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Original Article

Mitochondrial *m.1555A>G* Mutation, Prevalence and Clinical Features in a Hong Kong Chinese Cohort

LT ***Leung***, MMK ***Lee***, SSW ***Cheng***, HM ***Luk***, IFM ***Lo***

Abstract Mitochondrial *m.1555A>G* mutation in the 12S rRNA gene (MIM* 561000) is associated with maternally-inherited aminoglycoside–induced hearing loss and non–syndromic deafness. Herein we report 14 Hong Kong Chinese families with *m.1555A>G* mutation. Individuals in these families had variable hearing thresholds ranging from normal to profound hearing impairment with variable age of onset, which were consistent with typical findings of incomplete penetrance and variable expressivity for *m.1555A>G* mutation in previous studies overseas and among the Chinese population. No patient could recall previous history of exposure to aminoglycoside or antibiotic before onset of hearing impairment. It is hoped that through this study, the awareness of *m.1555A>G* mutation related hearing impairment could be raised among healthcare professionals and the general public so that carriers' exposure to potentially ototoxic drugs could be avoided.

Key words *Aminoglycoside–induced ototoxicity; Hearing impairment; Hong Kong Chinese; Mitochondrial m.1555A>G*

Introduction

Hearing impairment is associated with high socioeconomic cost both globally and locally in Hong Kong (WHO, 2017). It can be caused by environmental and/or genetic factors. Among the genetically related cases, up to 70% are non-syndromic while the remaining 30% are syndromic (involving ear and/or other organ malformations or dysfunctions).[1] Autosomal recessive variants especially in *GJB2* gene account for the majority (~80%) of cases with non-syndromic hearing loss, which are then followed by autosomal dominant, X-linked and mitochondrial variants.[2] Mitochondrial *m.1555A>G* mutation in the 12S rRNA gene (MIM* 561000) was first discovered to be the cause of maternally-inherited aminoglycoside–induced hearing loss and non–syndromic deafness in 1993.[3] The A-site of 12S rRNA is a highly conserved region. The *m.1555A>G* mutation results in a change of secondary structure of RNA so that it resembles the 16S rRNA of bacteria, resulting in susceptibility to ototoxicity during administration of antibiotics. In this study, the molecular and clinical characteristics of 14 Hong Kong Chinese families with *m.1555A>G* mutations were summarised. The findings coincided with the previous overseas and Chinese cohorts that *m.1555A>G* mutation demonstrates variable expressivity and incomplete penetrance. Even homoplasmic carriers could have normal to profound sensorineural hearing loss with a wide range in age of onset. Such variation was proposed to be related to different mitochondrial DNA (mtDNA) background haplogroups (A, B, DD, F, M7, N)[4] or nuclear DNA susceptibility loci discovered by linkage analysis.[5] However, the exact cause is yet to be determined by future

Clinical Genetics Service Unit, Hong Kong Children's Hospital, 1 Shing Cheong Road, Kowloon Bay, Kowloon, Hong Kong SAR, China

LT **Leung** (梁礪婷) *MRCPCH(UK)*
MMK **Lee** (李文傑) *BSc(Biomedical Science)(CUHK) student*
SZW **Cheng** (鄭斯穎) *FHKAM(Paed)*
HM **Luk** (陸浩明) *FHKAM(Paed)*
IFM **Lo** (盧輝文) *FHKAM(Paed)*

Correspondence to: Dr HM **Luk**
Email: lukhm@ha.org.hk

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studies due to the complex inheritance of mitochondrial diseases. It is hoped that future studies could shed light on the mechanisms of *m.1555A>G* mutation expression modification by mitochondrial DNA haplotypes and nuclear genome so that more accurate personalised genetic counselling could be offered to carrier families.

