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Phylogenetic analysis of mtDNA haplogroup TJ in a Finnish population

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Abstract An association between mitochondrial DNA (mtDNA) mutations 11778G>A and 14484T>C and mtDNA haplogroup J suggests that this haplogroup harbors substitutions capable of modifying the phenotype of Leber's disease. Our knowledge of the compilation of substitutions in haplogroup J is based on only a small number of complete mtDNA sequences, however. We constructed phylogenetic networks for mtDNA haplogroup TJ that were based on the sequence of the complete coding region and the hypervariable segment I, respectively, in 28 Finnish samples. The networks revealed a subdivision of the haplogroup into subclusters T1, T2, J1, and J2, while comparison of the two networks suggested nine fast evolving nucleotide sites in the hypervariable segment I. Genotypes of patients harboring 11778G>A or 14484T>C were obtained from the literature and were then placed in the network. Only four substitutions were found to be common to the patients, but none of these was unique to haplogroup J. If increased penetrance of the 11778G>A and 14484T>C mutations in patients belonging to haplogroup J is assumed, combinations of ancient substitutions must be implicated.

Key words Population genetics · Evolution · Mitochondrial disease · Length polymorphism · Single nucleotide polymorphism · mtDNA sequence · Leber's hereditary optic neuropathy

Introduction

Maternal inheritance and a high rate of new mutations make mitochondrial DNA (mtDNA) a useful tool in population genetics. Nucleotide variations detected by restriction fragment length polymorphism (RFLP) analysis of the coding region or by sequencing of the hypervariable control region have been used to define clusters of genotypes within populations, and practically all Europeans, for example, fall into the mtDNA haplogroups HV, UK, TJ, IWX, or M (Torroni et al. 1996, Richards et al. 1998). Nucleotide variation in mtDNA nevertheless causes difficulties in the identification of novel mutations among patients with mitochondrial diseases.

Many mutations have been associated with Leber's hereditary optic neuropathy (LHON) (Brown and Wallace 1994), three of which have been shown to be primary causes, namely 11778G>A in the *ND4* gene (Wallace et al. 1988), 14484T>C in the *ND6* gene (Johns et al. 1992, Mackey and Howell 1992) and 3460G>A in the *ND1* gene (Howell et al. 1991, Huoponen et al. 1991), while at least 15 others may play an etiological or pathogenic role (Brown and Wallace 1994). These secondary mutations are found in normal individuals, although at a lower frequency than in LHON patients.

The 11778G>A and 14484T>C mutations show an association with the European-specific mtDNA haplogroup J (Brown et al. 1997, Lamminen et al. 1997, Torroni et al. 1997), suggesting that a combination of polymorphisms specific to this haplogroup increases the penetrance of these two primary mutations or the risk of disease expression. Phylogenetic analyses of mtDNA harboring primary LHON mutations have been carried out using RFLP data or partial sequence data (Brown et al. 1992, 1995, 1997, Howell et al. 1995, Torroni et al. 1997), but it is still unclear whether haplogroup J harbors polymorphisms that contribute to the phenotype of LHON. We report here on the construction of phylogenetic networks of haplogroup TJ based on the nucleotide sequence of the entire mtDNA coding region and the hypervariable segment I (HVS-I), respectively, in 28 Finnish subjects. Previously reported partial sequences for patients with primary LHON mutations and belonging to haplogroup TJ (Brown et al. 1995, Howell et al. 1995, Lamminen et al. 1997, Torroni et al. 1997) were then incorporated into the network in an attempt to identify polymorphisms that could be considered secondary mutations.

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Patients and methods

Samples

Blood samples were obtained from 480 Finnish blood donors, with the requirement that they and their mothers should be free of diabetes mellitus, sensorineural hearing impairment, and neurological ailments. After obtaining this information the samples were anonymized. Total DNA was isolated using a QIAamp Blood Kit (Qiagen, Hilden, Germany), after which RFLP analysis of appropriate polymerase chain reaction (PCR) amplified fragments was used to define the haplogroups. Samples belonging to haplogroup TJ were identified by the gain of a *Nla*III restriction site at nucleotide (nt) 4216, and the gain of a *Bam*HI restriction site at nt 13366 was used to determine haplogroup T and the loss of a *Mva*I restriction site at nt 13704 was used to determine haplogroup J (Torroni et al. 1996).

