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Risk Factors Associated With Leber Hereditary Optic Neuropathy due to Rare Mutations in Mitochondrial DNA-Encoded Respiratory Complex I Subunits

Pilar Bayona-Bafaluy[1,2,3,4] | Javier Sanz-Pons[1] | Olivia Esteban[2,5] | Luca Bueno-Borghi[5] | Eduardo Ruiz-Pesini[1,2,3]

[1]Departamento de Bioquímica, Biología Molecular y Celular, Universidad de Zaragoza, Zaragoza, Spain | [2]Instituto de Investigación Sanitaria (IIS) de Aragón, Zaragoza, Spain | [3]Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid, Spain | [4]Instituto de Biocomputación y Física de Sistemas Complejos (BIFI), Universidad de Zaragoza, Zaragoza, Spain | [5]Servicio de Oftalmología, Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain

Correspondence: Eduardo Ruiz-Pesini (eduruiz@unizar.es)

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Keywords: incomplete penetrance | Leber hereditary optic neuropathy | mitochondrial deoxyribonucleic acid | rare mutation | respiratory complex I genes

ABSTRACT

An in-depth analysis of susceptibility factors modifying the penetrance of rare Leber hereditary optic neuropathy-causing mutations in respiratory complex I genes encoded in mitochondrial deoxyribonucleic acid has not been performed. To bridge this gap, we conducted a review of the literature on rare mutations associated with LHON, selected those with substantial evidence of pathogenicity, and performed an in-depth analysis of the various pedigrees. Examining the influences that modify the penetrance of the classical mutations associated with this disease may offer insights into susceptibility factors in individuals carrying the rare mutations.

1 | Introduction

Leber hereditary optic neuropathy (LHON, OMIM #535000) is a significant contributor to recorded cases of blindness. The majority of LHON cases, over 90%, are caused by one of the three classical pathogenic mutations in mitochondrial deoxyribonucleic acid (mtDNA): m.3460G>A, m.11778G>A, or m.14484T>C. These mutations occur within genes encoding subunits ND1, ND4 or ND6 of the oxidative phosphorylation (OXPHOS) respiratory complex I (CI) [1]. However, not all individuals carrying one of these mutations will develop the disease, a phenomenon known as incomplete penetrance. This highlights the involvement of additional factors in disease manifestation [2]. Studies on patients harboring these mutations have primarily defined the penetrance-modifying elements associated with this disease, including physiological, environmental, and genetic variables [1]. The remaining 10% of LHON cases are due to a variety of very rare mutations. While clinical features and some penetrance-modifying factors of these rare variants may resemble those associated with the more common LHON mutations, a comprehensive analysis of all pedigrees with these very rare mutations has not yet been undertaken. Here, we undertook this in-deep analysis.

2 | Materials and Methods

We used the combination of terms "Leber AND mutation AND mtDNA NOT review" on the PubMed website to retrieve publications concerning rare mutations in mtDNA genes for CI subunits linked to LHON. We obtained 1058 hits (August 16, 2023) and reviewed the abstracts to eliminate those

publications that were not in English, French or Spanish languages; dealt with LHON clinical aspects; were based on any of the three classical LHON mutations or on any mutation not belonging to mtDNA CI genes. This allowed us to reduce the number of items to be reviewed to 87. Subsequently, we included an additional publication from the "LHON mutations" section of Mitomap website, which was not included in our initial search. From the bibliography of these publications, we retrieved 33 additional reports, resulting in a total of 121 publications that meet the fundamental criteria for our study. As this study is based on published families, there is a potential risk of publication bias.

The population frequency of two of the classical mutations associated with LHON is relatively high, approximately 1 per 1000 individuals [3]. We have considered as probably pathological rare mutations causing LHON those genetic variants with a population frequency of ≤0.1 per 1000 individuals (Mitomap) and including some of the next pathogenicity criteria, such as reported in at least two patients with mitochondrial disease, with at least one of them suffering from LHON; absence from internal branches of a human phylogenetic tree (not defining a haplogroup); heteroplasmy and segregation with the phenotype at the individual level for heteroplasmic mutations; high evolutionary conservation of the affected amino acid as demonstrated by analysis of many protein sequences from very different organisms [4]; impact on an amino acid position previously associated with LHON; biochemical evidences from patient tissues/cells; or functional validation using cybrids or single fiber analysis, in which genetic backgrounds and environment are well controlled [5].

