

Spectrum of Pathogenic mtDNA Mutations in Leber's Hereditary Optic Neuropathy Families from Siberia

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Abstract—The results of clinical, genealogical and molecular investigation of eighteen families with Leber's hereditary optic neuropathy (LHON), identified on the territory of Siberia during the period from 1997 to 2005, are presented. Comprehensive analysis of mitochondrial genome variations in probands and their matrilineal relatives revealed the presence of relatively frequent (*G11778A*, *G3460A*, and *T14484C*), as well as rare and new mutations with the established or presumptive pathological effect (*T10663C*, *G3535A*, *C4640A*, and *A14619G*). The *G11778A* mutation was detected in nine pedigrees (50%), mostly in the families of ethnic Russians. In eight of these families *G11778A* was found in preferred association with the coding-region substitutions, typical of western Eurasian mtDNA lineage (haplogroup) TJ. On the contrary, the *G3460A* mutation was detected in the three families belonging to the indigenous Siberian populations (Tuvians, Altaians, and Buryats). It was associated with clearly different haplotypes of eastern Eurasian haplogroups, C3, D5, and D8. Unexpectedly, the *G3460A* de novo mutation was found in a large Tuvian pedigree. At the same time, in eleven out of fourteen families of Caucasoid origin pathogenic mutations in the ND genes were associated with the *T4216C* and *C15445A* coding-region mutations, marking the root motif of haplogroup TJ. It is suggested that phylogenetically ancient mutations could have provided their carriers with the adaptive advantages upon the development of Central and Northern Europe at the end of the last glaciation (10 000 to 9000 years ago), thereby, contributing to the preservation of weekly pathogenic LHON mutations, appearing at specific genetic background.

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INTRODUCTION

Leber's optic neuropathy (LHON, OMIM 535000) is the most common mitochondrial disease inherited along the maternal lineage. It classically presents acute or subacute disease with an age at onset of 16 to 40 years and simultaneous or subsequent loss of central vision due to degeneration of optical nerve along with focal degeneration of the retinal ganglion cell layer. The disease etiology is based on the mutations within the mtDNA coding region, which lead to biochemical defects in the mitochondria [1–4]. The probability of the disease occurrence and its clinical picture depends on the genotype, environment, and some other factors, especially heteroplasmy (percentage of the mutant and wild-type mtDNA molecules), determining the threshold of the mutation events expression in the tissues. The proportion of the affected males varies from pedigree to pedigree, constituting 50%, on average, while in women this value rarely exceeds 10% [5–7]. Usually,

all LHON mutations are concentrated within the ND genes (*ND1-6*, and *ND4L*), coding for 7 out of 42 complex I subunits of the mitochondrial respiratory chain (NADH dehydrogenase). Since the remaining 35 subunits are controlled by the nuclear genes, incomplete penetrance and gender bias are thought to be most probably associated with the segregation and incomplete inactivation of the nuclear genes linked to X chromosome [1, 8–10].

After the first description of the association between the *G11778A* mutation and the optical nerve neuropathy by Wallace *et al.* in 1988 [11], more than 30 new LHON mutations have been identified [12–16]. The three of these, *G11778A/ND4*, *G3460A/ND1*, and *T14484C/ND6*, prevail with the overall frequency of 95% in Europe, Asia, Australia, and North America [3, 15, 17–20]. These mutations are usually referred to as primary or basic, due to their undoubted pathogenic effect and the distribution in different continents. At the same time, increasing attention has been drawn to the

data indicating that some substitutions in the coding regions, found in the populations with variable frequencies, modify the phenotypic expression of the basic mutation [21]. Recently, the suggestion that the *G11778A* and *T14484C* mutations are manifested at an earlier age, or in relatively more severe clinical variants, if they are associated with the haplogroup J nucleotide variants (one of the nine western European mtDNA haplogroups [22, 23]), has received statistical support [24].

However, the mutation spectrum of Leber's hereditary optic neuropathy/atrophy in Russia remains poorly known. In 1997, we have initiated the joint project on the territory of Siberia, which provided first identification of six LHON families, where two main mutations (*G11778A* and *G3460A*) and three new ones, *G3635A*, *C4640A*, and *T10663C*, were identified [13, 14]. In the present study, the data on clinical, genealogical, and molecular screening of the twelve new LHON pedigrees are presented together with the earlier published data, which were extended during the current investigation.

