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ORIGINAL COMMUNICATION

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Spectrum of the mitochondrial DNA mutations of Leber's hereditary optic neuropathy in Koreans

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Abstract We investigated 14 primary mitochondrial DNA (mtDNA) mutations at nucleotide positions (nps) 3460A, 4160C, 5244A, 9101C, 9804A, 10663C, 11778A, 13730A, 14459A, 14482G, 14484C, 14495G, 14498T, and 14568T, and one common secondary mutation at np 15257A, in 82 Korean patients with suspected Leber's hereditary optic neuropathy (LHON). Only three kinds of LHON mutations were identified in 60 (73%) of the 82 probands, these being the 11778A, 14484C, and 3460A mutations with 46 (56%), 13 (16%), and 1 (1%) cases, respectively. None of the other mtDNA mutations was detected. Of the 60 probands with LHON positive mutations, 19 (32%) had relevant family histories. Heteroplasmy was determined in 2 (4%) of the 46 probands with the 11778A mutation and 1 (8%) of the 13 probands with the 14484C mutation. In conclusion, the 11778A mutation was the most common cause (56%), with a high prevalence of the 14484C and a lower prevalence of the 3460A mutations being characteristic of Korean patients with LHON. The 3460A mutation especially showed a remarkable racial difference from that in Caucasians. With the exceptions of the 3460A, 11778A, and 14484C, the mutations screened may not be involved in the pathogenesis of LHON in Koreans and may not have a synergistic effect on its clinical expression.

Key words Leber's hereditary optic neuropathy · mitochondrial DNA · mutation · prevalence · Koreans

Introduction

Leber's hereditary optic neuropathy (LHON; MIM 535000) is a maternally inherited disease, characterized by acute visual loss with varying degrees of recovery [1]. Wallace et al. first identified a mutation of the mitochondrial DNA (mtDNA), at the 11778 position, as a primary etiologic factor in LHON; subsequently more than 20 kinds of mtDNA mutations have been reported [2] (http://www.mitomap.org/). From detailed genetic analysis, over 95% of LHON pedigrees harbored one of four mtDNA mutations, these being the 3460A, 11778A, 14459A, and 14484C mutations, which all involve genes encoding complex I subunits of the mitochondrial respiratory chain [3, 4]. These primary LHON mutations share certain genetic features: (1) they alter complex I polypeptides; (2) they have been found in several unrelated LHON families; (3) they do not co-occur with each other; (4) they can be homoplasmic or heteroplasmic; (5) they have not been found in control mtDNAs [4]. In addition to the four primary LHON mutations, other primary variants, so called "provisional" primary mutations, have been found in more than one LHON family but are rare [4]. The secondary LHON mutations are ambiguous with respect to LHON etiology and some are likely to be unrelated to the disease [5].

The identification of a primary LHON mutation has

profound implications for the proband and his/her family. There are well-established prognosis and recurrence risks for matrilineal lineages that carry the 3460A, 11778A, 14459A, or 14484C mutations. However, their relative frequencies are reported to vary considerably worldwide. Furthermore, these four primary mutations have not been identified in a small minority of diagnosed LHON cases, and the most likely explanation is that rare pathogenic mtDNA variants are segregating in these pedigrees [4].

To identify the prevalence and spectrum of pathogenic mtDNA mutations in Korean LHON patients, we investigated the presence of primary mutations at 14 mtDNA nucleotide positions (nps), including 4 "well-known" and 10 "provisional" primary mutations, as well as one common secondary mutation in 82 unrelated probands with bilateral optic atrophy.