Previously reported partial sequences from LHON patients harboring either 11778G>A or 14484T>C and belonging to haplogroup TJ were included in the study (Brown et al. 1995, Howell et al. 1995, Lamminen et al. 1997, Torroni et al. 1997). None of these sequences covered the entire mtDNA genome and they were based on restriction fragment analysis (Brown et al. 1995, Torroni et al. 1997), on restriction fragment analysis and partial sequencing (Lamminen et al. 1997), or on partial sequencing covering 8300nts in the mtDNA coding region (Howell et al. 1995).

Conformation-sensitive gel electrophoresis

Thirty-four samples belonged to haplogroup TJ, and 17 haplogroup J samples and 11 haplogroup T samples were randomly selected for analysis by conformation-sensitive gel electrophoresis (CSGE), carried out as described previously (Finnilä et al. 2000). In short, 63 pairs of primers were designed for amplification of the mtDNA coding region (nts 523–16090). The heteroduplex formation was allowed to occur autogenously and, furthermore, each amplified fragment was mixed with the corresponding fragment amplified on a control template with known sequence (Finnilä et al. 2000). The amplified fragments were denatured at 95°C for 5min and the heteroduplexes were subsequently allowed to anneal at 68°C for 30min. The heteroduplex samples were electrophoresed through a polyacrylamide gel at a constant voltage of 400V overnight at room temperature. The bands in the gel were stained with ethidium bromide and visualized in UV light.

Sequencing

PCR fragments that differed in migration pattern from homoduplexes on CSGE and the HVS I of all samples were analyzed by automated sequencing (ABI PRISM 377 Sequencer, using the Dye Terminator Cycle Sequencing Ready Kit; Perkin Elmer, Foster City, CA, USA) after treatment with exonuclease I and shrimp alkaline phosphatase (Werle et al. 1994). The primers used for sequencing of the coding region were the same as those used in the amplification reactions for CSGE. The HVS I was amplified in a fragment spanning nts 15975–16555 and the sequence was determined between nts 16024 and 16400.

A median-joining network was constructed for the HVS-I sequence data by means of the program NETWORK 2.0 (Bandelt et al. 1995, Röhl 1997).

Subcloning of fragments harboring a 9-bp tandem repeat segment

The probable quasispecies in the intergenic region between the gene encoding cytochrome oxidase subunit II (CO II) and the $tRNA^{Lys}$ gene in two samples (6 and 8) belonging to haplogroup J were studied by subcloning a fragment spanning between nts 8100–8437 of mtDNA into a pCR2.1-TOPO vector (TOPO TA Cloning Kit; Invitrogen, Leek, The Netherlands). Positive colonies were cultured overnight in 2ml of Luria Bertani (LB) medium containing 250μg/ml ampicillin. A portion of 1μl from 60 cultures was then incubated for 10min at 94°C, in order to lyse the cells and to inactivate the nuclease, and amplified in the presence of the plasmid-specific primers M13F(−20) and M13R. The sequencing was carried out using plasmid-specific and insert-specific primers.

A 338-bp fragment with a sequence identical to the Cambridge reference sequence between nts 8100 and 8437 was subcloned into pCR2.1-TOPO vector. The vector was grown in host bacteria in a stepwise manner. Thirty colonies were analyzed straight from the bowl, while 30 other colonies were cultured further overnight in 2ml of LB medium containing 250μg/ml ampicillin. A portion of 1μl from these 30 cultures was then amplified with plasmid-specific primers and sequenced. Another portion of 1μl from the 30 cultures was then cultured further overnight in 2ml of LB medium containing 250μg/ml ampicillin, and amplified by PCR and sequenced.