MATERIALS AND METHODS

Detailed clinical examination of the probands with typical LHON symptoms, as well as of their clinically healthy relatives was carried out in the conditions of ophthalmologic hospital or out-patient clinic. In addition, to finish work with the pedigrees, and to collect blood samples from the families living in distant districts, expeditions were organized. Informed consent for participation in the research project was obtained in each case. Arranging of the pedigrees was accompanied by the elucidation of the ethnic history of the family down maternal and paternal lineages. The age of the disease onset varied greatly, from 10 to 42 years, both in males and females. The main contingent, however, was presented by young adults (16 to 28 years). Among clinical manifestations, asymptomatic, simultaneous or subsequent (with the interval of one to two months) bilateral loss of vision and the formation of central, or paracentral scotoma, should be mentioned. Fundus examination at the acute stage of the disease showed peripapillary microangiopathy, hyperemia, and diffused edges of the optic disk. At atrophic stage, 6 to 12 months after the disease onset, pallor of the whole disk or of its temporal part was observed [25]. General clinical investigation sometimes revealed psychoneurological defects and conducting system of heart abnormalities both in probands and their matrilineal relatives. Clinical manifestation and the disease progression patterns were in general similar to those described in the majority of the LHON cases, confirmed by molecular studies [6, 26–28].

For DNA analysis, 5 to 6-ml blood samples were taken from the ulnar vein. Genomic DNA was extracted from the thrombocytic-leukocytic suspension using the standard method (QIAamp Blood kit, Qiagen). At the first stage, in search of most frequent pathogenic

mtDNA mutations (*G11778A*, *G3460A*, and *T14484C*) standard PCR analysis was used. Identification of rare mutations, as well as determination of the mtDNA haplotypes was performed via complete sequencing of the samples including those obtained from previously untyped probands [13]. The mtDNA samples from all matrilineal pedigree members available were subjected to partial sequencing (HVS-I region) in combination with extended restriction analysis. For these purposes, double-stranded PCR products were fluorescent labeled using BigDye Terminator chemistry (ABI/Perkin-Elmer Cetus) with subsequent separation of the products using ABI Prism 3100 automated sequencer. The data obtained were analyzed using SEQUENCHER software package (version 4.2, GeneCode Corp). The list of restriction endonucleases and primers used for complete mtDNA sequencing and RFLP analysis was published earlier [29, 30]. Attribution of a haplotype to a phyletic lineage was determined based on the total number of specific polymorphic substitutions in the coding and control regions, according to the permanently renewing classification [31–34].

Heteroplasmy level was evaluated using denaturing high performance liquid chromatography (DHPLC) according to recommendations of the manufacturer (Wave Nucleic Acid Fragment Analysis System, Transgenomic).

RESULTS

Pedigrees containing the highest numbers of patients and their matrilineal relatives are demonstrated in Fig. 1. As expected [8, 17, 26, 35], males are affected 2.6 times more often than females (Table 1).

The exception was one pedigree (L26, the *G11778A* mutation), where clinical symptoms of the disease were observed in three males and seven females (0.4 : 1). Another distinguishing feature of this pedigree was high penetrance (56%): among 18 matrilineal relatives (four generations) 10 affected individuals were observed. The cases with atypical gender distribution along with the increased proportion of affected offspring, especially daughters, born by the women with clinical symptoms of LHON were already reported earlier [8, 36, 37]. Taking into consideration the data on preferable association of asthenozoospermia with haplogroup T2 [38, 39], increased concentration of infertile marriages in the pedigree (L01) carrying pathogenic mutation *G11778A*, deserves special attention. The unique finding was a large pedigree of Russian emigrants to Western Siberia (L30) with new mutation *G3635A*, which leads to the replacement of highly conservative serine by asparagine at amino acid position 110 of the ND1 subunit.

