

Prevalence and Genetics of Leber Hereditary Optic Neuropathy in the Danish Population

Thomas Rosenberg,^{1,2} Søren Nørby,² Marianne Schwartz,³ Juliette Saillard,⁴ Paulo J. Magalhães,⁵ David Leroy,⁶ Erik C. Kann,¹ and Morten Duno³

¹National Eye Clinic, Kennedy Center, Glostrup, Denmark

²Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

³Department of Clinical Genetics 4062, University Hospital Copenhagen, Copenhagen, Denmark

⁴Agence Nationale de Recherche sur le Sida (ANRS), Paris, France

⁵Department of Biomedical Sciences, University of Padua, Padua, Italy

⁶Centre d'Etude des Transformations des Activités Physiques et Sportives (CETAPS), Faculty of Sports Science, University of Rouen, Rouen, France

Correspondence: Morten Duno, Molecular Genetics Laboratory, Department of Clinical Genetics, University Hospital Copenhagen, 4062, Blegdamsvej 9, DK 2100 Copenhagen, Denmark; mdunoe@rh.dk.

Submitted: October 1, 2015

Accepted: December 17, 2015

Citation: Rosenberg T, Nørby S, Schwartz M, et al. Prevalence and genetics of Leber hereditary optic neuropathy in the Danish population. *Invest Ophthalmol Vis Sci*. 2016;57:1370–1375. DOI:10.1167/iov.15-18306

PURPOSE. In Denmark, the occurrence of Leber hereditary optic neuropathy (LHON) has continuously been monitored since 1944. We provide here a summary of 70 years of data collection including registered lines and subjects by the end of 2012.

METHODS. Affected individuals were identified from a national register of hereditary eye diseases at the National Eye Clinic (NEC), a tertiary low vision rehabilitation center for the entire Danish population. The assembling of LHON pedigrees was based on the reconstruction of published families and newly diagnosed cases from 1980 to 2012 identified in the files of NEC. Genealogic follow-up on the maternal ancestry of all affected individuals was performed to identify a possible relation to an already known maternal line. A full genotypic characterization of the nation-based LHON cohort is provided.

RESULTS. Forty different lines were identified. The number of live affected individuals with a verified mitochondrial DNA mutation was 104 on January 1, 2013, which translates to a prevalence rate of 1:54,000 in the Danish population.

CONCLUSIONS. Haplogroup distribution as well as mutational spectrum of the Danish LHON cohort do not deviate from those of other European populations. The genealogic follow-up reveals a relatively high turnover among families with approximately 15 newly affected families per century and the dying out of earlier maternal lines.

Keywords: Leber hereditary optic neuropathy, LHON, national cohort study

Leber hereditary optic neuropathy (LHON) is a rare, maternally-inherited dystrophy affecting mainly retinal ganglion cells and resulting in optic atrophy with subacute deterioration of central visual function leading to irreversible blindness in most of the affected.¹ Leber hereditary optic neuropathy is primarily caused by mutations in the mitochondrial genome affecting three subunits of respiratory complex I, nicotinamide adenine dinucleotide hydrogen-ubiquinone oxidoreductase, of the oxidative phosphorylation pathway. Three common mutations, m.3460G>A, m.11778G>A, and m.14484T>C in *MTND1*, *MTND4*, and *MTND6*, respectively, are known as the primary pathogenic mutations and account for approximately 90% of LHON patients of Caucasian origin.² A novel *MTND1* mutation, m.3395A>G, associated with the LHON phenotype has recently been identified in a Danish patient (Soldath P, written communications, 2015), and in approximately 1% of the LHON patients other mitochondrial (mt)DNA variations, primarily affecting the complex I genes, can be found. However, a stringent pathogenicity of such variants is often hard to establish.³ In a considerable fraction of clinically clear-cut patients, no causative mtDNA mutation have been found; and with an additional pronounced sex-biased penetrance, the genetic etiology of LHON remains complex.⁴ It

has been suggested that the specific haplogroup background of genetically verified LHON patients has an influence on disease manifestation,⁵ and several so-called secondary LHON-associated mtDNA variants have been claimed to play a role in penetrance and disease expression, though the significance of such variants still remains uncertain.⁶

The aim of this study is to present national prevalence data, genealogic workup, and molecular genetic data on mutations and haplogroup background of LHON patients in the Danish population of 5.6 million inhabitants.

