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No evidence of association between optic neuritis and secondary LHON mtDNA mutations in patients with multiple sclerosis

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ABSTRACT

Leber's Hereditary Optic Neuropathy (LHON) shares features with Multiple Sclerosis (MS). Both diseases develop optic lesions. Frequent secondary LHON mutations in MS patients may explain the optic damage. Here, we tested the hypothesis that secondary LHON mutations are associated with optic neuritis (ON) in MS patients. We recruited 56 MS subjects with ON and 47 MS subjects without ON. DNA was extracted by salting out, after sampling of peripheral blood from each participant. We completed Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis with appropriate primers and restriction endonucleases for seven secondary LHON mutations. Products were visualized using 3% agarose gel electrophoresis with the aid of DNA safe stain in a UV transilluminator. Accuracy of the genotyping procedure was confirmed by sequencing. Data was analyzed using chi square and Fisher exact tests and logistic regression analysis. There was no significant difference between the numbers of MS subjects with ON and without ON that carried secondary LHON mutations (T4216C [$P = 0.1$], A4917G [$P = 0.2$], G13708A [$P = 0.6$], G15257A [$P = 1$], G15812A [$P = 0.8$], G15927A [$P = 1$], G15928A [$P = 0.4$]). The evidence from the present study are not consistent with the hypothesis that secondary LHON mutations are associated with ON in MS subjects.

1. Introduction

Multiple Sclerosis (MS) is a debilitating disease of the central nervous system (Roudbary et al., 2017) that gives rise to physical and cognitive impairments (Benedict et al., 2005; Ghojazadeh et al., 2014). The etiology of MS remains uncertain, but it is known to arise from inflammatory processes (Andalib et al., 2015a), as well as conventional neurodegeneration. MS plaques are found in the central nervous system (CNS), especially in optic nerve, brain stem, basal ganglia, and spinal cord. The optic nerve involvement can manifest itself as optic neuritis (ON) (Talebi et al., 2013), with partial or full loss of vision. Sixty-five percent of patients with MS experience ON at some time in the course of the disease (Sørensen et al., 1999), more commonly in women. Acute ON is the primary symptom of MS that may develop anytime during the course of the disease and may lead to bilateral chronic optic atrophy. A slight recovery in visual acuity with time may happen (Beck et al., 2004b), but permanent residual defects of color vision and contrast and brightness sensitivity are the rule (Beck et al., 2004a).

ON is confirmed by physical examination, patients' history investigation, and visual evoked potential (VEP), and magnetic resonance imaging (MRI), assessments. Optic involvement is seen in other neurodegenerative diseases, such as Leber's Hereditary Optic Neuropathy

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(LHON) that is a painless central loss of vision stemming from maternally-inherited mitochondrial DNA (mtDNA) mutations. LHON presents with a central scotoma combined with a comparatively modest loss of peripheral vision, and it eventually leads to optic atrophy (Vanopdenbosch et al., 2000). It is a rare disease affecting roughly 1 in 30,000–50,000 citizens in England and other northern European populations (Man et al., 2003; Puomila et al., 2007; Spruijt et al., 2006).

There is evidence of mitochondrial dysfunction both in MS and LHON. Mitochondria, the sites of oxidative phosphorylation, contain multiple units of circular DNA with 37 intronless genes (Andalib et al., 2015b) that express 13 subunits of the electron transfer chain, 2 ribosomal RNAs (rRNAs), and 22 transfer RNAs (tRNAs). LHON is an important example of mtDNA disease (Andalib et al., 2017b), but many other neurological disorders such as stroke (Andalib et al., 2017a), Parkinson's disease (Andalib et al., 2014), and MS (Andalib et al., 2016), are known to be associated with mtDNA changes. Activity of the mitochondrial respiratory chain complex I is reduced in LHON (Howell, 1998). Primary (high risk) LHON mtDNA mutations at nucleotide positions (nps) 3460, 11778, and 14484, affecting complex I, play a causative role in the development of LHON. The role of secondary (low risk) LHON mutations remains unknown, as they can also occur in healthy individuals (Mayr-Wohlfart et al., 1996). Mitochondria play a pathogenic role also in the axonal degeneration of MS (Dutta et al., 2006). Diminished complex I activity is present in chronic active MS lesions, with reduced activities of complexes I and III throughout non-lesional motor cortex (Dutta et al., 2006; Lu et al., 2000).

