Point mutations associated with Leber hereditary optic neuropathy in a Latvian population

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Purpose: To study mutations associated with Leber hereditary optic neuropathy (LHON) in patients suspected of having this mitochondrial disorder in a Latvian population. Additional aims were to determine the heteroplasmacy status of all non-synonymous polymorphisms identified in the current study and to identify the mitochondrial haplogroups of the studied participants because these factors may contribute to the manifestation of LHON.

Methods: Twelve patients, including patients in two families, were enrolled in the current study. LHON was suspected based on the findings of ophthalmologic examinations. In clinically affected individuals, the presence of all previously reported LHON-associated mutations was assessed with sequencing analysis. Additionally, the SURVEYOR endonuclease assay was used to detect heteroplasmacy. The mitochondrial haplogroups were identified with restriction analysis and the sequencing of hyper-variable segment 1.

Results: In one family (mother and son), there was one primary LHON-associated mutation, G11778A. In addition, one rare previously reported LHON-associated polymorphism, A13637G, was detected in two unrelated patients. A non-synonymous polymorphism at T6253C was found in one individual. This mutation was reported in the background of the 3460 mutation among LHON patients in a Chinese population. No non-synonymous point mutations in mitochondrial DNA were found in five of the study participants.

Conclusions: Molecular analysis of 12 patients with suspected LHON confirmed the diagnosis in four patients and allowed the use of appropriate prophylactic measures and treatment. Further investigations and additional studies of different populations are necessary to confirm the role of the non-synonymous polymorphisms A13637G and T6253C in the manifestation of LHON and the associations of these polymorphisms with mitochondrial haplogroups and heteroplasmacy.

Leber hereditary optic neuropathy (LHON) is a mitochondrial disorder characterized by bilateral or, rarely, unilateral painless acute or subacute visual failure without a clear etiology. The incidence of LHON according to various authors varies from 1:50,000 to 1:31,000 [1,2]. The provisional diagnosis of this disease is based on ophthalmologic examinations that reveal swelling of the optic nerve head and vascular changes such as peripapillary telangiectasia, microangiopathy, and vascular tortuosity [3]. The clinical manifestations of LHON and the age of onset are highly variable. Both sexes can be affected, but the clinical symptoms of this disease most often appear in 20- to 30-year-old men [4]. Visual failure usually develops with visual blurring and impairment of the central visual field in one eye, and some months later, the same symptoms are found in the second eye. However, there have been rare cases of LHON in which the second eye remained unaffected for years [5]. This state can progress to atrophy of the optic nerve, causing blindness [6].

Approximately 90% of individuals affected by LHON have one of three point mutations in mitochondrial DNA (mtDNA): G3460A, G11778A, or T14484C [7]. Molecular analysis of individuals who do not harbor these mutations but exhibit clinical manifestations of LHON has revealed other point mutations in mtDNA. Currently, 69 non-synonymous polymorphisms in the MITOMAP database may contribute to this disease when the three strictly LHON-associated mutations are not present.

To cause phenotypic pathology, the amount of mutant mtDNA should exceed a critical threshold level, which may differ for different positions in the mitochondrial genome [8]. It is thought that heteroplasmic LHON-associated mutations in mtDNA can cause significant effects if the proportion of the mutant variant exceeds 60% [6].

When a mitochondrial disease as complex as LHON is studied, it should be kept in mind that the penetrance, phenotypic expression, and prognosis of this disorder may depend on many factors, e.g., the mtDNA haplogroup-defining polymorphisms (selectively neutral), the mutation type (e.g., patients with the 14,484 mutation have a better prognosis and a greater chance of partial vision recovery than patients...
with the 11,778 mutation), hormonal factors, environmental factors, and other factors. Moreover, the possible effects of the nuclear DNA background on the severity and penetrance of LHON have been widely discussed [2,9,10].

The epidemiological study of this rare mitochondrial disease is based on molecular analysis of the mitochondrial genome of patients suspected of having LHON based on ophthalmologic examinations. Only three common LHON-associated mutations are usually analyzed, and the rare LHON-associated polymorphisms have been comparatively poorly studied [11].

It may be of critical importance to analyze all non-synonymous polymorphisms in the mtDNA of patients with clinical manifestations of LHON in different populations to evaluate the role of these polymorphisms in the development of this mitochondrial disorder. The primary aim of this study was to detect LHON-associated mutations in patients suspected of having LHON in a Latvian population. A secondary aim was to report all new non-synonymous polymorphisms in the mitochondrial genome found in the study population. Additionally, the heteroplasmy status of each non-synonymous polymorphism was determined, and the mitochondrial haplogroups of the study participants were identified because these factors may also contribute to the manifestation of LHON.

