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Herawati Sudoyo · Helena Suryadi · Patcharee Lertrit
Patcharin Pramoonjago · Diana Lyrawati
Sangkot Marzuki

Asian-specific mtDNA backgrounds associated with the primary G11778A mutation of Leber's hereditary optic neuropathy

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Abstract We studied 19 patients of Southeast Asian (SEA) ethnic ancestry with Leber's hereditary optic neuropathy (LHON) to investigate the mtDNA haplotypes associated with the primary mutation(s). Eighteen patients carried a mitochondrial DNA (mtDNA) G11778A mutation (Arg340His in the respiratory complex I ND4 subunit), while one had a T14484C mutation (Met64Val in the ND6 subunit). One patient had a class II LHON mtDNA mutation, G3316A. Sequencing data of the *ND* genes showed many single-nucleotide polymorphisms (62 SNPs in 17 individuals; 10 LHON patients and 7 normal controls) not previously reported in Europeans or Japanese. The SEA G11778A LHON mutation was associated mostly with two mtDNA haplogroups, M (47%) and a novel lineage, characterized by the gain of a 10394 *Dde*I site but absence of the 10397 *Alu*I site, designated BM (37%). A significant association was observed between one SNP, A10398G, resulting in a Thr114Ala substitution in the ND3 subunit, and the primary LHON mutation. This SNP also characterizes haplogroup J, with which the European LHON 11778 and 14484 mutations show preferential association. The combination of A10398G and other SNPs, specific for the haplogroups J, M, or BM, might act synergistically to increase the penetrance of the LHON mutations, thus allowing their detection.

Key words Human mtDNA · mtDNA mutations · Leber's hereditary optic neuropathy · mtDNA SNP · mtDNA haplotypes

H. Sudoyo · H. Suryadi · P. Pramoonjago · D. Lyrawati · S. Marzuki (✉)
Eijkman Institute for Molecular Biology, Jl. Diponegoro 69, Jakarta 10430, Indonesia
Tel. +62-21-3148694; Fax +62-21-3147982
e-mail: smarzuki@eijkman.go.id

P. Lertrit
Department of Biochemistry, Mahidol University Medical School at Siriraj Hospital, Bangkok, Thailand

Introduction

While there has been major progress in the identification of a large number of disease-related mutations in the mitochondrial DNA (mtDNA), the pathobiology of these mutations in general is still poorly understood. The frequent lack of correlation between the mutations and their biochemical expression, their relatively tissue-specific clinical manifestation despite the mitochondrial oxidative energy metabolism being essential for the function of almost all cell types, and the incomplete and often low penetrance of the mutations all point to the complexity of the pathogenesis of the resulting disorders. Indeed, it might be more appropriate to consider these disorders as polygenic, with the clinical expression of the causal mtDNA mutations being modulated by other genetic factors, both in the nuclear and mitochondrial DNA, as well as by environmental factors.

Leber's hereditary optic neuropathy (LHON), a maternally inherited degenerative disorder characterized by late-onset visual failure resulting from a bilateral optic atrophy, epitomizes the above complexity. The disease is associated with three primary mutations in the mtDNA, G3460A (Howell et al. 1991; Huoponen et al. 1991, 1993), G11778A (Wallace et al. 1988), and T14484C (Johns et al. 1992; Mackey and Howell 1992), affecting subunits of respiratory enzyme complex I (ND1 Ala52Thr, ND4 Arg340His, and ND6 Met64Val, respectively). Two additional mtDNA mutations, G14459A (Jun et al. 1994; Shoffner et al. 1995) and G15257A (Brown et al. 1992), resulting in ND6 Ala72Val and Cyt*b* Asp171Asn amino acid replacements, are also considered primary LHON mutations. The first three primary or class I mutations account for about 90% of the LHON cases reported (Brown and Wallace 1994); about 50% of LHON cases in the European population and about 95% in the Japanese population carry the causal G11778A mutation (Mashima et al. 1993).

In addition to the above mutations at least 22 other mtDNA sequence variants (classified as class II mutations) are frequently found to occur together with the primary LHON mutations (Brown and Wallace 1994; Wallace

1995), leading to the suggestion that these, and possibly some other mtDNA mutations, might interact synergistically with and modulate the clinical expression of the predisposing class I mutations. The most extensive support for this suggestion came from high-resolution restriction fragment length polymorphism (RFLP) analyses of Caucasian LHON pedigrees from North America and Europe. These analyses demonstrated that while the G3460A mutation is distributed evenly among the major Caucasian mtDNA lineages or haplotype groups (haplogroups), the T14484C (80%) and, to a lesser extent, the G11778A (37%) mutations tend to be associated with one of these, i.e., the European-specific haplogroup J (Brown et al. 1995, 1997; Howell et al. 1995; Torroni et al. 1997; Lamminen et al. 1997). This finding has been interpreted to indicate that some of the mtDNA polymorphic variants that characterize haplogroup J are positive modifiers of the biochemical expression of the two primary LHON mutations, and thus presumably increase their penetrance, allowing their detection as disease mutations.

Like many disease-related mitochondrial mutations, however, the primary LHON mutations are found in patients from distantly related ethnic populations, and thus with clearly distinct spectra of mtDNA haplotypes. This observation raises the important question as to whether the molecular mechanism(s) responsible for the primary LHON mutations arose many times independently, but also has implications regarding how the presumably very different mtDNA haplotype backgrounds might influence and modify the clinical expression of the primary mutations. If there is indeed a specific set of mtDNA single-nucleotide polymorphisms (SNPs) that act as positive modifiers of the LHON primary mutations in Europeans, different sets of SNPs must play a similar role in other populations for the same primary LHON mutations to be found at such a high prevalence. The examination of the primary mtDNA mutations and their mtDNA backgrounds in populations that are phylogenetically distant from the European is important for the elucidation of the role of the mtDNA haplotype environment in the generation and clinical expression of mtDNA mutations.

In the present study, we have examined the mtDNA of 19 patients with Leber's hereditary optic neuropathy from Southeast Asian (SEA) ethnic backgrounds. We report here our finding of a surprisingly large number of previously unreported sequence variants in the mtDNA of Southeast Asians (62 in 17 mtDNAs), albeit in line with the distant phylogenetic relationship between these populations and those of Europe and Japan. Despite the significant difference in the mtDNA background, the G11778A mutation is also by far the main underlying mutation for LHON in Southeast Asia (18 out of 19). In contrast, only one of the Caucasian class II mutations (G3316A) was found. An Asian-specific haplogroup M and a novel mtDNA haplogroup BM (characterized by the gain of a 10394 *Dde*I site but the absence of the 10397 *Alu*I site) were found to be the most common haplogroups associated with the G11778A mutation, instead of haplogroup J as in Europeans. Our results indicate that a different set of mtDNA polymorphisms are involved in the clinical expression of the G11778A primary LHON mutation in Southeast Asians. An SNP in the *ND3* gene (A10398G), leading to a Thr114Ala amino acid replacement, which characterizes haplogroups M and BM (as well as the European haplogroup J), however, was found to be significantly associated with the primary mutation.