Taken together, pathogenic mtDNA mutations were found in 76 LHON patients and in 66 of their clinically healthy matrilineal relatives, where blood samples for the mtDNA analysis were available. Molecular analysis

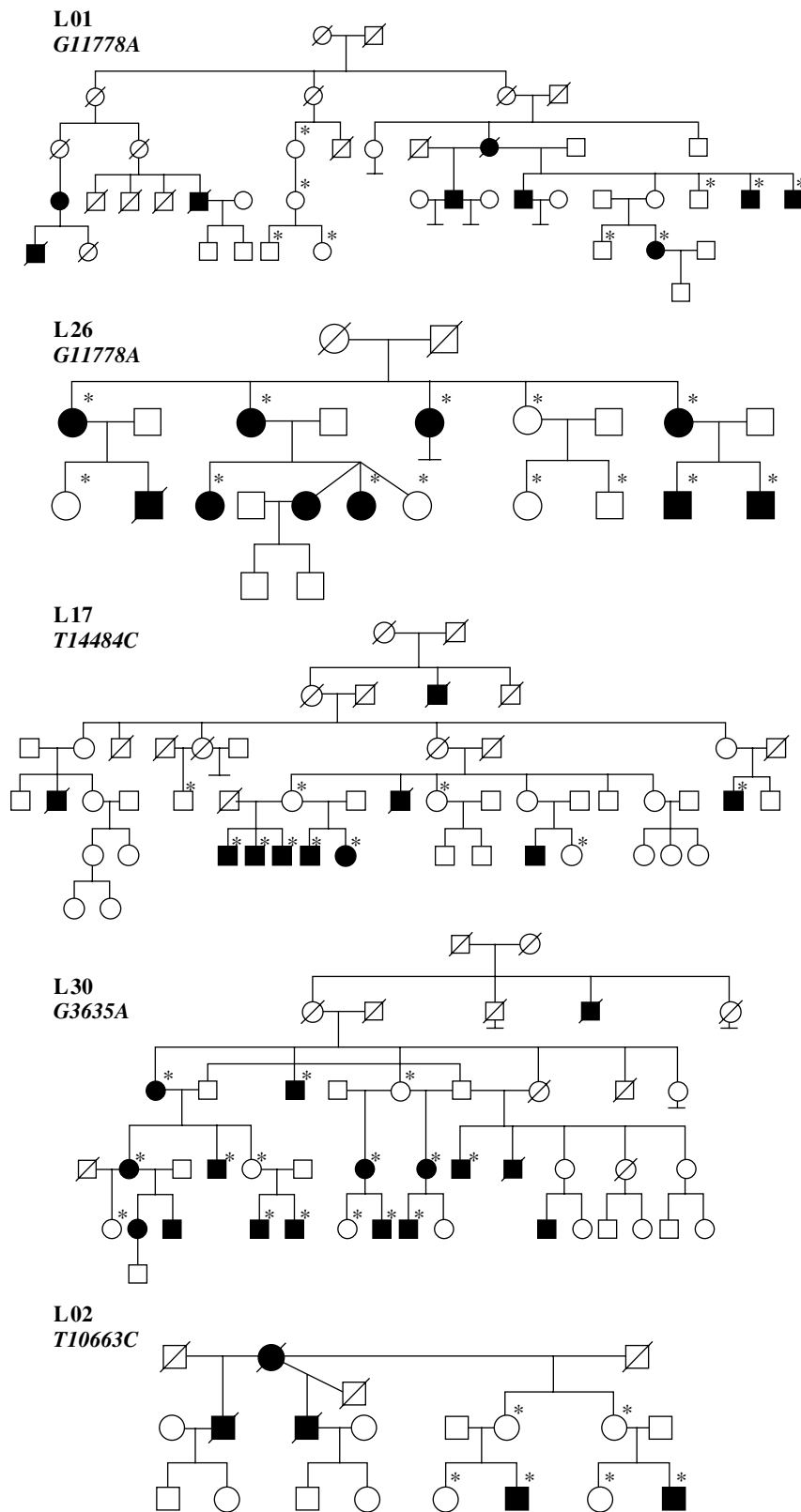


Fig. 1. Pedigrees of the LHON patients carrying the mutations *G11778A* (L01 and L26), *T14484C* (L17), *G3635A* (L30), and *T10663C* (L02). * The presence of mutation in the family members examined.