MATERIALS AND METHODS

Patients

Patients were recruited from the National Eye Clinic (NEC) for the Visually Impaired, a tertiary national referral center. Anamnestic information on hitherto unrecognized family members was noted and followed by a request of the files from local eye departments. Supplementary information was obtained from the Department of Clinical Genetics at the University Hospital, Rigshospitalet in Copenhagen, which until



TABLE 1. Population Overview and LHON Sex- and Age-Specific Prevalence Rates in the Danish Population of 5.6 Million Inhabitants (January 1, 2013)

Age Group, y	Population (P)			LHON, n			Prevalence, P:N		
	Males, n	Females, n	Total, n	Males	Females	Total	Males	Females	Total
0-9	329,266	313,210	642,476	0	0	0	0	0	0
10-19	355,271	338,073	693,344	4	0	4	1:89,000	0	1:173,000
20-29	345,797	336,050	681,847	6	1	7	1:58,000	1:82,000	1:97,000
30-39	350,817	349,217	700,034	2	4	6	1:175,000	1:87,000	1:117,000
40-49	412,036	403,858	815,894	14	4	18	1:29,000	1:101,000	1:45,000
50-59	364,797	362,954	727,751	25	6	31	1:15,000	1:60,000	1:23,000
60-69	341,978	351,538	693,516	17	6	23	1:21,000	1:59,000	1:32,000
70-79	193,604	221,479	415,083	8	1	9	1:24,000	1:221,000	1:46,000
80-89	74,785	117,266	192,051	4	0	4	1:12,000	0	1:48,000
90-99	10,335	29,288	39,623	2	0	2	1:5,000	0	1:20,000
100+	166	843	1,009	0	0	0	0	0	0
0-100+	2,778,852	2,823,776	5,602,628	82	22	104	1:34,000	1:123,000	1:54,000

recently was the only center offering genetic analysis for LHON in Denmark.

The clinical diagnosis was based on a combination of symptoms and signs; the presence of rapid and painless visual decline in one eye, mostly to Snellen acuities between 3/60 and 1/60, followed by a similar visual loss in the fellow eye within days or weeks. Examinations in the acute phase typically showed swelling of the nerve fibre layer, a circum-papillary teleangiectic microangiopathy, and large central scotomas with normal periphery followed by paleness of the papilla during the following months.

Family history was based on patient interviews and questionnaires, and affected maternal relatives supported the clinical diagnosis. Since 1989, mutation analyses have been performed in at least one member from each line.⁷ Several members of a single large multigenerational line (line no. 706) participated in a research project with the purpose of assessing heteroplasmy for the pathogenic mutation and the association between the mutational load and the clinical phenotype.

Prevalence data were calculated based on individuals with an identified mitochondrial mutation only. Genealogic tracing along the maternal ancestry line back to at least 1800 was conducted in every newly identified person manifesting LHON. Besides the primary mutation, haplogroups and their subclades were included as a guide for the clarification of the matrilineal genealogy. Population statistics were obtained through Statistics Denmark (<https://www.dst.dk/en>, in the public domain).

Molecular Genetics

We isolated DNA from an EDTA blood sample by standard methods. Since 1989, specific assessment of the three common LHON mutations, m.3460G>A, m.11778G>A, and m.14484T>C have been performed by various methods, ranging from PCR and restriction analysis, over direct sequencing to allelic discrimination through Taqman assays. The latter have been performed since 2007. Primers and PCR conditions are available upon request. All analyses were carried out in a clinical setting, with informed consent from the patients. The study adhered to the tenets of the Declaration of Helsinki.