Materials and methods

Subjects

Based on the surveys of the available web-based database (http://www.mitomap.org/; http:www.genetests.org/) and the published literature [4], we selected a total of 15 LHON mutations that we predicted would be found more frequently in Korean or Asian LHON patients. We performed mtDNA analyses for the 4 well-known LHON primary mutations (at nps 3460A/ND1, 11778A/ND4, 14459A/ND6, and 14484C/ND6), the 10 provisional primary mutations (at nps 4160C/ND1, 5244A/ND2, 9101C/ATP6, 9804A/CO3, 10663C/ND3, 13730A/ND5, 14482G/ND6, 14495G/ND6, 14498T/ND6, and 14568T/ND6), and one common secondary mutation (at np 15257A/CYB) in 82 unrelated probands referred from regional hospitals throughout South Korea with informed consent. Patients meeting the following criteria were selected: 1) history of acute or subacute bilateral optic neuropathy without ocular pain, 2a) at least one maternally related individual with bilateral optic neuropathy, 2b) telangiectatic microangiopathy of optic disc, and 2c) no response to treatment with corticosteroids. The patients who fulfilled criterion 1) and at least one of criteria 2) were subjected to DNA analysis. All other causes of optic neuropathy were excluded by ophthalmological and neurological examinations including brain magnetic resonance imaging. Nineteen of the 82 patients were diagnosed as definite LHON, viz., at least one maternally related individual also had bilateral optic atrophy [6] and the remaining 63 patients were suspected of having LHON with no apparent family histories.

DNA analysis

Total DNA was extracted from peripheral blood leukocytes using the standard method. Polymerase chain reaction amplifications (PCR) and restriction endonuclease digestion of the target mtDNA sequences, were performed using the protocols designed and/or modified from previous reports [7]. Amplified mtDNAs were digested with the appropriate restriction enzymes, and the products underwent electrophoresis on 2–4 % agarose gels. Diagnostic restriction site gain or loss was confirmed by direct sequencing using Thermo Sequenase radio-labeled terminator cycle sequencing kits (Amersham, Buckinghamshire, UK) and 6 % polyacrylamide sequencing gels. The family members of the positive LHON probands were recruited and were investigated the LHON mutation detected in the probands by PCR and restriction digestion.

Results

LHON mutations were determined in 60 of the 82 probands analysed (73.2 %). These mutations were identified at nps 11778A, 14484C, and 3460A in 46 (56.1 %), 13 (15.9 %), and one (1.2 %) probands, respectively. No other LHON mutations were identified. All of the probands with LHON exhibited only one primary mutation. Heteroplasmy was ascertained in 2 (4.3 %) of the 46 probands with the 11778A and 1 (7.7 %) of the 13 probands with the 14484C.

Of the 60 probands with positive mutations, 19 (31.7 %) had family histories: 16 with the 11778A, and 3 with the 14484C. In the family study, 65 family members with the 11778A mutation in 11 families, and 3 with the 14484C mutation in 3 families, were detected.

Discussion

Of the 15 LHON mutations investigated, only three, the 3460A, 11778A, and 14484C were determined in 73.2 % of Korean patients. The prevalence of those mutations among Korean LHON patients is similar to that in other ethic populations, but the mutation spectrum is quite different (Table 1).

The presence of the 11778A mutation for LHON has been identified in 40–90 % of patients, with diverse ethnic backgrounds, and is regarded as the most common cause of LHON [8]. This mutation arose many times at random in Caucasian populations and its frequency varies among ethnic groups [2, 9, 10]. Among the Asian populations, the 11778A mutation was reported in 73.8–82.4 % of Japanese [11, 12] and 48.9 % of Chinese LHON patients [13], and was also found to be the most common cause in Korean patients (56.1 %). The 14484C has been reported to occur several times at random and is found in Caucasians and Asians with the incidences of 10–13 % and 4.3–7.5 %, respectively [8, 10, 12, 13]. In Koreans, it was found to be the second most common cause of LHON mutation with an incidence of 15.9 %. The 3460A has been found in 8–25 % of Caucasian LHON patients, mainly in North America and Europe [8, 10]. But this mutation was very rare in Asians: only one case (1.2 %) of 82 was identified in Koreans and only three pedigrees were found in the Japanese [11, 12]. These data indicate a remarkable racial difference of the 3460A mutation as a founder effect.

