried blood spots by pre-existing newborn screening programmes is common to determine the initial cut-off values of the pilot service and to verify the performance of the assay. The cut-off value is set cautiously and will be revisited from time to time to balance sensitivity and recall rate as more data accumulate. Availability of resources is also a factor. Dried blood spots may first be tested in singlicate for TREC only, which if below a higher threshold, triggers re-test in singlicate or duplicate for TREC and control gene against a lower final threshold. An urgent threshold may also be defined to implicate a very high chance of SCID. Special neonate groups, e.g. those in neonatal intensive care or born preterm before 37 weeks may have a different threshold. Whenever the control gene level falls below a pre-defined threshold, the result is invalidated and necessitates a second dried blood spot be taken. In some programmes, a confirmed low TREC leads to immediate evaluation of the patient by immunologists with flow cytometry, but in others a second dried blood spot being taken, if not below the urgent threshold, if any. After flow cytometry, targeted sequencing or next generation sequencing confirm the diagnosis.

### Patients with Low TREC

The number of patients detected with low TREC depends highly on the cut-off level of TREC in populations without widespread consanguinity or known founder mutations for SCID. As shown in Table 2, percentage population with low TREC levels or referred to flow cytometry varies between 0.1% and 0.02%. All patients
with low TREC would be given flow cytometry, perhaps except in programmes with long delay in result confirmation in which patients may die before given a chance for laboratory testing.

T cell receptor excision circle is a clinical marker for naive T cell lymphopenia (TCL). Incidence of TCL revealed by TREC screening has been found to be between 1 in 1030 (Seville, Spain) and 1 in 15300 (California, US), which may be explained by the threshold for TREC levels that trigger immunological evaluation and different diagnostic thresholds for TCL. Having a higher threshold for TREC level may capture more cases of TCL, in particular non-SCID TCL, yet will inevitably increase the false positive rate. In contrast, SCID incidence is more similar amongst different populations at around 1 in 58000. In the California study, for every SCID newborn found, 3 cases of non-SCID TCL were found, and 6 had low TREC but normal T cell numbers.55

Causes of non-SCID TCL can be grouped into syndromic, preterm, secondary or idiopathic. DiGeorge syndrome (DGS), in which 22q11.2 is deleted in the infant is the predominant cause of syndromic non-SCID TCL.68 The chromosomal microdeletion syndrome causes conotruncal cardiac anomalies, hypocalcaemia and the most relevant in this case, thymic hypoplasia, which leads to variable T cell deficiency. Thymic aplasia happens in 1% of DiGeorge syndrome patients, dubbed complete DGS, causes severe combined immunodeficiency. Infants with DiGeorge syndrome may have very low to normal TREC depending on the level of thymic hypoplasia.69 Other known causes of non-SCID T lymphopenia syndromes include trisomy 21, trisomy 18, ataxia-telangiectasia, CHARGE syndrome, diabetic embryopathy, et cetera.63,68 These patients may benefit from immune interventions such as IgG infusion. Complete DGS patients may require thymic transplant or haematopoietic cell transplant. In the California study, 10 out of 72 cases of non-SCID TCL syndromes required immune interventions apart from live vaccine avoidance; 9 out of 72 cases died of non-immune causes and 0 of immune causes.68 Apart from syndromic causes, preterm T cell lymphopenia is an expected secondary target of TREC screening, and secondary causes would include third space displacement, chylothorax, maternal use of immunosuppressive agents during pregnancy, neonatal leukaemia and more.55,60 T cell deficiency in these scenarios are reversible. Idiopathic T cell lymphopenia is an incidental finding of TREC screening programs. In the New York cohort, patients have variable clinical features.70 They remain clinically stable but both the causes and outcomes of their conditions are unclear. Continued monitoring is needed to understand the condition, thus their detection by TREC screening programs may be of benefit.

**Implications for SCID Patients**

As shown by data reported in Table 2, TREC screening is effective in picking up SCID. Except for 2 infants with
leaky SCID missed by the California program, none of the other programs reported missed SCID patients, giving TREC screening programs 100% sensitivity on classical SCID. The 2 cases of leaky SCID missed by the Californian NBS had sufficiently hypomorphic mutations in IL2RG and ADA genes, causing TREC levels to be significantly above the screening cut-off in the neonatal period, and were successfully treated by haematopoietic cell transplant and gene therapy respectively. For the patients that have not been missed, prognosis is generally good with higher than 85% survival reported in most cohorts. SCID patients that are picked up early have better outcome in haematopoietic stem cell transplant as shown in several large studies as previously mentioned. As higher standard of care, better haematopoietic stem cell transplant conditioning regimen and more therapeutic options such as gene therapy, become available for SCID patients, the survival rate for SCID patients may approach 100%.

Live vaccines such as BCG vaccine and rotavirus vaccine form a part of universal childhood vaccination program in many countries, yet SCID patients exposed will be at risk of developing vaccine-related infections. SCID patients identified through newborn screening can avoid those vaccines, but only if the vaccines are not administered before screening results become available. For example, Taiwanese vaccination schedule once included the BCG vaccine on the first day of life, yet it was postponed to 1-4 weeks in 2012 for newborns participating in a new SCID TREC screening pilot at that time, and was further delayed to 5-8 months after it was found that postponing BCG vaccination in the population did not increase the cases of neonatal tuberculosis and did not slow down the downward trend of TB incidence.

**Financial and Ethical Justifications**

**Early SCID HSCT is Cheaper than Late**

Cost-effectiveness is one of the considerations when pursuing public health policy. Several papers evaluating the financial aspects of TREC newborn screening have been published, either evaluating real field data of patients with newborn screening or making estimations based on patients with early HSCT. Dutch analyses by Van der Ploeg et al estimated that newborn screening reduces the cost of treating SCID in a population of 100,000 by 187,000 EUR (207,000 USD; 1,620,000 HKD) while the cost of screening the same population is 609,800 EUR (676,000 USD; 5,300,000 HKD), which is estimated to lead to the gain of 11.7 quality-adjusted life years (QALYs) in the same population, resulting in a cost-utility ratio of 33,400 EUR/QALY (37,000 USD; 290,000 HKD) gained and may be considered cost-effective. The UK newborn screening council review conducted in 2017 had an even more favourable cost-utility ratio estimate of 17,600 GBP/QALY gained (23,000 USD; 179,000 HKD).

While the exact costs of screening and treatment vary among localities due to socioeconomic factors, experts generally agree that newborn screening can reduce cost of treating SCID due to shortened diagnostic journey, reduced cost of treating complications which could be prevented by prophylaxis and placing infants in protective isolation, and reduced cost of treating morbidities in the future because of better outcomes from early HSCT. Costs of screening may be further reduced by controlling turn-around time, which determines the timing of institution of prophylactic measures, thus the chances of infection before HSCT and therefore its cost; and the recall rate for immunological evaluation, reducing the diagnostic costs associated with false positive samples.

**Ethical Considerations**

The appropriateness of including SCID in any population newborn screening programme is well proven by experiences from all around the world. The epidemiology and natural history of SCID are now more well understood than ever – it is a severe disease that requires early aggressive treatment. There are no primary prevention measures available, other than carrier testing, which can only be done in minority of cases with family history of diagnosed SCID. TREC assay is a well-validated, 100% sensitive assay for typical SCID. The sample required, dried blood spots, can be collected from neonates with minimal risk. Haematopoietic stem cell transplant is a potentially curative treatment for SCID patients, and outcomes such as survival and immune reconstitution improve when HSCT is offered early.

As with any screening programmes, the specificity of the first-tier testing is low as the cut-off is set to increase sensitivity. Half of the newborns with low TREC have normal T cells. Yet, it is a reasonable sacrifice to save the lives of infants with serious conditions. Physicians and other medical professionals involved in the screening process must explain the implication of a positive TREC test to the family accurately. Patients with conditions other than SCID diagnosed by TREC screening should be referred
to appropriate specialties so they may benefit from early
detection as well. Studies on idiopathic T cell lymphopenia
should continue to better understand the pathogenesis and
optimal treatment for such patients.

**SCID Screening for Hong Kong**

**Local SCID Care Experience**

The Queen Mary Hospital is the tertiary and teaching
hospital affiliated with the University of Hong Kong and
has been the referral centre for patients diagnosed with
primary immunodeficiencies in the region. The Asian
Primary Immunodeficiency Network founded by the
authors offer free molecular diagnostics for suspected
primary immunodeficiency, including SCID. We have
diagnosed over 100 cases of SCID, and our experience has
culminated in several publications.

Between years 1991 and 2017, 15 infants with SCID
have been referred to Queen Mary Hospital, including 13
classical SCIDs and 2 leaky SCIDs. Six were X-SCID
(IL2RG), 3 classical Artemis SCID, 2 leaky RAG1 SCIDs,
and 1 each for JAK3 deficiency and MHC II deficiency
(RFXANK). Two infants have perished before molecular
diagnosis could be given. Only 12 of the 15 SCID patients
lived long enough to receive HSCT, and for those treated
we have an 83% long-term survival rate. Among them,
patients 2b and 9b were diagnosed early and received
HSCT early by 3.5 months of age due to the family history
of their siblings 2a and 9a. 2b and 9b are still alive as of
last follow up and do not require immunoglobulin therapy.