Comprehensive mtDNA sequencing was performed in eight individuals by standard next-generation sequencing approach on PCR products covering the mtDNA sequence,⁸ using commercial sequencing technology (IonTorrent; Thermo Fisher Scientific, Waltham, MA, USA) essentially as described in Sundaresan et al.⁹ A mean coverage of >1000× was achieved.

The haplogroup affiliations¹⁰ were based on direct sequencing of a PCR product covering HVS-1, using forward and reverse primers spanning m.15714 to 15733 and m.70 to 89, respectively. We used rCRS/NC_012920 as reference.

The level of heteroplasmy for m.3460G>A in members of line 706 (the mutational load—i.e., the percentage of mtDNA harboring the mutation), was determined by solid-phase minisequencing, essentially as described in Suomalainen and Syvanen.¹¹

RESULTS

Prevalence

By January 1, 2013, 111 persons still living had been diagnosed with LHON. In seven of these patients, no pathogenic mtDNA mutation was found. The 104 mutation-positive individuals correspond to an overall observed prevalence rate of 1:54,000 in the Danish population (Table 1).

The sex proportion was 82 males (1:34,000 male population) versus 22 females (1:123,000 female population). For males, the age at onset was distributed over 6.4 decades with a median value of 25 years and for females over 4.4 decades with a median value of 33 years (Fig. 1). Six of 82 males (7.3%) had disease onset in the first 10 years of life. The age distribution showed a maximum prevalence for both sexes in the age group 50 to 59 years, except for six males aged 80 years and more who had a higher age-specific prevalence.

A total of 40 maternal lines were represented in the cohort. In nine of these, only one live individual was recorded, additional affected members being deceased. In 15 lines, only one case of LHON has been detected (Table 2). In the present cohort, six lines had 6 to 11 affected individuals and nearly half of the 104 patients belonged to seven of the 40 maternal lines. In six of the largest genealogies, the eldest obligate carrier was born before 1850.

Mutations and Haplogroups

Seventy-eight individuals carried the common *MT-ND4* m.11778G>A mutation, while 15 and 10 harbored the *MT-ND1* m.3460G>A and *MT-ND6* m.14484T>C, respectively. In one isolated case, a novel *MT-ND1* m.3395A>G mutation was identified. The distribution among the 40 maternal lines according to mutation was 27 (67%) with *MT-ND4* mutation, 7 (18%) with *MT-ND6* mutation, 5 (13%) with the classic *MT-ND1* mutation, and 1 with the novel *MT-ND1* mutation (Table

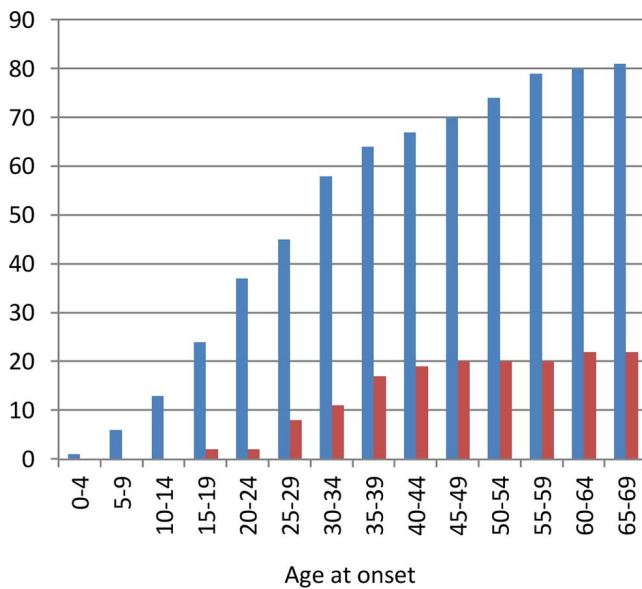


FIGURE 1. Age at onset among 104 LHON patients with known mutations. *Legend:* The age of patients at LHON onset shown as cumulated numbers (ΣN) in 5-year intervals. *Red bars, males; blue bars, females.* The median age at onset was 25 years in males and 33 years in females.