Subjects and methods

Subjects and sample collection

Nineteen LHON patients were included in the present study. They belonged to five different SEA ethnic backgrounds (Table 1): one Chinese-Indonesian (EIJbIII-4-94), two Javanese-Indonesian (EIPwII-7-97 and EICbII-6-97), five Sundanese-Indonesian (EISbIII-33-95, EIJkIV-7-99, EIKrIII-10-00, EISdIII-4-00, and EIBtIV-13-01), one Banjarese-Indonesian (EIBmV-9-98), and ten Thais (SR96-013, SR94-012, SR96-010, SR96-008, SR96-015, SR-97-038, SR98-020, SR98-043, SR98-044, and SR99-031). Samples of blood were collected as part of the diagnostic work-up of the patients, and further molecular genetic analysis was performed with informed consent. The nine samples from Indonesia were collected in private practices in Subang and Cirebon (West Java), and at Jambi Hospital in Sumatera, Karyadi Hospital in Semarang (Central Java), and the Eijkman Institute in Jakarta. The ten samples from Thailand were collected at Siriraj Hospital in Bangkok. A total of 67 healthy individuals were selected as controls for the mtDNA haplotype study. These individuals included 30 Thais, 33 Javanese-Indonesians, 3 Chinese-Indonesians, and 1 Balinese. In addition, another 70 individuals were recruited for a phylogenetic analysis based on the sequence of hypervariable region 1 (HVR1) of the mtDNA D-loop region. MtDNA samples were extracted from blood samples according to a standard procedure (Sudoyo et al. 1998).

Detection of class I and class II LHON mutations

The primary LHON mutations G3460A (*ND1* gene), G11778A (*ND4* gene), T14484C (*ND6* gene) and G15257A (*Cytb* gene) were detected by polymerase chain reaction (PCR)-RFLP. MtDNA fragments were amplified by PCR with the primer pairs shown in Table 2 in a programmable thermal cycler (Perkin Elmer 9700 Gene Amp PCR System, Foster City, CA, USA). The G3460A mutation was detected by using the primer pair L2826 and H3728 and followed by restriction endonuclease digestion with the restriction endonuclease *Bsa*HI (Table 2). The LHON mutations G11778A and T14484C were detected by employing the modified forward primers L11728* and L14436* and the reverse primers H11942 and H14701 to introduce restriction sites for *Bcl*I and *Apa*LI, respectively (Sudoyo et al. 1998). The G15257A mutation was amplified by using the primer pair L14820 and H15680 and

Table 1. Families investigated with Leber's hereditary optic neuropathy (LHON)

Family/ patient	Ethnic group	Family size (members [generations])	Primary LHON mutation	Penetrance (%)	Male/ female
With family study					
EIJb-94	Chinese	124 [4]	G11778A	18.3	10/3
EISb-95	Sundanese	70 [4]	T14484C	24.4	9/1
EIPw-96	Javanese	110 [5]	G11778A	33.3	15/13
EICb-97	Javanese	75 [4]	G11778A	10.7	3/0
EIBm-98	Banjarese	74 [4]	G11778A	9.8	4/1
Without family study					
SR94-012	Thai	n.i	G11778A	—	—
SR96-008	Thai	n.i	G11778A	—	—
SR96-010	Thai	n.i	G11778A	—	—
SR96-013	Thai	n.i	G11778A	—	—
SR96-015	Thai	n.i	G11778A	—	—
SR97-038	Thai	n.i	G11778A	—	—
SR98-020	Thai	n.i	G11778A	—	—
SR98-044	Thai	n.i	G11778A	—	—
SR98-043	Thai	n.i	G11778A	—	—
SR99-031	Thai	n.i	G11778A	—	—
EIJkIV-7-99	Sundanese	n.i	G11778A	—	—
EIKrIII-10-00	Sundanese	n.i	G11778A	—	—
EISdIII-4-00	Sundanese	n.i	G11778A	—	—
EIBtIV-13-01	Sundanese	n.i	G11778A	—	—

n.i., not investigated

Table 2. Detection of LHON mutations by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and sequencing

Mutation	Coding gene	Amino acid changes	Primer pairs	Restriction enzyme
Class I by PCR-RFLP and sequencing				
G11778A	*ND4*	R340H	L11728-H11942	*Bcl*I
G3460A	*ND1*	A52T	L2826-H3728	*Bsa*HI
T14484C	*ND6*	M64V	L14436-H14701	*Apa*LI
G15257A	*Cytb*	D171N	L14820-H15680	*Acc*I
G14459A	*ND6*	A72V	By sequencing	
Class II by PCR-RFLP				
G7444A	*COI*	Term514K	L7321-H8156	*Hpa*II
G15812A	*Cytb*	V356M	L15481-H16115	*Rsa*I
Class II by sequencing				
G3316A	*ND1*	A4T		
T3394C	*ND1*	Y30H		
G3496T	*ND1*	A64S		
C3497T	*ND1*	A64V		
A4136G	*ND1*	Y277C		
T4160C	ND1	L285P		
T4216C	*ND1*	Y304H		
A4917G	*ND2*	D150N		
G5244A	*ND2*	G260S		
T9101C	*ATP6*	I192T		
G9438A	*COIII*	G78S		
G9738T	*COIII*	A178T		
G9804A	*COIII*	A200T		
T10663C	*ND3*	V65A		
G13708A	*ND5*	A458T		
G13730A	*ND5*	G465E		
C14482G	*ND6*	M64I		
C14568T	*ND6*	G36S		

detected with the restriction endonuclease *Acc*I. Two class II LHON mutations, G7444A and G15812A, were also screened by using a PCR-RFLP strategy (Table 2). The other class II mutations were identified by direct sequencing. The PCR amplification was performed for 30 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 90 s, and extension at 72°C for 2 min 30 s. The mtDNA fragments digested by restriction endonucleases were then separated in 2% agarose gels containing ethidium bromide.

Haplotype analysis

Haplotype analysis was carried out by the amplification of five mitochondrial DNA fragments with the primer pairs L9773-H11325, L15760-H162, L12104-H13425, L100-H815, and L4476-H5620. The PCR fragments were digested with seven restriction endonucleases (*Dde*I, *Alu*I, *Hae*III, *Hae*II, *Hha*I, *Hin*cII, and *Hpa*I) for *Alu*I-10397, *Dde*I-10394, *Hae*III-16517, *Hin*cII/*Hpa*I-12406, *Hin*cII-13259, *Alu*I-13262, *Hae*III-663, *Hae*II-4830, *Hha*I-4831, and *Alu*I-5176 sites. In addition to the RFLP analysis, the COII-tRNA^{Lys} 9-bp deletion of the mtDNA was examined by amplifying this region using primer pair L8211-H8310. The mtDNA fragments were then separated by electrophoresis in 1.0%–4.0% agarose gels containing ethidium bromide.

Direct sequencing of PCR products

For the sequencing of the seven *ND* genes, mtDNA was amplified in six overlapping segments. The primer pairs used for the amplifications were L2322 and H5482, L4955 and H6854, L7901 and H10679, L9773 and H11942, L10977 and H13200, and L12376 and H16115. The PCR-amplified fragments were then purified using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany). Cycle sequencing reactions were performed as indicated by the manufacturer with ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems, Foster City, CA, USA), and analyzed in a model 377 automatic DNA sequencer (Applied Biosystems). A total of 26 internal primers were used for sequencing the mtDNA: L2826, L3015, H3185, L3311, L3636, L4049, L4476, L4955, L5223, L9773, L10126, L10348, L10515, L10997, L11228, L11580, L11891, L12104, L12367, L12706, L13145, H13200. L13571, L13977, L14241, and L14438.

