ONLINE MUTATION REPORT

Frequency of rare mitochondrial DNA mutations in patients with suspected Leber's hereditary optic neuropathy

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Leber's hereditary optic neuropathy (LHON (MIM 535000)) characteristically presents with subacute painless bilateral visual failure in young adults, with a predilection for males.[1 2] In the largest multicentre study of white people with LHON,[3] 97% of those affected were found to harbour one of three "primary" mitochondrial DNA (mtDNA) point mutations affecting genes that code for different subunits of complex I (NADH-ubiquinone oxidoreductase, or ND) of the respiratory chain, G11778A, G3460A, and T14484C, which affect the ND4,[4] ND1,[5 6] and ND6,[7] subunits respectively. This work had profound implications for the clinical investigation of patients with suspected LHON, indicating that a simple molecular genetic blood test would confirm the diagnosis in 19 out of 20 cases, and that a negative result reduces the likelihood of LHON to less than 1 in 20.

In the original multicentre study of Mackey *et al*,[3] cases were carefully selected to avoid the inclusion of pedigrees with autosomal dominant or autosomal recessive optic atrophy. The authors only analysed pedigrees where there were at least two affected people related through an unaffected woman, and most of the pedigrees spanned several generations displaying strict maternal inheritance.[3] Although this approach enriched their cohort for definite cases of LHON, it reduced the likelihood of including small pedigrees, which account for up to a third of genetically confirmed cases of LHON.[8] Several additional mtDNA sequence variants have been described in patients with LHON over the past 10 years. Some of these sequence changes are also found in healthy controls at a lower frequency than in cases of LHON,[9–11] and the role of these "secondary mutations" has yet to be established.[12] By contrast, some sequence changes have only been found in families with LHON and are likely to be primary pathogenic mtDNA mutations.[13–22] These new mutations often occur in small pedigrees that would not have been included in the original study.[3] This raises the possibility that rare primary LHON mtDNA mutations are more common than was previously thought.

To consider this issue it is necessary to carry out a population based genetic epidemiological study of LHON, looking for novel rare LHON mutations in patients not harbouring the G11778A, G3460A, or T14484C mutations. We recently carried out a rigorous population based genetic epidemiology study of LHON in a population of 2 173 800 people in the north east of England.[23] This established LHON as one of the most common inherited eye diseases. In this region, at least 1 in 14 067 males develop visual failure owing to the G11778A, G3460A, and T14484C mtDNA mutations, which were found in about 1 in 8500 of the general population. While carrying out this study we identified a cohort of patients with suspected LHON who did not harbour the G11778A, G3460A, or T14484C mutations. Here we report the results of further investigations carried out on these patients. This was done with three aims: (1) to determine the incidence of rare LHON mutations in the general population; (2) to determine the relative frequency of rare LHON mutations when compared with G11778A, G3460A, or T14484C; and (3) to determine the role of extensive mtDNA sequencing when investigating suspected LHON.

Key points

- Most patients with Leber hereditary optic neuropathy (LHON) harbour one of three mitochondrial DNA (mtDNA) point mutations, G11778A, G3460A, and T14484C, but the frequency of rare LHON mtDNA point mutations is not known
- We carried out mtDNA sequencing in 10 sporadic patients with suspected LHON who did not harbour G11778A, G3460A, or T14484C. We did not identify any rare or de novo mtDNA mutations.
- In our population based cohort of LHON, the G11778A, G3460A, and T14484C mutations were found in 94% of patients with LHON. Molecular genetic testing for G11778A, G3460A, and T14484C will confirm the diagnosis of LHON in 19/20 cases, and the yield of extensive mtDNA sequencing is low.

METHODS

The north east government office region of England is a stable, largely white population of 2 173 800 children and adults below 65 years of age for the mid-year period of 1998.[24] This region is served by a centralised genetics service based in Newcastle upon Tyne. Patients with suspected LHON in the northern region of England have been referred to the Mitochondrial Genetic Service in Newcastle by ophthalmologists, neurologists, and geneticists for over 10 years. All affected people were assessed clinically by an ophthalmologist or neurologist who documented subacute visual failure, and excluded structural, metabolic, toxic, and inflammatory causes before DNA analysis. We identified 37 cases of suspected LHON between 1997 and 2003 where the G11778A, G3460A, or T14484C mutations had been excluded by standard molecular genetic analysis.[23] Ten of these cases were selected for further investigation at random by laboratory scientists unaware of the clinical data. In all 10 cases the optic neuropathy was unexplained and LHON was considered to be the most likely clinical diagnosis. All were sporadic cases.