Based on 1.75 million accumulated births in that same
period (1991-2017) in Hong Kong, and on an estimated
population incidence of 1 in 60,000, one could calculate
that 29 SCID infants have been born in that period, only
15 of them received the diagnosis, and only 10 of them
were saved assuming all SCID infants diagnosed in Hong
Kong have been diagnosed by us and undiagnosed SCID
infants have 100% mortality. The long-term survival for a
SCID infant born in Hong Kong is therefore 34%. This
figure could be improved significantly with newborn
screening and rise to above 90%.

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**Figure 5** Diagnostic and therapeutic journey of 15 SCID patients diagnosed in 1991-2017 in Hong Kong.

Note: False positive cases refer to infants with low TREC but normal lymphocyte subset results.
The Swedish study is excluded due to inadequate information.
Proposed SCID Pilot

Our data prove that Hong Kong has a great need of screening for SCID, yet currently there is no SCID newborn screening in Hong Kong. Recently, the newborn screening pilot for inborn errors of metabolism (IEM) has been completed in Hong Kong, and it is based on mass spectrometry of dried blood spots. A territory-wide screening service for IEM is expected to begin in all public hospitals. There is a well-developed and mature service model which includes steps in universal newborn screening beginning from informed consent collection and recall and counselling. As SCID TREC screening programs typically use dried blood spots as well, adding severe combined immunodeficiency, and in the future other conditions such as spinal muscular atrophy to the IEM screening programme would be highly economical and add little additional workload to frontline postnatal staff. Follow-up of low TREC infants can be handled centrally in the Hong Kong Children’s Hospital by paediatric immunologists and clinical geneticists. The authors estimate that the annual budget for screening the expected birth cohort of 55000 infants will only increase by less than 3 million HKD if SCID TREC screening is included. With the many reports of success in saving lives of SCID patients with newborn screening worldwide, the time has come for Hong Kong to join the world in this respect.

Acknowledgements

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Declaration of Interests

The authors declare no competing interests.

References


75. Lau YL. Asian network for molecular diagnosis of primary immunodeficiencies. BMC Proceedings 2011;5(S1).


The First Case of Homocystinuria Picked Up by Newborn Screening in Hong Kong: A Case Report

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Abstract
Classical homocystinuria is a rare metabolic disease among the local Chinese population. We report a case of pyridoxine non-responsive classical homocystinuria who was picked up by the Hong Kong government's territory wide newborn screening programme which was started in 2015.

Key words
Cystathionine beta-synthase deficiency; Homocystinuria; Newborn screening; Pyridoxine unresponsive

Introduction
The success of the pilot newborn screening (NBS) programme conducted between October 2015 and March 2017 in two public obstetric units encouraged the extension of the programme to cover newborn babies born in all the public obstetric units in Hong Kong. The programme screened for 24 metabolic conditions. In this case report, we report the clinical course of a newborn baby who was flagged as a possible case of homocystinuria with elevated methionine level and was subsequently confirmed to have classical homocystinuria.

Case Report
The proband was the firstborn of a non-consanguineous Chinese couple who enjoyed good past health. There was no significant family history of note. She was born at 40 weeks of gestation after an uncomplicated antenatal course with a birth weight of 3.1 kg. Her first newborn screening at 48 hours of life showed an elevated methionine (Met) level of 84.8 µmol/L (reference range [RR] 11-32 µmol/L). Both methionine-to-phenylalanine (Met/Phe) ratio and methionine-to-sum of the isobaric amino acids leucine, isoleucine, hydroxyproline (Met/Xle) ratio were elevated at 1.44 (RR 0.22-0.51) and 0.77 (RR 0.1-0.32) respectively. Repeated screening on day 5 showed further elevation of Met to 214 µmol/L (RR 8-26 µmol/L). The baby remained asymptomatic all along with normal physical examination. Further workup confirmed an elevated methionine level of 292 µmol/L (RR 10-60 µmol/L) on the plasma amino acid profile and total homocysteine (Hcy) level was significantly elevated to 150.4 µmol/L (RR 2.9-10 µmol/L). Molecular genetic study by Sanger sequencing identified two novel compound heterozygous likely pathogenic mutations at c.439T>A p.(Ser147Thr) and c.912A>C p.(Glu304Asp) in the Cystathionine beta-synthase CBS gene. Both parents are heterozygous carriers of the CBS variants. These novel missense variants are classified as likely pathogenic according to the American College of Medical Genetics guideline as they exist in trans, are absent from the Genome Aggregation Database and predictions using multiple algorithms showed that both variants are pathogenic consistently. The proband was initially treated with pyridoxine and folic acid. Despite increasing to a high dose of 100 mg/day pyridoxine for a total of 3 weeks, there was no appreciable drop in the total homocysteine level. Upon the lack of response to the
medical treatment, the baby was started on a low methionine diet as prepared by mixing methionine-free and normal formula. Her methionine tolerance was gradually worked out through regular monitoring of the plasma total homocysteine and methionine levels. The patient was last seen at follow up at 18 months of age. She enjoyed normal growth and development.

Discussion

Classical homocystinuria (MIM 236200), otherwise known as Cystathionine beta-synthase deficiency is a methionine catabolic pathway disorder with variable prevalence amongst different ethnic backgrounds. It is most commonly reported in Qatar with a frequency up to 1:1800 but is considered exceedingly rare in the Chinese population. Homocysteine (Hcy) is a non-structural amino acid produced during the catabolism of methionine. Hcy will be further converted into cystathionine with the help of the enzyme Cystathionine beta-synthase (CBS). In classical homocystinuria, the absence of CBS leads to an ineffective Hcy conversion resulting in methionine and homocysteine accumulation.

Classical homocystinuria is a clinically heterogeneous condition. It mainly manifests with problems in the eyes, and skeletal, central nervous and vascular systems. As patients can present to different specialists for their clinical problems, diagnosis is often delayed. Clinical features include ectopia lentis, severe myopia, Marfanoid habitus, osteoporosis, bony deformities, thromboembolism, developmental delay, intellectual disability, seizure, psychiatric and behavioural problems. Great variations exist in terms of the age of onset, symptoms and progression of the disease. While some of the affected individuals can present in childhood with severe multisystemic disease, others remain asymptomatic throughout life. The phenotypical diversity may be partly related to the degree of pyridoxine-responsiveness although the exact pathophysiology of classical homocystinuria is yet to be fully understood. Mildly affected patients may present as adults with thromboembolism and are likely to respond to treatment with pyridoxine. The pyridoxine responsive patients could attain a normal homocysteine level after a small amount of pyridoxine supplementation. The largest cohort reported by far suggested only 44% of CBS deficiency patients are pyridoxine-sensitive. However as some of the individuals with pyridoxine-sensitive disease may remain asymptomatic, this mild spectrum of the disease might be considerably underdiagnosed. In contrast, the more severely affected patients usually present in childhood with ectopia lentis, learning difficulties and skeletal abnormalities. These patients are usually managed with a low-methionine diet and/or betaine. Early diagnosis is the key to reduce complications and long term morbidity among the affected patients. As symptomatic patients can present in a wide variety manner to a wide range of specialists including paediatricians, ophthalmologists, haematologists, neurologists, psychiatrists, orthopaedic surgeons, cardiologists, vascular specialists and clinical geneticists, it is very important that their symptoms be recognised and appropriate referrals and investigations follow.

The biochemical diagnosis of homocystinuria is made on the basis of an elevated plasma total homocysteine together with a high or borderline high level of plasma methionine. It is important to note that total homocysteine should be requested as a separate assay which is not routinely covered in the plasma amino acid profile. The availability of newborn screening enables homocystinuric patients to be picked up early and presymptomatically through an elevated methionine level on the dried blood spot cards. Majority of the individuals identified from newborn screening are subsequently proven to be pyridoxine unresponsive as is our patient. It is estimated that up to 50-80% of the homocystinuric patients picked up by newborn screening are pyridoxine unresponsive. Most pyridoxine unresponsive patients require a diet that is low in natural protein, with supplements of a Met-free special formula. This dietary treatment needs to be lifelong and is highly successful in preventing almost all the complications of classical homocystinuria, whilst maintaining normal growth and development.

Without treatment, life expectancy is markedly reduced in patients with classical homocystinuria especially the pyridoxine unresponsive patients. When life-long treatment is started early in life, most patients are observed to have good outcomes. The importance of early detection by newborn screening cannot be overemphasised. It is hoped that with the implementation of a universal newborn screening programme, affected individuals could be diagnosed at the earliest instance and have a good prognosis and lead healthy and normal lives. Classical homocystinuria will then no longer be a cause of intellectual disability in our community.
Conflict of Interest

The authors declare they have no competing interest.

References


Case Report

Clinical Course of Two Newborns Affected by Cobalamin C Deficiency Diagnosed in the Pre and Post Newborn Screening Era

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Abstract

Cobalamin C deficiency is the best understood and most common phenotype within the group of intracellular cobalamin disorders. The clinical phenotype can range from a disastrous, heterogeneous multisystem disease presenting in the newborn period, to a milder disease with acute or slowly progressive neurological symptoms and behavioural disturbances. Here we report 2 cases of confirmed cobalamin C deficiency. The first case was diagnosed in the pre and the second case in the post newborn screening (NBS) era in Hong Kong. The dramatic difference in the clinical courses of these two patients highlight the importance of early diagnosis and treatment in improving the outcomes of affected individuals through a successfully implemented NBS programme.

