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Leber's Hereditary Optic Neuropathy with 3460 Mitochondrial DNA Mutation

Leber's hereditary optic neuropathy (LHON) is a maternally transmitted disease causing acute or subacute, bilateral optic atrophy mainly in young men. It is found to be a mitochondrial disorder with the primary mitochondrial DNA (mtDNA) mutations at 11778, 3460, and 14484. The incidence of each mutation is reported to be race-dependent. Point mutations at mtDNA nucleotide position 11778 and 14484 have been reported in Korean patients with LHON, however there has been no report of mtDNA mutation at nucleotide position 3460. Molecular genetic analyses at four primary sites (11778, 14484, 15257, and 3460) of mitochondrial DNA using the polymerase chain reaction, restriction enzyme digestion, and direct sequencing were performed in a 35-yr-old man with severe visual loss. A point mutation in the mtDNA at nucleotide position 3460 was identified and a conversion of a single alanine to a threonine was confirmed. To our knowledge, this is the first report confirming mtDNA mutation at nucleotide position 3460 in Korean patients with LHON. Detailed molecular analyses would be very helpful for the correct diagnosis of optic neuropathy of unknown etiology and for genetic counseling.

Key Words : *Optic Atrophy, Hereditary, Leber; Nucleotides; Nucleotide position 3460; Korean*

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INTRODUCTION

Leber's hereditary optic neuropathy (LHON) is a maternally inherited disease characterized by acute or subacute loss of the central vision primarily in young men (1). Mitochondrial inheritance was first confirmed in 1988 by Wallace et al. with the identification of a mitochondrial DNA point mutation at nucleotide position 11778 in the NADH dehydrogenase subunit 4 gene in nine pedigrees with a clinical diagnosis of LHON (2). A second mutation at nucleotide position 3460 was identified in three pedigrees without the 11778 mutation by Huoponen et al. in 1991 (3). LHON is associated with three different point mutations of mitochondrial DNA (mtDNA) affecting nucleotide positions 3460 (3-5), 11778 (2), and 14484 (6). These mutations are estimated to account for 8-25%, 50-60%, and 10% of LHON pedigrees respectively (3-7). The incidence of each mutation is reported to be race-dependent (3-7).

Since the first identification of the 11778 mutation in Korean patients in 1995 (8), 60 patients with a 11778 mutation (9) and 12 patients with a 14484 mutation have hitherto been identified (10). LHON is now recognized as one of the most common causes of optic neuropathy in young males (11). However, among the known primary mutations, 3460 mutation has not been confirmed in Koreans.

CASE REPORT

A 35-yr-old man complained of visual disturbance in his left eye 10 months ago, followed by a decline in his right vision three months ago. The visual loss did not accompany any ocular or orbital pain. His medical history was unremarkable, and had no history of smoking or consuming alcohol. He did not admit to elicit drug use, nor had any visually symptomatic family members.

Corrected visual acuity was finger count OU, and color vision was 0/14 OU by Ishihara color test. Temporal pallor of both optic discs was observed. Goldmann perimetry examination revealed bilateral cecocentral scotoma (Fig. 1).

DNA analysis

MtDNA analysis was undertaken for four LHON mutations (at np 11778, np 3460, np 14484, and np 15257). DNA was extracted from peripheral white blood cells using the