Methods

Clinical history and diagnostic findings were acquired in the Clinical Genetics Service Unit of Hong Kong Children's Hospital during the period 5/2005 to 2/2023. A total of 484 unrelated Chinese families (580 individuals) with a proband presenting with non-syndromic hearing loss to our outpatient genetic counselling clinic were included. Among them, the clinical characteristics of families with *m.1555A>G* were summarised. For the detection of *m.1555A>G* mutation, Genomic DNA was extracted from peripheral blood samples. For analyses prior to 2008, restriction fragment length polymorphism (RFLP) analysis was carried out in which restriction enzymes were used to cleave the mitochondrial DNA (mtDNA) into fragments at specific recognition sites, which was followed by gel electrophoresis to separate and identify different fragments. After 2008, direct sequencing of the mtDNA fragment encompassing *m.1555A* (NC_012920.1) replaced the use of RFLP in our unit.

Results

During the study period 5/2005 to 2/2023, a total of 484 families were encountered in our outpatient clinic as one of their members (the probands) were referred for genetic counselling due to non-syndromic hearing loss. *m.1555A>G* mutation was detected among 14 out of the 484 families (~3%).

Among 11 out of the 14 families, two or more members were diagnosed with *m.1555A>G* mutation-related hearing loss, while the remaining 3 families had only single members affected. Pedigrees of those 11 families were charted in Figure 1. They all showed a typical maternal inheritance pattern of mitochondrial diseases with incomplete penetrance and variable expressivity.

A total of 43 individuals were revealed to be *m.1555A>G* mutation carriers during the study period. They ranged from 8 to 90 years old. The male-to-female ratio was 13:30. In the 14 families, 8 out of 43 individuals had *m.1555A>G* mutation detected by RFLP which could not accurately distinguish heteroplasmy from homoplasmy. Whereas in the remaining 35 individuals, only one was found to be heteroplasmic for *m.1555A>G* mutation by direct mtDNA sequencing. She was incidentally found to be *m.1555A>G* mutation carrier by family cascade screening at 6 years old and remained asymptomatic to date.

The clinical characteristics of all probands in the 14 families with *m.1555A>G* mutation were summarised in Table 1. The average age of diagnosis with genetic confirmation was 35 years old. The majority of them (57%) had prelingual onset of hearing loss with one individual (Proband 14) presented with congenital hearing loss failing newborn hearing screening. Whereas the remaining 6 probands had hearing loss onset at childhood. For the nature and severity of hearing impairment, 10 (71%) individuals had bilateral severe to profound hearing loss while the remainder had mild to moderate hearing loss. No proband could recall an onset of hearing impairment after aminoglycoside exposure. However, 5 (36%) recalled a history of febrile episodes prior to hearing deficit onset.

Discussions

A total of 484 unrelated Hong Kong Chinese families with 580 patients were included in the present study. *m.1555A>G* mutation was found in 14 out of the 484 families presented with non-syndromic hearing loss, with an estimated prevalence of ~3%. Such finding was comparable to the prevalence of 2-9% in previous overseas and cohort studies conducted in China and Taiwan among individuals with non-syndromic hearing loss.[4,6,7]

Among the 14 families, 11 had two or more members affected with hearing impairment, while the remaining 3 families had only a single member with *m.1555A>G* mutation-related hearing loss. However, ascertainment bias existed as 44% of *m.1555A>G* mutation carriers in our cohort did not undergo formal hearing assessment so subtle hearing impairment not affecting daily activities or function might not have been picked up. Furthermore, individuals with onset of sensorineural hearing loss at older age might also attribute their symptoms to the natural path of aging without further seeking medical attention. The age of onset of hearing loss was also highly variable with a proportion of individuals previously reported to have disease manifestation only at advanced age.

