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mtDNA Haplotype Analysis in Finnish Families with Leber Hereditary Optic Neuroretinopathy

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Abstract

The mitochondrial DNA (mtDNA) sequence variation of 24 Finnish Leber hereditary optic neuroretinopathy (LHON) probands was characterized by sequencing and restriction endonuclease analyses. All LHON-associated substitutions and Caucasoid haplogroup-specific mutations were screened in the families. Analysis of the mtDNAs revealed that the Finnish LHON families have two unique features: an absence of the ND6/14484 mutation and a high number of families (10/24) without the primary mutations ND1/3460 and ND4/11778. Furthermore, the LHON families showed considerable mtDNA heterogeneity: among 24 families 22 haplotypes were detected. Overall, the haplogrouping of LHON families was similar to other European populations. However, the frequency of ND4/11778-positive families in haplogroup J was high, which may indicate that background mutations in this haplogroup together with the ND4/11778 primary mutation promote the penetrance of LHON.

Introduction

Leber hereditary optic neuropathy (LHON) is an eye disease which usually causes permanent blindness in affected individuals. Patients are predominantly (over 80%) males. LHON is maternally inherited, and although the etiology of LHON is still partly unknown, it has been unquestionably shown that several point mutations of mitochondrial DNA (mtDNA) are associated with the disease [1, 2].

mtDNA mutations associated with LHON can be classified into primary and secondary mutations. Primary mutations are sufficient by themselves to cause the disease, are not detected in controls, usually change evolutionarily conserved amino acids, and are found in multiple LHON families. Accordingly, mutations at nucleotides (nts) 11778 in the ND4 gene [1], 3460 in the ND1 gene [3, 4] and 14484 in the ND6 gene [5] can be unreservedly classified as primary LHON mutations. In addition, a few other base substitutions such as ATPase6/9101 [6], COXIII/9804 [7], ND6/14459 [8], ND4/11696 and ND6/14596 [9] have also been suggested to be primary mutations in LHON.

The frequencies of the primary mutations vary between populations. In Caucasian LHON patients, mutation ND4/11778 covers 50–76%, ND1/3460 7–30% and ND6/14484 10–31% of cases [10–13].

Several mtDNA mutations are considered to have a secondary role in the pathogenesis of LHON. The mutations are also found at a low frequency in healthy control

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individuals. It has been suggested that the secondary mutations act synergistically either with a primary mutation or with the other secondary mutations [14, 15]. Secondary LHON mutations include base changes e.g. at the following nts: 3394, 4216, 4917, 9438, 13708, 15257 and 15812. The frequencies of these mutations are remarkably higher in LHON families [16].

Phylogenetic analyses of LHON families have recently provided new insight into the population history of LHON as well as into the etiological significance of the various mutations associated with the disease [16–18]. Most of the mtDNA variation is ethnic-specific, and for example, virtually all European mtDNAs are subsumed within nine mtDNA haplogroups (denoted as H, I, J, K, T, U, V, W and X) [19]. The phylogenetic studies have revealed a striking difference between the occurrence of the primary and secondary LHON mutations: the primary mutations have arisen several times independently, while the secondary mutations are lineage-specific [16–18]. Furthermore interesting distributions exist for the European haplogroups: haplogroup J seems to be more frequent among LHON families than in controls, and the pathogenic mutations ND4/11778 and ND6/14484 show significantly preferential association with the haplogroup J [16, 20]. Therefore it has been proposed that the expression of LHON is promoted when these primary mutations occur on haplogroup J background [20, 21].

A small founder population and isolated location have influenced the population structure of Finland. Studies of nuclear genes suggest that the early Finnish poulation has gone through a substantial bottleneck in size, indicated by the 'Finnish disease heritage' [22]. According to nuclear markers, the Finns are outliers when compared to other European populations [23]. However, mtDNA studies give different results: the same Caucasoid-specific lineages and haplogroups are found in Finns as in other European populations [19, 24, 25]. In the present study we have performed a phylogenetic analysis of 24 Finnish LHON pedigrees and evaluated the distribution of the Caucasoid haplogroups among them.

Material and Methods

Subjects

The material consisted of probands of 24 LHON families, out of which 3 families (families X, Y and Z) have not been included in the previous studies [3, 11]. 11 families (A, D, E, F, G, L, M, P, R, S and T) were ND4/11778-positive and 3 (families B, C and N) had the ND1/3460 mutation. Probands of families G, P and R were singleton cases. Furthermore, the probands of families G, M and P were heteroplasmic for ND4/11778 mutation. One family harbored the ATPase6/9101 mutation (family K). In 9 LHON families (H, I, O, Q, U, V, X, Y and Z) classified according to the criteria by Vilkki et al. [26] no known primary mutation was observed. Probands of families H, K, U, X, Y and Z were singleton cases.

