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Co-occurrence of A1555G and G11778A in a Chinese family with high penetrance of Leber's hereditary optic neuropathy

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ABSTRACT

Co-occurrence of double pathogenic mtDNA mutations with different claimed pathological roles in one mtDNA is infrequent. It is tentative to believe that each of these pathogenic mutations would have its own deleterious effect. Here we reported one three-generation Chinese family with a high penetrance of LHON (78.6%). Analysis of the complete mitochondrial genome in the proband revealed the presence of the LHON primary mutation G11778A in the NADH dehydrogenase 4 (ND4) gene and a deafness-associated mutation A1555G in the 12S rRNA gene. The other mtDNA variants in this family suggested a haplogroup status G2b. Although A1555G has long been confirmed to be a primary mutation for aminoglycoside-induced and non-syndromic hearing loss, none of the maternally related members in this family showed hearing impairment. It thus seems that the occurrence of A1555G in this family had no pathological manifestation. However, whether A1555G has a synergistic effect with G11778A and contribute to the high penetrance of LHON remained an open question. To our knowledge, this is the first report that identified the co-existence of a deafness mutation A1555G and a primary LHON mutation G11778A in one family.

Leber's hereditary optic neuropathy (LHON; MIM 535000) is the first genetically characterized mitochondrial disease, with a manifestation of painless, acute or subacute bilateral visual loss in midlife leading to blindness and central scotoma [1–5]. The affected patients are mainly young men. A wide range of mtDNA mutations have been reported to be associated with LHON (cf. http://www.mitomap.org/rimtab1.html). Among them, three primary mutations (G3460A in the NADH dehydrogenase 1 [ND1] gene, G11778A in the ND4 gene, and T14484C in the ND6 gene) are predominant and account for over 95% LHON cases [2–4]. However, the prevalence of each of these primary mutations varies from East Asian to European patients [6,7]. Mutation G11778A is much more frequent (up to 90.2%) in Chinese LHON patients [7] when compared to European patients (56.6%) [6]. Conversely, LHON patients from Europe have higher frequencies of T14484C (20.8%) and G3460A (22.6%) [6] than those of Chinese patients (T14484C, 8.7%; G3460A, 1.2%) [7]. All three mutations impair the function of the respiratory chain complex I, but the exact pathological mechanism of LHON has not been sufficiently elucidated [2–4]. Incomplete penetrance of LHON in subjects with primary mutations and a prevalence of disease manifestation in male subjects are two of the main difficulties in understanding the etiology and pathophysiology of LHON [2–4]. Many factors, such as mtDNA haplogroup background, nuclear genes, and environmental factors, can affect the disease expression of LHON [2–4].

To present date, there have been hundreds of mtDNA mutations that have been found to be associated with various diseases [8,9]. For instance, A1555G in the 12S rRNA gene has long been confirmed to be a primary mutation for aminoglycoside-induced and non-syndromic hearing loss [10–17], although more recent studies have shown that nuclear genetic background can also affect the phenotypic manifestations of the A1555G mutation [13,18]. Co-occurrence of mutations with differing pathologies in one family is a rare event. Whether each co-occurring pathogenic mutation would have its own deleterious effect or a synergistic effect has not been well discussed in previous studies.

In this study, we reported one Chinese family with double pathogenic mutations that were reported to affect vision (G11778A) and hearing (A1555G), respectively. However, the probands only presented LHON without hearing impairment. This seems to indicate that the co-occurrence of pathogenic mutations may not necessarily correlate with co-morbid pathologies.

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Materials and methods

Patient evaluation and sample collection. A large LHON family (Le696) from Hebei Province was physically evaluated and collected at the Pediatric and Genetic Clinic of the Eye Hospital, Zhongshan Ophthalmic Center. Informed consents conforming to the tenets of the Declaration of Helsinki and following the guidance of sample collection of Human Genetic Disease (863 program) by the Ministry of Public Health of China were obtained from each participant prior to the study. The institutional review boards of the Zhongshan Ophthalmic Center and the Kunming Institute of Zoology approved this study.

mtDNA genome sequencing. Total genomic DNA was isolated from blood using a standard phenol/chloroform method. The entire mtDNA genome of one proband (III:10) was amplified and sequencing using a modified method as described in our recent study. In brief, the mtDNA genome was amplified by four overlapping pairs of primers with the following condition: one cycle of 94 °C for 1 min; 30 cycles of 94 °C for 30 s, 65.6 °C for 6 min; one extension cycle of 72 °C for 10 min. The purified PCR products were then directly sequenced by using 66 inner primers [19].

Data analyses. Sequences were edited using DNAstar (DNASTAR Inc., Madison, WI, USA). Sequence variation was scored relative to the revised Cambridge Reference Sequence (rCRS) [20]. We followed the recently updated version of East Asian mtDNA tree [21] to classify the LHON mtDNA. Novelty of mtDNA variants were defined by an exhausted database search following the guidelines described in our recent study [22]. Potential risk of accidental amplification of nuclear mitochondrial pseudogenes (NUMTs) was evaluated and identified using the available guideline [23]. Sequence variation in the proband, together with one phylogenetically related mtDNA sequence (EWK28, GenBank Accession No. AY255139) [24] and two LHON mtDNAs (Le1244, EU545470; Le1269, EU545471) [19] from published sources, was presented in a tree. Evolutionary conservation analysis for a certain mtDNA variant was performed using the same approach in our recent study [19].

