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ORIGINAL ARTICLE

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Mitochondrial Variants in Leber's Hereditary Optic Neuropathy in Turkish Patients

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Keywords

Leber's hereditary optic neuropathy, LHON, mtDNA, mutation, Next Generation Sequencing

ABSTRACT • Leber's hereditary optic neuropathy (LHON) is the most common mitochondrial disease. There are more than 20 mtDNA mutations causing LHON. In this study we screened five LHON patients and 475 non-LHON patients suspected to have mitochondrial disorder other than LHON by Next Generation Sequencing (NGS). We found three different mutations (m.11778G>A, m.3460G>A, and m.14484T>C) in three different patients. We also found these secondary LHON mutations in 56 non-LHON patients in our cohort but there were not any primary mutations. In conclusion these secondary LHON mutations have high sensitivity but have low specificity in Turkish population.

INTRODUCTION

Leber's hereditary optic neuropathy (LHON) (MIM 535000) first described by Theodore Leber in 1871 (1, 2). LHON is the most frequent mitochondrial disorder and maternally inherited (3).

LHON is clinically characterized by mostly bilateral (sequential) subacute or acute painless loss of central vision associated with impaired color vision, and atrophy of the optic nerve, often resulting in a rapid and devastating loss of vision. LHON predominantly affects young adult males (male:female ratio of 5:1) (4). The median inter-eye delay is 6-8 weeks and the second eye is almost always affected within one year of disease onset (5, 6).

Homoplasmic m.11778G>A in the *MT-ND4,* m.3460G>A in the *MT-ND1*, or m.14484T>C in the *MT-ND6* mutations are the most common causes of the LHON (7, 8). These variants explain 80-90% of the cases. The m.11778G>A pathologic variation is the most common pathogenic mutation accounting for ~70% of all LHON cases worldwide (7, 8). These variations are defined primary mutations. Additionally there are secondary mutations have been found in LHON such as m.14482C>G, m.14482C>A, m.14568C>T, m.13708G>A, m.15257G>A, m.14831G>A and m.4216T>C (9, 10).

In this study, we screened the whole mitochondrial genome in 5 unrelated patients with LHON and 475 patients with suspected mitochondrial disorders other than LHON.

MATERIALS and METHODS

Subjects

Our study cohort consists of 5 probands with LHON and 475 probands with mitochondrial disorders

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without LHON from Turkey. The patients with a preliminary diagnosis of LHON were all referred to our center by different ophthalmologists after coming across diagnostic signs with slit-lamp examination. The other group consisting of 475 patients were suspected to have a mitochondrial disorder with clinical examination and biochemical tests but did not have eye involvement. DNA was extracted from peripheral leukocytes of each patient by by Iprep™ PureLink® gDNA Blood Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol.

DNA Sequencing

The mitochondrial genome was amplified in two large fragments by using Long PCR Enzyme Mix (Thermo-Fischer). We used Ion Xpress™ Plus Fragment Library Kit (Life Technologies, Guilford, CT, South San Francisco, CA), a ready-to-go analysis kit to be analyzed with the Ion PGM™ or Ion S5 platform (Life Technologies, Guilford, CT, South San Francisco, CA). Analysis was done by using an Ion Torrent 314 or 316 chip (Life Technologies, Guilford, CT, South San Francisco, CA). The analysis was successful with 100 % reads on target, 100× coverage of 99.99 % and 20× coverage of 99.99 %. The results were investigated with Integrative Genomics Viewer (11). The Revised Cambridge Reference Sequence was used as the reference. MITOMAP database were used to evaluate variants and their population frequency (MITOMAP).

RESULTS

In this study we found three different homoplasmic variants in mtDNA in our three patients that diagnosed with LHON (Table 1, Figure 1). We found m.4917A>G variant in the *MT-ND2* in patient M1, m.13708G>A variant in the *MT-ND5* in patient M2 and m.13708G>A and m.4216T>C variants in *MT-ND5* and *MT-ND1* in patient M3. We did not find any homoplasmic mtDNA variations in patient M4 and M5.

