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# GENOTYPES OF ALDEHYDE DEHYDROGENASE AND ALCOHOL DEHYDROGENASE POLYMORPHISMS IN PATIENTS WITH LEBER'S HEREDITARY OPTIC NEUROPATHY

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*Summary* To define whether alcohol drinking provides a risk for Leber's hereditary optic neuropathy (LHON), the genotypes of low $K_m$ aldehyde dehydrogenase (ALDH2) and alcohol dehydrogenase type 2 ($ADH_2$), major enzymes involving the alcohol metabolism, were examined in 29 unrelated Japanese patients with LHON associated with mitochondrial DNA 11778 mutation, 24 unrelated asymptomatic carriers with the mutation and 57 normal controls without the mutation. PCR-restriction detection revealed three genotypes of ALDH2 and $ADH_2$. The allele frequencies of either enzyme in LHON patients, asymptomatic carriers, or both, did not differ from those in normal controls. There is no association between LHON and genotypes of alcohol-metabolizing enzymes. However, six of the LHON patients had frequent alcohol consumption, while none of the asymptomatic carriers claimed frequent drinking habit. Thus, we could not make a denial of drinking effects on optic nerve damage in LHON.

***Key Words*** Leber's hereditary optic neuropathy, mtDNA 11778 mutation, aldehyde dehydrogenase, alcohol dehydrogenase, genotyping

## INTRODUCTION

Leber's hereditary optic neuropathy (LHON) is a genetic optic nerve disease that usually affects young adults, presents with acute or subacute central visual loss, and frequently results in poor visual outcome. Molecular genetic analyses have revealed a variety of mutations in mitochondrial DNA (mtDNA) that are evidently pathogenic for LHON and account for the unique inheritance of the

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disease (Wallace *et al.*, 1988). The mtDNA defect is, however, not the sole determinant of clinical disease as some 15% of males and up to 70% of females harboring the pathognomonic mtDNA mutation remain unaffected throughout life (Newman, 1993). It is, therefore, most likely that yet undefined environmental factors act as an additional risk in the development of LHON.

Many LHON patients consume heavy alcohol, and its incidence appears significantly high in view of cultural or socioeconomic standards, and the clinical features of LHON sometimes resemble those of alcohol amblyopia (Cullom *et al.*, 1993). Thus, alcohol has been proposed as one of the risk factors for clinical manifestations of LHON. Low $K_m$ aldehyde dehydrogenase (ALDH2), a mitochondrial enzyme, is believed to be responsible for the oxidation of most of the aldehyde generated during alcohol metabolism. Molecular genetic studies indicate that the gene for ALDH2 is polymorphic and that the capacities of alcohol metabolism are determined with a complete agreement between the ALDH2 genotype and phenotype (Hsu *et al.*, 1987).

Referring to these backgrounds, we studied whether the clinical manifestation of LHON is influenced by the genotypes of ALDH2. We also examined the gene for alcohol dehydrogenase type 2 ($ADH_2$). $ADH_2$ is another enzyme related to alcohol metabolism, and the relevant gene is also highly polymorphic among Orientals (Takeshita *et al.*, 1994). This paper is concerned with the frequency of the genotypes of these enzymes in LHON patients with mtDNA 11778 mutation and in persons carrying the same mutation but remaining asymptomatic.

## PATIENTS AND METHODS

The genotypes of ALDH2 and $ADH_2$ polymorphisms were determined in 29 unrelated Japanese patients (25 males and four females, ranged in age from 14 to 58 years) who had mtDNA 11778 mutation and clinical manifestations of LHON, 24 unrelated healthy individuals (10 males and14 females, ranged in age from 15 to 67 years) who had the same mtDNA mutation but remained asymptomatic, and 57 normal controls (29 males and 28 females, ranged in age from 20 to 57 years) from a population in Kagoshima prefecture who had only wild-type sequence at the nucleotide position 11778 in mtDNA. The mtDNA mutation was confirmed by the method described elsewhere (Isashiki *et al.*, 1992).