Sequence divergence and phylogenetic analysis

The mtDNA of 10 of the 17 LHON patients were subjected to sequence analysis. The mtDNA sequence divergence of 17 individuals, 10 LHON patients, and 7 normal subjects, were calculated from the *ND* gene sequences by using Kimura's two-parameter procedure and the PHYLIP Phylogeny Inference Package (Felsenstein 1993).

The phylogenetic relationships between the ten LHON patients and ten normal individuals (seven sequenced in the present study and three unpublished sequences from our laboratory) were inferred by a parsimony analysis on the basis of the sequences of the *ND* genes by employing the PHYLIP Phylogeny Inference Package. To evaluate the validity of the tree based on the actual data set, the data were bootstrapped with 1000 replicates and a consensus tree was produced by using an African mtDNA as an outgroup (GenBank accession no. D38112). To examine the validity of the mtDNA haplotype grouping, phylogenetic relationships between 98 individuals were also determined on the basis of the HVR1 D-loop region of the mtDNA by a neighbor-joining analysis.

Results

mtDNA mutations underlying LHON in SEA

Nineteen unrelated LHON patients were included in the present study, the details of whom are shown in Table 1. Eight of the patients belonged to related, Austronesian language-speaking populations of the Indonesian archipelago: Javanese of the central region of Java Island (EIPwII-7-97 and EICbII-6-97), Sundanese of West Java (EISbIII-33-95, EIJkIV-7-99, EIKrIII-10-00, EISdIII-4-00, and EIBtIV-13-01), and Banjarese of the island of Kalimantan (EIBmII-9-98). Ten patients (SR96-013, SR94-012, SR96-010, SR96-008, SR96-015, SR-97-038, SR98-020, SR98-043, SR98-044, and SR99-031) were Thais from Bangkok in central Thailand, and one was of Han Chinese ethnic ancestry (EIJbIII-4-94), although members of this Chinese family had lived in the province of Jambi on Sumatera Island of Indonesia for at least four generations. The phylogenetic relationships among the populations of East Asia, and their relationships to Europeans, have been previously investigated by high-density RFLP analyses of the mtDNA (Ballinger et al. 1992; Torroni et al. 1993, 1994a). The similarities in the spectra of mtDNA haplotypes in the Thai and Indonesian western island populations indicate a close relationship among those populations, justifying the pooling of the 19 SEA patients in a single population unit for the purpose of this study (see Lum et al. 1994; Fucharoen et al. 2001).

We first examined the patients by a PCR-RFLP analysis for the four primary LHON mutations reported in European, Black American, and Japanese patients, i.e., G11778A in the *ND4* gene, G3460A in the *ND1* gene, T14484C in the *ND6* gene, and G15257A in the *Cytb* gene. It is of significance that despite their phylogenetic distance, two of the primary LHON mutations observed in Caucasians, Black Americans, and Japanese were found also in our SEA LHON patients. Of the 19 patients examined, 18 (95%) were found to carry the G11778A mtDNA mutation, which accounts for approximately 50% to 70% of cases in Europe (Brown and Wallace 1994) and for 95% of cases in Japan (Mashima et al. 1993). The other patient (EISbIII-33-95) had the T14484C mutation.

We had extensive access to the families of the five Indonesian patients, four carrying the G11778A mutation and one with the T14484C mutation, and investigated these families to four generations (two have been reported in Sudoyo et al. 1998). As in Caucasians, the penetrance for the G11778A mutation varies in the SEA LHON families, ranging from 10%–33%, with a similar male preponderance (Table 1). Of interest is the observation that in the family with the T14484C mutation, which showed a penetrance of 24%, only one out of the ten family members who exhibited clinical expression of the mutation was female.

Haplotype analysis of the SEA LHON mtDNA

To examine the possibility that the mtDNA G11778A primary LHON mutation is also associated with a specific

Table 3. Mitochondrial haplotypes observed in Southeast Asia populations and LHON patients

Haplotype group	N	**10394 *Dde*I**	**10397 *Alu*I**	**16517 *Hae*III**	Δ 9 bp	**4830 *Hae*II**	**4831 *Hha*I**	12406 *Hinc*II	12406 *Hpa*I	13259 *Hinc*II	**13262 *Alu*I**	**663 *Hae*III**	5176 *Alu*I
CRS		−	−	−	−	−	−	−	−	−	−	−	−
B	10	−	−	+	+	−	−	−	−	−	−	−	−
B*	6	−	−	+	−	−	−	−	−	−	−	−	−
F	7	−	−	+	−	−	−	+	+	−	−	−	−
M	28	+	+	+/−	−	−	−	−	−	−	−	−	−
M-C	1	+	+	+	−	−	−	−	−	+	+	−	−
M-D	2	+	+	−	−	−	−	−	−	−	−	−	+
M-G	4	+	+	−	−	+	+	−	−	−	−	−	−
BM	18	+	−	−	−	−	−	−	−	−	−	−	−
Others	3	−	−	−	−	−	−	−	−	−	−	−	−

Sites are numbered from the first nucleotide of the recognition sequence according to the published sequence Anderson et al. (1981). The restriction enzymes used in the analysis were *Dde*I, *Alu*I, *Hae*III, *Hae*II, *Hha*I *Hinc*II, and *Hpa*I; site gain is indicate by boldface and site loss by nonboldface type. The polymorphic restriction sites are listed as either absent (−) or present (+) relative to the Cambridge reference sequence (CRS) except for the 9-bp deletion, where (+) indicates the presence of a 9-bp deletion between the *COII* and *tRNAlys* genes and a (−) indicates the absence of the deletion

Table 4. Haplogroups associated with the Southeast Asian LHON mtDNA

	Haplotype group (%)									
Populations	N	B	B*	F	M	M-C	M-D	M-G	BM	Others
Control population	60	15	8	12	32	2	2	7	18	5
LHON patients	19	5	5	0	47	0	5	0	37	0

Haplotype groups are as described in Table 3

haplogroup in the Southeast Asians, the mtDNA lineages of the SEA patients were determined with the help of 11 polymorphic restriction sites at nt 10394 (*Dde*I), 10397 (*Alu*I), 16517 (*Hae*III), 12406 (*Hinc*II and *Hpa*I), 13259 (*Hinc*II), 13262 *(Alu*I), 663 (*Hae*III), 4830 (*Hae*II), 4831 (*Hha*I), and 5176 (*Alu*I). Together with the COII-tRNA*Lys* 9-bp deletion in the mtDNA, these polymorphic sites define seven of the most common Asian mtDNA haplogroups (A, B, F, M, M-C, M-D, and M-G; Wallace et al. 1999), and distinguish these lineages from the I, J, K, and other haplogroups that characterize European populations (Torroni et al. 1994b). To obtain the frequency of the various haplogroups in the Austronesian language-speaking populations of Indonesia, and in the Thai population, 30 unrelated individuals from each of the above populations were also examined.