In families without a known primary mutation, the clinical picture was consistent with LHON. Shortly, all these patients had experienced acute or subacute painless visual loss due to bilateral optic neuropathy and peripapillary microangiopathy was observed in the acute stage of condition. Accordingly, all singleton cases included in this study had a clinical picture highly suggestive of LHON.

Clinical Studies

The data of ophthalmological, cardiological and neurological findings in ND4/11778 patients was obtained as described earlier [27–29].

mtDNA Analysis

Restriction Enzyme Digestions. Haplotype analysis was performed in 8 LHON probands (families R, S, T, U, V, X, Y, Z). The other 16 probands have been previously analyzed [29]. The analyses were made by amplifying the whole mitochondrial DNA in 9 overlapping fragments and digesting each fragment with 13 restriction enzymes (*AccI, ApaI, AvaII, BanI, BclI, BstNI, DraI, EcoRV, HaeII, HindII, MspI, StuI* and *XbaI*). Resulting fragments were separated on agarose gels and visualized after ethidium bromide staining in ultraviolet light.

In addition to the above-mentioned RFLP analyses, known mutations at nts 3394, 4025, 4136, 4160, 4216, 4633, 4732, 4917, 5244, 6464, 7444, 7476, 8286, 9438, 9804, 13637, 13730, 13966, 13967, 14459, 14470, 14484, 14766, 14798, 15257, 15693 and 15812 were separately screened in all families, either by sequencing (nts 4025, 4160, 4216, 13966, 13967, 14459, 14470, 14484, 14766 and 14798) or by digesting the PCR fragment encompassing the mutation site with a specific restriction enzyme (*HaeIII* nt 3394; *NlaIII* nt 4136; *BanI* nt 4633; *RsaI* nt 4732 and nt 15812; *BfaI* nt 4917; *MspI* nt 5244; *BclI* nt 6464; *AluI* nt 7476; *XbaI* nt 7444 and nt 8286; *StuI* nt 9438; *MaeIII* nt 9804; *TaqI* nt 13637; *ApoI* nt 13730; *AccI* nt 15257; *SspI* nt 15693).

The polymorphisms defining the European specific haplogroups H, I, J, K, T, U, V, W and X characterized by Torroni et al. [19] are defined as the following restriction site changes:

Haplogroup H: lack of *AluI* 7025 and *DdeI* 10394 sites; haplogroup I: presence of *AvaII* 8249, *DdeI* 10394, *AluI* 10028 and *MboI* 16389 sites and absence of *DdeI* 1715 and *HaeII* 4529 sites; haplogroup J: lack of *BstNI* 13704 and *HinfI* 16065 sites and presence of *DdeI* 10394 site; haplogroup K: absence of *HaeII* 9052 site, presence of *DdeI* 10394 site and A to G transition at 12308 (defined by sequencing); haplogroup T: presence of *BamHI* 13366 and *AluI* 15606 sites, lack of *DdeI* 10394 and *MspI* 15925 sites; haplogroup U: lack of *DdeI* 10394 site and A to G transition at 12308; haplogroup V: lack of *NlaIII* 4577 and *DdeI* 10394 sites; haplogroup W: absence of *AvaII* 8249 and *DdeI* 10394 sites and presence of *HaeIII* 8994 sites; haplogroup X: lack of *DdeI* 1715 and 10394 sites.

Sequencing of the polymorphic sites 4577, 7025, 10394, 13704 and 16065 revealed the accurate nucleotide changes. The nucleotide positions were 4580, 7028, 10398, 13708 and 16069, respectively, and they will be used in the text.

Sequencing. Total DNA was extracted from blood samples. 100 ng was used in a standard PCR reaction, and PCR-amplified

double-stranded fragments were directly sequenced using the Sanger dideoxy chain termination method [31] and Sequenase enzyme (USB, Amersham).

All the mitochondrial protein coding genes were sequenced in the 7 families with no confirmed primary mutation (families H, I, K, O, Q, U, V). The families X, Y and Z included in the course of the study were sequenced only for the genes of complexes IV and V.

To further extend the phylogenetic study, the first hypervariable region of the D-loop (HV-1) was sequenced in all families. PCR amplification was performed with the primers located at nt 15926–15952 (→) and 16498–16479 (←). The forward primer was used as a sequencing primer, except in the samples (E, G, K, L) which harbored the length polymorphism at the homopolymeric tract around the nt 16189 T → C mutation. In these families heavy strand sequencing was performed with the reverse primer.

Phylogenetic Analysis of LHON Families. Programs of the PHYLIP program package [32] and METREE (minimum evolution, ME, tree testing) [33] and the quartet maximum likelihood program PUZZLE [34] were used to analyze and construct phylogenetic trees of the families. The sequences were coded relative to the Cambridge standard sequence [35]. In addition to the mutation site both flanking nucleotides were included. Neighbor joining (NJ) and maximum likelihood (ML) trees were obtained using a transition: transversion rate 12.3 [18] with the categories option and the relative mutation rates set to 0, 5 and 10 with probabilities of 0.70, 0.29 and 0.01, respectively. To evaluate the validity of the tree based on the actual data set the data was bootstrapped at least 1,000 times and a consensus tree either based on NJ algorithm (PHYLIP) or on Tamura-Nei's evolutionary model [34] was constructed.