Key words

Cobalamin C; Homocysteine; Methylmalonic acidaemia; Newborn screening

Introduction

Cobalamin (or vitamin B12) is a cobalt-containing water soluble vitamin found in animal products such as milk and meat, and plays an essential part in amino acid metabolism.1 This involves complex absorption, transport systems and multiple intracellular conversions for cobalamin to form two active co-enzymes, methylcobalamin in the cytosol and adenosylcobalamin in the mitochondrion.

Cobalamin C deficiency results from mutations in the MMACHC gene which lead to impaired conversion of dietary cobalamin to its two metabolically active forms, methylcobalamin and adenosylcobalamin. They are essential coenzymes to methionine synthase and methylmalonyl-CoA mutase, whose functional deficiency lead to homocysteinaemia combined with methylmalonic acidaemia. An increase of homocysteine with low levels of methionine in combination with methylmalonic acidaemia are biochemical hallmarks of cobalamin C deficiency.2

Without active metabolites of cobalamin, accumulation of methylmalonic acid and homocysteine leads to neurotoxicity, nephrotoxicity and vascular damage. Depending on the severity of the deficiency, the clinical phenotype can range from a disastrous, heterogeneous multisystem disease presenting in the newborn period, to a milder disease with acute or slowly progressive neurological symptoms and behavioural disturbances. Treatment is with parenteral hydroxycobalamin, betaine and folic acid. Haemolytic uraemic syndrome (HUS) as a consequence of vascular damage is one of the known neonatal presentations of cobalamin C deficiency.3

Dried blood spot tandem mass spectrometry-based...
newborn screening (NBS) enables babies with cobalamin C deficiency to be picked up with an elevated propionylcarnitine (C3) acylcarnitine. This finding together with a high C3/C2 carnitine ratio suggest a variety of disorders of propionate metabolism, including methylmalonic acidaemia, propionic acidaemia, and congenital cobalamin defects including cobalamin C deficiency.

The NBS programme for inherited metabolic diseases started in Hong Kong as a pilot programme since 2015. Here we report a case of cobalamin C deficiency who presented symptomatically with HUS diagnosed before the era of NBS in Hong Kong. The second case was diagnosed pre-symptomatically when the baby's NBS showed an abnormal result with an elevated C3 carnitine.

Case 1

A male neonate was born in 2014 by normal spontaneous vaginal delivery with a birth weight of 3.45 kg as the second child of unrelated Chinese parents with uneventful antenatal and postnatal courses.

He presented to the emergency department at 45 days of age with one-week history of progressive worsening irritability, shortness of breath, choking and coughing during feeds. Upon admission, examination revealed tachycardia, hypertension, marked pallor and generalised oedema. A complete blood count revealed pancytopenia with Hb 4.6 g/dL, WBC 5.5x10^9/L, platelets 76x10^9/L. Serum chemistries showed metabolic acidosis with blood pH 7.25, pCO2 5.0, BE -10.0 and renal failure with severe hyponatraemia (Na 115 mmol/L), hyperkalaemia (K 8.2 mmol/L), and mildly elevated urea of 8.9 mmol/L and creatinine of 42 µmol/L. Urinalysis was positive for protein and blood. His electrocardiogram was normal. Sodium bicarbonate, calcium gluconate and resonium were given for control of the hyperkalaemia and metabolic acidosis.

A complete blood count revealed pancytopenia with Hb 4.6 g/dL, WBC 5.5x10^9/L, platelets 76x10^9/L. Serum chemistries showed metabolic acidosis with blood pH 7.25, pCO2 5.0, BE -10.0 and renal failure with severe hyponatraemia (Na 115 mmol/L), hyperkalaemia (K 8.2 mmol/L), and mildly elevated urea of 8.9 mmol/L and creatinine of 42 µmol/L. Urinalysis was positive for protein and blood. His electrocardiogram was normal. Sodium bicarbonate, calcium gluconate and resonium were given for control of the hyperkalaemia and metabolic acidosis.

Multiple packed cell transfusions were given for correction of anaemia. Other pertinent initial investigations showed an increased reticulocyte count of 4.6%, elevated D dimers, low haptoglobin of <0.2 g/L and elevated C3 carnitine of 7.9 µmol/L (ref <0.88 µmol/L). Clotting profile was normal. Blood film revealed increased schistocytes with mild polychromasia and moderate thrombocytopenia. The clinical picture was consistent with microangiopathic haemolytic anaemia. He was transfusion dependent, requiring packed cells, platelet and fresh frozen plasma transfusions every 1-2 days.

Despite maximal supportive measures the patient's renal condition deteriorated with development of oedema, renal failure and persistent hypertension requiring multiple antihypertensives including IV hydralazine boluses and a labetolol infusion. Glomerular filtration rate was calculated to be only 13 ml/min/1.73 m^2 with persistent haematuria and proteinuria.

Because of the baby's unresponsiveness to supportive therapies, plasmapheresis was initiated. Unfortunately, it was complicated with bilateral acute subarachnoid haemorrhage and hydrocephalus requiring urgent decompression and craniectomy. He went on to developed seizures requiring midazolam infusion, and cardiopulmonary compromise with heart failure requiring inotropic and mechanical ventilatory support.

With the early age of atypical presentation of HUS, inherited causes were considered likely and metabolic investigations were initiated. Urine metabolic screen revealed a moderate increase in methylmalonic and methylcitric acids. Plasma homocysteine was markedly elevated to 107 µmol/L (ref 0-1 µmol/L). This finding together with the elevated cystathionine and low methionine of 3.4 µmol/L (ref 9-42) on the plasma amino acid profile suggested possible cobalamin C deficiency. The diagnosis was finally confirmed with targeted genetic testing of the MMACHC gene. Heterozygous MMACHC NM_015506.2:c.398_399delAA, p.(Gln133Argfs*5) and heterozygous MMACHC NM_015506.2:c.609G>A, p.(Trp203*) mutations were detected. Both mutations are predicted to result in truncated proteins and are known to be pathogenic.

Following commencement of subcutaneous hydroxocobalamin, our patient's blood counts stabilised, blood pressure normalised, renal function and urine output improved, seizures stopped and he was successfully extubated. His total duration of intensive care stay was 45 days. He has remained stable since discharge with no relapse and stable serum total homocysteine and methionine levels. Current medications include hydroxocobalamin, betaine, folinic acid and levocarnitine. He was diagnosed with global developmental delay and severe visual impairment on subsequent follow up. At his last follow up at 4.5 years of age, he continues to show developmental progress with a latest developmental assessment of motor development of around 24 months and language development of around 12-15 months.
Case 2

A male neonate was born at full-term by normal vaginal delivery with a birth weight of 2.92 kg. He was the second child of non-consanguineous Chinese parents with unremarkable family history. His antenatal history was unremarkable. He had perinatal pneumonia with respiratory distress soon after birth, requiring non-invasive ventilatory support for two days and a course of intravenous antibiotics.

The baby boy underwent newborn screening with dried blood spot collected at 24 hours of life. He was found to have elevated C3-carnitine 6.6 µmol/L (ref 0.51-2.5 µmol/L), elevated C3/C2-carnitine ratio 0.31 (ref 0.03-0.12), and low methionine level 4.68 µmol/L (ref 11-32 µmol/L). Clinically, the baby was feeding well. He did not have lethargy or seizure. He was well hydrated and tone was normal. Further workup for metabolic diseases was performed. Cobalamin C deficiency was diagnosed based on abnormal biochemical findings including raised C3-carnitine level 10.5 µmol/L (ref <0.88 µmol/L), elevated total plasma homocysteine level 149.5 µmol/L (ref 2.9-10 µmol/L) and low methionine level <3 µmol/L (ref 13-44 µmol/L). Both vitamin B12 level of 868 pmol/L (ref 145-569 pmol/L) and folate level of >45.4 nmol/L (ref 12.0-30.0 nmol/L) were higher than the normal reference ranges and maternal vitamin B12 level was also normal.

Urine organic acids showed marked increase in methylmalonic acid and small amount of methylcitric acid. Complete cell count was normal except for borderline thrombocytopenia 129x109/L. He did not have any metabolic acidosis, and renal function was normal.

Targeted genetic analysis confirmed cobalamin C deficiency with homozygous MMACHC NM_015506.2: c.609G>A, p.(Trp203*) mutations.

The baby boy was started on intramuscular hydroxocobalamin 1 mg injections daily since day 11 of life and oral betaine since day 12 of life. Betaine was gradually titrated up from 100 mg/kg/day to 250 mg/kg/day as tolerated. His homocysteine levels dropped significantly and methionine levels normalised with treatment (Figure 1). He was continued on normal feeds with no protein restriction. Hydroxocobalamin 1 mg injections was reduced to three times per week since four weeks of life when his homocysteine levels remained below 50 µmol/L. Dried blood spot showed normal C3-carnitine 0.95 µmol/L (ref 0.25-1.32 µmol/L) and methionine 13 µmol/L (ref 8-26 µmol/L) on day 14 of life. He was progressing well at six months old on his last follow-up. His growth parameters including head circumference and weight were on track with head circumference at 25th-50th centile and weight at 75-90th centile. His development was age appropriate. He had normal neurological examination with no seizures.