2). The latter mutation was represented by a single simplex family. Seven males (from six lines) had a typical clinical course of LHON, but no mutation was identified in mtDNA in spite of comprehensive NGS.

The mitochondrial haplogroups represented in the 40 lines were H ($n = 14$), J ($n = 8$), K ($n = 6$), T ($n = 7$), U ($n = 4$), and A2 ($n = 1$). Among the 14 lines belonging to haplogroup H, the m.11778G>A mutation was present in 11, nine of which were multigenerationally affected. Only four lines with haplogroup H represented simplex cases. Sequencing the hypervariable HVS-1 region allowed classification into subclades, which by and large confirmed the assignment to separate maternal lines, and furthermore facilitated the genealogic workup on newly diagnosed patients (Supplementary Table S1).

The results of the investigation of individuals of line 706, including the mutational load in the cases of heteroplasmy, are presented in Figure 2.

DISCUSSION

The present data were collected over a 70-year period, including the work by Ruth Lundsgaard—whose dissertation on Leber's disease appeared in 1944¹²—and later follow-up by Seedorff,¹³ who also added new maternal lines. Since 1980, a genealogic reconstruction of already known families has been carried out and together with newly diagnosed cases, integrated in a running and currently updated register of hereditary eye conditions in Denmark. The data set is characterized by a high degree of completeness regarding affected individuals and their pathogenic mutation(s), which until recently is mainly ascribed to a centralized care for visually impaired people in Denmark.

Limitations

Prior to the introduction of molecular genetic verification, an LHON diagnosis was solely based on family history and clinical criteria. Pertinent genealogic studies sometimes were able to document ancestral relations to a maternal LHON carrier

generations back without any known disease manifestation in the intervening period. Thus, genealogy was an important part of the diagnostic process. Optic neuropathy is a common cause of visual impairment among patients referred to NEC. In most instances, however, extensive neurologic assessment was carried out before referral, excluding a primary disease. Historically, LHON has been confused with dominant optic atrophy, which is prevalent in Denmark. Patients with LHON have also been wrongly diagnosed as suffering from toxic amblyopia, tobacco-alcohol amblyopia, or optic neuritis. It was important for a reliable clinical diagnosis of LHON that patients were examined from early visual loss to late stage. Since the pioneering work by Ruth Lundsgaard in the 1940s, the studies by Tove Seedorff during the 1960s, and the renewed attention from the 1980s and onward, there has been an awareness of LHON among Danish ophthalmologists. Combined with a relatively easy access to ophthalmologic service in this country, we have been able to go through the results of the clinical examinations from the early phase in all patients. Mutation analyses were performed in all patients and supported the diagnosis in 104 of 111 subjects. The remaining seven subjects were not included in the study. In conclusion, we consider the risk of false positive LHON cases as minimal. During the last 20 years, mtDNA mutation analysis is performed in otherwise unexplained cases of optic neuropathy. This has increased the fraction of solitary LHON cases including patients, which otherwise would have escaped recognition. However, it is likely that some cases were overlooked due to unawareness, atypical clinical course, erroneous diagnosis, or because of missing referral or reporting to our clinic. This implies that the prevalence calculations may be considered as minimum figures.