After informed consent, peripheral blood was obtained, and genotypes of ALDH2 and $ADH_2$ were determined by the methods described (Takeshita *et al.*, 1994; Xu *et al.*, 1988). In brief, to identify the genotype of ALDH2, exon 12 of the ALDH2 gene was amplified using a mutated primer pair. Amplified products were digested with *Ksp* 632I and separated by 3% NuSieve-agarose gel electrophoresis, which enabled one to distinguish homozygote of typical allele ($ALDH2^1$/$ALDH2^1$), heterozygote of typical and atypical alleles ($ALDH2^1$/$ALDH2^2$), and homozygote of atypical allele ($ALDH2^2$/$ALDH2^2$). To identify the genotype of

$ADH_2$, 5′- end of exon 3 of the $ADH_2$ gene was amplified using a wild-type primer pair, followed by digestion with *Mae*III and by separation by 3% NuSieve-agarose gel electrophoresis, which enabled one to determine three genotypes, $ADH_2^1$/$ADH_2^1$, $ADH_2^1$/$ADH_2^2$ and $ADH_2^2$/$ADH_2^2$.

## RESULTS AND DISCUSSION

Table 1 shows the frequency distribution of three genotypes of ALDH2 in LHON patients, asymptomatic carriers and normal controls. Gene frequencies of typical and atypical alleles were calculated to be 0.776 and 0.224 for symptomatic patients, and 0.813 and 0.187 for asymptomatic carriers, respectively. Deviation from the Hardy-Weinberg's prediction was not significant in either group (chi-square: 0.0238 and 1.278, df=1, $p>0.5$). Also, differences in the allele frequencies were not significant between patients and asymptomatic carriers (chi-square: 0.035, df=1, $p>0.5$). Furthermore, the allele frequencies of either patients or carrier group did not differ from those of controls.

Table 2 shows the frequency distribution of genotypes of $ADH_2$. Statistical analyses did not show any significant difference in the $ADH_2$ genotype between LHON patients and asymptomatic carriers, or between either group and controls.

Enzyme activities related to alcohol metabolism are highly polymorphic in Orientals with corresponding polymorphic genotypes. Persons with typical genotype and active enzymes, in particular ALDH2, are prone to frequent large-dose alcohol drinking. On the other hand, persons with atypical genotype are deficient in alcohol metabolism and do not consume alcohol because of overt symptoms following drinking (Crabb *et al.*, 1988). Thus, it would be expected that either typical or atypical genotype is increased in frequency as compared with the control population; typical allele would cause chronic influence of alcohol because of high-dose drinking habit, and atypical allele could be relevant to part

Table 1. Genotypes of ALDH2 in 53 individuals with mtDNA 11778 mutation.

| | n | $ALDH2^{1/1}$ | $ALDH2^{1/2}$ | $ALDH2^{2/2}$ |
|---|---|---|---|---|
| LHON | 29 | 17 (58.6) | 11 (37.9) | 1 (3.5) |
| Carrier | 24 | 15 (62.5) | 9 (37.5) | 0 (0) |
| Control* | 57 | 32 (56.1) | 21 (36.9) | 4 (7.0) |

* A normal Japanese population without mtDNA 11778 mutation; ( ): %.

Table 2. Genotypes of $ADH_2$ in 53 individuals with mtDNA 11778 mutation.

| | n | $ADH_2^{1/1}$ | $ADH_2^{1/2}$ | $ADH_2^{2/2}$ |
|---|---|---|---|---|
| LHON | 29 | 3 (10.4) | 9 (31.0) | 17 (58.6) |
| Carrier | 24 | 1 (4.2) | 10 (41.6) | 13 (54.2) |
| Control* | 57 | 5 (8.8) | 22 (38.6) | 30 (52.6) |

* A normal Japanese population without mtDNA 11778 mutation; ( ): %.

Table 3. Clinical and genetic findings of LHON patients with daily alcohol-drinking habitation (over 40 g per day).

| No. | Age | Sex | Age at onset | Optic nerve disorder | ALDH2 | $ADH_2$ |
|---|---|---|---|---|---|---|
| 1 | 48 | F | >40 | chronic progressive | 1/1 | 2/2 |
| 2 | 52 | M | 52 | acute | 1/1 | 2/2 |
| 3 | 46 | M | 45 | acute | 1/2 | 2/2 |
| 4 | 58 | M | 58 | acute | 1/2 | 1/2 |
| 5 | 18 | M | 18 | acute | 1/1 | 1/1 |
| 6 | 17 | M | 17 | acute | 1/2 | 2/2 |

of alcohol-associated symptoms after a single drinking. Also, asymptomatic carriers are expected to have an increased frequency in atypical alleles through which they have no drinking habit. It is, however, remarkable that the present observations do not support either of these expectations, as the genotype frequency distribution in LHON patients or asymptomatic carriers does not differ significantly from that in normal controls.