Of the 79 individuals examined (Table 3), 10 were found to be of haplogroup B (absence of *Dde*I and *Alu*I sites at nt 10394 and 10397; gain of *Hae*III site at nt 16517; and the COII-tRNA^{Lys} 9-bp deletion), 7 of haplogroup F (absence of 10394 *Dde*I and 10397 *Alu*I sites; gain of 16517 *Hae*III and loss of 12406 *Hinc*II/*Hpa*I sites), and 28 of haplogroup M (gain of 10394 *Dde*I and 10397 *Alu*I sites). In addition, 1 individual was found to be of haplogroup M-C (gain of 16517 *Hae*III and 13262 *Alu*I, in addition to the 10394 *Dde*I and 10397 *Alu*I sites; loss of 13259 *Hinc*II site), 2 were of haplogroup M-D (loss of 5176 *Alu*I, in addition to the gain of 10394 *Dde*I and 10397 *Alu*I), while 4 were of haplogroup M-G (gain of 4830 *Hae*II and 4831 *Hha*I, in addition to the 10394 *Dde*I and 10397 *Alu*I sites) (Table 3). A total of 27 individuals, however, could not be classified easily into the haplogroups as defined in the detailed description of continent- and haplogroup-specific mtDNA polymorphisms (refer to the human mtDNA database MITOMAP: http://www.mitomap.org), in agreement with previous reports (Torroni et al. 1994a; Wallace et al. 1999), confirming the distant phylogenetic relationship between the SEA populations and those of northern East Asia, which have been studied more extensively.

Of these 27 individuals, 6 individuals were characterized by the absence in their mtDNA of the *Dde*I and *Alu*I sites at nt 10394 and 10397, and, as in haplogtype B, by the gain of the *Hae*III site at nt 16517; unlike haplogroup B, however, the mtDNA did not have the COII-tRNA^{Lys} 9-bp region V deletion and thus was designated as belonging to haplogroup B*. A significant number of individuals (18) appearred to belong to a novel haplogroup, characterized by the gain of the 10394 *Dde*I sire but the absence of the 10397 *Alu*I site. We propose to designate this novel haplotype group (see verification below), which is apparently a major mtDNA lineage for the SEA populations, as haplogroup BM. The last 3 individuals lacked both the 10394 *Dde*I and 10397 *Alu*I sites, and did not show any variation at the other sites examined, compared with the reference Cambridge sequence. It was not clear at this stage whether the mtDNA of these individuals represented a distinct lineage, one not distinguished by the limited sites analyzed.

The control population was distributed among haplogroups B, B*, F, M, M-C, M-D, M-G, and BM with frequencies of 15%, 8%, 12%, 32%, 2%, 2%, 7%, and 18%, respectively (Table 4). It is of significance that, in contrast to the distribution of the control mtDNAs into the eight haplogroups, 47% of the LHON patients were found to be

Table 5. Divergence of the mitochondrial *ND* genes

	SR94-012	SR96-010	SR96-008	SR96-015	EISbIII33-95	EIJbIII-4-94	EIPwII-7-97	EICbII-6-97	EIBmV-9-98	EIJv01-96	EIJv02-96	EIJv03-96	EIBal034-96	EICh01-98	EICh02-98	EICh03-98
SR96-013	0.25	0.25	0.22	0.24	0.33	0.30	0.32	0.27	0.24	0.25	0.25	0.27	0.33	0.38	0.25	0.28
SR94-012		0.28	0.25	0.39	0.33	0.33	0.16	0.30	0.27	0.25	0.25	0.27	0.33	0.41	0.28	0.32
SR96-010			0.13	0.36	0.24	0.30	0.35	0.17	0.20	0.19	0.19	0.30	0.27	0.32	0.28	0.25
SR96-008				0.30	0.17	0.20	0.28	0.11	0.14	0.13	0.13	0.20	0.20	0.28	0.22	0.16
SR96-015					0.41	0.38	0.39	0.32	0.28	0.30	0.30	0.38	0.38	0.43	0.36	0.33
EISbIII33-95						0.35	0.33	0.13	0.25	0.20	0.17	0.28	0.22	0.33	0.30	0.27
EIJbIII-4-94							0.36	0.28	0.25	0.27	0.27	0.28	0.35	0.43	0.30	0.27
EIPwII-7-97								0.33	0.30	0.35	0.35	0.33	0.39	0.47	0.32	0.39
EICbII-6-97									0.16	0.20	0.17	0.28	0.22	0.30	0.24	0.20
EIBmV-9-98										0.20	0.20	0.22	0.25	0.33	0.24	0.17
EIJv01-96											0.03	0.20	0.14	0.33	0.25	0.16
EIJv02-96												0.20	0.14	0.22	0.25	0.16
EIJv03-96													0.28	0.39	0.24	0.24
EIBal034-96														0.33	0.30	0.24
EICh01-98															0.38	0.32
EICh02-98																0.22

Pairwise comparisons were carried out on the *ND* genes of mtDNA sequences of the ten LHON patients and seven normal subjects included in this study. Values of the mean pairwise divergence between individuals appear on the diagonal. Results were expressed as the number of nucleotide differences observed between pairs of individuals per 100 nucleotides. Average for LHON Patients (±SD) = 0.27 ± 0.08. Average for LHON Patients vs normal control = 0.28 ± 0.06. Average for normal control vs normal control = 0.24 ± 0.09

of haplogroup M, while another 37% were of haplogroup BM (compared with 32% and 18%, respectively, for these haplogroups in the control population). Thus, the LHON mtDNA in the SEA populations also clusters in specific lineages. Different from the European patients, however, the clustering is in the two haplogroups M and BM, instead of in the European-specific haplogroup J.

Sequence variants and the divergence of the human mtDNA in SEA

The clustering of the SEA LHON patients mtDNA in haplogroups M and BM suggests that, as in the European patients, a particular group of mtDNA SNPs might be involved in the generation or the clinical expression of the G11778A causal mutation, albeit of a different set. However, whereas a large volume of sequence information has been generated for the mtDNA of Europeans, Black Americans, and Japanese, the mtDNA of the SEA populations has never before been sequenced. Sequence data from such phylogenetically distant populations are also of interest as they could potentially reveal important missing information regarding the divergence of human mtDNA. In the present context, such sequence data might allow the definition of the critical polymorphisms, common to the M and BM haplogroups, responsible for modulating the generation or the clinical expression of the primary LHON mutations. We thus completely sequenced the *ND* genes of the mtDNA, coding for subunits of respiratory complex I, from 10 of the 19 LHON probands (EIJbIII-4-94, EIPwII-7-97, EICbII-6-97, EISbIII-33-95, EIBmV-9-98, SR96-013, SR94-012, SR96-010, SR96-008, and SR96-015) and from 7 normal controls of Javanese (EIJv01-96, EIJv02-96, and EIJv03-96), Balinese (EIBal034-96), and Chinese ethnic origin (EICh01-98, EICh02-98, and EICh03-98).

A total of 93 single-nucleotide polymorphic (SNP) sites were observed in the seven *ND* genes coding for subunits of mitochondrial respiratory complex I (Fig. 1). Of these SNPs, 62 are new SNPs not previously observed in Europeans, Africans, or Japanese. Forty-four of the new SNPs were found in the mtDNA of the ten SEA LHON patients (Fig. 1), and only two were observed in the normal subjects. Eighteen new SNPs were found in the normal subjects but not the LHON patients. Pair-wise comparison of the mtDNA sequences of 17 individuals showed a wide variation in sequence divergence, ranging from 0.03 differences per 100 nucleotides (0.03%) between the mtDNA sequences of the two normal Javanese individuals EIJv01-96 and EIJv02-96, to 0.47% between the mtDNA sequences of the Javanese LHON patient EIPwII-7-97 and a Chinese normal control EICh01-98 (Table 5). The mean sequence divergence for the seven *ND* genes of the mtDNA (±SD) was 0.28 ± 0.06% (0.27 ± 0.08% and 0.24 ± 0.09% for the LHON patients and the normal individuals, respectively). This divergence is higher than that estimated for Australians of European origin (0.22 ± 0.08%; data derived from Marzuki et al. 1991), but similar to that for Japanese (0.26%; Horai and Matsunaga 1986).