METHODS

Patients: Twelve patients, including patients in two families, F1 and F2 (Figure 1), were enrolled in this study. In the first family, the mother and son were affected, and in the second family, visual failure was observed in only one family member (LHON-4) and not in her offspring (LHON-6 and LHON-7). The current study was approved by the Ethics Committee and complied with the Declaration of Helsinki [12]; all participants provided written informed consent. Two samples (LHON-1 and LHON-2) were obtained in 2007, and other samples were obtained in 2011–2012. Information on the health state and family history of the studied participants was collected using health and hereditary questionnaires.

Ophthalmologic examination: The ophthalmologic examinations were performed in the Ophthalmology Clinic of Pauls Stradins Clinical University Hospital, Riga. The following tests were used: visual acuity and visual field examinations, fundus photography, and optical coherence tomography (OCT). Other possible causes of visual failure were excluded, such as metabolic diseases (e.g., diabetes mellitus), toxicity (alcohol), trauma, and inflammation.

Molecular analysis of the mitochondrial genome: Whole DNA was extracted from blood leukocytes using the standard phenol chloroform method [13]. A total of 72 point mutations in mtDNA that had been previously reported to be associated with LHON according to the MITOMAP database (last update in August 2012) were analyzed. The polymorphism phenotyping (PolyPhen) and Sorting Tolerant From Intolerant (SIFT) databases were used to evaluate the interspecies conservation of these mutations and the possible or probable damaging status of each mutation (PolyPhen, SIFT). This information was considered when the results were interpreted. Twenty-two mtDNA fragments were amplified using oligonucleotide primers as described previously [14-17] (Appendix 1). PCR was performed in a final volume of 12.5 µl. The master mix contained (per tube) 1.25 µl of 10x Taq Buffer containing (NH₄)₂SO₄ (Thermo Scientific, Vilnius, Lithuania), 1.25 µl of 25 mM MgCl₂ (Thermo Scientific), 0.125 µl of dNTPs (10 mM each; Thermo Scientific), 0.5 µl of 10 µM forward primer (Metabion, Martinsried, Germany), 0.5 µl of 10 µM reverse primer (Metabion), 0.25 µl of Taq DNA Polymerase (5 U/µl; Thermo Scientific), and 7.63 µl of nuclease-free water (Thermo Scientific). To each tube was added 1 µl of 20 ng/µl DNA. The conditions of the

Figure 1. Two families (F1 and F2) involved in the current study.
PCR were as follows: one cycle at 95 °C for 5 min; 30 cycles of 95 °C for 30 s, primer annealing for 30 s (temperatures are given in Appendix 1) and 72 °C for 30 s (for long fragments, 13 and 15, the elongation time was 2 min); and a final elongation at 72 °C for 5 min. Each fragment was evaluated using an agarose gel stained with 10 mg/ml ethidium bromide and then was purified using the ExoI and FastAP enzymes (Thermo Scientific). The fragments were sequenced in both directions using the forward and reverse primers and a 3130×l Genetic Analyzer (Applied Biosystems, Carlsbad, CA) according to the manufacturer’s protocol. For PCR fragments 13 and 15, long reads were used, and the capillary length was changed from 50 cm to 80 cm.

Analysis of mitochondrial DNA haplogroups: The mtDNA haplogroups of the participants were determined by sequencing hypervariable segment I (HVS-I) and with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis [15].

Detection of mtDNA heteroplasy using the SURVEYOR assay: Because sequencing is not sufficiently sensitive to detect heteroplasm (the limit of detection is approximately 20% [18,19]), the SURVEYOR mismatch endonuclease assay was used. The limit of detection for heteroplasm by this method is 3% [14]. The mismatch endonuclease is able to digest a DNA heteroduplex on the 3′-side of the mismatch site formed by heating and slow cooling if heteroplasm is present in the studied mtDNA fragment. All PCR products were treated using the SURVEYOR mismatch endonuclease and analyzed with 4% polyacrylamide gel electrophoresis with staining with ethidium bromide. The SURVEYOR Mutation Detection Kit (Transgenomic, San Jose, CA) was used in this study. The reactions were performed according to the manufacturer’s protocols and Bannwarth’s method of detection of heteroplasmic mutations in a whole mtDNA using SURVEYOR mismatch endonuclease [14].