To test whether support for the phylogenies of the families could be obtained by data nonrelated to the LHON mutations, 57 HV-1 sequences from an unrelated Finnish population sample consisting of 360 nts (16024–16383) were included in addition to the 24 LHON HV-1 sequences. The genetic distances were estimated by the quartet method (PUZZLE) using a transition: transversion rate estimated from the data, bootstrapped 1,000 times and a tree based on actual computed distances constructed with NJ (PHYLIP).

Results

ND4/11778-Positive Haplotypes

The ND4/11778 mutation was observed in 11 families (table 1). Four of the ND4/11778-positive families (A, M, D and T) formed a separate cluster by sharing several mutations including secondary mutations at nts 4216 and 13708, and polymorphisms at nts 7028, 10398 and 16069. Families D and T had an identical haplotype. They were distinguished from A and M by having additional base changes at nts 6464 and 14798. Furthermore, A and M both harbored the nt 15257 and nt 7476 mutations, and A had secondary mutation at nt 15812.

Seven of the ND4/11778-positive families harbored no secondary mutations, but in 4 of them, a common neutral variant at nt 7028 was observed and 2 families carried the 14766 polymorphism. In addition, several other polymorphisms were detected in these families as listed in table 1.

ND1/3460-Positive Haplotypes

Families B, C and N harbored the ND1/3460 mutation together with the nt 14766 polymorphism. In addition, family B carried mutations at nts 4580, 7028 and 7444. Families C and N had otherwise identical mtDNA haplotypes except for the D-loop region where family C had a mutation at nt 16162.

Other Haplotypes

Ten families representing 9 haplotypes did not have any previously confirmed primary mutation. However, family K carried the ATPase6/9101 mutation which was recently suggested to be of primary importance in the pathogenesis of LHON [6].

Two of these families carried secondary mutations. Family I belonged to the cluster of ND4/11778-positive families A, M, D and T, which has nt 4216 and 13708 secondary mutations and polymorphisms at nts 7028, 10398 and 16069. In addition, base changes at nts 4025 and 11413 were detected in this family. Family Z harbored mutation at nt 4216 but not at nt 13708. Furthermore, this family carried the secondary mutation nt 4917 and polymorphisms at nts 7028, 12629, 13366, 15606 and 15925.

The families H, O, Q, U, V, X and Y harbored neither primary nor secondary LHON mutations. They represented 6 different haplotypes, of which 1 was shared by families H and U. Also these families frequently carried polymorphisms at nts 7028 and 14766 either simultaneously or separately. In addition, several other base changes were detected (table 1).

D-Loop Mutations

The distribution of D-loop mutations is presented in table 1. The results indicate a high heterogeneity between families. Pairwise identical D-loop haplotypes were observed only in families D and T, and H and U, respectively. Only few mutations occurred repeatedly in different haplotypes, most common being those at nts 16069 and 16189. It is notable that out of 6 families with the nt 16189 T→C mutation, heteroplasmy in the length of the homopolymeric C-tract was observed in 4 families (E, G, K, L). The families S and Z with the nt 16189 mutation had another substitution (C→T) either at nt 16192 or 16186, respectively, which disrupted the C-tract.

Table 1. Mutations in LHON families

Family	Primary mutations	Secondary mutations	Polymorphisms	D-loop mutations	Haplo-group
A	11778	4216, 13708, 15257, 15812	7028, 7476, 10398	16069, 16126, 16193, 16278	J
M	11778[a]	4216, 13708, 15257	7028, 7476, 10398	16069, 16126, 16145, 16231, 16261, 16344	J
D, T	11778	4216, 13708	6464,[b] 7028, 10398, 14798	16069, 16261	J
E	11778		1715,[c] 7028, 13966, 14470	16183,[b] 16189,[a] 16194,[b] 16223, 16248, 16255, 16278	X
F	11778			16162	H
G	11778[a]		4633,[b] 7028, 12308	16093, 16189,[a] 16270, 16465	U
L	11778		14766	16093, 16183,[b] 16189[a]	H
P	11778[a]		7028, 8249,[c] 8994[c]	16223, 16292, 16295	W
R	11778		14766	16129	H
S	11778		7028, 12308	16189, 16192, 16270	U
B	3460		4580, 7028, 7444, 14766	16153, 16298	V
C	3460		14766	16162	H
N	3460		14766		H
K	9101		14766	16183,[b] 16189,[a] 16400	H
I		4216, 13708	4025, 7028, 10398, 11413, 14798	16069, 16126, 16145, 16311	J
Z		4216, 4917	7028, 12629,[c] 13366,[c] 15606[c] 15925[c]	16126, 16163, 16186, 16189, 16294	T
H, U			4580, 7028, 14766	16298	V
O			12811, 13967, 14766	16311	H
Q			4732, 7028, 8705, 12308, 13637		U
V			8286, 14766	16477	H
X			14766	16209	H
Y			7028, 7299, 9777, 12308	16146, 16342	U

Nucleotide changes are transitions, unless otherwise noted.