Results

Construction of median networks of haplogroup TJ

Sequence data on the mtDNA coding region in the 28 samples belonging to haplogroup TJ were used to construct a median-joining network (Fig. 1a). Six mtDNA substitutions in the coding region were found in all the samples. The polymorphisms at nts 2706, 7028, and 11719 are common to all the other haplogroups, except for haplogroup H (Richards et al. 1998, Macaulay et al. 1999), and therefore only the polymorphisms at nts 4216, 11251, and 15452 were specific to haplogroup TJ. Haplogroup J was characterized by polymorphisms at nts 10398, 12612, and 13708, but 10398A>G is also found in haplogroups I, J, K,

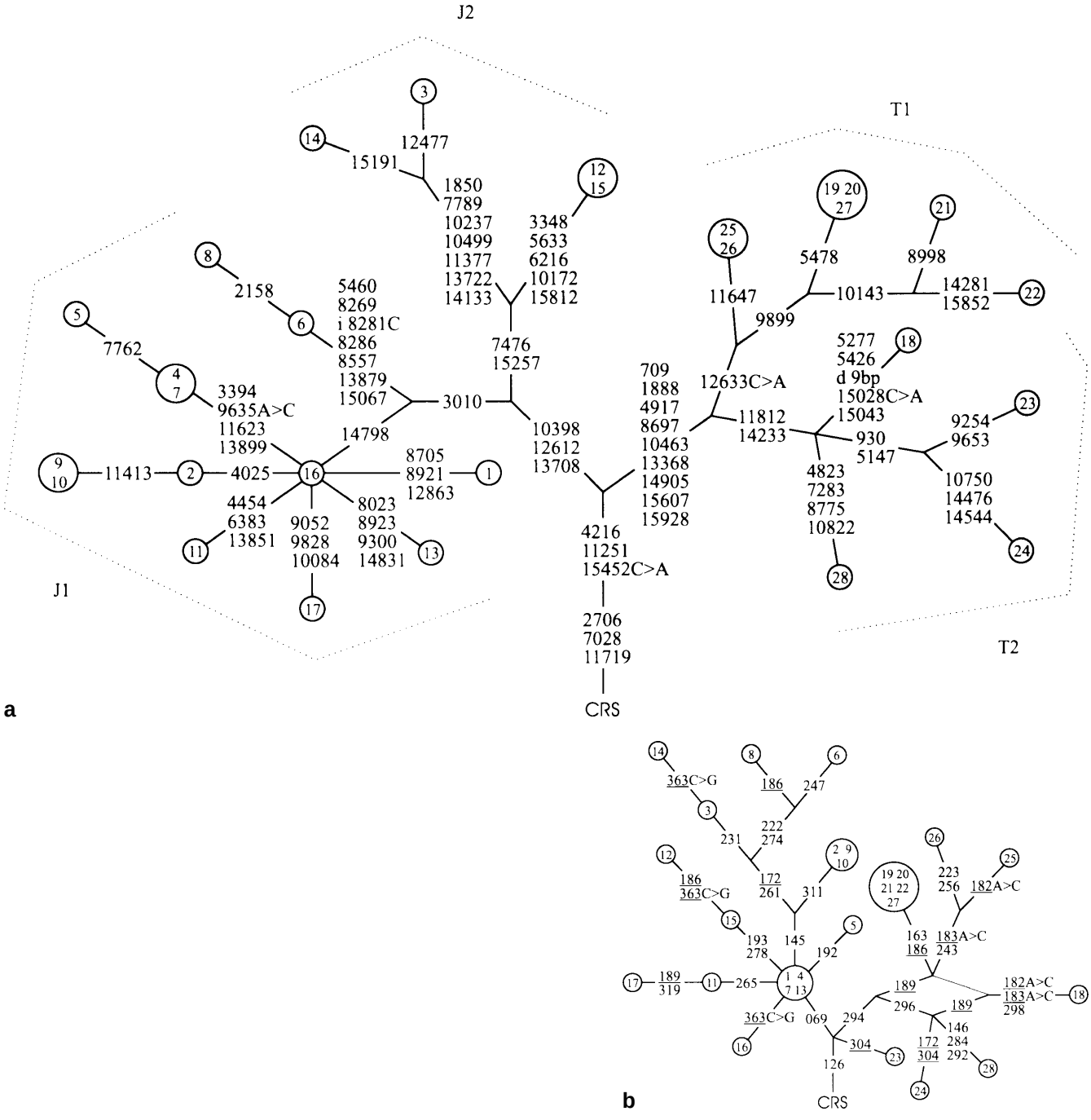

Fig. 1. **a** Phylogenetic network of haplogroup TJ based on variation in the coding sequence. *Numbers inside the nodes* denote samples. The polymorphic variants are shown on the lines connecting the nodes and are transitions unless otherwise marked. The following substitutions were common to all samples: 750A>G, 1438A>G, 3423G>T, 4769A>G, 4985G>A, 8860A>G, 9559G>C, 11335T>C, 13702G>C, 14199G>T, 14272G>C, 14365G>C, 14368G>C, 15326A>G, and deletion 3106C. *Broken lines* around the branches show the locations of subclusters J1, J2, T1, and T2. *CRS*, Cambridge reference sequence; *i8281C*, insertion of variable number of cytosine; *d 9bp*, 9-bp deletion in the CO II-tRNA^{Lys} intergenic region. **b** Phylogenetic network of haplogroup TJ based on variation in the hypervariable segment I (HVS-I) sequence (nucleotides 16024–16400). The *numbers inside the nodes* denote samples and *numbers on the lines* connecting the nodes denote polymorphic nucleotides, with the first two digits, 16, omitted. Polymorphisms are transitions unless otherwise marked. *Underlined digits* indicate parallel mutations. *CRS*, Cambridge reference sequence

and M at least (Torroni et al. 1996, Richards et al. 1998, Macaulay et al. 1999), and 13708G>A is found in some samples belonging to haplogroup X (Macaulay et al. 1999). Nine polymorphisms appeared to be specific to haplogroup T.