As regards drinking habit, six of the present 29 LHON patients continued a frequent alcohol consumption with daily amount of over 40 g. On the other hand, none of the 24 asymptomatic carriers claimed frequent drinking habit. Thus, we could not make a denial of drinking effects on optic nerve damage in LHON. Table 3 shows clinical and genotypic data in the six alcohol-habitant LHON patients, who did not show atypical homozygote in ALDH2 polymorphism. These patients may be able to drink ordinarily, because frequency and dose of habitual alcohol drinking has been shown to be influenced by ALDH2 activity (Takeshita *et al.*, 1994). Four of the alcohol-habitant LHON patients had the onset of optic nerve disease at an unusually old age over 40 years. In Japanese LHON patients with mtDNA 11778 mutation, the average age of onset was 23.4 years (Hotta *et al.*, 1995). Activities of NADH CoQ reductase, one of mitochondrial respiratory enzymes, have been decreased in platelets from mtDNA 11778 mutation-positive LHON patients and healthy carriers (Parker *et al.*, 1989). And, alcohol intake induces a secondary defect of respiratory enzyme in alcoholic brain and liver (Pratt *et al.*, 1990). Therefore, it is suggested that alcohol intake could accelerate optic nerve stress, where the mitochondrial function is decreased associated with mtDNA 11778 mutation, especially in elderly persons.

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## REFERENCES

Crabb DW, Edenberg HJ, Bosron WF, Li TK (1988): Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity: the inactive $ALDH^2$ allele is dominant. J Clin Invest **83**: 314-316

Cullom ME, Heher KL, Miller NR, Savino PJ, Johns DR (1993): Leber's hereditary optic neuropathy masquerading as tobacco-alcohol amblyopia. Arch Ophthalmol **111**: 1482-1485

Hotta Y, Fujiki K, Hayakawa M, Nakajima A, Kanai A, Mashima Y, Hiida Y, Shinoda K, Yamada K, Oguchi Y, Ishida M, Yanashima K, Wakakura M, Ishikawa S, Nakamura M, Sakai J, Yamamoto M, Hayashi T, Mitani I, Miyazaki S, Shimo-oku M, Imachi J, Kuniyoshi N, Nagataki S, Isashiki Y, Ohba N (1995): Clinical features of Japanese Leber's hereditary optic neuropathy with 11778 mutation of mitochondrial DNA. Jpn J Ophthalmol **39**: 96-108

Hsu LC, Bendel RE, Yoshida A (1987): Direct detection of usual and atypical alleles on the human aldehyde dehydrogenase-2 ($ALDH_2$) locus. Am J Hum Genet **41**: 996-1001

Isashiki Y, Ohba N, Uto M, Nakagawa M, Nakano T, Kitahara K, Hotta A, Okamura R, Ozaki M, Futami Y, Sawada A (1992): Nonfamilial and unusual cases of Leber's hereditary optic neuropathy identified by mitochondrial DNA analysis. Jpn J Ophthalmol **36**: 197-204

Newman NJ (1993): Leber's hereditary optic neuropathy. New genetic considerations. Arch Neurol **50**: 540-548

Parker WD, Oley CA, Parks JK (1989): A defect in mitochondrial electron-transport activity (NADH-Coenzyme Q oxidoreductase) in Leber's hereditary optic neuropathy. New Engl J Med **320**: 1331-1333

Pratt OE, Rooprai HK, Shaw GK, Thomson AD (1990): The genesis of alcoholic brain tissue injury. Alcohol Alcohol **25**: 217-230

Takeshita T, Morimoto K, Mao XQ, Hashimoto T, Furuyama J (1994): Characterization of the three genotypes of low $K_m$ aldehyde dehydrogenase in a Japanese population. Hum Genet **94**: 217-223

Wallace DC, Singh G, Lott MT, Hodge JA, Shurr TG, Lezza ANS, Elsas LJ II, Nikoskelainen K (1988): Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science **242**: 1427-1430

Xu Y, Carr LG, Bosron WF, Edenberg HJ (1988): Genotyping of human alcohol dehydrogenase at the ADH2 and ADH3 loci following DNA sequence amplification. Genomics **2**: 209-214