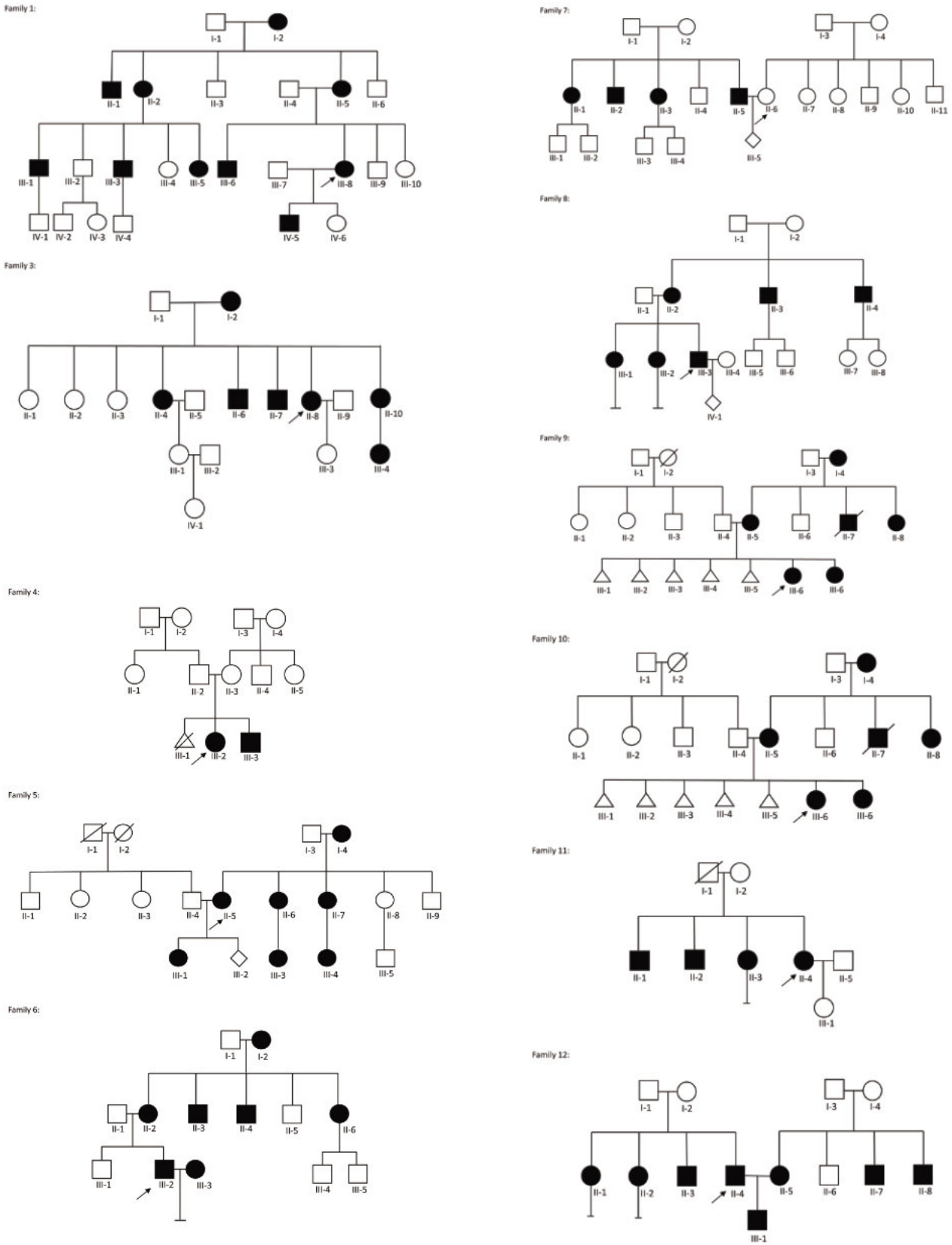

Figure 1 Pedigrees of 11 out of the 14 families with more than one family member presented with *m.1555A>G*-related hearing impairment. (Families 2, 13 and 14 were not included as they only had one isolated case of hearing loss in the family)

Table 1 Clinical characteristics of probands in the 14 families with *m.1555A>G* mutation related hearing loss