Validation of mtDNA haplotype grouping

We recognize that the lack of the 10397 *Alu*I site in our proposed haplogroup BM does not necessarily define a new haplogroup, and further evidence is needed to confirm that the Asian mtDNA haplogroup characterized by 10394+ *Dde*I and 10397− *Alu*I is in fact monophyletic. To confirm the validity of the mtDNA haplotype groupings, we examined 98 individuals (which included the 19 LHON patients)

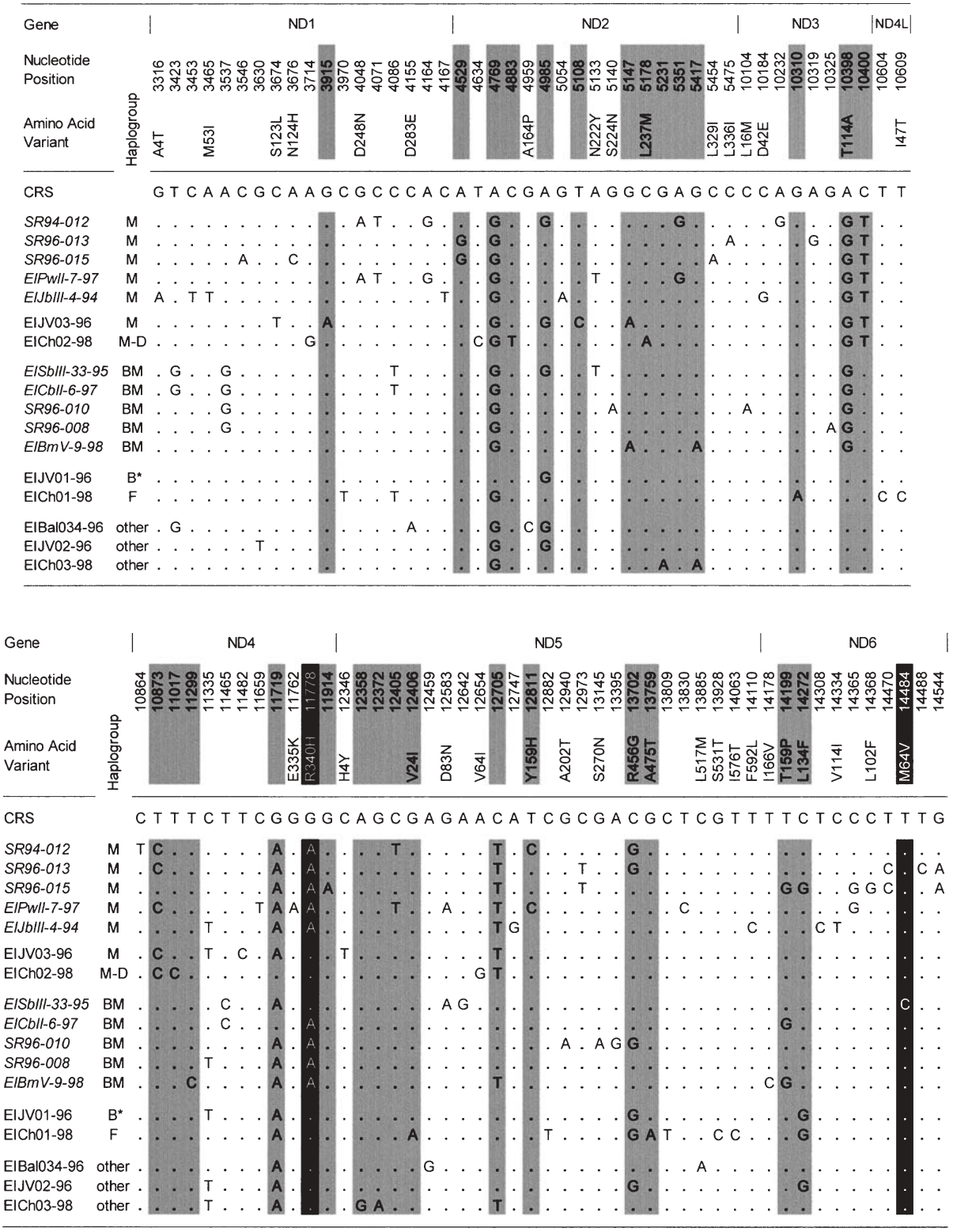

Fig. 1. New single-nucleotide polymorphisms detectable in the Southeast Asian (SEA) mtDNAs. Blood samples were obtained from ten Leber's hereditary optic neuropathy (LHON) patients and seven normal controls (EIJv01-96, EIJv02-96, EIJv03-96, EIBal034-96, EICh01-98, EICh02-98, and EICh03-98). Regions of the mtDNA containing the *ND* genes coding for subunits of respiratory complex I were amplified by polymerase chain reaction (PCR) and sequenced. A total of 93 nucleotide sequence variants were oibserved in the genes coding for the ND subunits of respiratory complex I, of which 44 were new variants in the ten LHON patients observed. The positions of the variant nucleotides in the mtDNA (position numbering as in Anderson et al. 1981), together with the resulting amino acid variants, are indicated. *Dots* represent nucleotides that are identical to those of the Cambridge reference sequence (*CRS*, Anderson et al. 1981), which has been corrected for errors (Andrews et al. 1999) with the variant nucleotides indicated by the base substitutions. Sixty-two of the variants (*not shaded*) have not been observed previously in Caucasian or Japanese individuals. LHON patients are indicated by *italics*

for the sequence of the HVR1 region of the mtDNA D-loop, together with their mtDNA haplogroups, as determined by the 11 polymorphic restriction endonuclease sites employed in the present study. The phylogenetic tree constructed by a neighbor-joining analysis of the HVRI sequence showed the clustering of the mtDNAs into four groups (I to IV, Fig. 2). These groups (from I to IV) were clearly identified as corresponding to haplogroups B, BM, F, and M, respectively, and thus confirmed that haplogroup BM is indeed monophyletic. A similar phylogenetic tree was constructed by the parsimony method (not shown), with mtDNAs belonging to haplogroup BM showing a distinct cluster.

An mtDNA lineage (designated haplogroup Y), also characterized by 10394+ *Dde*I and 10397− *Alu*I, has been reported recently in Kamchatkan populations (Schurr et al. 1999). This haplogroup is also defined by the gain of an *Mbo*I site at nt 7933 and the loss of an *Hae*III site at nt 8391. These last two markers were not found in our populations, confirming that the Southeast Asian haplogroup BM is distinct from haplogroup Y of north Asia.

The sequencing data generated in the present study allowed us also to further confirm the validity of our mtDNA haplotype grouping. For this purpose, a phylogenetic tree was constructed based on the sequences of the *ND* genes of the 17 mtDNAs obtained in this study, plus sequences from 3 other Indonesian patients to be published elsewhere; one Balinese with neurosensorial deafness associated with the A1555G mutation and two Javanese with preeclampsia. The parsimony tree in Fig. 3, clearly confirms our classification of the mtDNA haplotypes into the three groups, B, M, and BM, with the lineages being defined by the absence or presence of the 10394 *Dde*I and 10397 *Alu*I sites.