3 | Results

3.1 | Rare LHON Mutations

The pathogenicity criteria previously commented have allowed us to remove some of the genetic variants commonly cited as LHON associated (Table S1). Moreover, the pathogenicity predictor AlphaMissense defines most of these mutations (89%) as likely benign (AlphaMissense- May 6, 2024). As an example, m.14502T>C is considered one of the rare primary mtDNA mutations causing LHON in the Mitomap database. Nevertheless, this variant has been reported 684 times among 313 585 mtDNA sequences (0.2%) (Mitomap—September 11, 2023) and defines different mtDNA haplogroups, including M10 [6]. The prevalence of this haplogroup is 1.3% to 5.9% in the Han Chinese population [7], meaning a substantial number of individuals harbor the m.14502T>C transition, thereby raising doubts about its pathogenicity [8].

Finally, we included 42 pathogenic mutations, none of which correspond to the three classical LHON mutations (Table S2). AlphaMissense defines most of these mutations (81%) as likely pathogenic. Among these, the population frequency for 8 mutations was >0.01 but <0.05 per 1000, with cybrid analysis confirming the pathogenicity of 6. The remaining 34 mutations (81.0%) occurred at frequencies <0.01 per 1000, falling below the threshold considered as supporting evidence

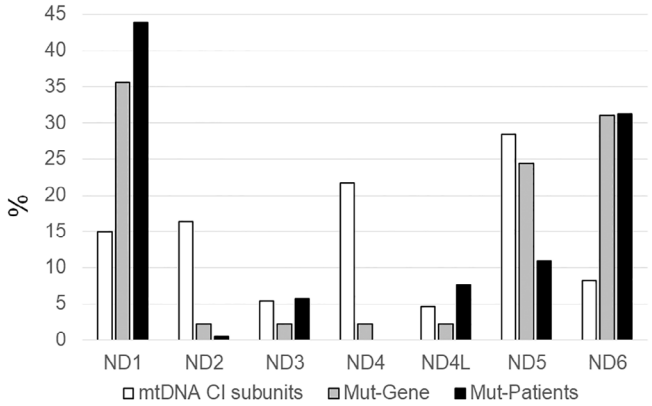

FIGURE 1 | Leber hereditary optic neuropathy mutations in mitochondrial deoxyribonucleic acid (mtDNA)-encoded genes for respiratory complex I (CI) subunits. The figure illustrates the percentage, according to its size, of each mtDNA-encoded gene coding for CI subunits (mtDNA CI subunits, white), the percentage of different mutations in each mtDNA-encoded CI gene (Mut-Gene, gray), and the percentage of patients with mutations in mtDNA-encoded genes coding for CI subunits (Mut-Patients, black).

for pathogenicity [9]. Hence, the majority of these mutations are exceptionally rare. The *MT-ND1* gene includes the highest number of mutations (15 plus m.3460G>A), followed by *MT-ND6* (13 plus m.14484T>C), and *MT-ND5* (11). Each of the other mtDNA-encoded CI genes carries only one pathogenic mutation. An analysis of the number of mutations per gene, adjusted for the proportion of mtDNA-encoded CI represented by each gene, reaffirms that *MT-ND1* and *MT-ND6* are mutational hotspots for LHON (Figure 1), consistent with previous findings [10–12].

3.2 | LHON Patients With Rare Mutations

On the other hand, demographic data were collected from 384 individuals affected by LHON (Table S3). Remarkably, eight mutations collectively account for 68.3% of all cases. As for the percentage of patients, *MT-ND1* is implicated in 44.0% of cases followed by *MT-ND6* in 31.3%. Despite the number of mutations, the *MT-ND5* gene corresponds to only 10.9% of patients, while *MT-ND4*, which includes the most prevalent classical mutation (m.11778G>A), is not mutated in this LHON patient cohort (Figure 1). Thus, mutations occurring in the antiporter-like subunits (ND2, ND4 and ND5) demonstrate infrequent association with LHON, suggesting the potential for these mutations to give rise to alternative phenotypes.

