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Original article

Mitochondrial DNA G13708A variation and multiple sclerosis: Is there an association?

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ABSTRACT

Background. – Multiple sclerosis (MS) is considered a pathogenetic enigma. Recently, efforts to implicate genetics in human susceptibility to MS have identified an important role of mitochondrial DNA (mtDNA). G13708A is a common mtDNA variation associated with MS in specific populations. This study tested the hypothesis that the mtDNA G13708A variation is associated with MS in an Iranian population.

Materials and methods. – Blood samples were collected from 100 MS patients and 100 unrelated healthy controls. DNA was extracted using a salting-out method, followed by polymerase chain reaction (PCR) amplification. For assessment of restriction fragment length polymorphism (RFLP), PCR products were restricted by restriction enzyme Mva I. Thereafter, the restriction products were assessed by means of an ultraviolet (UV) transilluminator following electrophoresis with 3% agarose gel. Accuracy of the genotyping procedure was assessed by direct sequencing.

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Results. – The mtDNA G13708A variation was found in 17 cases (17%) and 19 controls (19%) ($P = 0.7$, OR: 0.8, 95% CI: 0.3–1.9).

Conclusion. – The findings of the present study fail to support the hypothesis that the G13708A mtDNA variation is associated with MS in the selected Iranian population.

1. Introduction

Multiple sclerosis (MS), a painful and potentially debilitating disease, is also the most common chronic disease of the central nervous system (CNS) in young adults. At present, MS is incurable, and there is still a long way to go for any decisive cure. The traditional neurological perspective views MS as a chronic inflammatory disease of the white matter, with demyelination as the cause of physical disability [1]. However, MS has recently been considered a neurodegenerative disease in which axonal injury, neuronal loss and atrophy of the CNS bring about permanent neurological and clinical disability [1]. Classically, MS patients present with sensory loss [2], motor [3] and autonomic (bladder, bowel and sexual) [4] dysfunctions, cerebellar symptoms, optic neuritis [5], fatigue [6], pain [7], cognitive dysfunction [8], depression [9] and fear of disease progression [10]. The diagnosis is established by clinical findings and supporting evidence from ancillary tests, including magnetic resonance imaging (MRI) [11] and lumbar puncture [12]. Evoked potential analysis [13] and optical coherence tomography (OCT) [14] can add further valuable information about MS.

While the etiology of MS has not been fully established, it is increasingly difficult to ignore the role of genetics. Siblings, parents and children of patients with MS have a higher risk of the disorder [15]. Multiple nuclear genes, such as human leukocyte antigen (HLA) classes I and II, T-cell receptor β, cytotoxic T-lymphocyte-associated protein 4 (CTLA4), intercellular adhesion molecule (ICAM)-1 and the SH2 domain containing 2A (SH2D2A) appear to be associated with MS [16]. Mitochondrial deficiency is also likely to be an important factor in the pathophysiology of MS [17], as deficient mitochondria may give rise to axonal loss, leading to MS [18]. In addition, changes in nuclear genes such as mitochondrial transcription factor A (TFAM), peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α and nuclear respiratory factor 1 (NRF1), which all contribute to the maintenance of mitochondrial DNA (mtDNA) content, are associated with MS [19,20]. In fact, mtDNA changes are likely to be causes of neurological diseases, with Leber's hereditary optic neuropathy (LHON) being an example of an mtDNA disorder. More recently, attention has focused on the association of mtDNA alterations leading to a susceptibility to other neurodegenerative diseases, such as Alzheimer's disease [21,22], Parkinson's disease [23,24] and MS [25–31]. It is known that mtDNA encodes enzymes involved in oxidative phosphorylation, transfer RNA (tRNA) and ribosomal RNA (rRNA), which are all subject to maternal inheritance, and has an unusually high rate of mutation [32]. In terms of axonal damage in MS, gene products of the mitochondrial electron transport chain are decreased in the brain, and are particularly manifested in the cerebral cortex as a diminished capacity of respiratory chain complexes I and II [33].