[a] Heteroplasmic mutation.

[b] Transversion.

[c] Mutation screened with restriction enzyme.

Table 2. Haplogroup distribution of LHON families

	n	H n	I n	J n	K n	T n	U n	V n	W n	X n	Others n
11778-positive	11	3		4			2		1	1	
3460-positive	3	2						1			
Other	10	4		1		1	2	2			
Total	24	9 (37.5%)		5 (20.8%)		1 (4.2%)	4 (16.7%)	3 (12.5%)	1 (4.2%)	1 (4.2%)	
Controls[a]	49	20 (40.8%)	1 (2.0%)	7 (14.3%)	2 (4.1%)	3 (6.1%)	8 (16.3%)	2 (4.1%)	2 (4.1%)	2 (4.1%)	2 (4.1%)

[a] Data taken from Torroni et al. [19].

Haplogrouping of the LHON Families

The Caucasoid haplogroup-specific mutations, defined by Torroni et al. [19], were screened for the 24 LHON families. The haplogroup distribution of the Finnish LHON families and 49 Finnish control individuals [19] is presented in table 2. The most common haplogroup both in controls and in LHON families was H, represented by 9 out of 24 of LHON families and 20/49 in controls. The ND1/3460-positive families were found in haplogroups H and V. Haplogroups T, U, W and X were observed at equal numbers in controls and in LHON families, while haplogroup J was represented more often among the ND4/11778-positive families (4/11) than among the controls (7/49). Although the difference did not reach statistical significancy, the p value 0.087 in χ^2 testing was suggestive. Interestingly, the same clustering has also been observed in the LHON families belonging to other ethnic groups [20, 21].

Table 3. Clinical features of ND4/11778 families

Haplogroup comparison	Haplogroup J (families A, M, D, T)[a]	Others (families E, F, G, L, P, R, S)[a]
Visual field defect		
Absolute	13	12
Relative	6	2
Onset interval between eyes		
1–2 months	3	5
2–6 months	6	6
Over 6 months	3	
Tempo of LHON		
Acute	1	3
Subacute	11	8
Slow		1
Mean age of onset, years		
Male	24.7 (range 17–39, n = 13)	22.2 (range 6–31, n = 11)
Female	23, 24, 43	36, 58
Sex ratio male/female	34/9 (79% males)	22/7 (76% males)
Penetrance, %		
Male	49.2 (n = 63)	36.8 (n = 38)
Female	15.3 (n = 65)	14.7 (n = 34)
Peripapillary microangiopathy	47/67 (70%)	32/45 (71%)
Recovery[b]	4/19	2/14
Neurological abnormalities	11/19	5/12
Preexcitation syndrome		
WPW	3/18	1/12
LGL		1/12

[a] Unless otherwise specified, values are numbers.
[b] Visual acuity over 20/50.

Clinical Features of ND4/11778-Positive Families in Haplogroup J and Other Groups

It has been proposed that the expression of LHON is promoted when primary mutations ND4/11778 and ND6/14484 occur on haplogroup J background [20, 21]. Therefore, we studied if the ND4/11778 families in haplogroup J differ in clinical features from ND4/11778 families belonging to other haplogroups. The ophthalmological, cardiological and neurological findings [27–29] (table 3) were gathered from our previous reports, and therefore the number of patients studied for different parameters are not the same. Ophthalmological findings and the number of patients with Wolff-Parkinson-White (WPW) and Lown-Ganong-Levine (LGL) preexcitation syndromes showed no major differences between the two groups of ND4/11778 families. The onset age in group J is little higher in males than in the other group, but in females the onset ages vary greatly in both groups. Due to a small sample size, the mean values were not calculated for females. The frequency of neurological findings and penetrance of optic atrophy in males are higher in group J, but statistical significance could not be shown. In conclusion, no major clinical differences distinguish these two family groups.

Phylogenetic Study

Several approaches were used in the phylogenetic analysis of LHON families. The quartet, ML, ME or NJ methods were used to estimate the phylogenetic relationships among the LHON families and they all resulted in similar topologies of the trees. Figure 1 shows an example of an ML run. All coding region mutations and haplogroup J-specific D-loop mutation at nt 16069 were included. It is notable that in all analyses the cluster of families A, M, D, T and I was maintained. The cluster is shown also in the insert picture of figure 1, which presents a phylogenetic tree of D-loop mutations only. Also, the inclusion of D-loop sequences of 57 Finnish controls maintained the clustering topology (data not shown). When the data was bootstrapped the topology favoring the clustering of LHON D-loop sequences was largely lost as there appeared not to be significant D-loop sequence differences between LHON and those representing the general Finnish population.