Results

Clinical features

The overall penetrance of LHON in the family Le696 was strikingly high and reached 78.6% (11/14). Particularly, all four offspring (including three females and one male) in the second generation were affected with severe vision loss. Seven of the 10 maternally related subjects in the third generation were affected. The penetrance of LHON was comparable in male (8/10) and female (3/4) descendants (Fig. 1). It is worth noting that the estimate for the penetrance of LHON might be biased due to the small sample size. None of the family members showed symptoms of hearing loss.

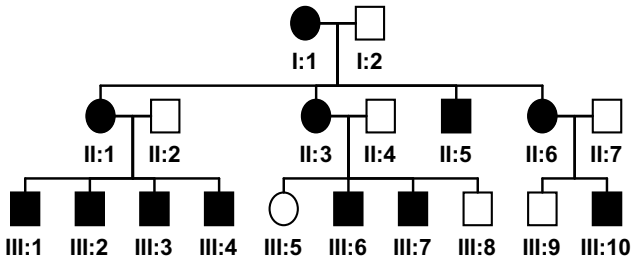

Fig. 1. Pedigree information for a Chinese LHON family (Le696) with A1555G and G11778A. Affected individuals are marked by filled symbols. The proband that was analyzed for the entire mtDNA sequence was marked by an arrow.

mtDNA mutation analysis

The proband's mtDNA harbored a string of sequence variants that suggested a haplogroup status G2b (Fig. 2), which is a rare haplogroup and mainly presented in Northern China. This mtDNA had five private substitutions A1555G, T11353C, G11778A,

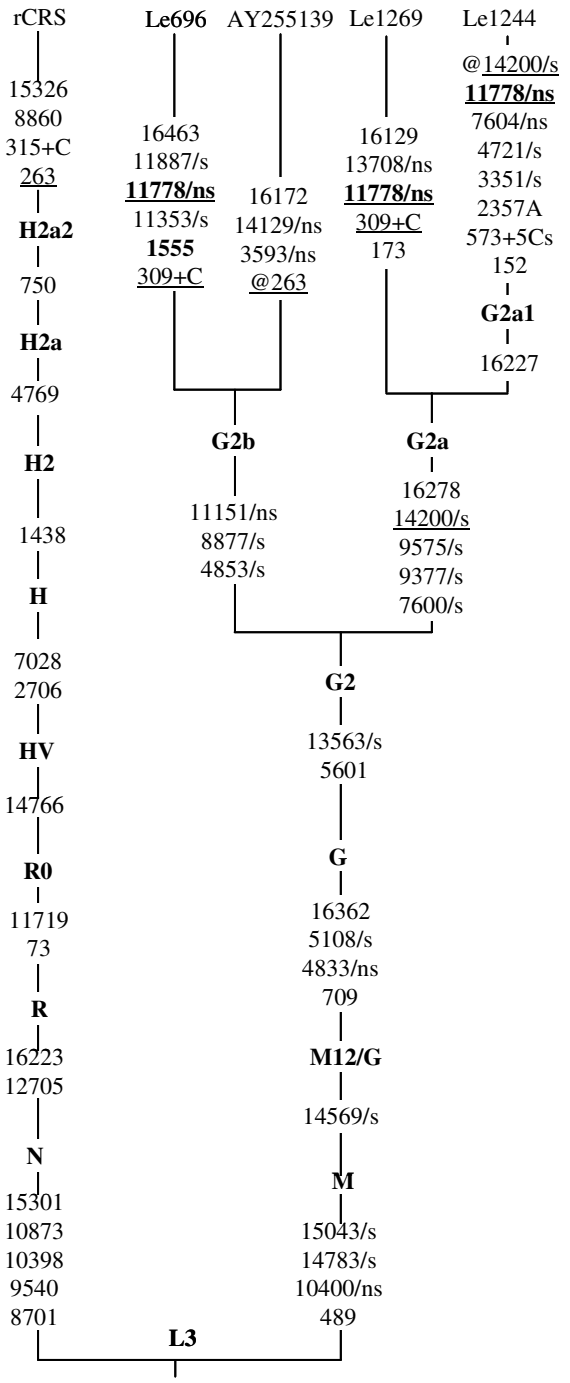

Fig. 2. Classification tree of the proband mtDNA in family Le696 and three reported mtDNAs, plus the revised Cambridge reference sequence (rCRS) [20]. The reported LHON families Le1244 (EU545470) and Le1269 (EU545471) and sequence AY255139 were adopted from Wang et al. [19] and Kong et al. [24], respectively. The order of mutations on each uninterrupted branch section is arbitrary. Recurrent mutations are underlined and back mutations at sites 263 and 14,200 were marked by "@". Suffix "A" refers to transversion and '+C' indicates an insertion of cytosine. Previously confirmed pathogenic mutations are in bold. The synonymous and non-synonymous coding-region variants (relative to the root of M) in the four mtDNAs are further denoted by "/s" and "/ns", respectively.