The analysis also gave us the needed information on the probable lineages of the unidentified mtDNA group, in the earlier RFLP haplotyping study, that lacked both the 10394 *Dde*I and 10397 *Alu*I sites, but also did not show any polymorphism at the other diagnostic sites examined. MtDNAs of EIJv02-96 and EIBal034-96 clustered together with those of haplogroup B, and thus presumably belong to this haplogroup; the absence of the 16517 *Hae*III site and the COII-tRNA^{Lys} 9-bp deletion that typify haplogroup B in these two cases is probably due to back mutations in these two hot spots, which are subject to many recurrent mutations (Macaulay et al. 1999; Finnilä and Majamaa 2001). MtDNA of EICh03-98, which also lacked all of the above RFLP markers, on the other hand, clearly belongs to haplogroup BM, and the absence of the *Hae*III site at nt 16517 is again due to a back mutation at this site.

MtDNA SNPs involved in the clinical expression of the LHON G11778A mutation

To identify the mtDNA SNPs that could play a role in the penetrance of the primary LHON mutation, we have examined the sequences of the mtDNA *ND* genes, which code for subunits of respiratory complex I, more closely. The finding of a large number (62) of SNPs that are specific to the SEA populations is of interest for several reasons. Of these coding SNPs in the *ND* genes, 35 are silent. The other 27, however, are associated with amino acid changes (Fig. 1), some of which might be functionally significant. Of these polymorphic amino acid residues, six were in ND1, five in ND2, two in ND3, one in ND4L, one in ND4, nine in ND5, and three in ND6 subunits. One amino acid variant, ND3 Thr114Ala, corresponding to the A10398G SNP, was found in 90% of the LHON patients tested (Fig. 1). Three coding SNPs that have not been reported before, G4048A, A5133T, and G12583A, were found twice. The rest appeared only once. Two coding SNPs, A3676C in *ND1* and G12583A in *ND5*, result in significant changes in conserved amino acid residues, Asn124Lys and Asp83Asn, respectively.

The sequencing data also revealed information regarding the class II LHON mutations reported in European patients. Of the 20 previously reported class II LHON mutations, 18 (G3316A, T3394C, G3496T, C3497T, T4160C, A4136G, and T4216C in the *ND1* gene; A4917G and G5244A in the *ND2* gene; T9101C in the *ATP6* gene; G9438T, G9738T, and G9804A in the *COIII* gene; T10663C in the *ND3* gene; G13708A and G13730A in the *ND5* gene; and C14482G and C14568T in the *ND6* gene) could be identified from the sequencing data. Only one of these class II mutations (G3316A) was found in a single patient (Chinese patient EIJbIII-4-94). The examination of the remaining two class II mutations (G7444A in the *COI* gene and G15812A in the *Cytb* gene; Table 2) by PCR-RFLP confirmed the absence of these mutations in the Southeast Asian populations.

The nucleotide sequence data reported are available in the GenBank databases under the accession numbers AF447710–AF447726 and AY063315–AY063416.

Discussion

In European LHON patients, both the G11778A and the T14484C primary LHON mutations have been shown to exhibit a certain degree of clustering on mtDNA haplogroup J (Lamminen et al. 1997; Brown et al. 1997; Torroni et al. 1997) typified by the T4216C and G13708A class II mutations, suggesting a pathogenic role for the subset of mtDNA sequence variants associated with this haplogroup, presumably in promoting the clinical expression of the primary LHON mutations. In the present study we have tested the hypothesis that, since the primary LHON G11778A mutation is found in distantly related ethnic populations with clearly distinct mtDNA haplotypes, different sets of SNPs must be able to play the same role either as predisposing factors for the generation of the primary LHON mutations, or as positive modifiers of the mutations allowing their detection as disease mutations.

Results of our mtDNA haplotyping study by PCR-RFLP and of the SNP analysis of the *ND* genes by direct sequencing confirm the distant phylogenetic relationship between the SEA populations and the European populations, from which most of the information regarding the molecular ba-