Table 1. Gender distribution of the LHON patients

Mutation	No. of families	No. of patients		Gender ratio
		males	females	
<i>G3460A</i>	3	8	3	2.7 : 1
<i>G11778A</i>	9	25	14	1.8 : 1
<i>T14484C</i>	2	13	1	13.0 : 1
<i>T10663C</i>	1	4	1	4.0 : 1
<i>G3635A</i>	1	11	5	2.2 : 1
<i>C4640A</i>	1	4	1	4.0 : 1
<i>A14619G</i>	1	2	0	–
Total	18	67	25	2.6 : 1

of mitochondrial genomes of the probands showed the presence of the already known, as well as some new mutations in the ND genes coding for complex I subunits of the mitochondrial respiratory chain, namely, *G11778A*, *G3460A*, *T14484C*, *G3635A*, *T10663C*, *C4640A*, and *A14619G* (Table 2). It was demonstrated that in eight out of nine families the prevalent mutation *G11778A* was associated with the polymorphic coding-region substitutions, typical of western European haplogroups J1, J2, and T2. On the contrary, the second prevalent pathogenic mutation, *G3460A*, was found exclusively in the families of the indigenous populations of Southern Siberia, Tuvians, Altaians, and Buryats. It was expressed at the background of one of eastern Eurasian haplogroups, C3, D5, or D8. Judging by the heteroplasmy level, mutation *G3460A*, found in the Tuvian pedigree (L24) (Fig. 2), was restricted to the germline cells, originating from one of the nine sisters of the second generation. This means that *G3460A* is a de novo mutation. Identity of the control region sequences (HVS-I) in all members of this large pedigree confirms their descent from one ancestor mtDNA. These are the reasons to exclude acquisition of *G3460A* from possible adopted children and their subsequent transmission down the mtDNA lineage.

Interesting finding, similarly to that reported in Southeast Asia [40], was a pedigree (L10), in which mutation *T144484C* was associated with haplogroup M9. In addition, in one of the families a youth with clinical symptoms of Leber's optic neuropathy and carrying the *A14619G/ND6* mutation, resulting in the replacement of phenylalanine for leucine, was detected. Although mutation *A14619G* is not described in literature and is absent from the known databases [41], before the establishment of the biochemical defect in the complex I respiratory chain, it should be considered as a candidate [42].

DISCUSSION

Analysis of the mtDNA mutation spectrum is of crucial importance for Leber's hereditary optic neuropathy diagnostics, since it provides differentiation of LHON from the other ophthalmologic and neurodegenerative disorders [1, 6, 27]. Furthermore, application of the method of complete sequencing of mtDNA extends the possibilities of identification of rare and new mutations, associated with Leber's disease [15, 28]. Indeed, in the total sample of pedigrees currently distinguished in Southwestern Siberia ([13, 14, 43], and the present study) a total of five (21%) new mutations (*G3635A/ND1*, *C4640A/ND2*, *T10663C/ND4L*, *A14619G/ND6*, and *G14279A/ND6*), associated with clinical manifestation of LHON were described.

At the same time, the data on the LHON epidemiology are important not only for differential diagnostics and the genetic counselling. Leber's hereditary optic neuropathy represents a natural model suitable for investigating the role of selection on phylogenetically old and young mutations in the ND genes [21]. In addition, mutations *G11778A* and *T14484C*, if they happen in the individuals of the Caucasoid origin, are not randomly distributed along the phylogenetic tree, but display a tendency to preferable association with the coding-region polymorphic variants, namely, *T4216C* in the *ND1* gene and *G13708A* in the *ND5* gene, marking haplogroup J [22–24, 44]. It should be noted that monophyletic cluster TJ belongs to the group of western Eurasian mtDNA clusters, which at different times (and, probably, on different territories) diverged from supercluster N after the outlet of the modern humans from Africa [31, 33, 45].