Proband	Sex/Age	Onset of hearing loss	Age at genetic diagnosis	Onset after febrile episode	Onset after aminoglycoside exposure	Nature and severity of hearing impairment	Outcome
1	F/45	Prelingual	27	Yes	N/A	Bilateral profound	Deaf and dumb
2	M/44	Prelingual	33	N/A	N/A	Bilateral profound	Deaf and dumb
3	F/55	Childhood	38	Yes	N/A	Bilateral severe SNHL	On hearing aids
4	F/42	Prelingual	28	Yes	N/A	Bilateral profound	Deaf and dumb
5	F/49	Prelingual	36	Yes	N/A	Bilateral profound	Deaf and dumb
6	M/42	Prelingual	31	No	N/A	Bilateral severe	Deaf and dumb
7	M/60	Prelingual	49	No	N/A	Bilateral profound	Deaf and dumb
8	M/64	12 years old	58	No	N/A	Bilateral profound SNHL	On hearing aids
9	F/24	Childhood	18	No	N/A	Bilateral high tone loss	No need for hearing aid
10	M/52	Prelingual	47	Yes	N/A	Right dead ear, left severe SNHL	On hearing aids
11	F/59	Childhood	56	No	N/A	?Mild	No need for hearing aid
12	M/61	~10 years old	58	N/A	N/A	Bilateral profound	Communicate by writing
13	M/9	4 (presented with disarticulation)	7	No	No	Bilateral mild high tone SNHL	No need for hearing aid
14	F/8	Congenital, failed newborn screening	7	N/A	N/A	Right mild SNHL, left mild to moderate SNHL	No need for hearing aid

Therefore, children in our cohort would need to have continuous longitudinal follow up to assess their hearing function. The patient's referral pathway may also contribute to selection bias as most probands were referred to our genetic counselling clinic by paediatricians or otorhinolaryngologists when genetic testing was deemed necessary.

Figure 1 showed the pedigrees of families with more than one individual presenting with *m.1555A>G* mutation-related hearing impairment. All pedigrees showed a typical maternal inheritance of mitochondrial disorders with risk to offspring only determined by the maternal lineage. All offspring of paternal *m.1555A>G* mutation carriers remained unaffected by the disease. Incomplete penetrance and variable expressivity were demonstrated among all families. Large intrafamilial variation existed with individuals in the same family having normal to profound hearing loss irrespective of whether they are homoplasmic or heteroplasmic for *m.1555A>G* mutation. The penetrance of *m.1555A>G* might have been previously overestimated in other studies due to recruitment bias. There was a report of *m.1555A>G* mutation carriers with normal hearing despite repeated definite exposure to aminoglycosides.[8] A recent study non-selectively screened 3,555,336 neonates born in China under the Chinese Newborn Concurrent Hearing and Genetic Screening cohort for any GJB2, SLC26A4, GJB3 or mitochondrial mutations. It was found that the *m.1555A>G* allele frequency among the large cohort of newborn to be 0.239%. Provided that the allele frequency did not change significantly among populations of the same ethnicity, the prevalence of *m.1555A>G* mutation-related hearing loss was less than expected from postulation with an allele frequency of 0.239%. This further suggested that a number of non-penetrant carriers of *m.1555A>G* mutation remained asymptomatic and undetected in the general population.[9] It is hoped that future studies could understand more in depth on the mechanisms of *m.1555A>G* mutation expression modification by mitochondrial DNA haplotypes and nuclear genome so that more accurate personalised genetic counselling could be provided to carriers and their families.

A small proportion (5 out of 14; 36%) of probands recalled a history of hearing loss following an episode of febrile illness. However, none of them could recall the use of antibiotics especially aminoglycosides preceding the onset of hearing impairment. It was likely related to the low level of awareness of mitochondrial-inherited aminoglycoside–induced ototoxicity among the Hong Kong general population and even among health professionals. It is hoped that through this study, the awareness of *m.1555A>G* mutation related hearing impairment could be raised so that carriers' exposure to potentially ototoxic drugs could be avoided.

Compliance with Ethical Standards

Conflict of Interest

All authors have disclosed no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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