A median-joining network was also constructed for the HVS-I sequence data (Fig. 1b). Haplogroup J was defined by polymorphisms at nts 16069 and 16126, and haplogroup T by polymorphisms at nts 16126 and 16294, with the exception of one sample. The haplogroup T network included

parallel mutations at nts 16172, 16182, 16183, 16186, 16189, 16304, and 16363, and one reticulation. Comparison of the HVS-I network with the coding region network suggested that the polymorphisms creating the reticulation in sample 18 had arisen in the order 16296 → 16189 rather than the reverse. In the case of haplogroup T, the HVS-I network correlated well with the network based on the coding region sequence, with the exception of sample 23, which was discrepant between the two networks. On the other hand, parallel mutations at nts 16145, 16172, and 16261 had to be assumed in order to obtain concordant networks for haplogroup J.

Expansion of the 9-bp tandem repeat in the *CO II-tRNA^{Lys}* intergenic region

An autogenous formation of heteroduplexes in a fragment spanning between the nts 8100 and 8437 was observed in two samples belonging to haplogroup J, but not in 478 other samples suggesting haplogroup-specificity. A heteroplasmic mutation within the fragment would explain the heteroduplex formation and, therefore, these two samples were studied further in order to obtain complete sequence data. PCR amplified fragments were subcloned and sequence data were obtained for 50 (sample 6) and 39 (sample 8) clones. All clones were found to harbor 8269G>A and 8286T>C excluding contamination. Furthermore, the *CO II-tRNA^{Lys}* intergenic region was found to harbor two heteroplasmic mutations. The degree of the 8288T>C mutant heteroplasmy was 12% in sample 6 and 2% in sample 8. The second heteroplasmic mutation was a length variation in the 3′-copy of the 9-bp tandem repeat (Table 1). The repeat was expanded by 1 to 4 cytosines in sample 6 and by 2 to 6 cytosines in sample 8. Together with 8288T>C this length variation gave rise to cytosine tracts consisting of 8–11 and 9–13 cytosines, respectively. The modal number of cytosine residues was 8 in sample 6, while the corresponding value was 12 in sample 8 (Table 1).