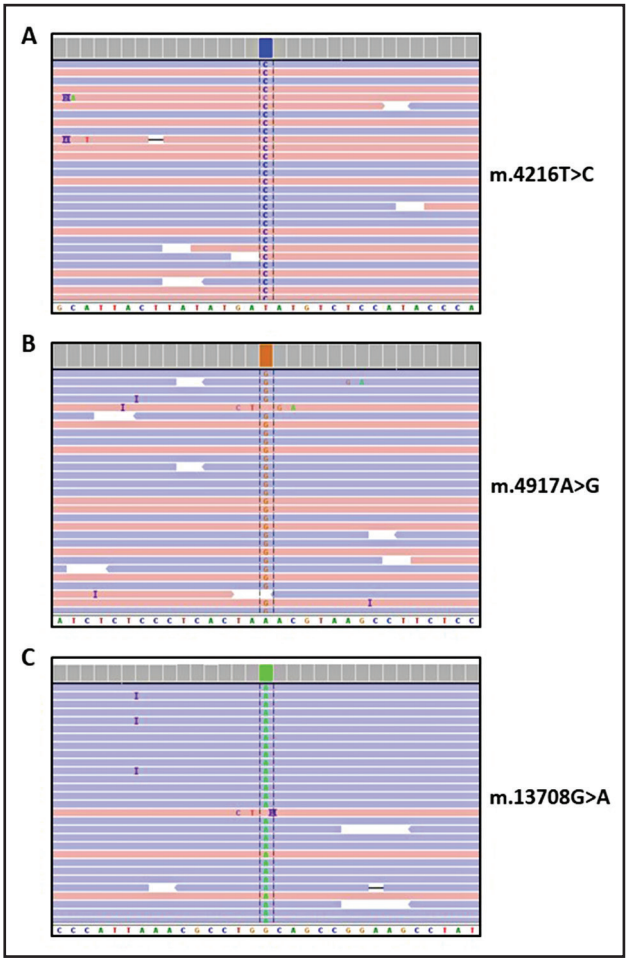

Figure 1 Detection of mutations in LHON patients. The sequence alignments were viewed using the Integrative Genomics Viewer (IGV). **(A)** m.4216T>C mutation, **(B)** m.4917A>G mutation, (C) m.13708G>A mutation in mtDNA.

Table 1 mtDNA variants in LHON patients.

Probands	mtDNA Variants	Gene
M1	m.4917A>G	MT-ND2
M2	m.13708G>A	MT-ND5
M3	m.13708G>A and m.4216T>C	MT-ND5 and MT-ND1

We also screened our mitochondrial disorder cohort that were not diagnosed with LHON for homoplasmic m.4917A>G, m.13708G>A and m.4216T>C secondary LHON variants and we found these variants in 56 different Turkish patients (11.8%) All mutations were homoplasmic (Table 2).

Table 2 The number of m.4917A>G, m.13708G>A and m.4216T>C variants in mitochondrial disorder patients.

mtDNA Variants	# of patients
m.4216T>C and m.4917A>G	25
m.4216T>C and m.13708G>A	19
m.4216T>C	8
m.13708G>A	3
m.4917A>G, m.13708G>A and m.4216T>C	1
Total # of Patients	56

DISCUSSION

All three variants (m.4917A>G, m.13708G>A and m.4216T>C) found in our patients are reported to cause LHON (Table 1). Previously m.4216T>C variant in two patients, m.4917A>G and m.4216T>C in one patient and m.13708G>A variant in two patients were reported in Turkish LHON patients (10).

Yu et al. have identified that m.13708G>A variant susceptibility allele for multiple sclerosis (MS) (12). Zonouzi et al. has showed association between MS and mtDNA encoded complex1 subunit genes (13). We found m.13708G>A variation in one LHON patient and 22 other patients that have other mitochondrial disorders. This variation varies in different populations (12).

The m.4917A>G and m.4216T>C secondary variants are commonly constransmitted. We found co-existance of m.4917A>G and m.4216T>C variants in 25 patients, only m.4216T>C variant in 8 patients where as only one patient had all m.4917A>G, m.13708G>A and m.4216T>C variants in our mitochondrial disorder cohort without LHON (Table 2). We only found one LHON patient that have m.4917A>G (patient M1). The m.4917A>G and m.4216T>C secondary variants are associated with insulin resistance, Parkinson disease, the diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) syndrome (14-16).

The most prevalent LHON mutation in Northern Europe, Austria and the Far East is m.11778G>A (17-20). The m.3460G>A and m.14484T>C are also common primary mutations in East Asian and European patients. Interestingly, we did not find the most common mutations, which cause approximately 90% of the LHON, in our LHON patients. Gurkan et al. screened six LHON patients and they found homoplasmic m.11778G>A mutation in one patient (21). The absence of the most common variations in our patients shows that these secondary three variants are more important for the Turkish population and Turkish ethnical population is unlike from the other ethnic populations.

We consider four different statements for this status that our patients have only secondary LHON variations; (1) in our study we only indicate homoplasmic variations however there are heteroplasmic variations that can affect the patients in maternally inherited disorders, (2) we performed all experiments in the blood samples and it is required to perform in different tissues, (3) genetic modifiers and mutations in nuclear DNA (nDNA) also may cause mitochondrial disorders (4) incomplete penetrance still poorly understood in mtDNA mutations in LHON.