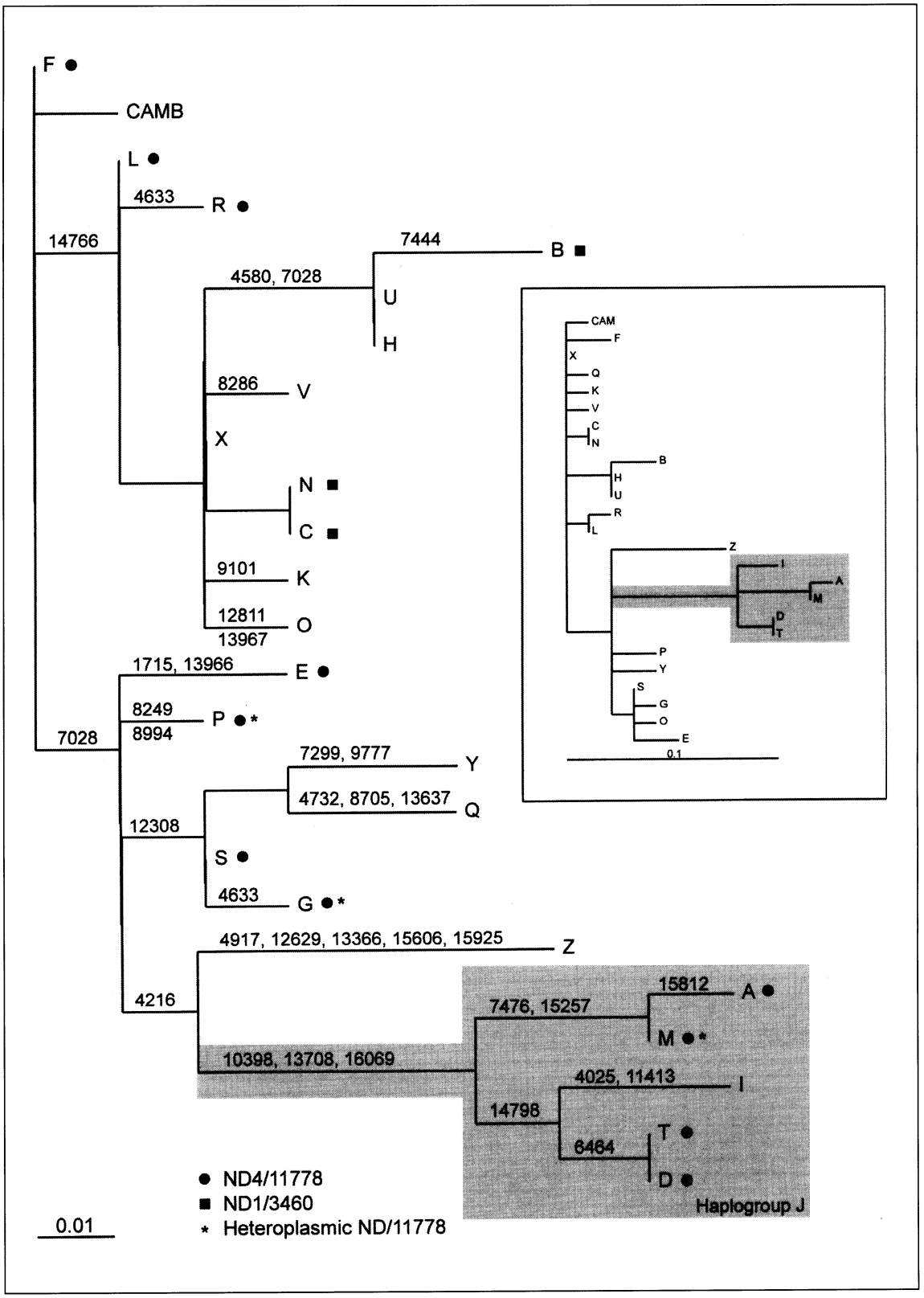

Fig. 1. Phylogenetic tree has been prepared using PHYLIP software package, DNA ML program. The figure illustrates a tree with all coding region mutations presented in table 1 and D-loop mutation at nt 16069. Inserted figure shows the phylogenetic tree including only D-loop material of LHON families using the PUZZLE program.

Discussion

All Finnish LHON families were subsumed within the 9 mtDNA haplogroups (H, I, J, K, T, U, V, W and X) [19]. Although the number of LHON families was small in different haplogroups, the frequencies showed roughly the same distribution of haplogroups as found in the controls. The exception was haplogroup J, which was more frequent among the ND4/11778-positive families than in controls. The 3 ND1/3460-positive families were found in haplogroups H and V. Haplogroups T, W and X were rare in both LHON families and in controls.