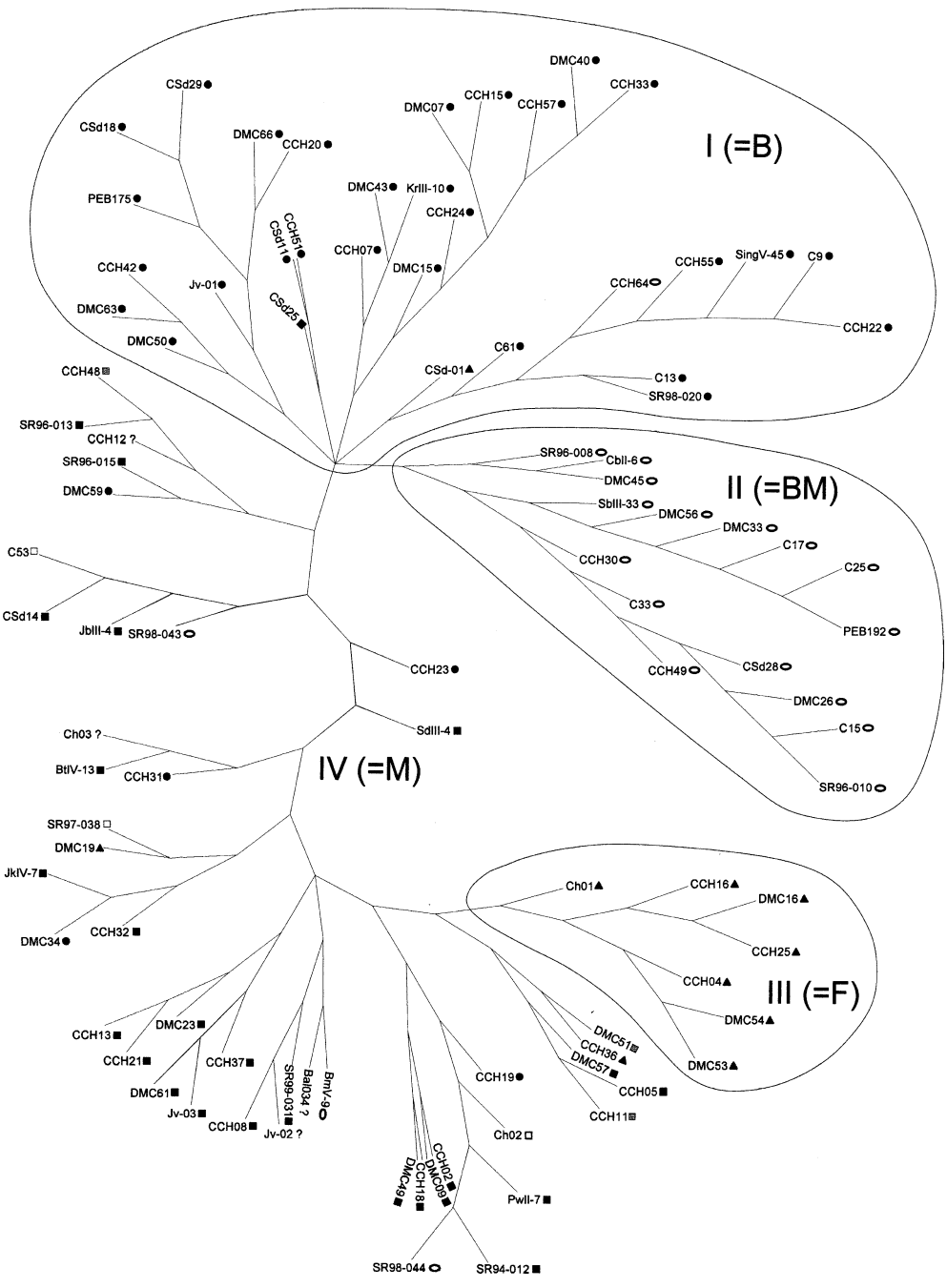

Fig. 2. A neighbor-joining tree of the hypervariable region 1 (HVR1) D-loop control region sequences from 19 LHON patients and 79 Southeast Asian control individuals was constructed from genetic distances estimated with the Kimura two-parameter model in DNADIST (Felsenstein 1993). The haplogroup of each mtDNA sample as determined by the combination of 11 restriction endonuclease sites is indicated next to the sample numbers as follows: B, *black circle*; F, *black triangle*; M, *black square*; M-G, *gray square*; M-D, *white square*; BM, *oval*; unidentified, *question mark*. The HVR1 D-loop control region sequences of the samples cluster into four groups: *I*, *II*, *III*, and *IV*. These groups correspond to haplogroups *B*, *BM*, *F*, and *M*, respectively

sis of LHON has been derived. At least 34% of the SEA mtDNA samples cannot be classified into the documented Asian haplogroups (A, B, F, M, M-C, M-D, and M-G). Further, as many as 62 novel coding SNPs not previously reported in Europeans, Africans, or Japanese were identified in the *ND* genes of the 17 mtDNA samples sequenced. Despite this phylogenetic distance, however, the G11778A (Arg340His in the ND4 subunit of respiratory complex I) mutation was found in 18 out of the 19 (95%) patients examined. The other patient also carries a primary LHON mutation that has been reported in Europeans, the T14484C substitution leading to a Met64Val amino acid replacement in the ND6 subunit of respiratory complex I.

RFLP analysis of the mtDNA in the 19 LHON patients and 60 control subjects demonstrated that the LHON mtDNAs in SEA populations cluster in two major haplogroups, haplogroup M and a novel mtDNA haplogroup BM (characterized by the gain of a 10394 *Dde*I site and the absence of the 10397 *Alu*I site). These two haplogroups represent 47% and 37% of the LHON mtDNAs, respectively, compared with frequencies of only 32% and 18% in the control population. Thus, the incidence of the G11778A mutation in these two major Southeast Asian haplogroups was no more than twice what would be expected if this mutation had occurred randomly in all mtDNA backgrounds, but a two-tailed Fisher's exact analysis confirmed

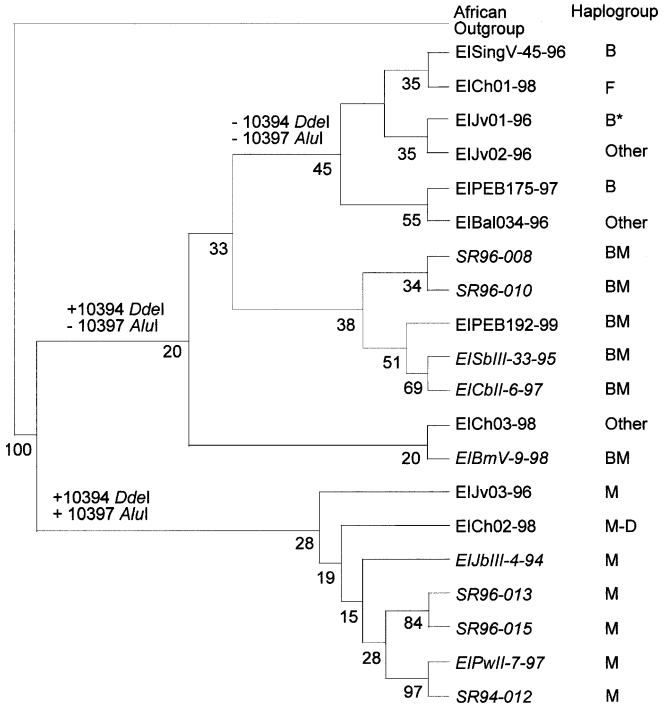

Fig. 3. Parsimony phylogram based on the *ND* gene sequences. A phylogenetic tree relating ten LHON patients and ten control individuals (seven sequenced in the present study and three unpublished sequences from our laboratory), five individuals of Sundanese/Javanese origin, two Balinese-Indonesians, and three Chinese-Indonesians, was constructed, by parsimony analysis using the PHYLIP: Phylogeny Inference Package. The data were bootstrapped with 1000 replicates (bootstrapped value shown on nodes). The consensus tree was unrooted, and an African *ND* gene sequence was used as the outgroup. mtDNAs of the LHON patients (indicated by *italics*) cluster together as haplogroups *M* and *BM*

that this difference was significant ($P = 0.04$ and $P = 0.004$ for haplogroups M and BM, respectively). This result compares very well with the clustering of the same LHON mutations in haplogroup J in European patients (Brown et al. 1997), although it was slightly weaker in comparison with the two- to threefold increase in frequency observed in the Europeans.

The clustering of the LHON G11778A in the SEA patients in the two haplogroups is consistent with the hypothesis that a different set of mtDNA polymorphisms is involved in the clinical expression of the G11778A primary LHON mutation in Southeast Asians. A closer look at the *ND* gene sequences of the LHON mtDNAs did not reveal any common SNP, except for the coding SNP A10398G associated with a Thr114Ala amino acid replacement in the ND3 subunit of respiratory complex I. This SNP is the one responsible for the *Dde*I RFLP detected at nt 10394, and it is found in 90% of the LHON mtDNAs examined, including EISbIII-33-95, which has mtDNA T14484C in the *ND6* gene (Met64Val) as the underlying causal mutation. The difference in the prevalence of this SNP in the LHON patients (90%) compared with the combined normal Thai and Austronesian–Indonesian populations (60%) is highly significant ($P < 0.0001$; two-tailed Fisher's exact analysis). It is of significance to note in this regard that the A10398G SNP also characterizes the European-specific haplogroup J. The potential role of the A10398G SNP as a positive modifier of the LHON G11778A mutation has been considered previously, but from two opposing viewpoints. Torroni et al. (1997) suggested that this SNP played a role, but others (Brown et al. 1992; Finnilä and Majamaa 2001) dismissed that possibility because of the SNP's presence in numerous haplogroups. The finding in our study of the common presence of this SNP in European and SEA LHON patients, however, has provided further supporting evidence in support of a role for the A10398G SNP in the clinical expression of the G11778A mutation.

The Thr114Ala substitution in the ND3 subunit of respiratory complex I associated with the A10398G SNP is not a drastic amino acid change. Such a conservative amino acid change, however, has been reported to be associated with one class I (G3460A) and three class II (G3316A, G9738T, and G9804A) mutations, and thus could be functionally significant. The Thr114Ala amino acid change in the ND3 subunit could, for example, influence the biochemical manifestation of the causal Arg340His amino acid replacement in the ND4 subunit associated with the LHON G11778A mutation. The presence of the A10398G SNP alone, however, is apparently not sufficient for the positive modifying effect of the mtDNA background on the primary LHON mutation observed in the European-specific haplogroup J and in the SEA-specific haplogroups M and BM. Perhaps, interactions among a number of SNPs in the *ND* genes characteristic of the three haplogroups, with A10398G being a major and common one, are responsible for the proposed increased penetrance of the primary LHON mutations. The ancient combination (Torroni et al. 1997) of T4216C (ND1 Tyr304His) and G13708A (ND5 Ala458Thr), together with A10398G is a good candidate for such an interaction. A different interaction involving A10398G might be responsible in the SEA patients, although no candidate SNP is immediately apparent from the SEA mtDNA sequence data that we have collected.