All of the established primary pathogenic mtDNA mutations that have been described to date were found in mtDNA complex I (*ND*) genes, with the exception of one mutation in

Abbreviations: LHON, Leber's hereditary optic neuropathy; mtDNA, mitochondrial DNA; ND, NADH-ubiquinone oxidoreductase or complex I; PCR, polymerase chain reaction; rCRS, revised Cambridge reference sequence

Table 1 Sequence analysis of suspected cases of LHON not harbouring one of the three primary pathogenic LHON mtDNA mutations (G11778A, T14484C, or G3460A)

Patient No	Changes from reference sequence
1	C3107del; T4216C; A4769G; A4917G; G5147A; G9947A; **C10175T**; T10463C; A11251G; G11719A; A11812G; T12441C; G13368A; A13563G; T13743C; A14233G; C14766T; G14905A; A15326G
2	C3107del; T3197C; A4769G; A11467G; G11719A; A12308G; G12372A; T13617C; T14182C; C14766T; A15326G
3	C3107del; A4769G; **C9911T**; G13759A; A15326G
4	C3107del; A4769G; A10771G; A15326G
5	C3107del; A4769G; A13098G; T14956C; A15326G
6	C3107del; T4733C; A4769G; G13708A; **A15052G**; A15326G
7	C3107del; G4580A; A4769G; A15326G
8	C3107del; A3720G; A4769G; A5390G; T5426C; A10876G; A11467G; G11719A; A12308G; G12618A; G12372A; T13020C; T13734C; C14766T; A15326G
9	C3107del; T3197C; A4769G; T5495C; A11467G; G11719A; A12308G; G12372A; A12612G; T13617C; C14766T; A14793G; A15218G; A15326G
10	C3107del; T4216C; A4769G; A10398G; A11251G; G11719A; G13708A; G14323A; C14766T; T14798C; **C15199T**; A15326G

Bold, unique changes not previously reported. All of these changes were synonymous.

the mtDNA cytochrome b gene (G15257A). We therefore sequenced these mtDNA genes for the 10 patients with suspected LHON. Thirteen regions of mtDNA were amplified by polymerase chain reaction (PCR) using M13 forward and M13 reverse tagged primer pairs.[25] Products of the PCR were purified (Qiagen, Germany) and sequenced bidirectionally on an ABI 377 DNA sequencer by the standard dideoxy chain termination procedure, using Big-Dye terminator kits (Perkin-Elmer, UK). The raw sequence data were compared with the revised Cambridge reference sequence (rCRS),[25] using Factura (Ver 1.2) and Sequence Navigator (Ver 1.0) softwares (Perkin Elmer, UK). Sequence variants were compared with our own database of 66 complete mitochondrial genomes, and known polymorphisms on the Mitomap database (http://www.mitomap.org/), and 646 human mtDNA sequences held by the University of Uppsala, Sweeden (http://www.genpat.uu.se/mtDB/index.html).

RESULTS

Sequence differences from the rCRS are shown in table 1. Most of the base changes had been previously described in controls. Four base changes had not been described previously (bold in table 1), and all of these had no effect on the corresponding amino acid sequence. Only one of the 10 patients had the characteristic polymorphisms of haplogroup J (patient 10). This would be expected by chance because 14% of the general population in the north east of England have haplogroup J mtDNA.[26]

DISCUSSION

We found no rare mtDNA mutations in this cohort of patients with suspected LHON. This supports the findings of Fauser *et al*,[20] who also concluded that detailed mtDNA sequencing is likely to have a low yield after excluding G11778A, G3460A, and T14484C, particularly when there is no family history of similarly affected people.

We previously described a novel ND6 mtDNA mutation in an LHON family living in the north east of England, and confirmed pathogenicity by identifying the same mutation in a Canadian patient on a different mtDNA background.[18] When we combine these data with our previous epidemiological study, it is possible to estimate the relative frequency of the primary and rare LHON mutations within a defined geographical region (table 2). The frequency of these groups was strikingly similar to the relative frequency reported in the original study of Mackey *et al*.[3] Although the relative frequency may differ in some populations because of a population founder effect,[27] the results of our population based epidemiological study of molecular genetically confirmed LHON support the findings of the original large multicentre study: the G11778A, G3460A, and T14484C mtDNA mutations are found in about 95% of cases, and rare mutations occur in fewer than 1 in 20 cases.

Table 2 The relative frequency of mtDNA mutations causing LHON

	Pedigrees (n (%))	
Pathogenic mtDNA mutation	North east of England	Mackey *et al*[β]
G11778A	9 (56)	110 (69)
G3460A	5 (31)	21 (13)
T14484C	1 (6)	23 (14)
All 3 "primary"	15 (94)	154 (97)
Non-primary	1 (6)	5 (3)
Total	16	159

There was no significant difference between the frequency of the different groups in the north east of England and those of Mackey *et al*[β] (χ^2=4.67, p>0.25).

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