3.2.1 | Heteroplasmy

Heteroplasmy, a mixture of mutated and wild type mtDNA, occurs in approximately 10%–15% of individuals with LHON classical mutations [1]. In this cohort of LHON patients harboring rare mutations, heteroplasmy is observed in 16.7% of individuals. Notably, 8.3 and 21.7% of patients with rare mutations in *MT-ND1* and *MT-ND6* show heteroplasmy, respectively. This

frequency increases to 50.0% among those with rare mutations in *MT-ND5*. Furthermore, heteroplasmy percentages differ significantly between *MT-ND1* [56.1 ± 22.9 (9)] and *MT-ND6* [57.1 ± 25.5 (18)], in one side, and *MT-ND5* [25.1 ± 18.0 (15)], in the other ($p = 0.001$, Kruskal–Wallis test. *MT-ND5* exhibits a notable discrepancy from *MT-ND1* or *MT-ND6*, $p < 0.005$). The conservation index of amino acid positions affected by *MT-ND5* mutations [93.7 ± 9.0 (8)] is higher than that of *MT-ND1* [80.8 ± 25.2 (11)] and *MT-ND6* [35.4 ± 29.7 (11)]. In a pedigree with the rare m.13094T>C mutation, low mutation percentages are associated with LHON, whereas higher percentages lead to more severe phenotypes, such as Leigh syndrome (LS) [13]. Furthermore, a comparison between the heteroplasmy percentages of rare *MT-ND5* mutations associated with LHON [25.1 ± 18.0 (15)] and those of the same heteroplasmic mutations in LS patients [49.9 ± 20.9 (31)] (Table S4) reveals significant differences ($p = 0.0007$, Mann–Whitney U test). These finding corroborate the earlier observation suggesting that *MT-ND5* mutations are usually associated to more severe no-LHON phenotypes.

3.2.2 | Risk Factors

By definition, individuals carrying rare variants responsible for monogenic conditions will be uncommon. As we have already seen, the majority of rare mutations associated with LHON have a frequency < 0.01 per 1000. Even fewer individuals share risk modifiers capable of elucidating the incomplete penetrance observed in these rare mutations [8]. Consequently, identification of risk modifiers for monogenic conditions remains a challenge.

3.2.3 | Sex

Sex and age of onset are two variables frequently reported in articles on patients with rare LHON mutations. Among the cases analyzed, the gender distribution was 254 males and 126 females. If we add the numbers of males and females with LHON due to classical mutations in one study of numerous populations worldwide, except Australia (Males, 1067; Females, 341), to those exclusively of Australian population (Males, 430; Females, 120), we obtain 1497 males and 461 females. This results in a collective ratio of 3.3:1 [14, 15]. This ratio is significantly higher than the ratio observed in our study for rare mutations (2.02:1; $p = 0.0001$, Fisher exact test). The 380 LHON patients with rare mutations represent 16.3% of the total LHON patient population, with classical or rare mutations, a proportion very similar to the commonly quoted figure of 10%.

3.2.4 | Age

For males, symptom onset dramatically peaks during the second decade (accounting for 63.6% of male cases) (Figure 2). For females, age of onset is more equally distributed across all ages, also similar to the classical mutations [14], but a peak was also observed during the second decade (accounting for 35.2% of female cases). The mean age at onset for females is significantly later than that for males [30.2 ± 18.0 (71) vs. 19.7 ± 9.1 (165), $p = 0.0001$, unpaired *t*-test], and the median age at onset was 27 years for females and 17 for males, a little lower than the 30 and 20, respectively, for the LHON classical mutations [14]. Conversion to the affected state, similar to classic mutations [14, 15], is lower in girls ≤ 10 years old with rare mutations. A 7.9% of males (13/165) and 7.0% of females (5/71) lost vision age ≤ 10 years old. Conversion to the affected state is higher in women with rare mutations > 50 years. 1.2% of males (2/165) and 12.7% of females (9/71) lost vision age > 50 years old. Only 2.9% of affected individuals experienced LHON onset after 50 years of age. For classical mutations, 6.6% or 10% of affected individuals experienced LHON onset ≥ 50 [15] or > 50 years of age [14]. A 5.4% of males (14/259) and 11.8% of females (7/59) lost vision age 50 years or over [15].

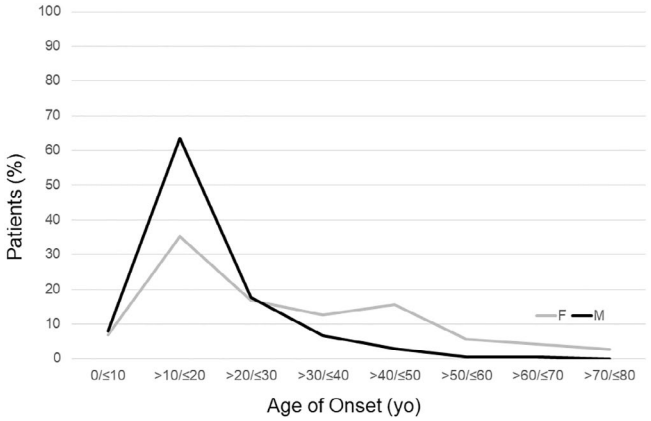

FIGURE 2 | Age of onset (yo, years old) of patients with rare mutations in mtDNA-encoded genes for respiratory complex I subunits causing Leber hereditary optic neuropathy. F, female; M, male.