A control experiment revealed that the length variation in the *CO II-tRNA^{Lys}* intergenic region was not generated as a cloning artifact. A fragment with a sequence identical to the Cambridge reference sequence was subcloned and the colonies were passaged three times. No length polymorphisms were found in either repeats of the 9-bp segment when 30 colonies were examined at each passage.

Discussion

Comparison of the HVS-I network constructed here for mtDNA haplogroup TJ with similar networks based on a large number of European samples (Richards et al. 1998, Macaulay et al. 1999) revealed a good concordance, suggesting that the haplotypes found in the Finnish population are similar to those in other European populations. Therefore, the present network may be used for the comparison of sequences from any European population. The proposed subcluster T1 (Richards et al. 1998) was found to be defined by the coding region variant 9899T>C and could be enlarged to include samples with 12633C>A. We suggest that the remaining part of haplogroup T may be regarded as forming subcluster T2, defined by coding region polymorphisms 11812A>G and 14233A>G. Such a subdivision into subclusters T1 and T2 is also supported by a previous network (Macaulay et al. 1999).

The haplogroup J network based on sequence variation in the coding region could be divided into two subclusters that conformed to the subdivision proposed previously (Torroni et al. 1997). Accordingly, 7476C>T and 15257G>A were found to define subcluster J2, whereas subcluster J1, which has been defined by the lack of 15257G>A (Torroni et al. 1997), was found to be characterized by the polymorphism 3010G>A. A major part of this subcluster was further determined by 14798T>C in the cytochrome b gene.

CSGE analysis suggested the presence of a heteroplasmic mutation in samples 6 and 8 that belonged to haplogroup J. The *CO II-tRNA^{Lys}* intergenic region was found to harbor two heteroplasmic mutations, one being 8288T>C and the other being a length variation in the 3′-

Table 1. Sequence of the *CO II-tRNA^{Lys}* intergenic region in mtDNA between nts 8269 and 8294 in clones from samples 6 and 8

		8	8	8	8
	Frequency	2	2	2	2
	of clones	6	8	8	9
	(%)	9	0	8	4
CRS		G C A C C C C C T C T A C C C C C		T C T A G A G C C	
Sample 6	10	A C A C C C C C T C T A C C C C C		C C C A G A G C C	
	70	A C A C C C C C T C T A C C C C C C		C C T A G A G C C	
	14	A C A C C C C C T C T A C C C C C C C		C C T A G A G C C	
	2	A C A C C C C C T C T A C C C C C C		C C C A G A G C C	
	4	A C A C C C C C T C T A C C C C C C C C C		C C T A G A G C C	
Sample 8	8	A C A C C C C C T C T A C C C C C C C		C C T A G A G C C	
	13	A C A C C C C C T C T A C C C C C C C C		C C T A G A G C C	
	28	A C A C C C C C T C T A C C C C C C C C C		C C T A G A G C C	
	2	A C A C C C C C T C T A C C C C C C C C C		C C C A G A G C C	
	44	A C A C C C C C T C T A C C C C C C C C C C		C C T A G A G C C	
	5	A C A C C C C C T C T A C C C C C C C C C C C		C C C T A G A G C C	

CRS, Cambridge reference sequence; nts, nucleotides

copy of the 9-bp tandem repeat (Table 1). The length variation was not generated as a cloning artifact, as our results did not reveal any new length variation in clones passaged several times. Furthermore, the length variation was observed exclusively in the 3′-copy of the tandem repeat, and none of the clones revealed instability in the 5′-copy. These analyses enabled us to obtain complete sequence data also for these two subjects.