Figure 1  Homocysteine and methionine level.
Discussion

The incidence of cobalamin C deficiency estimated through newborn screening from the state of California in the United States of America is in the range of 1:67,000.\textsuperscript{4} California is known to have an ethnically diverse population. When compared to ethnic Chinese population from Mainland China, the incidence of cobalamin C deficiency in China appears much higher with an estimated incidence of 1:16833.\textsuperscript{5,6} It is the more prevalent type of methylmalonic acidemia.\textsuperscript{5} The availability of tandem mass spectrometry based newborn screening enables babies with cobalamin C deficiency to be picked up through elevated levels of propionylcarnitine (C3 carnitine) and low methionine. With the implementation of universal NBS for inherited metabolic diseases in Hong Kong, early diagnosis and early initiation of treatment can prevent irreversible neurological damage as well as many other disease related complications. This point was well demonstrated by the relatively uneventful clinical course of our second patient in contrast to the catastrophic clinical course of our first patient who was only diagnosed after symptomatic presentation.

Out of all the different types of cobalamin disorders, cobalamin C deficiency is the most prevalent among the Chinese population. The MMACHC gene mutation NM_015506.2:c.609G>A, p.(Trp203*) identified in both of our patients is the most frequent cobalamin C mutation among Chinese patients, affecting more than 50% of MMACHC alleles and may be associated with the early-onset phenotype.\textsuperscript{7,8} With its high prevalence in the Chinese population, direct mutation analysis can be used for rapid confirmatory testing as well as potential development of targeted drug therapies in the future.

Though the overall incidence of cobalamin C deficiency is low, our 2 cases well demonstrated the benefit of NBS in improving the prognosis of these patients through early diagnosis and treatment. At the start of the pilot NBS programme in Hong Kong, only methylmalonic acidemia due to methylmalonyl-CoA mutase deficiency was included among the 24 screened conditions. With the known higher incidence of cobalamin C deficiency among the Chinese population and the identification of local cases, cobalamin C deficiency will be officially added to the list of conditions screened in Hong Kong from October 2019 onwards.

Conflict of Interest

The authors have no conflicts of interest to disclose.

References

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Case Report

Newborn Screening Pitfalls: A Missed Case of Salt-losing Type of Congenital Adrenal Hyperplasia

WY Wong, LM Wong, KM Belaramani

Abstract Congenital adrenal hyperplasia (CAH) is a rare group of inherited disorders of enzymes mediating adrenal steroidogenesis resulting in inadequate production of cortisol, aldosterone or both by the adrenal glands. Newborn screening for CAH using a dried blood spot sample to measure 17-hydroxyprogesterone (17-OHP) is employed for early detection of severe type of CAH. In this report, we describe a patient with classical CAH who had a normal newborn screening including second tier testing for CAH, but subsequently presented in salt-losing crisis at 1 month of age. Clinicians should not be falsely reassured by a negative newborn screening result, and they should proceed to diagnostic testing in clinical suspicious cases.

Key words Congenital adrenal hyperplasia; Missed screening case; Newborn screening

Introduction Congenital adrenal hyperplasia (CAH) is a rare group of inherited disorders of enzymes mediating adrenal steroidogenesis resulting in inadequate production of cortisol, aldosterone or both by the adrenal glands. Majority of the cases are due to 21-hydroxylase deficiency resulting from mutations or deletions in the CYP21A gene. Salt-losing type of CAH accounts for two thirds of the classical CAH cases, the remaining being non-salt losing type or non-classical CAH. Salt-losing crisis are life threatening and newborns may present as hyponatraemia, hyperkalaemia, shock and even death. Hormonal replacement therapy can result in correction of the electrolyte imbalance. In view of the possibility of such a morbid presentation in the newborn period and treatment being readily available, CAH has been included in most western newborn screening programs. The Hong Kong newborn screening for inborn errors of metabolism (NBSIEM) program has been implemented in stages for all babies born in government hospitals. CAH screening is included in the screening program. Newborn screening for CAH relies on using a filter paper blood spot sample to measure 17-hydroxyprogesterone (17-OHP). Many factors affect the measurement of 17-OHP and thus second tier testing using 17-OHP and androstenedione/cortisol ratio is being used to increase the reliability of the test. Despite having second tier testing, screening tests have limitations. In this report, we describe a case of classical CAH who passed second tier testing for CAH, but subsequently presented in salt-losing crisis at 1 month of age.

Case A female baby was born at 41 weeks of gestation with a birth weight of 3.07 kg at 25th percentile for age and sex. Antenatal and postnatal history was unremarkable.
NBSIEM was performed on day two of life, showing a minimal elevation in 17-OHP at 25.4 nmol/L (reference interval for full term babies: <25.0 nmol/L). Second tier testing was subsequently performed and showed 17-OHP + androstenedione/cortisol ratio was 0.37 ng/ml (reference interval: <2.0 ng/ml), which was considered normal.

On day five of life, she was admitted to a local hospital for excessive weight loss of 13.5% with hypernatraemic dehydration. Sodium on admission was 151 mmol/L (Reference range: 137-144 mmol/L) and potassium was 4.3 mmol/L (Reference range: 3.5-5.0 mmol/L). With rehydration therapy, sodium gradually normalised but potassium was found to be on the high side 5.6-6.4 mmol/L. This was attributed to difficult blood taking in a newborn. Physical examination was normal. She fed well and regained weight, and was subsequently discharged.

One month later, she was admitted again for fever to another hospital. She had no gastrointestinal nor respiratory symptoms. Feeding was reported to be satisfactory all along without any vomiting. However, body weight on admission was 3.56 kg which was at 3rd percentile for age and sex. Vitals were stable with a blood pressure of 99/51 mmHg and a pulse rate of 154 per minute but skin mottling was noted. Physical examination of respiratory, cardiovascular and abdominal systems were unremarkable. When saving urine sample, isolated cliteromegaly was noted. There was no associated skin hyperpigmentation. Initial investigation showed hyponatraemia with a sodium level of 126 mmol/L (Reference range: 139-146 mmol/L) and hyperkalaemia with a potassium level of 5.4 mmol/L (Reference range: 4.1-5.3 mmol/L). Serial plasma glucose ranged between 5-6 mmol/L. A salt-losing crisis was suspected despite a normal NBSIEM result. Further work up for CAH was performed. The 17-OHP was grossly elevated to 718 nmol/L (Reference range: <8 nmol/L). Testosterone was 2.13 nmol/L (Reference range: <2.15 nmol/L). Cortisol was 167 nmol/L (Reference range: 101-536 nmol/L). Aldosterone was 578 pmol/L (Reference range: <488 pmol/L). Renin was 116.28 ng/ml-h (Reference range 0.08-3.84 ng/ml-h). Urine steroid profile was performed and showed significant elevation of metabolites if 17-hydroxyprogesterone, moderate elevation of ratio of 16-alpha-hydroxyprogrenolone/16-alpha-hydroxydehydroepiandrosterone. This pattern was suggestive of 21-hydroxylase deficiency. Genetic test showed compound heterozygous mutation of CYP21A2 gene, with CYP21A2 c. 518T>A p. (Ile173Asn) and CYP21A2 c. 1069C>T p. (Arg357Trp) mutation, confirmed by parental analysis.

Empirical antibiotics were given after initial sepsis workup. Stress dose intravenous hydrocortisone was started on day 3 of admission, which was subsequently changed to oral hydrocortisone, fludrocortisone and sodium chloride supplement. She was stable and then discharged.

Upon follow-up visit, she had good drug compliance and was thriving well. Her body weight and body height were at 75th percentile and 50th percentile for age and sex now. She has also been referred to paediatric surgeon for assessment and reconstruction of external genitalia.

**Discussion**

CAH owing to 21 hydroxylase deficiency occurs with an incidence of 1:15000 in Canada and the United States combined. The incidence in the Chinese population is even lower as reported by Lee et al. Classical CAH fulfills the criteria for newborn screening because it has a fairly high incidence and tests are available for its diagnosis. Besides, effective treatment and timely treatment reduced mortality.

Measuring 17-OHP concentration is the mainstay of newborn screening for CAH. However, 17-OHP can be affected by multiple factor including – sex, race, birth weight, gestational age and timing of newborn screening. All these factors make false positive results a long-standing problem of newborn screening for CAH. Wisconsin state in United States of America has reported the sensitivity of CAH newborn screening to be 83% in male infants but only 60% in female infants. Whereas New York state in United States of America reported a sensitivity of 95% and a specificity of 99.9% for the CAH screening program. It also reports that the positive predictive value of the screening test was higher in full-term infants than in pre-term. This has also been noted by Kopacek et al in Brazil who noted a higher number of false positive results among newborns with a birth weight <2000 g.

Many strategies have been used in the past two decades to reduce the false positive rates. Adjustment of diagnostic levels of 17-OHP according to birth weight tiers was one proposed strategy. Birth weight data is still being used to determine 17-OHP cut-offs in many places including New York State. However, Van der Camp et al and Streigert et al both suggested that the efficacy of 17 OHP screening can be improved by adjusting cut-offs to gestational age rather than to birth weight. Our newborn screening program, similar to the Netherlands and Switzerland, also adjusts cut-
offs to gestational age rather than birth weight.