3.2.5 | Environment

On the other hand, although gene–environment interactions, such as toxic exposure, are likely to be widespread, they are often extremely hard to prove. The comprehensive and systematic collection of an individual's environment is nearly unfeasible, and detailed relevant exposure data are seldom available alongside genetic data [8]. In our patient cohort, environmental exposures were only reported in 32 individuals of 384 (8.3%) and not in great detail. However, certain findings suggest that these toxics, in particular smoking, can contribute to the conversion to LHON. For instance, in the case of the rare mutation m.3734A>G, there is a non-statistically significant excess of affected smokers (14/33—42.4%) compared to affected non-smokers (12/44—27.3%) [16].

3.2.6 | mtDNA Copy Number

The mtDNA content in blood samples from healthy carriers of classical LHON mutations is higher than in affected subjects [17]. Consequently, mtDNA copy number could play a crucial role in determining disease conversion among mutation carriers. In fact, numerous other risk factors influence mtDNA levels [18]. Unfortunately, similar to the discussion on drug exposure,

this variable is almost never reported in articles on rare LHON mutations.

3.2.7 | Blood Mutation Load

Previous research has linked the frequency of blindness in males to the load of the m.11778G>A mutation in their blood [19]. In patients with rare mutations associated with LHON, we have observed a negative correlation, although no significant ($p = 0.09$), between the percentage of heteroplasmy in blood samples, including buffy coats and leucocytes, and LHON age of onset (Figure 3). Indeed, patients with the m.14495A>G rare mutation exhibited higher levels of heteroplasmy compared to healthy carriers and those who developed LHON at a younger age showed increased heteroplasmy level. This correlation was statistically significant [20]. In addition, for another rare mutation, m.13094T>C, a rapid recovery of vision coincided with a decrease in the mutation percentage in the blood [21].

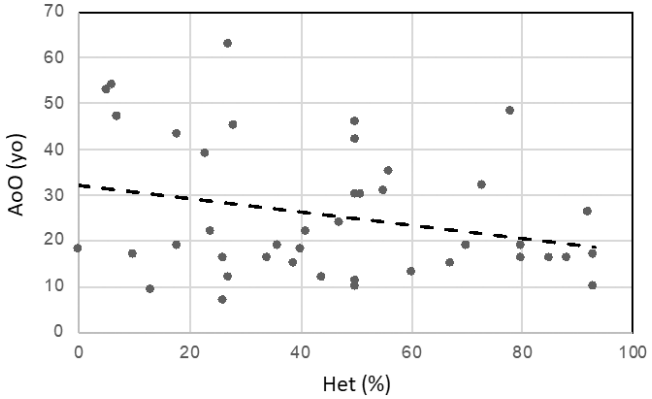

FIGURE 3 | Correlation between the age of onset (AoO) and the percentage of heteroplasmy (het) for all rare LHON-associated variants. Yo, years old.

3.2.8 | mtDNA Genetic Background

The limited number of pedigrees available for each mutation (a mean of 3.1) presents a significant challenge in studying mtDNA genetic factors that modify phenotype. The risk of visual failure associated with LHON classical mutations is greater when they are found within specific mtDNA haplogroups, groups of phylogenetically related mtDNA genotypes. For example, Western Eurasian haplogroup J increases the penetrance of m.11778G>A and m.14484T>C mutations [22], while the Asian haplogroup M7b1′2 increases penetrance for m.11778G>A [23]. Interestingly, a Western Eurasian haplogroup has been reported in 127 individuals from 45 pedigrees within this collection of LHON patients with rare mutations, with haplogroup J comprising 26.7% of pedigrees (Figure 4). Haplogroup J frequencies range from 3% to 15% in European populations [22]. Conversely, Asia-American haplogroups has been reported in 138 individuals from 36 pedigrees within this collection of patients, with a frequency of M7b of 19.4% (Figure 4). In Asia, M7b frequency is reported to be 7.0% [24]. Thus, the same haplogroups that seem to increase susceptibility to LHON in individuals with classical mutations also appear to heighten the risk of developing the disease in patients with rare mutations.