Prevalence

Lundsgaard's original data set comprised 101 familial cases distributed among 20 families and five clinically well-documented solitary cases, which translates to a prevalence of 1:37,000 based on the size of the Danish population in 1944 (Statistics Denmark). The updated 2013 data set approximates a considerably lower prevalence of 1:54,000. In the same period, the overall size of the Danish population rose from 4 to 5.6 million inhabitants. The number of affected individuals in the Danish population, however, was strikingly similar (106 in 1944 vs. 104 in 2013). Earlier, a familial occurrence was an important diagnostic criterion and Lundsgaard demanded a minimum of two affected members to define a LHON family. For practical reasons we considered also isolated cases where genealogic research failed to establish any connection to other cases. To a large degree, the genealogic conclusions were corroborated by haplotype analyses. In Lundsgaard's terms, our 40 maternal lines consist of 25 familial cases and 15 isolated cases. A closer look at the pedigrees shows a quite high turnover among families with approximately 15 newly affected families per century and the dying out of earlier maternal lines. Live affected descendants or known mutation carriers were present in only nine of the families described by Lundsgaard. The introduction of mutation screening in cases of optic neuropathies without known etiology has led to a genetic diagnosis in many simplex cases, which were missed before the mutational era. The latter factor explains the rise in the number of isolated cases. The increase in the number of immigrants, in whom genealogic tracking down along the female line was not possible, may have contributed to an overestimation of this number.

The number of epidemiologic studies in LHON is still limited. A Finnish study showed a nearly identical number of affected in a population size comparable to the Danish.¹⁴ In

TABLE 2. Overview of Lines With Known Mutation and Still Alive Affected Members

Line No.	Year of Birth Eldest Obligate Carrier	Generations Traced Back, <i>n</i>	Total Affected, M:F	Living Affected, <i>n</i> (M:F)	Gene	Haplogroup
701	1721	10	49:7	11 (9:2)	<i>ND4</i>	K
702	1745	9	15:1	4 (3:1)	<i>ND6</i>	J
703	1799	8	23:2	6	<i>ND4</i>	T
706	1842	7	11:5	8 (6:2)	<i>ND1</i>	K
707	Unknown	9	15:6	3 (2:1)	<i>ND1</i>	T
708	1810	6	3:1	1	<i>ND4</i>	K
710	~1750	10	14:1	4	<i>ND4</i>	H
711	1773	7	6:2	1	<i>ND4</i>	H
712	1801	8	16:2	5	<i>ND4</i>	H
725	1826	7	11:4	8 (7:1)	<i>ND4</i>	J
727	1852	5	13:1	3 (2:1)	<i>ND4</i>	H
732	1794	8	12:10	11 (7:4)	<i>ND4</i>	H
733	1855	6	6:1	6 (5:1)	<i>ND4</i>	H
736	1864	4	7:0	2	<i>ND4</i>	J
737	1877	5	4:0	3	<i>ND4</i>	H
738	~1870	5	2:0	1	<i>ND4</i>	U
739	1785	7	4:2	1	<i>ND4</i>	T
741	1925	3	1:0	1	<i>ND6</i>	H
742	1956	2	1:0	1	<i>ND4</i>	J
743	1933	3	0:2	1	<i>ND6</i>	H
745	1925	3	0:1	1	<i>ND4</i>	H
746	1928	3	1:0	1	<i>ND6</i>	K
747	1919	3	0:1	1	<i>ND6</i>	T
772	1896	3	2:0	1	<i>ND4</i>	U
773	1913	3	1:1	2 (1:1)	<i>ND4</i>	J
774	1921	4	1:0	1	<i>ND4</i>	U
778	1882	6	1:2	1	<i>ND4</i>	U
782	1933	3	1:0	1	<i>ND4</i>	H
792	1852	5	3:0	2	<i>ND4</i>	T
793	1872	5	2:1	1	<i>ND4</i>	H
796	1937	3	1:0	1	<i>ND1</i>	J
797	1874	5	0:2	1	<i>ND4</i>	H
111	1960	2	2:0	2	<i>ND1</i>	K
115	1933	2	1:0	1	<i>ND1</i>	T
138	1934	2	1:0	1	<i>ND4</i>	J
172	Unknown	2	1:0	1	<i>ND6</i>	J
182	1923	2	0:1	1	<i>ND4</i>	T
183	1963	2	0:1	1	<i>ND1*</i>	A2a
184	Unknown	2	1:0	1	<i>ND4</i>	K
213	1912	2	1:0	1	<i>ND6</i>	H

* Atypical *ND1* mutation.