Another strategy that our laboratory utilises to reduce the false positive rates is to perform a second tier test using liquid chromatography tandem mass spectrometry (LC-MS) to measure the levels of adrenal steroid in dried blood spots. Sarafoglou et al have reported that despite using the second tier testing, the positive predictive value for CAH testing remains relatively low. In fact, the Minnesota program has the longest experience in using a single sample two-tier screening algorithm with steroid profiling by LC-MS/MS as the second tier in specimens that exceeded the first-tier 17-OHP cut-off. It showed that second tier testing can reduce false positive rate but the false negative rate are doubled. Our newborn screening program has only recently started and it is too early to draw a conclusion about the usefulness of second tier testing in CAH newborn screening.

With implementing all of the above strategies, we have managed to reduce the number of false positives but not the false negative cases. The aim of newborn screening for CAH is to detect the severe form of CAH, especially the salt-wasting form, in order to prevent life-threatening adrenal crisis. From literature, false negative cases are usually the non-salt losing type, also known as the simple virilising CAH or the non-classical CAH. The false negative cases are also usually female because they present with virilised external genitalia but such signs are not clear in males. A 2016 study from New York reviewed two million newborns screened by the New York State Newborn Screening Programme from 2007 to 2014 which diagnosed 105 babies with CAH. Three other confirmed CAH cases were reported with false negative newborn screening results. All 3 cases were female, and they all suffered from simple virilising CAH. In another European study in 2005, researchers had analysed newborn screening cards of 110 patients with CAH in five middle European countries between 1988 and 2000 and compared with the newborn screening cards of 920 normal controls. It was found that 17-OHP level of all patients with salt-wasting CAH were largely above cutoff level, while 10 out of 33 patients with simple virilising CAH had 17-OHP values below cutoffs. They were likely to be missed if screening was done. Both these studies show that false negatives cases in CAH newborn screening are usually simple virilising CAH.

Our patient has classical salt-losing CAH which was missed by newborn screening despite using gestational age adjusted cut-offs and using second tier testing. False negative cases of salt-losing CAH are uncommon but they have been reported in literature. In a study by the University of Wisconsin in 2015, screening data were collected from 2 one-screen states and 5 two-screen states in USA over 3 to 5 years. Four million babies were screened and 374 of them were diagnosed with CAH. Ten cases of salt-wasting CAH were not detected by newborn screening or had an initial specimen unsatisfactory for screening. In another Minnesota study, 838241 babies were screened over 12 years from 1999 to 2010 and identified 52 cases with classical CAH. Fifteen cases were missed and five of them had salt-wasting type. Table 1 shows the comparison of screening protocols, test essays and cutoff values of Hong Kong, New York and Minnesota centres.

One possible method to reduce the number of false negative cases is to adopt a two-screen test in which the first blood sampling is taken between 48-72 hours and the second between day 8 to day 14 of life. A delayed rise in 17-OHP level is seen in some newborns with CAH and this group of patients will be missed by single screening. In a 2015 Wisconsin study, by using the 2-screen method, 6.5% of classical salt-wasting CAH were detected in the second screen versus the first screen.

Hong Kong employs a one-screen test based on measurement of 17-OHP level on from heel-prick blood sample collected on filter paper taken at 24 to 72 hours of life. Second tier test utilising tandem mass spectrometry and measurement of steroid ratios on the same blood sample is performed on cases with borderline results. Since two-screen testing is not available in Hong Kong, postponing the timing of first blood sampling to beyond 48 hours may be another option to improve sensitivity of the screening test. However, it may prolong hospital stay of the mother and baby. The default rate may increase if the screening is to be performed after discharge.

In conclusion, measurement of 17-OHP level is commonly used in newborn screening of CAH, and was thought to have good sensitivity for salt-losing CAH. However, false negative cases of salt-losing CAH were reported in literature and in local experience. Newborn screening has been implemented in Hong Kong recently and more experience is needed to assess whether second-tier testing is useful. More studies are required to establish whether second screening or postponing collection of blood sample to beyond 48 hours is applicable and cost-effective in Hong Kong. We would like to raise awareness amongst clinicians to suspect CAH in clinically suspicious patients, even they passed the newborn screening, and not to delay effective treatment which may be life-saving.
Table 1

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<td>Second tier test:</td>
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| Protocol | One-screen, two-tier test     | One-tier test                   | One-screen, two- tier test             |
|          | Heel-prick blood sample       | Heel-prick blood sample         | Heel-prick blood sample collected on   |
|          | collected on filter paper at  | collected on filter paper at    | filter paper at 24-48 hours of life    |
|          | 24-72 hours of life           | 24-48 hours of life             |                                        |

| Cut off  | Term: 17-OHP <25 nmol/L       | Any age:                        | Weight-based cutoff:                   |
|          | Preterm: We are adopting     | <22.4 ng/ml (67.8 nmol/L)       | 17-OHP (F): <4.0 ng/ml                 |
|          | gestational age cutoff provided by the International Society for Neonatal Screening) | | 17-OHP (M): <7.0 ng/ml |
|          |                               | by the International Society for Neonatal Screening | 17-OHP+4A Cortisol >2.5 |
|          |                               |                                 | 11-deoxycortisol>10.0 ng/ml           |
|          |                               |                                 | 21-deoxycortisol >1.6 ng/ml           |

| Babies screened | 50,649* | 1,962,433 | 838,241 |
| CAH picked up by NBS | 1 (Classical) | 105 | 52 |
| CAH missed by NBS | 1 (Classical) | 3 (SV) | 15 (SW-CAH: 5, SV-CAH: 10) |

CAH: Congenital adrenal hyperplasia; 17-OHP: 17- hydroxyprogesterone; NBS: Newborn screening
* October 2018-June 2019

Conflict of Interest

The authors have no conflicts of interest to disclose.

References

Case Report

Classical Neonatal Propionic Acidaemia: Diagnostic and Management Pitfalls

TYS LUI, GPG FUNG, KM BELARAMANI

Abstract
Propionic acidaemia is an inborn error of metabolism classified under the category of organic acidaemias. This disease is caused by the deficiency of propionyl-CoA carboxylase enzyme. Initial presentation can be subtle with subsequent rapid deterioration. Newborn Screening for inborn error of metabolism can provide an early diagnosis and thus prompt treatment can be given, preventing mortality and morbidity.

Key words
Hyperammonaemia; Inborn error of metabolism; Propionic acidaemia, newborn screening

Introduction
Propionic acidaemia (PA) is an inborn error of metabolism (IEM) classified under the category of organic acidaemias. This disease is caused by the deficiency of propionyl-CoA carboxylase (PCC) enzyme. Severe forms of the disease can present within the first few days of life with deterioration of general condition, metabolic acidosis and hyperammonaemia. It can be fatal if diagnosis and treatment is delayed. Early recognition and timely treatment can be achieved by universal newborn screening for IEM. We present a case of PA with a non-specific clinical presentation to illustrate the importance of Newborn Screening for IEM (NBSIEM).

Case Presentation

History
Baby S was a female baby born at a gestation of 41 weeks by spontaneous vaginal delivery, weighing 3.2 kilograms. Apgar scores were 9 at 1 minute and 10 at 5 minutes. Antenatal history was uneventful.

Breast-feeding was initiated after birth, but she developed vomiting and respiratory distress on Day 1 of life. She was transferred to the special care baby unit (SCBU) for management. Chest radiography revealed a small spontaneous pneumothorax. Blood gas showed no acidosis, and sepsis screen was negative. Respiratory distress improved with oxygen supplement. She was kept nil by mouth from Day 2-4 of life due to suboptimal respiratory condition. Feeding was resumed on Day 5 of life, but she still had intermittent vomiting one to two times per day. Therefore, feeding could only be advanced cautiously. Full feeds was eventually attained on Day 15 of life, but intermittent vomiting around once a day persisted.

Despite adequate caloric intake, she continued to have weight loss (maximum weight loss 10.8%). Repeated investigations including blood gas, glucose, renal function test, CRP and abdominal radiographs on Day 15-18 of life were normal. However, feeding performance continued to deteriorate and by Day 21 of life, she developed drowsiness, lethargy with poor sucking effort. Physical examination at this juncture showed a sleepy, hypotonic baby with normal
power and reflexes, and no evidence of raised intracranial pressure. She had no seizures all along.

Both parents were Pakistani by ethnicity, and they had a consanguineous fifth degree family relationship. Father's brother and mother's sister were married and had given birth to six children in Pakistan, of which two died due to unknown cause during the neonatal period (Figure 1).

Further investigations were performed in view of neurological deterioration. Complete blood count, electrolytes, liver function test, glucose and lactate were normal. Venous blood gas showed no acidosis (pH 7.45; HCO3 24 mmol/L; BE 0.2 mmol/L). Urine ketone and reducing substances were negative. Electrocardiogram, Echocardiogram, Computer tomography of the brain and electroencephalography were normal. However, ammonia was markedly elevated to 353 µmol/L. With the available results, it was difficult to differentiate the cause of hyperammonaemia since both urea cycle defects and organic acidaemia could present with a similar picture.