3.2.9 | Nuclear DNA Modifiers

The general lack of extensive pedigrees containing multiple affected individuals is a significant obstacle to identifying genetic modifiers in nuclear DNA for LHON attributed to rare mutations. However, it is plausible that phenotype-modifying genetic factors influencing LHON classic mutations may exert similar effects on LHON rare mutations. Notably, a modifying genetic factor has been described, which enhances the penetrance of the LHON classical mutation m.11778G>A. This factor consisted of a c.572G>T substitution, p.Gly191Val, in the mitochondrial tyrosyl-tRNA synthetase (*YARS2*) gene [25]. Interestingly, this modifying factor has also been found to increase the penetrance of the LHON rare mutation m.3635G>A [26].

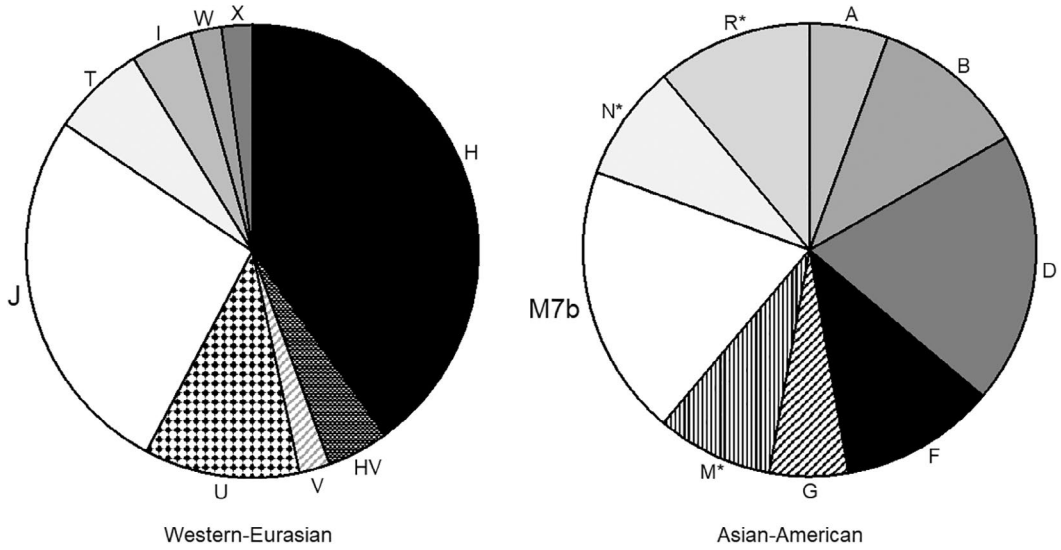

FIGURE 4 | Fraction of Western-Eurasian and Asian-American haplogroups among patients with rare mutations in mtDNA-encoded genes for respiratory complex I subunits causing Leber hereditary optic neuropathy.

4 | Discussion

Many mutations have been published as etiologic agents of LHON. However, for many of them, and particularly for many of the rare ones, there is insufficient evidence of pathogenicity. Moreover, mutations associated with LHON show incomplete penetrance. This makes assigning pathogenicity to LHON-associated genetic variants quite challenging, requiring special considerations [27]. Thus, the process of variant classification is still more art than science [28], and additional evidence will be required to confirm as etiologic factors all the mutations proposed herein as LHON rare mutations.

Rare LHON-causing mutations, so far not analyzed as a whole, appear to affect functionally more important amino acids than classical mutations, as they are much less frequent and affect generally more evolutionarily conserved positions, which could indicate greater negative natural selection; they tend to cause phenotypes at slightly younger ages; they are less sex-dependent for phenotypic manifestation; and the frequency of heteroplasmy is also slightly higher.

On the other hand, the rest of the susceptibility factors appear to be very similar to those of the classical mutations and this suggests that identifying risk factors that modify the penetrance of classical LHON mutations, which are more common, will facilitate their subsequent analysis in pedigrees featuring rare LHON mutations. Our observations also suggest that, although the m.11778G>A mutation in the ND4 subunit is the most frequent mutation in LHON, mutations in anticarrier-type subunits are less frequently associated with LHON and generally cause more severe phenotypes. These facts will have an impact on better genetic counseling for carriers of these mutations.

Author Contributions

All authors contributed to the study conception and design, material preparation, data collection and analysis. The first draft of the manuscript was written by Eduardo Ruiz-Pesini and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the supporting information of this article.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/cge.14683.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.