Phylogenetic analysis revealed that samples 6 and 8 belonged to a specific subcluster of haplogroup J and that sample 8 departed from a common node by substitutions at nt 2158 in the coding region and at the frequently mutated site 16186 in the control region, while sample 6 departed from the node by a substitution at nt 16247 in the control region. These differences suggest that haplotype 8 is genetically more distant than haplotype 6 from their common ancestor and, interestingly, the expansion of the 3′-copy was also larger in sample 8 than in sample 6. This finding suggests that the length variation in the 9-bp repeat sequence in the *CO II-tRNA*^{Lys} intergenic region is polymorphic in nature. Support for this assumption has been provided by demonstration of similar expansions in members of two tribes in India (Watkins et al. 1999). Interestingly, two samples from the Khonda Dora tribe have been found to harbor an expansion in the 3′-copy of the tandem repeat and the 8288T>C (Watkins et al. 1999), thus being identical to our samples 6 and 8. On the other hand, samples from the Santal tribe harbored an expansion in the 5′-copy but not in the 3′-copy of the 9-bp repeat (Watkins et al. 1999).

An association has been reported between haplogroup J and the primary LHON mutations 11778G>A and 14484T>C (Brown et al. 1997, Lamminen et al. 1997, Torroni et al. 1997), suggesting that polymorphisms in this haplogroup increase the penetrance of the mutations or the risk of disease expression. The relative risk of LHON appears to be highest among those belonging to subcluster J1 (Torroni et al. 1997). Six of the substitutions that have been considered to be secondary mutations in LHON were found to be scattered in the haplogroup TJ network, but only two of them, 4216T>C and 13708G>A, were common to the 31 LHON patients belonging to haplogroup J and harboring 11778G>A or 14484T>C (Brown et al. 1995, Howell et al. 1995, Lamminen et al. 1997, Torroni et al. 1997) (Fig. 2).

In addition, the polymorphisms 10398A>G and 15452C>A were common to the patients with LHON. The nt 10398 is a variable one (Macaulay et al. 1999), and therefore was not considered further. The transversion 15452C>A leads to replacement of a moderately conserved leucine by isoleucine in cytochrome b and 4216T>C to replacement of a poorly conserved tyrosine by histidine in the *ND1* subunit. Prediction of the protein domains by means of the Profile fed neural network systems from HeiDelberg (PHD) program (Rost et al. 1995) suggested that both replacements are located in a putative transmembrane region. The transition 13708G>A leads to the replacement of a moderately conserved alanine by threonine in the *ND5* subunit. This creates a novel phosphorylation site KRLT for cyclic adenosine monophosphate

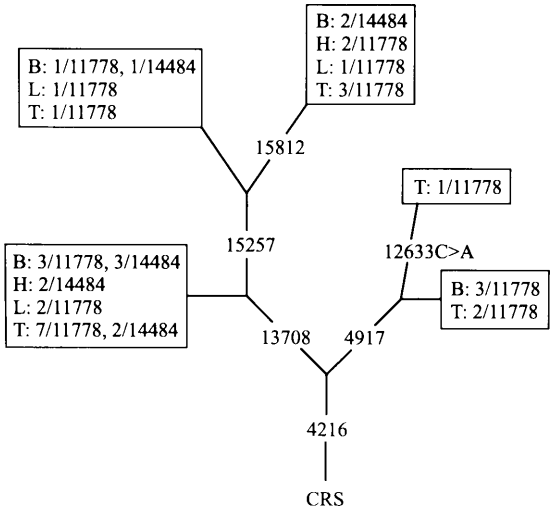

Fig. 2. Mitochondrial DNA genotypes of patients with Leber's hereditary optic neuropathy and with one of the primary mutations 11778G>A or 14484T>C, placed in the network skeleton. The original publications are indicated by the letters *B* (Brown et al. 1995), *H* (Howell et al. 1995), *L* (Lamminen et al. 1997), and *T* (Torroni et al. 1997). The number of patients is shown before the mutation. As only partial mtDNA sequences and restriction fragment length polymorphism (RFLP) data on the coding region are available for these patients (Brown et al. 1995, Howell et al. 1995, Lamminen et al. 1997, Torroni et al. 1997), their genotypes are placed in a network skeleton rather than in the complete network

(cAMP)-dependent protein kinase (Bairoch et al. 1997), which is known to phosphorylate a number of mitochondrial proteins (Papa et al. 1999). The substitutions 3010G>A and 14798T>C associated with subcluster J1 were not considered secondary mutations, because they have been detected in population controls belonging to haplogroups other than J (our unpublished observations).