While awaiting further metabolic investigation results, acute management of hyperammonaemia was nevertheless initiated without further ado. Sodium Benzoate, arginine, levocarnitine and biotin were started. Patient was kept fasted. Protein was withheld, and a high calorie regime (100 to 110 kcal/kg/d) was provided by parenteral nutrition. Ammonia level decreased from 353 µmol/L to 199µmol/L within 6 hours after initiation of treatment. The first free Carnitine level was 982.7 µmol/L (Reference 19.3-53.9 µmol/L), however it was only taken after commencement of Levocarnitine.

Sodium benzoate was further increased from 250 mg/kg/d to maximum dose (500 mg/kg/d) and two doses of Carglumic acid (100 mg/kg/dose) were given. Ammonia further decreased to 71 µmol/L the within the next 15 hours. As the ammonia levels decreased, clinically the baby's drowsiness and lethargy improved, and she became much more alert and responsive.

Dried blood spot test (DBS) became available within 24

Figure 1   Family Tree. [a] Father's mother & mother's GM are sister. [b] Father's brother married mother's sister. [c] Deceased at 3-4 days and 6-7 days respectively.
hours and showed a significantly elevated Glycine (634 µmol/L) and C3 carnitine (20 µmol/L). The DBS result was positive for either PA or methylmalonic aciduria (MMA) and the baby was managed accordingly. Vitamin B12, folate, beta-hydroxybutyrate, homocysteine were not identified. Plasma amino acids showed elevated glycine (881 µmol/L), with mild elevations in leucine (169 µmol/L), lysine (356 µmol/L) and valine (242 µmol/L). Urine organic acid showed significant elevation of methylcitric, propionylglycine, tiglyglycine, and 3-hydroxypropionic acids which is diagnostic of PA. This diagnosis was genetically confirmed and a homozygous mutation in the beta subunit of the PCC gene (PCCB) was detected (NM_000532.4:c.1498+2T>C). Genetic screening of the parents has been done, and their carrier status was confirmed.

The baby was subsequently managed by a low protein diet, and carnitine and biotin supplement. She is currently 11 months old.

Discussion

PA was first described in 1961. It is a rare organic aciduria with an autosomal recessive inheritance. It results in the deficiency of propionyl CoA carboxylase which impairs the metabolism of branched-chain amino acids.

Worldwide incidence is estimated to be 1:100,000 to 1:150,000, with a higher incidence of up to 1:2000-5000 in some populations (e.g. Saudi Arabia). Currently, there is no local data on incidence or prevalence of PA in Hong Kong. However, incidences in the Asia-Pacific region have well been reported with the incidence of PA in China and Taiwan being reported to be 1 in 116,000 and 1 in 464,000 which is comparable to the world-wide incidence. Japan, however, has an unexpectedly high incidence of PA of 1 in 41,000 due to the presence of a common mutation (Y435C) in the PCCB gene which causes a milder phenotype.

Majority of PA cases present in the neonatal period. Other clinical presentations have been described in infants, children and adults. The reason for the phenotypic variation is likely related to genotype and propionyl CoA carboxylase activity. The classical neonatal PA usual presents within the first few days of life with non-specific symptoms including deterioration of general condition, unstable temperature, weight loss, dehydration, vomiting, irritability, hypotonia, hypertension, lethargy, or even coma and seizure. Grünert et al reported that 78% of PA cases present within the first 5 days of life. Pena et al on the other hand reported that 39% of PA cases were diagnosed within the first week of life and an additional 25% were diagnosed within the first month of life. This discrepancy between presentation and diagnosis is likely due to the lack of newborn screening, non-specific nature of presentation, presentation being attributed to sepsis which is a more common entity in neonates, and lack of awareness amongst frontline staff. In our case, Baby S too presented clinically with poor feeding and respiratory issues within the first 5 days of life but her diagnosis was delayed and made at 3 weeks of life.

In addition to non-specific clinical symptoms, non-specific laboratory results could also result in delayed diagnosis. Organic acidaemias usually show severe metabolic acidosis, ketosis and hyperammonaemia. In our case, Baby S had hyperammonaemia but no metabolic acidosis or ketosis. Although hyperammonaemia is present in 90% of PA cases at presentation, ammonia is usually not the first-line investigation ordered by most paediatricians when encountering a neonate with non-specific symptoms. Blood gas analysis is a more routine investigation in neonates but this too was not so useful in our case because it was repeatedly normal. Studying neonates with PA, Grünert et al, found that one third of the cases had no metabolic acidosis or ketosis. Another retrospective review, Al-Makadma et al, also found one sixth of patients without metabolic acidosis. Absence of metabolic acidosis may be falsely reassuring to a paediatrician and hinder early recognition.

It was well described that the variation of presentation and absence of metabolic acidosis in organic acidaemia may be associated with the variation of genotype. In Baby S, we also postulate that the absence of metabolic acidosis may also be caused by inadequate protein intake, which is a frequent presentation in babies suffering from IEM. However, more observation or further studies are needed to prove our postulation. Therefore, we advocate that in neonates with insidious deterioration of feeding or general condition, especially with family history of unexplained neonatal deaths or consanguinity, suspicion of metabolic conditions and their work-up should be initiated early.

There are well written guidelines on management of neonatal hyperammonaemia. They usually include stopping protein intake, starting 10% dextrose infusion, initiation of ammonia scavenging drugs, and collection of urine and blood sample for diagnosis.

Sodium benzoate is the first ammonia scavenger that has been used for many years. It lowers serum ammonia
levels by conjugating with glycine to form Hippurate, which will then be excreted by kidney. Sodium phenylacetate works by a similar mechanism as sodium benzoate. Hemodialysis is considered if hyperammonaemia is not controlled by medical treatment. However, dialysis services may not be readily available. Recent literature suggest that carglumic is a more effective in treating hyperammonaemia in organic acidaemias and may reduce the need for hemodialysis. Propionyl Co A suppresses N-acetylglutamate synthase (NAGS) which is an activator of carbamoyl phosphate synthetase 1, which converts ammonia to urea in the first step of urea cycle. Carglumic acid is a synthetic structural analogue of NAGS and as such is a specific intervention for hyperammonaemia in PA. Chakrapani et al studied 98 hyperammonaemia episodes and concluded that ammonia scavengers when used in combination with carglumic acid is more effective in rapidly reducing ammonia levels at a dose of greater than 100 mg/kg, generally well tolerated and can be given orally or via nasogastric tube. The latter being very important since intravenous access can be a problem when PA patients are in a state of acute decompensation. In Baby S, the response to sodium benzoate was suboptimal initially. However, after addition of carglumic acid, ammonia levels decreased rapidly to normal range. Although more large-scale studies are needed before carglumic acid can be recommended for initial management of organic acidaemias, this modality of treatment may be considered in cases of hyperammonaemia refractory to conventional therapy like this case.

Since it was first described, the mortality of neonatal onset PA has been decreasing from 85% in the 1980s to 41.5% in the 1990s to 8% in more recent studies. The reasons for this include increased awareness amongst paediatricians regarding organic acidaemias, readily available laboratory testing of ammonia, and newborn screening. We postulate that newborn screening is pivotal in the reduction in mortality.

NBSIEM varies worldwide in terms of the disease coverage and the turnaround time but most tandem-mass spectroscopy based NBSIEM cover organic acidaemias and have a turnaround time of 7 days. Both MMA and PA show elevated C3 levels on the NBSIEM DBS and this will alert paediatricians of the possibility of organic acidaemias. In many cases the turnaround time of NBSIEM is suboptimal for organic acidaemias, because as mentioned before 78% cases of PA present within the first 5 days of life and decompensate before the availability of the NBSIEM result. Despite this delay, Grunert et al reported that there was tendency towards a lower mortality rate in the NBS screened babies versus selectively screened patients (0% vs 12%). This is probably because although the baby presents before 5 days of life, not all have had severe decompensations like in the case of Baby S. Baby S would have been picked up and treated much earlier had there been NBSIEM. Although NBSIEM reduces mortality, long term data is needed to show any benefit for clinical outcomes including neurocognitive development and other long term complications.

Universal NBSIEM is still being implemented in Hong Kong in phases beginning 2015 and will be made available territory-wide in all government birthing units by 2020. We hope that universal NBSIEM will be able to pick up newborns with not only classical but those with atypical or non-specific presentation, of not only PA, but other IEM as well. Early recognition will result in earlier treatment which can reduce the mortality and morbidity.

Conclusion

In PA, the diagnosis is often easily delayed due to three main reasons, one that it is an uncommon disease, two that its clinical presentation is rather non-specific, and three the lack of awareness amongst paediatricians about organic acidaemias and thus delayed use of carglumic acid. With the availability of NBSIEM, there is hope that the time-lag between presentation and diagnosis will be shortened and this in term will improve long-term outcome of this rare group of disorders.

Declaration of Interest

The authors declare that they have no financial or other conflicts of interest in relation to this publication.