contrast, a study from Northeast England found a higher prevalence of 1:31,000.¹⁵ This frequency, however, was based on the adult population younger than 65 years and consequently not directly comparable with other prevalence data. Based on an analysis of the families collected by van Senus¹⁶ in The Netherlands, a maximum prevalence, corrected for bias due to a high fraction of individuals from a m.14484T>C founder family, reached an estimate of 1:39,000.¹⁷ A meta-analysis of the LHON prevalence in Europe arrived at a frequency of 1:45,000.¹⁸ Thus, the prevalence of affected LHON patients in different European countries is of the same magnitude, and geographic differences seem mainly to be due to different representation of very large pedigrees mimicking founder effects. In this sense, it is noteworthy that almost half of the confirmed patients in Denmark represent only 18% of the maternal lines (7/40). Five of the seven lines stem from original Lundsgaard families, while a sixth is represented by a descendant of one of Lundsgaard's isolated cases.

Several genetic and environmental factors have been proposed to influence the penetrance of LHON, but the underlying factors are still poorly understood. Members of maternal line no. 706 (Fig. 2) strikingly illustrate an association between mutational load and clinical manifestation. The level of mutational load in one branch, which did not exhibit LHON, did not reach above 11%; while in another branch, with several affected individuals, homoplasmy for the mutation is found. It is clear, however, that the association is not complete, which is likely to reflect that the leukocytes of peripheral blood are not fully representative for the target cells in LHON. A case in question is the branch marked by an asterisk (Fig. 2): The individual with a 100% mutational load is clinically unaffected and has two children with mutational loads below 100%. This individual is obviously heteroplasmic in the germline and, since clinically unaffected, is likely also to be heteroplasmic in the retinal target cells. This figure also illustrates the reversibility in mutational load within a maternal line.