It should be noted that both frequent and rare pathogenic and candidate mtDNA mutations found in 11 out of 14 Russian pedigrees of the Caucasoid origin cor-

Table 2. Spectrum of mtDNA mutations in case of Leber's hereditary optic neuropathy

Pedigree	Gene	Mutation	Heteroplasm	Amino acid substitution	Conservatism level	Haplotype of the proband		Haplogroup	Ethnicity of the maternal lineage
						coding region	control region, HVS-I (minus 16 000)		
L18	<i>ND1</i>	<i>G3460A</i>	-	A52T	C	5178A 8414 8701 10398 10400 12705 15106	223 362	D8	Altaians
L24	"	"	+	"	"	8584 8701 10398 10400 12705 13263 14318 15080	093 223 228 288 298 327 390 519	C3	Tuvinians
L25	"	"	+	"	"	5178A 5301 8701 10397 10398 10400 12026 12705	172 182ë 183ë 189 223 266 362	D5	Buryats
L30	"	<i>G3635A</i>	-	S110N	B	4216 5633 7476 8551 10398 13708 15257 15452A 15812	069 126 193 286 319	J2	Russians
L31	<i>ND2</i>	<i>C4640A</i>	-	I57M	C	1811 11467 12308 12372 14139 15454	343 362	U3	"
L02	<i>ND4L</i>	<i>T10663C</i>	-	V65A	H	3010 4216 10398 12083G 13708 14798 15452A	069 126	J1	"
L01	<i>ND4</i>	<i>G11778A</i>	+	R340H	B	1888 4216 4917 6261 10463 15452A 15928	126 294 296 304 519	T2	"
L03	"	"	-	"	"	1888 4216 8875 10463 12373 13708 15452A 15928	126 294 519	T2	"
L05	"	"	-	"	"	3010 4216 10398 13708 14798 15452A	069 126 147 242	J1	"
L12	"	"	-	"	"	4216 5633 7476 9921 10398 13708 15257 15452A 15812	069 126 193 278	J2	"
L14	"	"	-	"	"	1888 4216 4917 10463 15452A 15928	126 294 296 304 519	T2	"
L23	"	"	-	"	"	4216 7476 10398 13708 15257 15452A 15812	069 129 193	J2	"
L26	"	"	-	"	"	3010 4216 10398 13708 15452A	069 092 126 261	J1	"
L28	"	"	-	"	"	1888 3338 4216 4917 10463 15452A 15468 15924 15928	126 147 294 296 297 304 390 519	t2	"
L27	"	"	-	"	"	1438 2706 4769 7028 8843 13708	519	H2	"
L10	<i>ND6</i>	<i>T14484C</i>	-	M64V	H	3394 4491 8701 10398 10400 14417	223 234 316 362	M9	Altaians
L17	"	"	-	"	"	3010 4216 10398 13708 13933 14798 15452A	069 126 213 519	J1	Russians
L04	"	<i>A14619G</i>	-	F19L	"	1811 4646 5999 6047 11332 12308 12372 14620 15693	294 519	U4	"

Note: The level of the amino acid sequence conservatism was conditionally designated as high (B), middle (C), and low (H). Transversions detected upon the haplotyping were designated by the letters. In the previous publications [13, 14] pedigrees L25, L30, L31, L28, L27, and L02 were designated by the single-letter codes, S, E, M, I, D, and C, respectively.