RESULTS

Five affected men, five affected women, and two unaffected women participated in the study. The average age of onset was 38.5 years. The results of the ophthalmologic examinations and molecular analysis are summarized in Table 1 and Table 2, respectively.

Analysis of individual cases:

First family: Mother (LHON-1) and son (LHON-2)—In the first family (F1), mother and son had significant visual impairment. The clinical manifestation was more severe in the son (LHON-2) than in his mother (LHON-1). A significant decrease in visual acuity and central scotoma occurred in LHON-2 at the age of 21 years, but 1 year later, an ophthalmological examination revealed a decrease in the size of the central scotoma. Both individuals harbored a primary LHON-associated homoplasmic mutation in the MT-ND4 gene: G11778A (p.Arg340His).

Patient LHON-3—The family history was not available for this patient. At the age of 39 years, this patient experienced significant visual loss in both eyes (visual acuity, 0.01) and

| Code of the sample | Gender | Family history of visual failure | Age of onset | Age of enrollment into the genetic study | Visual acuity OD | Visual acuity OS | Visual fields OD | Visual fields OS |
|-------------------|--------|---------------------------------|--------------|----------------------------------------|-----------------|-----------------|-----------------|-----------------|
| LHON-1            | F      | No                              | 35           |                                        | 50              | 0.06            | CS              | CS              |
| LHON-2            | M      | Yes (LHON-1; mother)            | 21           |                                        | 27              | 0.03            | CS              | CS              |
| LHON-3            | M      | NA                              | 39           |                                        | 43              | 0.01            | CS              | CS              |
| LHON-4            | F      | No                              | 40           |                                        | 55              | 0.05            | CCS             | CCS             |
| LHON-5            | M      | No                              | 53           |                                        | 54              | 0.05            | CCS             | N               |
| LHON-6            | F      | Yes (LHON-4; mother)            | -            |                                        | 24              | 0.75            | N               | N               |
| LHON-7            | F      | Yes (LHON-4; mother)            | -            |                                        | 32              | 1               | N               | N               |
| LHON-8            | F      | No                              | 30           |                                        | 49              | 0.1             | CS              | CS              |
| LHON-9            | F      | No                              | 51           |                                        | 53              | 0.4             | CCS             | CCS             |
| LHON-10           | M      | No                              | 58           |                                        | 58              | 0.1             | CCS             | CCS             |
| LHON-11           | F      | No                              | 41           |                                        | 45              | 0.3             | CCS             | CCS             |
| LHON-12           | M      | No                              | 26           |                                        | 36              | 0.2             | CCS             | N               |

Abbreviations: CS – central scotoma; CCS – cecocentral scotoma; N – normal; NA – not available; OD – oculus dexter (right eye); OS – oculus sinister (left eye).
**Table 2. Summary of molecular data on analyzed samples.**