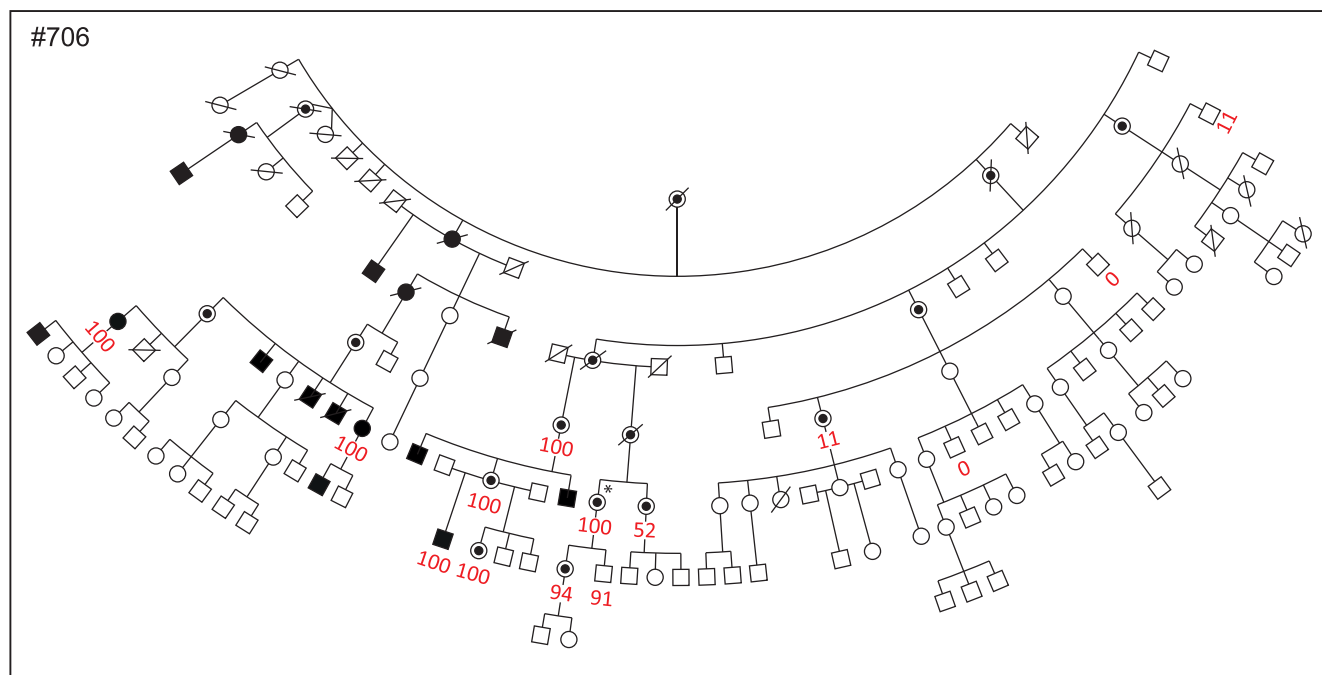


FIGURE 2. Pedigree of maternal line 706. *Legend:* This line demonstrates a striking difference in penetrance among family members in two separate branches. The percentage of mutational load in tested family members is shown in red below individual symbol. *Circles:* Females. *Squares:* Males. *Filled symbols:* Clinically affected. *Circles with dot:* Obligate and/or mutation-verified carrier state; see explanation in the Discussion.

Mutational Spectrum

The distribution of the three primary LHON mutations among the genetically verified patients did not deviate significantly from previously published distributions in Caucasians,^{2,15} although the common m.11778G>A is found at a slightly higher fraction (75% of patients) in the present material. The most frequent haplogroup among the m.11778G>A lines was H, which was found in 10 out of the 27 families. The H haplogroup background has been associated with a reduced risk of visual failure.⁵ On the other hand, the J haplogroup background has been proposed to exacerbate the severity of both m.11778G>A and m.14484T>C. However, this background was only observed in 5 of 27 of m.11778G>A families and two of seven of the m.14484T>C families, indicating that the J haplogroup does not contribute significantly to the clinical expression in the Danish population. The patient with a haplogroup A2 background is of Greenland Inuit descent and belongs to the common Inuit subhaplogroup A2a.¹⁹ The overall haplogroup distribution of the remaining 39 LHON lines shows no striking deviation from the haplogroup distribution in the general Danish population or in Europe as a whole.²⁰ At present, it seems justified to conclude that we still do not have reliable prediction tools for the risk of disease manifestation in genetic counseling.

In seven patients with a clear-cut LHON phenotype, we were unable to identify any mtDNA alteration. This is not uncommon. Achilli and colleagues³ sequenced the entire mtDNA of 174 patients highly suspected for LHON, but harboring none of the three common mutations, and only detected 16 with a possibly pathogenic mtDNA mutation.

In our search for a genetic basis of newly diagnosed cases of LHON, we have so far focused solely on mutations in mtDNA, and especially in the genes encoding subunits of complex I harboring the three primary LHON mutations worldwide. However, since the majority of complex I subunits (38 out of 45) are encoded by the nuclear genome, the existence of nuclear LHON mutations seems likely. In fact, for years

Lundsgaard's family U, consisting of two affected brothers, has appealed to us as a possible candidate family for Mendelian inheritance of LHON and lately this hunch has been substantiated by a male-to-male transmission in the following generation of an otherwise unaffected pedigree. Current methods for genome sequencing have finally made it possible to pursue the search for nuclear mutations causing Leber hereditary optic neuropathy.

Acknowledgments

Supported by grants from the Danish Society of the Blind (JS), the Portuguese National Research Council (PJM), and the Erasmus Student Mobility Program (DV), respectively, and were involved in LHON mtDNA analyses in 1996, 1991 to 1995, and 1993, respectively. Laboratory equipment was partly obtained through a research grant from the Toyota Foundation, Denmark.