ND4/11778 Mutation

The ND4/11778 is the most common primary mutation in Finland, covering 46% (11/24 families) of all the families. This is in accordance with the reports from other populations [12, 16, 17]. Seven out of the 11 Finnish ND4/11778-positive mtDNA types do not harbor any secondary mutations, but in 4 families (A, M, D and T) secondary mutations were present (table 1). These families belong to the previously reported mtDNA lineage [36], which is identical to the European-specific haplogroup J [19]. Since the accumulation of the mutations at nts 4216, 13708, 15257 and 15812 along this lineage correlated with the increased probability of LHON, Brown et al. [36] suggested that this was due to a synergistic effect of the secondary mutations. Our studies show no such correlation between the number of secondary mutations and high expression of optic atrophy, except in family A with 4 secondary mutations [29]. The expression in families M, D and T with 2 or 3 secondary mutations was similar to that in other ND4/11778-positive families without any secondary mutation.

The clustering of families A, M, D and T in haplogroup J made this haplogroup more common among the LHON patients than in controls. Although the number of LHON families in haplogroup J was small, and the difference of frequencies of LHON families and controls did not reach statistical significancy, the resulting p value was suggestive. A similar accumulation of LHON families in haplogroup J has been observed in other ethnic groups [20, 21]. The occurrence of secondary mutations exclusively in haplogroup J families suggests that the background mutations (secondary mutations and polymorphisms) may promote the expression of LHON, i.e. increase the probability of blindness when the ND4/11778 mutation arises at this mtDNA background [21]. The higher expression may be caused by extensive reduction of cell fitness in the presence of secondary mutations as compared to cells with primary mutation only [37]. A synergistic effect therefore may operate and lead to high expression of LHON in ND4/11778-positive families belonging to haplogroup J. No clinical finding, however, distinguished these families from other ND4/11778-positive families.

Phylogeny of the ND4/11778 Mutation

The different programs gave similar topologies of the phylogenetic trees all indicating substantial heterogeneity of the Finnish LHON families. The ND4/11778 mutation seems to have arisen several independent times in the Finnish LHON population. A single ancestral ND4/11778 mutation is improbable for several reasons. First, the D-loop analysis shows that except for D and T, all families have different D-loop sequence. Even by assuming the highest estimate for D-loop mutation rate [38], it is very unlikely that the sequence diversity in the ND4/11778-positive families has arisen in one founder lineage. Secondly, the ND4/11778-positive families are seen in several haplogroups, defined by the ancient haplogroup-specific mutations [19], implying that ND4/11778 mutations have occurred independently in different lineages. Thirdly, among 11 ND4/11778-positive families there are heteroplasmic families, indicating recent occurrences of the mutation. In families G and P the primary mutation can be traced back to the maternal relative in whom the mutation has arisen [39].

NDI/3460-Positive Families. Three Finnish LHON probands harbored the ND1/3460 primary mutation. Usually the primary mutation ND1/3460 appears alone, but in family B it is present together with the nt 7444 mutation. This haplotype has been described earlier in 1 LHON family [16]. Occasionally ND1/3460 has been found in combinations with nts 4216 + 4917 or 4216 + 13708 mutations [16–18], but in our survey no combinations with secondary mutations were found.

Families C and N with ND1/3460 mutation had similar coding region haplotypes characterized by polymorphism at nt 14766, but they had differences in the D-loop region. Family B differed from these families both in coding region and D-loop sequence. Thus the ND1/3460 mutation has probably arisen twice in the Finnish LHON families: once in the lineage with families C and N, assuming that the separating D-loop mutation is younger than the ND1/3460 mutation, and a second time in the lineage represented by family B. The ND1/3460-positive families were found in haplogroups H and V, which were also represented in controls. Due to the small number of ND1/3460 families, no conclusions could be drawn from

the haplogrouping. The scattering of ND1/3460 mutation in different mtDNA backgrounds without preference for any haplogroup has been suggested in other studies as well [20, 21].

ND6/14484 Mutation. Thus far, the ND6/14484 mutation has not been observed in Finnish families. This is notable, since this mutation covers approximately 15% of LHON patients in other populations being as common as the ND1/3460 mutation [16]. Most of the ND6/14484-positive patients harbor also secondary mutations at nts 4216, 13708 and 15257 [16, 17]. In the Finnish haplotypes, these secondary mutations occur only with the ND4/11778 primary mutation.

Families without Primary Mutations

The diagnosis of LHON in the Finnish families is based on careful ophthalmological examination, performed by the same ophthalmologist. All 24 probands included in this study are clinically confirmed LHON patients, although 9 of them have no known primary mutations. Five of the families are singletons (H, U, X, Y, Z), but in I, O, Q and V several affected persons are seen in maternal lineages. Microangiopathy in unaffected family members has been observed in families H, I and O.