| Code of the sample | Detected polymorphisms* | Change of amino acid | Prediction of pathogenicity | Other mtDNA coding region polymorphisms | HVS-I haplotype | Polymorphisms that determine haplogroup and RFLP restriction site | HG |
|-------------------|-------------------------|----------------------|-----------------------------|------------------------------------------|----------------|---------------------------------------------|----|
| LHON-1            | G11778A (MT-ND4)       | Arg340His            | Damaging                    | Polymorphisms that determine haplogroup and RFLP restriction site | Not checked due to discovery of the primary with LHON-associated mutation | rCRS                         | 7028C (−7025 AluI) H |
| LHON-2            | G11778A (MT-ND4)       | Arg340His            | Damaging                    | Polymorphisms that determine haplogroup and RFLP restriction site | Not checked due to discovery of the primary with LHON-associated mutation | rCRS                         | 7028C (−7025 AluI) H |
| LHON-3            | T6253C (MT-COXA1)      | Met117Thr            | Benign                      | A4769G, T5267C, G8860A, G11914A, T14953C, A15326G | 16,126-16184 | 7028C (−7025 AluI) H |
| LHON-4            | A13637G (MT-ND5)       | Gln434Arg            | Benign                      | A12308G, G12372A, T13617C, T14182C         | 16,126-16189–16325 | 7028T (+7025 AluI); A12308G (+12308) |
| LHON-5            | A13637G (MT-ND5)       | Gln434Arg            | Benign                      | T3197C, A7768G, C9477T, A11467G, T12308G, G12372A, T13617C, T14182C | 16,126-16258–16270–16292–16362 | 7028T (+7025 AluI); A12308G (+12308) |
| LHON-6            | A13637G (MT-ND5)       | Gln434Arg            | Benign                      | T3197C, A7768G, C9477T, A11467G, T12308G, G12372A, T13617C, T14182C | 16,126-16189–16325 | 7028T (+7025 AluI); A12308G (+12308) |
| LHON-7            | A13637G (MT-ND5)       | Gln434Arg            | Benign                      | T3197C, A7768G, C9477T, A11467G, T12308G, G12372A, T13617C, T14182C | 16,126-16189–16325 | 7028T (+7025 AluI); A12308G (+12308) |
| LHON-8            | A13637G (MT-ND5)       | Gln434Arg            | Benign                      | T3197C, A7768G, C9477T, A11467G, T12308G, G12372A, T13617C, T14182C | 16,126-16189–16325 | 7028T (+7025 AluI); A12308G (+12308) |
| LHON-9            | Not revealed           |                      |                             | A8860G, G9477A, G9622A, A10283G, A12612G, G12372A, T13617C, T14182C | 16,189-16270 | 7028C (−7025 AluI) H |
| LHON-10           | Not revealed           |                      |                             | A15326G                                      | 16304         | 7028C (−7025 AluI) H |
| LHON-11           | Not revealed           |                      |                             | G4580A, A4793G, G8860A, T11899C, A15326G | 16,153–1623–16298 | A4580G (−4577) NlaIII |
| LHON-12           | Not revealed           |                      |                             | T3552C, A4793G, G4924C, A11467G, A11530G, G11719A, T14110C, A15326G | 16,192–16256–16270–16304–16399 | 7028T (+7025 AluI); A12308G (+12308) |

*Non-synonymous polymorphisms that are already reported to be associated with LHON disease or novel mutations that are not associated with patient mitochondrial haplogroup; Abbreviations: rCRS – revised Cambridge Reference Sequence [34]; HG – mitochondrial haplogroup; HVS-I – Hypervariable Segment I; F – Female; M – Male; NA – not available; RFLP – Restriction Fragment Length Polymorphism.
atrophy of the optic nerve. Analysis of the mtDNA revealed one non-synonymous mutation, T6253C (p.Met117Thr), in the MT-COX1 gene, which is not noted as associated with LHON in the MITOMAP database. To assess an interspecies conservation and the possible pathogenicity of the T6253C mutation, the PolyPhen and SIFT databases were used. These databases indicated that this mutation may be benign and tolerated, respectively.

**Second family: Affected mother (LHON-4) and two unaffected daughters (LHON-6 and LHON-7):** In this family (F2), only the mother (LHON-4) exhibited clinical features of LHON, which appeared at the age of 40 years. The rare LHON-associated mutation A13637G was found in the MT-ND5 gene of this patient. Then the two daughters (LHON-6 and LHON-7) of this patient were examined ophthalmologically to assess the state of their vision. At the examination, they were 24 and 32 years old, respectively. In addition, fragment number 14 of each daughter was sequenced to determine if there was a mutation at position 13,637. The same mutation was found in both daughters even though they were phenotypically healthy at the time of the study. In the PolyPhen database, the A13637G mutation is listed as benign, and in the SIFT database, it is listed as “predict tolerated.”

**Patient LHON-5:** This male patient, who was 53 years old, had progressive visual impairment; over a period of 1 month, his visual acuity decreased from 0.3 to 0.05 in his right eye and from 1.0 to 0.75 in his left eye. The ophthalmological examination revealed edema, atrophy of the optic nerve, and cecocentral scotoma only in the right eye. Sequencing of the MT-ND5 gene revealed the presence of a secondary LHON-associated point mutation, A13637G, the same as that found in LHON-4.

**Patients LHON-8, LHON-9, LHON-10, and LHON-11:** These unrelated patients were suspected of having LHON due to significant visual impairment with an unclear etiology, but the molecular analysis of mtDNA did not confirm this diagnosis, as no LHON-associated mutations were found.

**Patient LHON-12:** The sex and age of onset of this study participant were characteristic of LHON. The patient was male and experienced significant visual impairment at the age of 26 years. This patient’s decrease in visual acuity was not as severe as that for the patients discussed above. Visual field impairment (ceccentric scotoma) occurred only in the right eye. Molecular analysis of the mtDNA did not reveal any LHON-associated mutations.