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References

Anderson S, Bankier AT, Barrell BG, De Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147

Ballinger SW, Schurr TG, Torroni A, Gan YY, Hodge JA, Hassan K, Chen KH, Wallace DC (1992) SEA mitochondrial DNA analysis

reveals genetic continuity of ancient Mongoloid migrations. Genetics 130:139–152

Brown MD, Wallace DC (1994) Spectrum of mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Clin Neurosci 2:138–145

Brown MD, Voljavec AS, Lott MT, Torroni A, Yang CC, Wallace DC (1992) Mitochondrial DNA complex I and III mutation associated with Leber's hereditary optic neuropathy. Genetics 130:163–173

Brown MD, Torroni A, Reckord CL, Wallace DC (1995) Phylogenetic analysis of Leber's hereditary optic neuropathy mitochondrial DNA indicates multiple independent occurrences of the common mutations. Hum Mutat 6:311–325

Brown MD, Sun F, Wallace DC (1997) Clustering of Caucasian Leber hereditary optic neuropathy patients containing the 11778 or 14484 mutations on an mtDNA lineage. Am J Hum Genet 60:381–387

Felsenstein J (1993) PHYLIP: Phylogeny Inference Package version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle

Finnilä S, Majamaa K (2001) Phylogenetic analysis of mtDNA haplogroup TJ in a Finnish population. J Hum Genet 46:64–69

Fucharoen G, Fucharoen S, Horai S (2001) Mitochondrial DNA polymorphisms in Thailand. J Hum Genet 46:115–125

Horai S, Matsunaga E (1986) Mitochondrial DNA polymorphism in Japanese. II. Analysis with restriction enzymes of four or five base pair recognition. Hum Genet 72:105–117

Howell N, Bindoff LA, McCullough DA, Kubacka I, Poulton J, Mackey D, Taylor L, Turnbull DM (1991) Leber hereditary optic neuropathy: identification of the same mitochondrial ND1 mutation in six pedigrees. Am J Hum Genet 49:939–950

Howell N, Kubacka I, Halvorson S, Howell B, McCullough DA, Mackey D (1995) Phylogenetic analysis of the mitochondrial genomes from Leber hereditary optic neuropathy pedigrees. Genetics 140:285–302

Huoponen K, Villki J, Aula P, Nikoskelainen EK, Savontaus ML (1991) A new mtDNA mutation associated with Leber hereditary optic neuroretinopathy. Am J Hum Genet 48:1147–1153

Huoponen K, Lamminen T, Juvonen V, Aula P, Nikoskelainen E, Savontaus JL (1993) The spectrum of mitochondrial DNA mutations in families with Leber hereditary optic neuropathy. Hum Genet 92: 379–384

Johns DR, Neufeld MJ, Park RD (1992) An ND6 mitochondrial DNA mutation associated with Leber hereditary optic neuropathy. Biochem Biophys Res Commun 187:1551–1557

Jun AS, Brown MD, Wallace DC (1994) A mitochondrial DNA mutation at np 14459 of the *ND6* gene associated with maternally inherited Leber's hereditary optic neuropathy and dystonia. Proc Natl Acad Sci USA 91:6206–6210

Lamminen T, Huoponen K, Sistonen P, Juvonen V, Lahermo P, Aula P, Nikoskelainen E, Savontaus M (1997) MtDNA haplotype analysis in Finnish families with Leber hereditary optic neuroretinopathy (LHON). Eur J Hum Genet 5:271–279

Lum JK, Rickards O, Ching C, Cann RL (1994) Polynesian mitochondrial DNAs reveal three deep maternal lineage clusters. Hum Biol 66:567–590

Macaulay V, Richards M, Hickey E, Vega E, Cruciani F, Guida V, Scozzari R, Bonné-Tamir B, Bryan S, Torroni A (1999) The emerging tree of west Eurasian mtDNAs: A synthesis of control region sequences and RFLPs. Am J Hum Genet 64:232–249

Mackey D, Howell N (1992) A variant of Leber hereditary optic neuropathy characterized by recovery of vision and by an unusual mitochondrial genetic etiology. Am J Hum Genet 51:1218–1228

Marzuki S, Noer AS, Lertrit P, Thyagarajan D, Kapsa R, Utthanapol P, Byrne E (1991) Normal variants of human mitochondrial DNA and translation products: the building of a reference database. Hum Genet 88:139–145

Mashima Y, Hiida Y, Oguchi Y, Kudoh J, Shimizu N (1993) High frequency of mutations at position 11778 in mitochondrial *ND4* gene in Japanese families with Leber's hereditary optic neuropathy. Hum Genet 92:101–102

Schurr TG, Sukernik RI, Starikovskaya YB, Wallace DC (1999) Mitochondrial DNA variation in Koryaks and Itel'men: population replacement in the Okhotsk Sea–Bering Sea region during the Neolithic. Am J Phys Anthropol 108:1–39

Shoffner JM, Brown MD, Stugard C, Jun AS, Pollok S, Haas RH, Kaufman A, Koontz D, Kim Y, Graham J, Dixon J, Wallace DC (1995) Leber's hereditary optic neuropathy plus dystonia is caused by a mitochondrial DNA point mutation in a complex I subunit. Ann Neurol 38:163–169

Sudoyo H, Sitepu M, Poesponegoro HD, Malik S, Marzuki S (1998) Leber's hereditary optic neuropathy — two Indonesian families with the mtDNA 11778G>A and 14484T>C mutations. Hum Mutat (suppl) 1:S271–S274

Torroni A, Sukernik RI, Schurr TG, Starikorskaya YB, Cabell MF, Crawford MH, Comuzzie AG, Wallace DC (1993) MtDNA variation of aboriginal Siberians reveals distinct genetic affinities with Native Americans. Am J Hum Genet 53:591–608

Torroni A, Miller JA, Moore LG, Zamudio S, Zhuang J, Droma T, Wallace DC (1994a) Mitochondrial DNA analysis in Tibet: implications for the origin of the Tibetan population and its adaptation to high altitude. Am J Phys Anthropol 93:189–199

Torroni A, Lott MT, Cabell MF, Schen Y, Laverge L, Wallace DC (1994b) MtDNA and the origin of Caucasian-specific haplogroups, one of which is prone to a recurrent somatic duplication in the D-loop region. Am J Hum Genet 55:760–776

Torroni A, Petrozzi M, D'Urbano L, Sellitto D, Zeviani M, Carrara F, Carducci C, Leuzzi V, Carelli V, Barboni P, De Negri A, Scozarri R (1997) Haplotype and phylogenetic analysis suggest that one European-specific mtDNA background plays a role in the expression of Leber hereditary optic neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. Am J Hum Genet 60:1107–1121

Wallace DC (1995) 1994 William Allan Award Address. Mitochondrial DNA variation in human evolution, degenerative disease and aging. Am J Hum Genet 57:201–223

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Elsas LJ, Nikoskelainen EK (1988) Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242:1427–1430

Wallace DC, Brown MD, Lott MT (1999) Mitochondrial DNA variation in human evolution and diseases. Gene 238:211–230