We found only four substitutions to be common to patients with primary LHON mutations, but none of these was unique to haplogroup J. If increased penetrance of the 11778G>A and 14484T>C mutations in patients belonging to haplogroup J is assumed, then combinations of ancient substitutions must be implicated. Complete mtDNA sequences from patients with LHON will be required in order to clarify the role of putative secondary mutations any further.

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References

Bairoch A, Bucher P, Hofmann K (1997) The prosite database, its status in 1997. Nucleic Acids Res 25:217–221

Bandelt HJ, Forster P, Sykes BC, Richards MB (1995) Mitochondrial portraits of human populations using median networks. Genetics 141:743–753

Brown MD, Wallace DC (1994) Molecular basis of mitochondrial DNA disease. J Bioenerg Biomembr 26:273–289

Brown MD, Voljavec AS, Lott MT, Torroni A, Yang CC, Wallace DC (1992) Mitochondrial DNA complex I and III mutations 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 and 14484 mutations on an mtDNA lineage. Am J Hum Genet 60:381–387

Finnilä S, Hassinen IE, Ala-Kokko L, Majamaa K (2000) Phylogenetic network of the mtDNA haplogroup U in northern Finland based on sequence analysis of the complete coding region by conformation-sensitive gel electrophoresis. Am J Hum Genet 66:1017–1026

Howell N, Kubacka I, Xu M, McCullough DA (1991) Leber hereditary optic neuropathy: involvement of the mitochondrial *ND1* gene and evidence for an intragenic suppressor mutation. Am J Hum Genet 48:935–942

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, Vilkki 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

Johns DR, Neufeld MJ, Park RD (1992) An *ND-6* mitochondrial DNA mutation associated with Leber hereditary optic neuropathy. Biochem Biophys Res Commun 187:1551–1557

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

Macaulay V, Richards M, Hickey E, Vega E, Cruciani F, Guida V, Scozzari R, Bonné-Tamir B, Sykes B, 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

Papa S, Sardanelli AM, Scacco S, Technikova-Dobrova Z (1999) cAMP-dependent protein kinase and phosphoproteins in mammalian mitochondria. An extension of the cAMP-mediated intracellular signal transduction. FEBS Lett 444:245–249

Richards MB, Macaulay VA, Bandelt HJ, Sykes BC (1998) Phylogeography of mitochondrial DNA in Western Europe. Ann Hum Genet 62:241–260

Röhl A (1997) Network 2.0: a program package for calculating phylogenetic networks. Mathematisches Seminar, University of Hamburg, Hamburg, Germany

Rost B, Casadio R, Fariselli P, Sander C (1995) Transmembrane helices predicted at 95% accuracy. Protein Sci 4:521–533

Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R, Obinu D, Savontaus ML, Wallace DC (1996) Classification of European mtDNAs from an analysis of three European populations. Genetics 144:1835–1850

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 (1997) 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 60:1107–1121

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

Watkins WS, Bamshad M, Dixon ME, Bhaskara Rao B, Naidu JM, Reddy PG, Prasad BVR, Das PK, Perry PC, Gai PB, Bhanu A, Kusuma YS, Lum JK, Fischer P, Jorde LB (1999) Multiple origins of the mtDNA 9-bp deletion in populations of South India. Am J Phys Anthropol 109:147–158

Werle E, Schneider C, Renner M, Volker M, Fiehn W (1994) Convenient single-step, one tube purification of PCR products for direct sequencing. Nucleic Acids Res 22:4354–4355