**Analysis of the mtDNA haplogroups of the studied patients:** The majority of the participants belonged to haplogroup H or U, which are abundant mtDNA genotypes in the Latvian population [20] (Table 2).

**Analysis of mtDNA heteroplasmy:** As described previously, all non-synonymous point mutations in the mtDNA found in the current study were present only in the homoplasmic state. The homoplasmic state of these polymorphisms was suggested by the analysis of the sequencing chromatograms and was confirmed using the SURVEYOR mismatch endonuclease assay (Figure 2).

**DISCUSSION**

The results of studies on the prevalence of LHON and reports of individual cases of this disease are available for some European and Asian populations [21,22]. The current study provides additional information about the distribution of LHON-associated mutations in a Latvian population.

**Prevalence of Leber hereditary optic neuropathy in European populations:** As described previously, there is the great probability of identifying of one of the most common with LHON-associated mutations (G11778A, G3460A, or 728 bp

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**Figure 2.** Detection of mitochondrial deoxyribonucleic acid heteroplasmy using SURVEYOR mismatch endonuclease on 4% polyacrylamide gel. Abbreviations: Lanes A, D, F, H, J, undigested PCR products; lanes B, E, G, I, K, digested PCR products with SURVEYOR mismatch endonuclease (Transgenomic); lane C, size marker (100 bp Ladder plus, Thermo Scientific). Lane A, a negative control for heteroplasmy (homoplasmic sample); lane B, a positive control for heteroplasmy (heteroplasmic sample). Stars indicate the location of cleavage fragments. Lanes D, E: LHON-1, 15 amplified fragment; lanes F, G: LHON-2, 15 amplified fragment; lanes H, I: LHON-3, 4 amplified fragment; lanes J, K: LHON-4, 14 amplified fragment.
T14484C) among individuals suspected of having LHON. In
the current study, the members of one family (two affected
members) with distinct clinical features of LHON harbored
the G11778A mutation. None of the studied individuals
possessed the G3460A or T14484C mutation. However,
the low prevalence of this disease in European populations
should be considered. The absence of other primary LHON-
associated mutations among the studied participants may
be due to the small number of patients enrolled in the current
study. Furthermore, the current project started recently and
represents data collected over a comparatively short period
of time. These data may be insufficient for evaluating the
prevalence of such a rare disease. However, the project is
ongoing, and all collected data may be used to develop a
LHON registry in Latvia. In Finland, which is geographically
close to Latvia, 36 families with LHON were identified over
a period of 34 years. The most common LHON-associated
mutation was G11778A (19 families with LHON). The
A3460G and C14484T mutations in the mtDNA were found
less frequently (four families and one family, respectively).
In the remaining 12 families with clinical manifestations of
LHON, none of the three primary LHON-associated muta-
tions were found [1]. Sixteen families with LHON were
identified in an epidemiological study performed in northeast
England. One of the three primary LHON-associated muta-
tions was found in each family by sequencing of the entire
mtDNA. The most common LHON-associated mutation in
this study was G11778A (60%), as observed in the Finnish
population. The G3460A mutation was found in 33% of
families with LHON, and the T14484C mutation was found
in 7% [23]. Another epidemiological study was performed in
the Netherlands, and among the 63 analyzed Dutch families
suspected of having LHON, 56 carried one of the primary
LHON-associated mutations. In 33 cases, it was the G11778A
mutation, in 11 cases, it was G3460A, and in 12 cases, it was
T14484C. Seven patients did not harbor any primary LHON-
associated mutations [24].

Haplogroup association with LHON: In some studies of
European populations, an association between LHON and
haplogroup J has been found [1,15]. Moreover, in some cases,
the clinical manifestations and penetrance of LHON have
been found to be milder in families belonging to haplogroup
H than among families belonging to haplogroup J [1,25].
Additionally, one clinical study showed that rare LHON-
associated point mutations may be deleterious or, conversely,
beneficial in the context of different mitochondrial haplogroup backgrounds [26]. It is important to consider the
distribution of the mitochondrial haplogroups in the region
under study to evaluate the mitochondrial genetic background
and its possible influence on the penetrance of LHON.