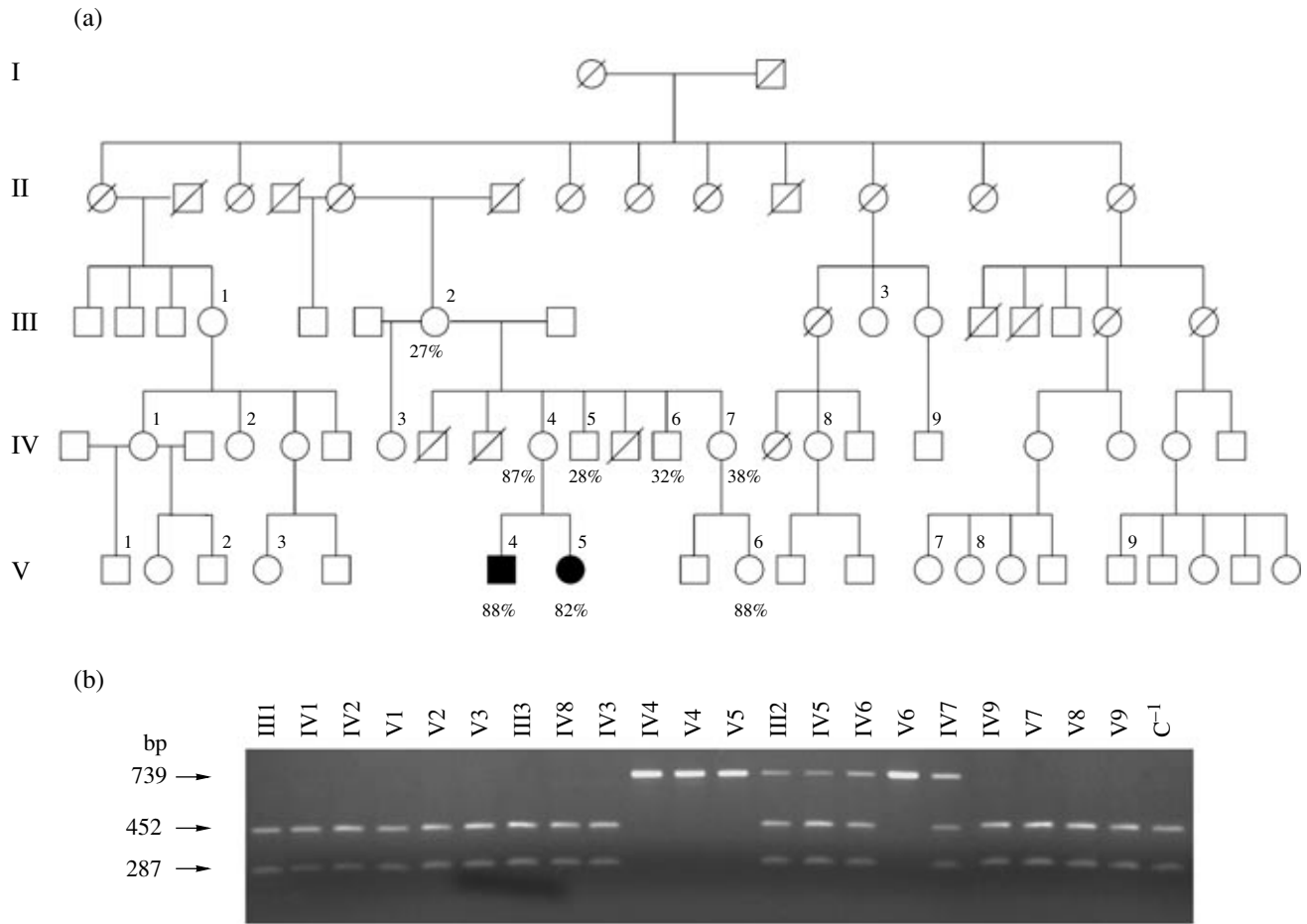


Fig. 2. A fragment of pedigree (a) carrying the *G3460A* mutation (pedigree L24); the level of heteroplasmy in mutant mtDNA determined by use of DHPLC is shown in percents; in the remaining examined pedigree members wild-type mtDNA was observed) and (b) electropherogram of the restriction products yielded after digestion of the mtDNA fragment carrying the *G3460A* mutation (the numbers of the lines correspond to the pedigree members numbers presented in (a): IV4, V4, V5, and V6, mutant genotype, homoplasmy; III2, IV5–IV7, mutant genotype, heteroplasmy; III1, III3, IV1–IV3, IV8, IV9, V1–V3, V7–V9, “wild-type” genotype; C⁻¹, negative control). The differences in heteroplasmy levels determined by use of the DHPLC method and RFLP analysis are explained in terms of lower sensitivity of the RFLP analysis.

minated with two coding-region mutations, *T4216C* and *C15452A*, marking the root motif of haplogroup TJ (Table 2). On the contrary, the detected in Asia “classic” mutations (*G11778A*, *G3460A*, and *T14484C*) are expressed independently on the background of eastern Eurasian haplogroups C, D, F, B5, and M ([20, 46, 47], and the present study). It is suggested that at the end of the last glaciation (10 000 to 9000 years ago) phylogenetically more ancient mutations could have provided their carriers with the adaptive advantages upon the development of Central and Northern Europe, and positive selection could contribute to the fixation of weakly pathogenic LHON mutations, appearing at specific genetic background [31, 38].

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