G11887A, and A16463G, besides an insertion of cytosine at site 309 that is located in a well-known mutational hotspot. None of these variants can be described as "novel" mutations based on the database (e.g. mtDB (www.genpat.uu.se/mtDB)) and web-based searches [22]. Among these variants, A1555G and G11778A are well-known pathogenic mutations that have been reported for more than a decade. Variants T11353C and G11887A are located in the ND4 gene. Both variants are synonymous mutations and cause no amino acid change. Variant T3593C in the ND1 gene (which changes the amino acid from valine to alanine at the 96th codon) was regarded as one of the characteristic mutations for haplogroup G2b in our previously published study [21], however, this variant was not detected in the proband mtDNA. Based on Fig. 2, the motif of G2b can be redescribed as G4853A-T8877C-C11151T. Conservation analysis indicated that the coding-region variants at sites 11353 and 11887 in family Le696 were not conserved and should be categorized as polymorphisms. The complete proband mtDNA was deposited in GenBank under Accession No. FJ015040.

Discussion

Co-existence of confirmed pathogenic mutations that affect vision and hearing in one mtDNA is a rare incident. It is speculative that each of these pathogenic mutations could have a deleterious effect, causing both vision loss and hearing impairment. In this study, we identified the deafness-associated mutation A1555G and the LHON primary mutation G11778A in a family from Northern China. To our knowledge, this is the first report that identified the co-existence of A1555G and G11778A in one family. In a recent report, Wei et al. reported a co-existence of A1555G and another LHON primary mutation T14484C in a Chinese family [25] belonging to haplogroup C4a1. It should be noted that this complete mtDNA was wrongly classified as H2 by Wei et al. [25] and had at least four reading errors, which are common in their previous studies [15,26].

There are some similarities and differences in regard to the disorders observed in these two families with double pathogenic mutations. On one hand, the penetrance of LHON in both families is incomplete and the apparent defect is vision loss; on the other hand, the disease manifestation varies in the two families. Statistically, there is a significantly higher penetrance of LHON in the family described in this study (78.6%; 11/14) compared to the family (30.8%; 4/13) reported by Wei et al. [25] (Fisher's exact test, two-tailed, $P = 0.021$; Chi-square with Yate's correction, 4.452, $P = 0.035$). Moreover, none of the maternally related members in our study showed hearing impairment, whereas one of 14 matrilineal relatives (including the female subject in the first generation) in the reported family exhibited mild hearing loss in addition to LHON [25]. Based on these results, it seems that co-occurrence of multiple pathogenic mutations may not necessarily result in co-morbidity of their independent pathologies.

One interesting question remains unresolved is whether the co-existent pathogenic mutation (e.g. A1555G) would enhance the symptom (e.g. vision loss) that was caused by the LHON primary mutation? The strikingly high penetrance of LHON in family Le696 could be in theory explained by a potentially synergistic effect stemming from the A1555G mutation. However, this explanation would seemingly run into conflict with the pattern observed in the LHON family reported by Wei et al. [25], in which the overall penetrance of LHON and hearing loss was very low. Therefore, based on the two families discussed here, we cannot conclusively determine whether a synergistic effect between these two co-existing mutations exists. It is worthwhile to have a long-term follow-up survey for the disease expression in these matrilineal relatives and their offspring.

Penetrance and phenotypic manifestation of LHON in subjects with primary LHON mutations is very complex. Additional genetic factors, such as nuclear genes, mtDNA haplogroup background, and environmental factors have been found to be involved in this process [1–4,6,27–29]. In a recent study, we observed a strikingly different penetrance pattern of LHON in two Chinese families with G11778A, in which family A had a significantly higher penetrance (53.3%) than family B (15.0%) [19]. We presented these two reported pedigrees together with the one described in this study in Fig. 2. Based on the tree pattern, it is quite obvious that G11778A occurred independently in these LHON families, notwithstanding that they shared same haplogroup status G2. Evidently, additional factors should be involved in the manifestations of LHON in these families.

Similar to the LHON primary mutations, phenotypic expression of A1555G has long been found to be modulated by nuclear genes and other factors [13,18]. This could account for the absence of hearing loss in family Le696 and the low penetrance of hearing loss in the reported family [25]. Intriguingly, the penetrance of LHON in male and female subjects in family Le696 was similar and was in sharp contrast to a predominantly-male pattern as observed in a large cohort of patients [6,7]. Whether this deviated pattern of LHON penetrance was caused by A1555G or other nuclear gene modulation deserves further study.

In short, we report a co-existence of pathogenic mutations A1555G and G11778A in one Chinese family for the first time. The disease expression pattern in this family suggested that the two mutations may not enact their noxious roles independently. More family and experimental data will be needed to further clarify a potentially synergistic effect of the co-existent pathogenic mutation.

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