Haplogroup J is more common in the Italian population than
in the Latvian population (14.3% versus 6.4%, respectively)
[20,27]. Haplogroup J is found in the Finnish population at
the same proportion as in the Latvian population (6.3% and
6.4%, respectively) [20,28]. Although haplogroup J is not
common in these populations, it could be overrepresented
among individuals with LHON due to the possible influ-
ence on the clinical manifestation of this disease. Based on
phylogenetic analysis, in regions where haplogroup J is not
common, there is an association between LHON and other
definitive geographic region-specific haplogroups, e.g., M7b
for the Chinese population [29]. This result may indicate that
various combinations of selectively neutral polymorphisms in
mtDNA may be associated with the penetrance of this disease.
However, the more abundant haplogroups in the region under
study should also be considered because of the possibility of
masking the association of LHON with comparatively rare
haplogroups, especially if the sample size is not large enough.
For example, haplogroups H and U are more frequent in the
Latvian population, and therefore, there is a high probability
that individuals with LHON in this region may belong to
these haplogroups instead of haplogroup J, which is rare
[20]. Published data on the association of LHON with specific
haplogroups are more abundant for the three common LHON-
associated mutations, especially for G11778A [15]. Currently,
there are insufficient data on the effect of the mitochondrial
haplogroup background on the severity and age of onset of
LHON in patients harboring the only rare LHON-associated
mutation in European populations. In the current study, the
majority of affected individuals belonged to haplogroups H
and U, and none of subjects belonged to haplogroup J; there-
fore, it is not possible to compare the penetrance and severity
of LHON between the two mtDNA phylogenetic branches:
 haplogroup H and haplogroup J.

In epidemiology studies of LHON in European popu-
lations, there were many sporadic cases of this disease and
families without primary LHON-associated mutations; these
patients harbored other non-synonymous polymorphisms in
the mitochondrial genome [1,24]. Many of these newly identi-
fied polymorphisms are included in the MITOMAP database
as predictably associated with the development of LHON. The
majority of these polymorphisms are localized in MT-ND
genes (49 (71%) out of 69 mutations), which encode the
subunits of NADH dehydrogenase, which is part of OXPHOS
(oxidative phosphorylation) respiratory chain complex I.

The previously reported rare LHON-associated muta-
tion in the MT-ND5 gene A13637G was found in two unre-
lated individuals with LHON in the current study. These
individuals belonged to the mitochondrial haplogroup U5b,
which is listed as associated with this polymorphism in the PhyloTree database. According to the PhyloTree database, the A13637G polymorphism is also associated with two Asian haplogroups: M1a3 and N1c. This association may indicate that this polymorphism arose in different populations due to independent mutational events [30]. In addition, the A13637G polymorphism was found in one Chinese family that harbored the primary LHON-associated mutation G3460A. This family belonged to the haplogroup M10a1, which has not been shown to be associated with the A13637G polymorphism according to the PhyloTree database [31].

In the study of LHON in a Chinese population, the non-synonymous polymorphism T6253C was also observed [29]. This same polymorphism was found in patient LHON-3 in the current study. According to the MITOMAP database, this point mutation was previously reported to be associated only with prostate cancer [32,33]. According to the PhyloTree database, the T6253C polymorphism is also associated with various mitochondrial haplogroups: L1c2, M13’46’61, D5b1, and H15.

The PolyPhen and SIFT databases, which are widely used to predict probable deleterious effects of non-synonymous polymorphisms, did not confirm the pathogenicity of the A13637G and T6253C mutations. In light of all this information, these non-synonymous polymorphisms in the mtDNA may not be sufficient for the development of LHON. However, we could not exclude the possibility that these polymorphisms may be possible risk factors for LHON that could contribute to the clinical manifestation of this disease under certain conditions (e.g., stress, trauma) or when accompanied by other risk factors (e.g., a definitive nuclear background, excessive alcohol consumption). Further investigations and additional reports on the association between the molecular and clinical characteristics are necessary to evaluate the significance of these mutations in the development of this complex mitochondrial disease.

Conclusions: In a Latvian population, one family affected with LHON with a primary LHON-associated mutation, G11778A, was identified. Additionally, the A13637G mutation was identified in the mtDNA of two affected unrelated participants in this study, and the T6253C mutation was found in one patient. The analysis of the A13637G and T6253C mutation using the SIFT, PolyPhen data analysis software, and PhyloTree databases revealed that these polymorphisms are not conserved among species and are widely distributed among different mitochondrial haplogroups. Further investigations and additional reports are necessary to confirm the roles of these polymorphisms in the manifestation of LHON.

APPENDIX 1. PRIMER PAIRS USED FOR AMPLIFICATION AND SEQUENCING ANALYSIS IN THE CURRENT STUDY.

To access the data, click or select the words “Appendix 1.”

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