Families I and Z harbored only secondary mutations. Family I belonged to the cluster of families which share 5 mutations (nts 4216, 7028, 10398, 13708 and 16069), but unlike other families in this cluster, family I does not harbor the ND4/11778 mutation. This was intriguing and therefore we studied the proband of family I for possible low level heteroplasmy of the ND4/11778 mutation in blood, hair, urinary epithelium and buccal mucosa samples by restriction site analysis and solid-phase minisequencing [39]. No sign of heteroplasmy for the ND4/11778 mutation was observed in any of the tissues studied (data not shown). This still leaves us with a possibility, though never reported, that the actual target tissue might carry the ND4/11778 mutation in its mtDNA. This of course may be true for all known or undetected LHON primary mutations. The haplotype characteristic of family I with secondary mutations 4216 and 13708 without ND4/11778 has also been observed in other studies [12, 17].

The 9 families without a primary mutation were dispersed in several haplogroups with approximately similar frequencies as the controls, except in haplogroup V. Due to the possibility that probands H and U are members of the same family, the number of families in haplogroup V may actually be 1 instead of 2.

Conclusions

The occurrence of LHON-associated mutations in the Finnish LHON families is similar to other populations: the primary mutations have arisen several times independently while the secondary mutations are haplogroup-specific. Most of the secondary mutations occur in haplogroup J. It is also notable that the ND4/11778-positive families have a tendency to cluster in haplogroup J, while the ND1/3460 families show no preferential association with any haplogroup. The study of clinical parameters showed no major differences between ND4/11778 families belonging to haplogroup J or to another haplogroup.

The Finnish LHON families show considerable variety in mtDNA haplotypes. The European-specific haplogrouping indicates a European origin of mtDNA lineages in LHON pedigrees. On the other hand, the absence of ND6/14484 mutation and the high number of families without the 3 common primary mutations are features typical of the Finnish LHON population.

References

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

2 Howell N: Primary LHON mutations: Trying to separate 'FRUIT' from 'CHAF'. Clin Neurosci 1994;2:130–137.

3 Huoponen K, Vilkki J, Aula P, Nikoskelainen E, Savontaus M-L: A new mtDNA mutation associated with Leber hereditary optic neuroretinopathy. Am J Hum Genet 1991;48:1147–1153.

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

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

6 Lamminen T, Majander A, Juvonen V, Wikström M, Aula P, Nikoskelainen E, Savontaus M-L: A mitochondrial mutation at nt 9101 in the ATP synthase 6 gene associated with deficient oxidative phosphorylation in a family with Leber hereditary optic neuroretinopathy. Am J Hum Genet 1995;56:1238–1240.

7 Johns DR, Neufeld MJ: Cytochrome c oxidase mutations in Leber hereditary optic neuropathy. Biochem Biophys Res Commun 1993;196:810–815.