Disclosure: **T. Rosenberg**, None; **S. Nørby**, None; **M. Schwartz**, None; **J. Saillard**, None; **P.J. Magalhães**, None; **D. Leroy**, None; **E.C. Kann**, None; **M. Duno**, None

References

1. Man PY, Turnbull DM, Chinnery PF. Leber hereditary optic neuropathy. *J Med Genet.* 2002;39:162-169.
2. Mackey DA, Oostra RJ, Rosenberg T, et al. Primary pathogenic mtDNA mutations in multigeneration pedigrees with Leber hereditary optic neuropathy. *Am J Hum Genet.* 1996;59:481-485.
3. Achilli A, Lommarini L, Oliveri A, et al. Rare primary mitochondrial DNA mutations and probable synergistic variants in Leber's hereditary optic neuropathy. *PLoS One.* 2012;7:e42242.
4. Meyerson C, van Stavern G, McClelland C. Leber hereditary optic neuropathy: current perspectives. *Clin Ophthalmol.* 2015;9:1165-1176.

5. Hudson G, Carelli V, Spruijt L, et al. Clinical expression of Leber hereditary optic neuropathy is affected by the mitochondrial DNA-haplogroup background. *Am J Hum Genet.* 2007;81:228-233.
6. Yu-Wai-Man P, Chinnery PF. Leber hereditary optic neuropathy. In: Pagon RA, Adam MP, Ardinger HH, eds. *GeneReviews*. Seattle, WA: University of Washington, Seattle; 2013.
7. Rosenberg T, Kann E, Nørby S. Hereditary optic nerve atrophy. A clinical-genealogical status over Danish families with Leber disease [in Danish]. *Ugeskr Laeger.* 1995;157:2707-2711.
8. Kleinle S, Wiesmann U, Superti-Furga A, et al. Detection and characterization of mitochondrial DNA rearrangements in Pearson and Kearns-Sayre syndromes by long PCR. *Hum Genet.* 1997;100:643-650.
9. Sundaresan P, Simpson DA, Sambare C, et al. Whole-mitochondrial genome sequencing in primary open-angle glaucoma using massively parallel sequencing identifies novel and known pathogenic variants. *Genet Med.* 2015;17:279-284.
10. van Oven M, Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum Mutat.* 2009;30:E386-E394.
11. Suomalainen A, Syvanen AC. Quantitative analysis of human DNA sequences by PCR and solid-phase minisequencing. *Mol Biotechnol.* 2000;15:123-131.
12. Lundsgaard R. Leber's disease. A genealogic, genetic and clinical study of 101 cases of retrobulbar optic neuritis in 20 Danish families. *Acta Ophthalmol (Copenh).* 1944;21(suppl): 1-306.
13. Seedorff T. The inheritance of Leber's disease. A genealogical follow-up study. *Acta Ophthalmol (Copenh).* 1985;63:135-145.
14. Puomila A, Hamalainen P, Kiviojs S, et al. Epidemiology and penetrance of Leber hereditary optic neuropathy in Finland. *Eur J Hum Genet.* 2007;15:1079-1089.
15. Man PY, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF. The epidemiology of Leber hereditary optic neuropathy in the North East of England. *Am J Hum Genet.* 2003;72:333-339.
16. van Senu AH. Leber's Disease in the Netherlands. *Doc Ophthalmol.* 1963;17:1-162.
17. Spruijt L, Kolbach DN, de Co RF, et al. Influence of mutation type on clinical expression of Leber hereditary optic neuropathy. *Am J Ophthalmol.* 2006;141:676-682.
18. Mascialino B, Leinonen M, Meier T. Meta-analysis of the prevalence of Leber hereditary optic neuropathy mtDNA mutations in Europe. *Eur J Ophthalmol.* 2012;22:461-465.
19. Saillard J, Forster P, Lynnerup N, Bandelt HJ, Norby S. mtDNA variation among Greenland Eskimos: the edge of the Beringian expansion. *Am J Hum Genet.* 2000;67:718-726.
20. Mikkelsen M, Sorensen E, Rasmussen EM, Morling N. Mitochondrial DNA HV1 and HV2 variation in Danes. *Forensic Sci Int Genet.* 2010;4:e87-e88.