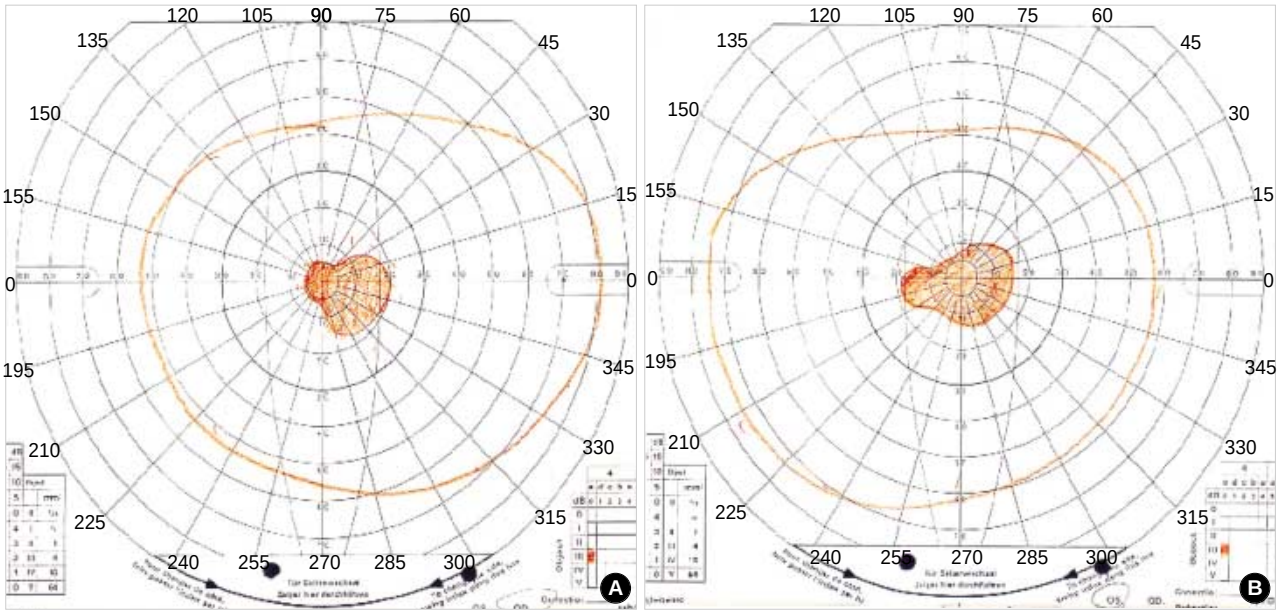

Fig. 1. Visual field testing. A cecocentral scotoma was detected in the right (A) and left (B) eyes.

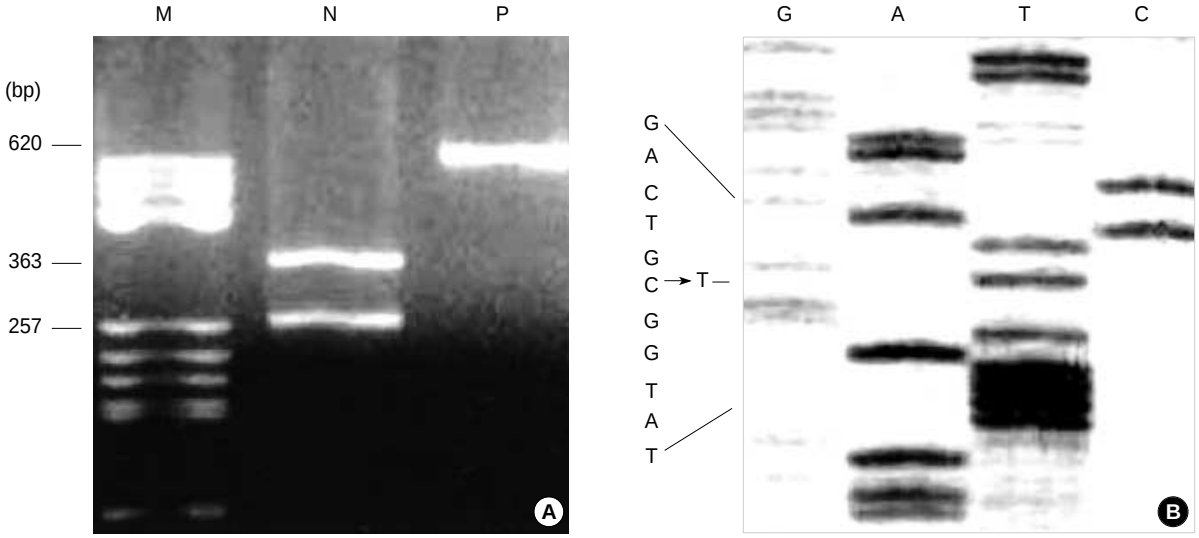

Fig. 2. (A) Restriction analysis of the PCR product amplified mtDNA around the np3460 area from a normal person (N) and the patient with LHON (P), showing a 620 bp band of *BsaHI* site loss in the patient due to MTND1*LHON3460A, M: molecular marker. (B) Sequencing result of mtDNA around the np 3460 area in the patient with MTND1*LHON3460A, showing C to T mutation in the antisense strand and G to A mutation in the sense strand.