8 Jun AS, Brown MD, Wallace DC: A mitochondrial DNA mutation at nucleotide pair 14459 of the NADH dehydrogenase subunit 6 gene associated with maternally inherited Leber hereditary optic neuropathy and dystonia. Proc Natl Acad Sci USA 1994;91:6206–6210.
9 De Vries DD, Went LN, Bruyn GW, Scholte HR, Hofstra RMW, Bolhuis PA, van Oost BA: Genetic and biochemical impairment of mitochondrial complex I activity in a family with Leber hereditary optic neuropathy and hereditary spastic dystonia. Am J Hum Genet 1996; 58:703–711.
10 Howell N, Halvorson S, Burns J, McCullough DA, Poulton J: When does bilateral optic atrophy become Leber hereditary optic atrophy? Am J Hum Genet 1993;53:959–963.
11 Huoponen K, Lamminen T, Juvonen V, Aula P, Nikoskelainen E, Savontaus M-L: The spectrum of mitochondrial DNA mutations in families with Leber hereditary optic neuroretinopathy. Hum Genet 1993;92:379–384.
12 Oostra RJ, Bolhuis PA, Wijburg FA, Zorn-Ende G, Bleeker-Wagemakers EM: Leber's hereditary optic neuropathy: Correlations between mitochondrial genotype and visual outcome. J Med Genet 1994;31:280–286.
13 Newman NJ: Leber's hereditary optic neuropathy: New genetic considerations. Arch Neurol 1993;50:540–548.
14 Johns DR, Berman J: Alternative, simultaneous complex I mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Biochem Biophys Res Commun 1991;174: 1324–1330.
15 Brown MD, Vojavec AS, Lott MT, Macdonald I, Wallace DC: Leber's hereditary optic neuropathy: a model for mitochondrial neurodegenerative diseases. FASEB J 1992;6:2791–2799.
16 Brown MD, Torroni A, Reckord CL, Wallace DC: Phylogenetic analysis of Leber's hereditary optic neuropathy mitochondrial DNA's indicates multiple independent occurrences of the common mutations. Hum Mutat 1995;6: 311–325.
17 Obermeier-Kusser B, Lorenz B, Schubring S, Paprotta A, Zerres K, Meitinger T, Meire F, Cochaux P, Blankenagel A, Kommerell G, Jaksch M, Gerbitz K-L: Features of mtDNA mutation patterns in European pedigrees and sporadic cases with Leber hereditary optic neuropathy. Am J Hum Genet 1994;55:1063–1066.
18 Howell N, Kubacka I, Halvorson S, Howell B, McCullough DA, Mackey D: Phylogenetic analysis of the mitochondrial genomes from Leber hereditary optic neuropathy pedigrees. Genetics 1995;140:285–302.
19 Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R, Obinu D, Savontaus M-L, Wallace DC: Classification of European mtDNAs from an analysis of three European populations. Genetics 1996;144: 1835–1850.
20 Brown MD, Sun F, Wallace DC: Clustering of Caucasian Leber hereditary optic neuropathy patients containing the 11778 or 14484 mutations on an mtDNA lineage. Am J Hum Genet 1997;60:381–387.
21 Torroni A, Petrozzi M, D'Urbano L, Sellitto D, Zeviani M, Carrara F, Carducci C, Leuzzi V, Carelli V, Barboni P, De Negri A, Scozzari R: Haplotype and phylogenetic analyses 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 1997;60:1107–1121.
22 de la Chapelle: Disease gene mapping in isolated human populations: The example of Finland. J Med Genet 1993;30:857–865.
23 Cavalli-Sforza LL, Menozzi P, Piazza A: Europe: Major outliers; in The History oand Geography of Human Genes. Princeton, Princeton University Press, 1994, pp 272–273.
24 Sajantila A, Lahermo P, Anttinen T, Lukka M, Sistonen P, Savontaus M-L, Aula P, Beckman L, Traneblaerg L, Gedde-Dahl T, Issel-Tarver L, DiRienzo A, Pääbo S: Genes and languages in Europe: An analysis of mitochondrial lineages. Genome Res 1995;5:42–52.
25 Lahermo P, Sajantila A, Sistonen P, Lukka M, Aula P, Peltonen L, Savontaus M-L: The genetic relationship between the Finns and Finnish Saami (Lapps): Analysis of nuclear DNA and mtDNA. Am J Hum Genet 1996;58:1309–1322.
26 Vilkki J, Savontaus M-L, Nikoskelainen E: Genetic heterogeneity in Leber hereditary optic neuroretinopathy revealed by mitochondrial DNA polymorphism. Am J Hum Genet 1989; 45:206–211.
27 Nikoskelainen EK, Savontaus M-L, Huoponen K, Antila K, Hartiala J: Pre-excitation syndrome in Leber's hereditary optic neuropathy. Lancet 1994;344:857–858.
28 Nikoskelainen EK, Marttila RJ, Huoponen K, Juvonen V, Lamminen T, Sonninen P, Savontaus M-L: Leber's 'plus': Neurological abnormalities in patients with Leber's hereditary optic neuropathy. J Neurol Neurosurg Psychiatry 1995;59:160–164.
29 Nikoskelainen E, Huoponen K, Juvonen V, Lamminen T, Nummelin K, Savontaus M-L: Ophthalmologic findings in Leber hereditary optic neuropathy, with special reference to mtDNA mutations. Ophthalmology 1996;10: 504–514.
30 Vilkki J, Savontaus M-L, Kalimo H, Nikoskelainen E: Mitochondrial DNA polymorphism in Finnish families with Leber's hereditary optic neuroretinopathy. Hum Genet 1989;82: 208–212.
31 Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 1977;74:5463–5467.
32 Felsenstein J: PHYLIP (phylogeny inference package), version 3.5c. Seattle, University of Washington, 1993.
33 Rzhetsky A, Nei M: Theoretical foundation of the minimum-evolution method of phylogenetic inference. Mol Biol Evol 1993;10:1073–1095.
34 Strimmer K, von Haeseler A: Quartet puzzling: A quartet maximum likelihood method for reconstructing tree topologies. Mol Biol Evol 1996;13:964–969.
35 Anderson S, Bankier AT, Barraell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG: Sequence and organization of the human mitochondrial genome. Nature 1981;290:457–465.
36 Brown MD, Voljavec AS, Lott MT, Torroni A, Yang C-C, Wallace DC: Mitochondrial DNA complex I and III mutations associated with Leber's hereditary optic neuropathy. Genetics 1992;130:163–173.
37 Vergani L, Martinuzzi A, Carelli V, Cortelli P, Montagna P, Schievano G, Carrozzo R, Angelini C, Lugaresi E: MtDNA mutations associated with Leber's hereditary optic neuropathy: Studies on cytoplasmic hybrid (cybrid) cells. Biochem Biophys Res Commun 1995;210: 880–888.
38 Howell N, Kubacka I, Mackey DA: How rapidly does the human mitochondrial genome evolve? Am J Hum Genet 1996;59:501–509.
39 Juvonen V, Huoponen K, Syvänen A-C, Nikoskelainen E, Savontaus M-L: Quantification of point mutations associated with Leber hereditary optic neuropathy by solid-phase minisequencing. Hum Genet 1994;93:16–20.