The etiological role of the 15257A mutation in terms of disease expression is not clear. In Caucasians, it is generally associated with the haplogroup J and can co-occur with a common primary LHON mutation [4, 14, 15], but in a recent report a Turkish patient with this mutation carried none of the 3460A, 11778A, or 14484C mutations [16]. In our study we found no patients carrying the 15257A mutation. Therefore, the 15257A may not be

Table 1 Comparison of the frequency of mtDNA mutations in LHON families

Mutation	Asians						Caucasians					
	Present study		Mashima et al. [12]		Yen et al [13]		Mackey et al. [3][a]		Huoponen et al. [15]		Johns et al. [19]	
	n	%	n	%	n	%	n	%	n	%	n	%
11778	46	56.1	59	73.8	23	48.9	110	69.2	13	56.5	86	55.1
14484	13	15.9	6	7.5	2	4.3	23	14.5	0	0	17	10.9
3460	1	1.2	3	3.8	0	0	21	13.2	3	13.0	14	9.0
15257[b]	0	0	0	0	0	0	NT	NT	2	8.7	15	9.6
Pos./Total[c]	60/82	73.2	68/80	85.0	25/47	53.2	154/159	96.9	16/23	69.6	117/156	75.0

[a] Only definite LHON families were analysed
[b] The 15257 mutation is excluded in the calculation of total incidence. Two families reported by Huoponen et al. [15] and four of 15 families reported by Johns et al. [19] also harbored the 11778 and the 14484 mutations, respectively
[c] Pos./Total: Number of patients with the positive mutations (excluding the 15257 mutation)/Total number of patients studied
NT Not tested

involved, as a primary mutation, in the pathogenesis of LHON in Koreans and may not have a synergistic effect on the clinical expression of LHON as a secondary mutation.

The 10 provisional primary mutations were not determined in any of our patients. This finding suggests that each of these mutations originates from a single founder and is then specific to that ethnic background. A significant number of individuals suspected of having LHON do not harbor any of the four primary mtDNA mutations. They may have a novel mutation unique to the family. Virtually all of the four primary LHON mtDNA mutations alter polypeptides that are components of complex I [4, 17]. Therefore, it would seem logical that mtDNA complex I genes should be sequenced in all LHON patients not harboring one of the four common mutations.

While LHON has been traditionally considered to be family related, many cases appear to be isolated. In Caucasians, the proportions of individual mutations with a family history have been reported as 71–78 % for the 3460A, 43–53 % for the 11778A, and 65–100 % for the 14484C [1, 9, 18, 19]. However, these incidences were much lower in the Japanese, where a total incidence with a family history was 45.6 %, and the incidences of family history for the 3460A, 11778A, and 14484C mutations were 33.3 %, 47.5 %, and 33.3 %, respectively [12]. In our study, the total frequency of LHON mutations with family history was 31.7 %, and the incidences of family history for the 11778A and 14484C were 34.8 % and 23.1 %, respectively. These incidences are much lower than those of Caucasians, but similar to those of the Japanese. This low level of family cases in Asian populations could be either a true estimate of the occurrence of sporadic cases and/or no knowing or concealing of the existence of affected relatives.

Heteroplasmy in LHON has been reported variously to be between 0–14 % and 0–17 % at nps 11778A and 14484C, respectively [1, 9, 20–22]. In this study, the rates of heteroplasmy were 4.3 % and 7.7 % in the probands with the 11778A and the 14484C mutations, respectively. All of the heteroplasmic patients did not show any peculiar clinical findings distinct from the homoplasmic ones. Indisputably, heteroplasmic families are often sporadic and most likely reflect a recent mutational event dating back only a few generations [20, 23].

In conclusion, the total prevalence of known primary LHON mutations was 73.2 %, with only three mutations, 3460A, 11778A, and 14484C, being identified in Koreans. With the exception of these mutations, no other provisional primary mutations were found to be involved in pathogenesis of LHON in Korean patients. There is a possibility of novel mtDNA mutations, especially in the complex I genes, which account for the visual loss in the Korean LHON patients not associated with the known mutations. Further study designed to recognize unidentified pathogenic mitochondrial mutations may provide a new spectrum and a diagnostic tool for LHON mutations in Koreans.

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