In conclusion, we need to screen more patients with LHON to understand mechanism of mitochondrial disorders such as LHON. Furthermore, NGS studies, cell and animal models have significant insights because these new techniques obtain new solutions for finding mutations in mitochondrial disorders easily and correctly. These variations (m.4917A>G, m.13708G>A and m.4216T>C) seem to have considerable high sensitivity but also seem to have low specificity.

WEB Resources
https://www.mitomap.org/

REFERENCES

1. Fraser JA, Biousse V, Newman NJ. The neuro-ophthalmology of mitochondrial disease. Surv Ophthalmol. 2010;55:299-334.
2. Newman NJ. Hereditary optic neuropathies: from the mitochondria to the optic nerve. Am J Ophthalmol. 2005;140:517-23.
3. Leruez S, Amati-Bonneau P, Verny C, et al. Mitochondrial dysfunction affecting visual pathways. Rev Neurol (Paris). 2014;170:344-54.
4. Farrar GJ, Chadderton N, Kenna PF et al. Mitochondrial disorders: aetiologies, models systems, and candidate therapies. Trends Genet. 2013;29:488-97.
5. Yu-Wai-Man P, Votruba M, Moore AT, et al. Treatment strategies for inherited optic neuropathies: past, present and future. Eye (Lond). 2014;28:521-37.
6. Meyerson C, Van Stavern G, McClelland C. Leber hereditary optic neuropathy: current perspectives. Clin Ophthalmol. 2015;9:1165-76.
7. Yu-Wai-Man P, Griffiths PG, Brown DT, et al. The epidemiology of Leber hereditary optic neuropathy in North East of England. Am J Hum Genet. 2003;72:333-9.
8. Finsterer J, Mancuso M, Pareyson D, et al. Mitochondrial disorders of the retinal ganglion cells and the optic nerve. Mitochondrion. 2017;S1567-7249(17)30022-3.
9. Fauser S, Luberichs J, Besch D et al. Sequence analysis of the complete mitochondrial genome in patients with Leber's hereditary optic neuropathy lacking the three most common pathogenic DNA mutations. Biochem Biophys Res Commun. 2002;295:342-7.
10. Dogulu CF, Kansu T, Seyrantepe V, et al. Mitochondrial DNA analysis in the Turkish Leber's hereditary optic neuropathy population. Eye (Lond). 2001;15:183-8.
11. Robinson JT, Thorvaldsdóttir H, Winckler W et al. Integrative genomics viewer. Nat Biotechnol 2011;29:24–26
12. Yu X, Koczan D, Sulonen AM et al. mtDNA nt13708A variant increases the risk of multiple sclerosis. PLoS One. 2008;3:e1530.
13. Poursadegh Zonouzi A, Ghorbian S, Abkar M et al. Mitochondrial complex I gene variations; as a potential genetic risk factor in pathogenesis of multiple sclerosis. J Neurol Sci. 2014;345:220-3.
14. Crispim D, Canani LH, Gross JL et al. The European-specific mitochondrial cluster J/T could confer an increased risk of insulin-resistance and type 2 diabetes: an analysis of the m.4216T > C and m.4917A > G variants. Ann Hum Genet. 2006;70:488-95.
15. Ross OA, McCormack R, Maxwell LD et al. mt4216C variant in linkage with the mtDNA TJ cluster may confer a susceptibility to mitochondrial dysfunction resulting in an increased risk of Parkinson's disease in the Irish. Exp Gerontol. 2003;38:397-405.
16. Hofmann S, Bezold R, Jaksch M et al. Wolfram (DIDMOAD) syndrome and Leber hereditary optic neuropathy (LHON) are associated with distinct mitochondrial DNA haplotypes. Genomics. 1997;39:8-18.
17. Chan C, Mackey DA, Byrne E. Sporadic Leber hereditary optic neuropathy in Australia and New Zealand. Aust N Z J Ophthalmol. 1996;24:7-14.
18. Mashima Y, Yamada K, Wakakura M et al. Spectrum of pathogenic mitochondrial DNA mutations and clinical features in Japanese families with Leber's hereditary optic neuropathy. Curr Eye Res. 1998;17:403-8.
19. Yen MY, Wang AG, Chang WL et al. Leber's hereditary optic neuropathy–the spectrum of mitochondrial DNA mutations in Chinese patients. Jpn J Ophthalmol. 2002;46:45-51.
20. Yu-Wai-Man P, Griffiths PG, Chinnery PF. Mitochondrial optic neuropathies - disease mechanisms and therapeutic strategies. Prog Retin Eye Res. 2011;30:81-114.
21. Gürkan H, Ozal SA, Esgin H. Results of Mitochondrial DNA Sequence Analysis in Patients with Clinically Diagnosed Leber's Hereditary Optic Neuropathy. Balkan Med J. 2012;29:306-9.