LHON may be difficult to distinguish from ON in MS (Mojon et al., 1999). Inasmuch as the symptoms of LHON are seen in MS (Lees et al., 1964), it is likely that they can occur in combination (Vanopdenbosch et al., 2000). The occurrence of ON in MS-like cases with pathogenetically significant LHON mutations and a higher incidence of maternal transmission in familial cases of MS indicate that mtDNA genes raise the susceptibility to MS (Ohlenbusch et al., 1998). In contrast to primary LHON mutations (Kalman et al., 1995), secondary LHON mutations have been reported to be associated with MS, some in connection with ON (Andalib et al., 2013a), and the secondary LHON mutation at np 4216 is associated with MS in a Bulgarian population (Mihailova et al., 2007). In German MS patients with optic involvement, secondary LHON mutations have been detected at nps 4216, 4917, 15257, 15812, 13708, 15927 and 15928 (Mayr-Wohlfart et al., 1996). A high frequency of secondary LHON mtDNA mutations was found in the American patients with MS but lack of primary LHON mtDNA mutations with primary pathogenic significance for blindness in the patients (Kalman et al., 1995). Secondary LHON mutations at nps 4216, 4917, and 13708 were seen in 24, 1, and 24% of the Caucasian MS patients, respectively, and mutations of Haplogroup J (nps 4216 and 13,708) were shown to be a risk factor for ON in MS (Reynier et al., 1999). The role of secondary LHON mutations in MS has been the topic of active research. Yet little is known about the association of these mutations with loss of vision or ON in patients with MS. Here, we tested the hypothesis that common secondary LHON mutations (Table 1) are associated with ON in MS patients.

2. Materials and methods

2.1. Study design, study size, setting and participants

The present case-control study design was approved by the Ethics Committee of Tabriz University of Medical Sciences (ECTBZMED). From therapeutic centers of Tabriz University of Medical Sciences, Tabriz, in the East Azerbaijan province of Iran, with written informed consent from each participant, we selected unrelated Azerbaijani subjects with relapsing-remitting (RR) MS according to the McDonald criteria. Subjects with a family history of neurodegenerative disorder or inherited diseases were excluded from the investigation. We obtained demographic data and confirmed the diagnosis of ON by physical examination, patients' history, and VEP and MRI assessments. We recorded the side of the involved eye and the number of ON attacks and divided the subjects into two MS groups with (case) or without (control) ON.

Table 1
Primers and restriction endonucleases utilized for assessment of the secondary LHON mutations in the MS subjects.

Mutation	MtGene	Forward primer 5′-3′	Reverse primer 3′-5′	Mutation detection
T4216C	ND1	3817-3837	4242-4262	Endonuclease *Nla*III
A4917G	ND2	4815-4835	5138-5158	Endonuclease MaeI
G13708A	ND5	13301-13321	13780-13800	Endonuclease MvaI
G15257A	$tRNA^{Thr}$	15141-15161	15580-15600	Endonuclease *Acc* I
G15812A	$tRNA^{Thr}$	15601-15621	16040-16060	Endonuclease *Rsa*I
G15927A	$tRNA^{Thr}$	15803-15821	16114-16133	Endonuclease HapII + sequencing
G15928A	$tRNA^{Thr}$	15803-15821	16114-16133	Endonuclease HapII + sequencing

2.2. MtDNA genotyping

Five ml of peripheral blood was obtained from each participant. DNA was extracted using the salting-out method (Miller et al., 1988). To confirm optimal DNA extraction, DNA quantification was carried out using DNA spectrophotometry. MtDNA was amplified by Polymerase Chain Reaction (PCR) with a previously used standard protocol (Andalib et al., 2013b; Motavallian et al., 2013), using appropriate forward and reverse primers (Table 1). PCR temperatures and cycling times were optimized prior to PCR amplification using a gradient thermocycler (PEQLAB Biotechnologie GmbH, Erlangen,Germany). We assessed 7 secondary mtDNA mutations (Table 1) that are commonly detected in MS patients in previously published studies. The PCR products were subjected to Restriction Fragment Length Polymorphism (RFLP) analysis, using appropriate endonucleases (Thermo Fisher Scientific, Waltham, MA, USA, [Table 1]). The restriction products were separated by 3% agarose gel electrophoresis with the aid of DNA safe stain. Electrophoresis gels were thereafter visualized in a UV transilluminator. Homo- and heteroplasmies were identified by detection of predefined electrophoresis band lengths in the transilluminator. Accuracy of the genotyping procedure was confirmed by sequencing of selected DNA samples. For this reason, samples were purified by the QIAquick Spin^{R} Purification Kit and sequenced by Macrogen Inc. (an online biotech company based in Seoul, South Korea) by means of an automated ABI Prism 3730XL DNA sequencer (Perkin-Elmer, Waltham, MA, USA).

2.3. Statistical analysis

Assessment of the association of secondary LHON mutations with ON in the MS subjects used the chi square and Fisher's exact tests (Stata data analysis and statistical software: version 12). Odds ratio (OR) and 95% confidence interval (95% CI) were calculated by logistic regression analysis.

3. Results

We tested the hypothesis in 56 MS subjects with, and 47 MS subjects without, ON. The demographic summary of the subjects is listed in Table 2. The ratio of women to men was significantly higher in MS subjects with ON (2.7) than in the MS subjects without ON (1.04) ($P = 0.004$) (Table 2), due to choice of inclusion. Twenty-five of the MS subjects suffered ON in both eyes, the number being higher than the number of patients presenting with ON in one eye only.

There were no statistical differences between the numbers of MS subjects with and without ON carrying the secondary LHON mtDNA