standard method. Polymerase chain reaction amplifications and restriction digestion of the target mtDNA sequences were performed using modifications of previously reported protocols (12). The mtDNA region bracketing the 3460 mutation was amplified with PCR using the following primers; forward: -TTCAAATTCCTCCCTGTACG, reverse: -CCACAAGCTTGGCTACTGCTCGCAGTG, and the PCR product was digested with *BsaHI* (New England Biolabs, MA, U.S.A.) (Fig. 2A). Verification of the 3460 mutation in the patient who lost the *BsaHI* site was carried out by direct sequencing using a Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham, Buckinghamshire, U.K.) and 6% polyacrylamide sequencing gels (Fig. 2B).

DISCUSSION

Huoponen et al. found a point mutation at mtDNA position 3460 coding for subunit 1 of complex I in three indepen-

dent Finnish families with LHON (3). Guanine is replaced by adenine and this converts the 52nd codon of the ND1 protein from alanine to threonine. To date, the 3460 mutation is observed only in LHON and thus its presence deserves to be a rational basis of the diagnosis of LHON (3, 4). Since 1995, none of over 150 control subjects or 74 patients positive for the 11778 or 14484 mutation had tested positive for the 3460 mutation (9-11).

The frequency of the 3460 mutation among LHON pedigrees without the 11778 mutation ranges from 13% (13) to 50% (six of 12) in a series performed in the United Kingdom and Australia (4). Another study of 10 Finnish pedigrees without the 11778 mutation identified three families (30%) with the 3460 mutation (3). These differences in the frequency of the mutation may reflect true population variability, or they may be simply due to sampling error related to the relatively small number of patients examined. The three large series have combined a total of 74 pedigrees without the 11778 mutation, of which 18 (24%) pedigrees were tested positive for the 3460 mutation (3-5). In Asians, the incidence of mutations at 3460, 11778, and 14484 were reported to be 4%, 87%, and 9%, respectively among 80 Japanese LHON pedigrees (14).

The patients with the 3460 mutation have a higher incidence of visual recovery, a higher percentage of pedigrees with more than one affected family member, and a greater frequency of tobacco and alcohol abuse than the patients with the 11778 mutation (5). The difference in visual prognosis of these two mutations and the need for the modification of possible risk factors provide added significance to genetic testing for LHON.

Of clinical importance is the fact that the visual prognosis differs according to the mutations. The rate of visual recovery with the 3460 mutation was reported to be 20% (5), which is much higher than the 4% with the 11778 mutation (15), and lower than 37-58% with the 14484 mutation (6, 10, 16). However, other series did not show any significant difference in visual outcome between the 11778 and 3460 mutation groups (17). As for the present case, visual acuity did not improve. However, our patient had only 10 months or less of follow-up and may yet recover vision.

The percentage of family with more than one visually symptomatic family member has been reported to be higher in pedigrees with the 3460 mutation (78%) (3-5) than in those with the 11778 mutation (43%) (14). In other words, the 3460 mutation may be more likely to result in visual loss than the 11778 mutation (5). These findings emphasize the value of molecular genetic testing in patients with suspected LHON because of the implications for prognosis with respect to visual recovery and the potential for risk factor modification in patients and their maternal relatives.

To our knowledge, this is the first detailed description on a Korean LHON patient with the 3460 mutation. The present case report demonstrates the possible mitochondrial genetic heterogeneity existing among Korean LHON pedigrees, and emphasizes the necessity for identifying mutations other than the 11778 for confirmative diagnosis, appropriate counseling for risk-factor management, visual outcome, and family planning.

In conclusion, whenever LHON is clinically suspected, detailed molecular analyses not only for the detection of the 11778 mutation but also for that of the other three primary mutations should be undertaken.

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