References


先天性腎上腺皮質增生症新生兒篩查中二次檢測的臨床應用：香港經驗


本研究目的是評價液相色譜（LC-MS/MS）類固醇分析用於新生兒篩查先天性腎上腺皮質增生症（CAH）二次檢測的應用價值。方法：本研究納入2016年4月至2019年3月共40,754例通過新生兒篩查CAH的檢測結果。第一次檢測利用離解增強體系熒光免疫分析法（DELFIA）測定幹血濾紙片中的17- 羥孕酮（17-OHP）。對一次篩查陽性的病例進行二次檢測，使用LC-MS/MS檢測同樣幹血片中的17-OHP、雄烯二酮和皮質醇。結果：在篩查的40,754例新生兒中，422名（1.04%）一次篩查陽性，需二次檢測。其中4例（0.01%）經二次檢測陽性，並被召回作進一步檢查。其中兩例被診斷患有CAH。此外，一名篩查陰性的新生兒逐漸出現發熱、低鈉血症和高鈉血症，隨後被診斷為CAH。二例為真陰性，一例為假陰性，均為失鹽型21- 羥化酶缺乏。結論：CAH在篩查人群中的預估發生率為1:13,585。結論：LC-MS/MS二次檢測類固醇分析可以顯著降低新生兒篩查CAH的假陽性率，避免不必要的召回。然而，假陰性篩查仍然存在。無論新生兒篩查結果如何，任何具有CAH臨床表現的患者都應該接受診斷性檢測。

關鍵詞：先天性腎上腺皮質增生症、假陽性、新生兒篩查、二次檢測

香港新生兒篩查專案幹血濾紙片的儲存和二次使用的倫理問題


高解析度串聯質譜法的進展，使運用幹血濾紙片（DBS）新生兒篩查的應用得到重大的擴展。在DBS採集時，血液收集量往往足夠用於初步檢查及復查確認。因此，餘下的DBS可用於其他二次用途。目前剩餘的DBS有多種用途，包括：質量保證及證實試驗，新篩查專案的發展和評估，生物醫學研究和公共衛生流行病學研究。因敏感資訊可從DBS中獲得，DBS的儲存和在科研方面的二次使用問題有很多倫理問題需要考慮。本篇論文討論了DBS剩餘樣本的儲存和二次使用，包括：知情同意、隱私、保密性、科研結果返回和公共透明度。

關鍵詞：生物倫理學、幹血濾紙片、擴展新生兒篩查、香港、知情同意
香港"新生兒先天性代謝缺陷病篩查"18 個月的初步研究評價

The Task Force on the Pilot Study of Newborn Screening for Inborn Errors of Metabolism. Evaluation of the 18-month "Pilot Study of Newborn Screening for Inborn Errors of Metabolism" in Hong Kong. HK J Paediatr (new series) 2020;25:16-22

介紹：2015 年香港行政長官發表施政報告後，一項關於在公共衛生系統中實行新生兒先天性代謝缺陷病（IEM）篩查可行性的初步研究開始計劃及實施。經過 6 個月的籌備，這項初步研究於 2015 年 10 月開始在 2 所公立分娩醫院（伊利沙白醫院和瑪麗醫院）實行。研究經歷兩個階段，歷時 18 個月：第一階段：2015 年 10 月 - 2016 年 3 月（包括 21 例 IEM）；第二階段：2016 年 4 月至 2017 年 3 月（包括 24 例 IEM）。目的：旨在回顧事件的過程及討論前期研究相關的臨床發現。結果和結論：研究在關於父母的受教育程度、樣本的採集及準備和派送等方面進展順利。不同參與機構（包括嬰兒召回、後期調查安排、臨床管理等）之間的溝通和協作有明顯的成效。在 15361 名合格的嬰兒中，有 15138 名嬰兒父母簽訂了參與研究的同意書，佔比 98.5%，其中有 9 例確診了 IEM（發生率 1:1682，可信區間 1:909-1:3333）。有 2 名母親偶然被發現分別是肉瘤攝取障礙（CUD）和典型苯丙酮尿症（PKU）患者，同時發現 2 例瓜氨酸血症（CIT）II 型假陰性病例。如果包括這兩例假陰性病例，發生率就會上升到 1:1376，比別的國家和地區要高。總的來說，IEM 在香港並不罕見。

關鍵詞：香港、先天性代謝缺陷性疾病、新生兒篩查、初步研究

NLS Tang, J Hui. 20 Years After Discovery of the Causative Gene of Primary Carnitine Deficiency, How Much More Have We Known About the Disease? HK J Paediatr (new series) 2020;25:23-29

關鍵詞：xx、xxx、xxx、xxxx
T细胞受體刪除環檢測應用於新生兒篩查重症聯合免疫缺陷病的10年國際經驗綜述


重症聯合免疫缺陷病（SCID）是一組多樣性先天性單基因免疫缺陷疾病，以T淋巴細胞數量或功能嚴重缺失為特徵，或伴有B和NK淋巴細胞數量異常。多種嚴重感染可導致早期嬰兒死亡。感染病前的SCID嬰兒看起來狀態良好，在新生兒篩查年代之前，就導致他們錯失進行造血幹細胞移植的黃金時機。T淋巴細胞受體刪除環是首次T淋巴細胞數量的一種臨床指標，可用於SCID的高敏感度篩查。國際經驗顯示：在篩查檢測中，至今沒有經典SCID病例被遺漏。因為通過篩查檢測到的SCID病人用早期造血幹細胞移植術取得非常良好的療效（90%長期生存），用T細胞受體刪除環檢測進行篩查具有巨大經濟價值。香港應該參與到這項SCID篩查的全球趨勢，挽救SCID兒童的生命。

關鍵詞：新生兒篩查、原發性免疫缺陷、重症聯合免疫缺陷病、T細胞受體刪除環檢測

香港首例通過新生兒篩查專案檢出的高胱氨酸尿症：一例病例報告

S Ho, J Hui. The First Case of Homocystinuria Picked Up by Newborn Screening in Hong Kong: A Case Report. HK J Paediatr (new series) 2020;25:42-44

經典型高胱氨酸尿症在當地中國人口中是罕見的代謝疾病。我們報導一例吡多醇無反應經典型高胱氨酸尿症，由新生兒篩查專案檢出，該專案自2015年開始在全港開展。

關鍵詞：胱硫醚β合酶缺乏、高胱氨酸尿症、新生兒篩查、吡多醇無反應

新生兒篩查開放時代前後兩例鈷胺C缺乏症新生兒的臨床病程


鈷胺C缺乏症是細胞內鈷維生素疾病組中研究理解最好和最常見的表型。臨床表型的範圍可以非常嚴重，在新生兒期及後期多個系統，也可以表現輕微、急性或慢性進展性神經症狀和行為異常。我們在此報導兩例確診的鈷胺C缺乏症。第一例在香港未展開新生兒篩查前診斷，第二例在新生兒篩查（NBS）開展後診斷。兩個病例在臨床病程中的顯著差異，表明成功實施NBS專案，可以達到早期診斷和治療，從而改善病人的預後。

關鍵詞：鈷胺C、同型半胱氨酸、甲基丙二酸血症、新生兒篩查
新生兒篩查的缺陷：失鹽型先天性腎上腺增生症漏診一例


先天性腎上腺增生症（CAH）為一組腎上腺激素生成相關酶類的罕見遺傳性疾病，導致腎上腺的皮質醇、醛固酮、或兩者同時出現不足。對一份幹血滴標本檢測 17- 羥孕酮（17-OHP）進行新生兒 CAH 篩查，可用於對重型 CAH 的早期檢測。本報告中，作者描述一例典型 CAH 病人，在新生兒篩查和 CAH 二次檢測結果均為正常，而後在 1 月齡時出現失鹽的危險情況。臨床醫生不應以一份新生兒篩查陰性報告而被誤導，對臨床可疑病例應進行診斷性檢查。

關鍵詞：先天性腎上腺增生症、篩查漏診病例、新生兒篩查

經典新生兒丙酸血症：診斷和治療缺陷


丙酸血症為一種先天性代謝病，分類歸屬於有機酸血症。本病由丙醯輔酶 A 羧化酶（PCC）缺乏引起。初發表現可不明顯，隨後快速惡化。針對先天性代謝病的新生兒篩查可提供早期診斷，從而給予及時治療，預防發病和死亡。

關鍵詞：高氨血症、先天性代謝病、新生兒篩查、丙酸血症
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) Clinical Utility of Second-tier Testing in Newborn Screening for Congenital Adrenal Hyperplasia: The Hong Kong Experience

1. What methodology is adopted for first-tier newborn screening test of measuring 17-hydroxyprogesterone (17-OHP) levels in dried blood spots in Hong Kong?
   a. High performance liquid chromatography
   b. Inductively coupled plasma mass spectrometry
   c. Gas chromatography with flame ionization detector
   d. Next generation sequencing
   e. Immunoassay

2. Which of the following is NOT associated with elevated 17-hydroxyprogesterone (17-OHP) levels in newborn?
   a. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency
   b. Low birth weight
   c. Prematurity
   d. Congenital adrenal hyperplasia due to 11 beta-hydroxylase deficiency
   e. Antenatal use of exogenous corticosteroid to enhance fetal lung maturation

3. First-tier screening test of measuring 17-hydroxyprogesterone (17-OHP) levels in dried blood spots suffers from poor specificity. Which of the following strategies can BEST improve the positive predictive value of congenital adrenal hyperplasia screening?
   a. Second-tier test by steroid profiling using liquid chromatography-tandem mass spectrometry (LC-MS/MS)
   b. Ensure adequate feed before blood taking
   c. Blood taking in the morning
   d. Improvement in analytical specificity of antibodies employed in immunoassay
   e. Lower the screening cutoff values for 17-OHP

4. What analytes are currently being measured in second tier test of congenital adrenal hyperplasia in Hong Kong?
   a. 17-hydroxyprogesterone (17-OHP), 11-deoxycortisol and 21-deoxycortisol
   b. 17-hydroxyprogesterone (17-OHP), cortisol and androstenedione (4-AD)
   c. 17-hydroxyprogesterone (17-OHP), cortisol and dehydroepiandrosterone sulfate (DHEA-S)
   d. 17-hydroxyprogrenolone, cortisol and androstenedione (4-AD)
   e. 17-hydroxyprogrenolone, dehydroepiandrosterone sulfate (DHEA-S) and androstenedione (4-AD)

5. Which of the following statements is WRONG?
   a. False negative result can occur in both first- and second-tier test, and a negative newborn screening result does not rule out all possibility of CAH, even for severe classical salt wasting type.
   b. Incorporation of second tier test for CAH can reduce false positive rate and reduce unnecessary follow up testing, healthcare expenditures and worry of parents.
   c. For first tier testing for CAH, a universal cutoff of 17-hydroxyprogesterone (17-OHP) is adopted for both term and preterm newborns.
   d. Newborn screening tests for CAH are performed on dried blood spots samples collected from newborns.
   e. In Hong Kong, for neonates less than 34 weeks' gestation or birth weight less than 2,000 grams or requiring neonatal intensive care, a second dried blood spot sample will be collected on day 28 of life or at discharge whenever comes first.
(B) Ethical Issues of Dried Blood Spot Storage and Its Secondary Use After Newborn Screening Programme in Hong Kong

1. What is/are the benefit(s) of establishing a local-wide dried blood spot storage programme?
   a. To evaluate quality assurance and control of the newborn screening programme
   b. To enable early disease diagnosis and prevent child mortality
   c. To foster biomedical research and public health programme development
   d. To facilitate research to develop new technologies and new treatments
   e. All of the above

2. Which of the following is NOT of clinical and ethical concern in the dried blood spot storage and its secondary use?
   a. Data management and handling
   b. Approach to obtain informed consent
   c. Researcher’s responsibility to return incidental findings
   d. Privacy
   e. None of the above

3. Which of the following(s) is/are the essence of informed consent procedures?
   a. Informedness
   b. Incentives
   c. Voluntariness
   d. (A) and (C)
   e. (A), (B), and (C)

4. What of the following is/are the best approach(es) to alleviating privacy and confidentiality concerns?
   a. Do not collect personally identifiable information at all
   b. Store personal identifier separately from the residual sample
   c. Apply a unique number to each sample
   d. (A) and (C)
   e. (B) and (C)

5. Which of the following is a true statement?
   a. DBS storage and its secondary use does not entail ethical concerns because it does not involve invasive procedures
   b. Obtaining parental consent is not mandatory as newborn screening is part of the standard of care
   c. Completely removing all personal identifiers from biospecimens is not an ideal approach to avoid privacy and confidentiality concerns in research involving residual DBS
   d. (A) and (C)
   e. (B) and (C)

(C) Evaluation of the 18-month "Pilot Study of Newborn Screening for Inborn Errors of Metabolism" in Hong Kong

1. Which type of specimen was taken from newborn babies for inborn errors of metabolism (IEM) screening?
   a. urine
   b. cord blood
   c. dried blood spots
   d. hair
   e. stool

2. In Phase II of the Pilot Study, which group of newborn babies needed to have more than one specimen tested?
   a. ≥34 weeks of gestation
   b. ≥34 weeks of gestation OR birth weight >2000 g
   c. ≥34 weeks of gestation AND birth weight >2000 g
   d. <34 weeks of gestation OR birth weight <2000 g OR being admitted to Neonatal Intensive Care Unit (NICU)
   e. <34 weeks of gestation AND birth weight <2000 g AND being admitted to Neonatal Intensive Care Unit (NICU)

3. Which of following condition or group of IEM disorders was NOT included in the Pilot Study?
   a. organic acid disorders
   b. lysosomal storage disorders
   c. fatty acid oxidation disorders
   d. congenital adrenal hyperplasia
   e. amino acid disorders
4. In the Pilot Study, what was the commonest IEM disorder picked up?
   a. Carnitine uptake deficiency (CUD)
   b. Phenylketonuria (not classic PKU) (Mild PKU)
   c. Citrullinaemia type II (CIT type II)
   d. Methylmalonic acidemia (MMA)
   e. Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD)

5. Which IEM condition was commented to be easily missed in the Pilot Study?
   a. Carnitine uptake deficiency (CUD)
   b. Phenylketonuria (not classic PKU) (Mild PKU)
   c. Citrullinaemia type II (CIT type II)
   d. Methylmalonic acidemia (MMA)
   e. Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD)

(D) 20 Years After Discovery of the Causative Gene of Primary Carnitine Deficiency, How Much More Have We Known About the Disease?

1. Which of the following is NOT a common fatty acid oxidation defect in Southern Chinese?
   a. Primary carnitine deficiency
   b. Carnitine-acylcarnitine translocase (CACT) deficiency
   c. Glutaric acidemia type II (GA2)
   d. Carnitine uptake defect
   e. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency

2. Which of the following is a founder mutation in SLC22A5 (OCTN2) gene causing primary carnitine deficiency in Southern Chinese?
   a. R254X
   b. S467C
   c. F17Q
   d. P478X
   e. R169C

3. Which of the following mutation is commonly associated with maternal carnitine uptake defect?
   a. R254X
   b. S467C
   c. F17X
   d. P478X
   e. R169C

4. Which of the following is the cellular location of carnitine transporter encoded by SLC22A5 gene?
   a. plasma membrane
   b. cytoplasm
   c. mitochondria
   d. nucleus
   e. peroxisome

5. Which of the following is NOT a feature in fatty acid oxidation defect?
   a. Hypoglycaemia
   b. Low ketone (hypoketotic)
   c. High ketone (ketosis)
   d. Negative for urine reducing substances
   e. High plasma ammonia

Answers of October issue 2019

(A) 1. b; 2. e; 3. d; 4. a; 5. a
(B) 1. c; 2. d; 3. a; 4. e; 5. d
(C) 1. b; 2. a; 3. b; 4. d; 5. a
(D) 1. b; 2. e; 3. a; 4. e; 5. d
Our Fond Memory
of the Late Dr. Sam LAU Sum Ping (劉森坪醫生)

M.D. (Germany), L.R.C.P. (U.K.), M.R.C.S. (London), M.R.C.P. (U.K.),

President of the Hong Kong Paediatric Society (1986-88)
Honorary Life Member of the Hong Kong Paediatric Society (1994)
Editor-in-Chief, Hong Kong Journal of Paediatrics (1989-94)
Member of Organizing Committee of the 9th Asian Congress of Paediatrics (1997)

Dr. Lau attended Pui Ching High School in Hong Kong and received a scholarship to study medicine in Germany. He graduated from the Munich University, Germany with distinction in 1970. Subsequently he received paediatric training in the U.K. where he gained extensive working experience before returning to Hong Kong in 1978. He was a teaching staff of the Paediatric Department, University of Hong Kong and Physician in-charge of the Neonatal Unit at Queen Mary Hospital and Tsan Yuk Maternity Hospital. His main research interests included Neonatology, Perinatal Statistics and Epidemiology, Growth and Lung Functions of Hong Kong Children.

He was Council member (1983-85), Member of the Organizing Committee of the 9th Asian Congress of Paediatrics (1997), President (1986-88) of the Hong Kong Paediatric Society, Executive editor (1984-88), Chief Editor (1989-94) of the Hong Kong Paediatric Journal and Member of the HKPS Child Advocacy Committee (1999-2018). There are more than fifty scientific papers published in international and local journals to his credit. He was a private Paediatrician since 1986.

Besides being a competent Paediatrician, Dr. Lau was also a well-known Classic Singer locally and internationally!

We are all sad that Dr. Lau has left us. To the profession, we have lost a great leader, a respected senior, a close friend and a great multi-talented colleague. To our children, they will forever miss this great paediatrician who has devoted his lifetime to the promotion of child health and children's welfare. He had indeed established a strong infrastructure for his successors to promote and demonstrated clear directions for his colleagues to follow. He will be eternally remembered by all of us at the medical profession.

Dr. Lau passed away peacefully at the age of 77 years old on 25th October 2019 in Hong Kong. He is survived by his wife and two children. Memorial Service will be held at 6-7pm on 10th November 2019 at the Universal Funeral Parlor at 10 Cheong Hang Road, Hung Hom, Hong Kong. To his family, we would like to convey our most sincere condolences. To the medical professionals, we shall forever miss him!

Dr. Chok-wan CHAN
President of the Hong Kong Paediatric Society (1982-85)
President of International Pediatric Association (IPA) (2007-2010)
Honorary President of Asia-Pacific Pediatric Association (APPA)
5th November 2019, Hong Kong.
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- **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.
- **Clinical Quiz** The clinical quiz should be educational. It should i) include the description of a case in no more than 250 words and 3 clinical photos or figures, and ii) provide answers on the diagnosis, clinical features and findings, and management of the condition in no more than 1,000 words, 10 references, and 3 photos, figures or tables.
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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.
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**The manuscript should usually be arranged as follows:**

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Each table should begin on a separate page. Number tables consecutively in the order of their first citation in the text and supply a brief title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in the heading. Vertical rules and horizontal rules should be omitted.

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