The G13708A variation is a secondary LHON mutation of the ND5 (NADH dehydrogenase 5) gene found in MS patients in specific populations. Yu et al. [34] showed that this variation is associated with susceptibility to MS in a European population, and Mayr-Wohlfart et al. [35], who assessed the mtDNA G13708A variation in a German population, also found that such variations may contribute to susceptibility to MS. However, another German study carried out by Hanefeld et al. [36] was unable to demonstrate such an association.

These equivocal findings heighten the need for assessments of the variation in individual populations. Therefore, the present study was designed to test the hypothesis of an association of the mtDNA G13708A variation with MS in an Iranian population.

2. Materials and methods

2.1. Study design, setting and participants

In this case–control study, to avoid confounding risk factors, relapsing-remitting MS patients were recruited from several therapeutic centers of Tabriz University of Medical Sciences. McDonald criteria were applied for the diagnosis of MS. The exclusion criterion of having a family history of neurodegenerative and inherited diseases was applied in the selection of cases, while control subjects were selected from the general population. As well as matching individuals for gender, frequency matching was done for age in cases and controls to restrict their confounding effects. Informed written consent was obtained from each participant, and the study was approved by the ethics committee of Tabriz University of Medical Sciences.

2.2. Variables and study size

Considering the sample sizes of previous published research and using STATA software (version 12), the present study sample size for a test power of 80% was determined to be 200 participants (100 cases and 100 controls [case/control ratio = 1/1]); therefore, 100 relapsing-remitting MS patients and 100 unrelated healthy controls were recruited. The confounding effect of heterogeneity of the subjects' genetic backgrounds, which could result in false-positive or false-negative results, was offset by the selection of a genetically admixed population from the province of Azerbaijan.

2.3. mtDNA genotyping

Blood samples were obtained from each subject, and mtDNA extracted by salting out [37]. Quantitation of the extracted

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DNA, indicating optimal DNA extraction, was performed by spectrophotometry. Using previously designed primers (Table 1), polymerase chain reaction (PCR) was completed according to a previously applied standard PCR protocol [38,39]. PCR temperatures and cycling times were optimized by using a gradient thermocycler (PEQLAB Biotechnologie GmbH, Erlangen, Germany). PCR products were then digested by restriction endonuclease enzyme reaction (Thermo Fisher Scientific, Waltham, MA, USA; Table 1) for restriction fragment length polymorphism (RFLP) analysis. Restriction products were assessed using electrophoresis (3% agarose gel) and visualized by Safe DNA gel staining, along with the use of QIAquick Spin^{R} purification kits (QIAGEN GmbH, Hilden, Germany). Sequencing of mtDNA was carried out of selected samples, to confirm the accuracy of mtDNA genotyping, by Macrogen Inc. (an online biotech company based in Seoul, South Korea), using an automated ABI Prism 3730xl DNA sequencer (PerkinElmer, Waltham, MA, USA).

2.4. *Statistical methods*

Data management and analyses were performed by chi-square tests using STATA (version 12.0) software, and a *P* value $\leq$0.05 was considered statistically significant. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated by means of bivariate logistic regression analysis.

3. Results

The mtDNA G13708A variation was identified in 17 of the 100 cases (17%) and in 19 of the 100 healthy controls (19%). No heteroplasmy was seen for the G13708A variation (Fig. 1). Fig. 2 displays the wild-type and variant alleles of the mtDNA G13708A, and Fig. 3 compares allelic frequencies of the mtDNA G13708A variation between the case and control groups. Chi-square analysis revealed no significant association between MS and G13708A variation (*P* = 0.7), while the bivariate logistic regression analysis yielded an OR of 0.8.

4. Discussion

In recent years, much attention has focused on the role of mtDNA variations in the pathology of MS, and G13708A is a frequently studied mtDNA variation in MS patients in specific populations. Our present study tested the hypothesis that this variation is associated with MS in an Iranian population, and the findings showed that the mtDNA G13708A variation is present in 17% and 19% of patient and control cases, respectively, with no significant association between an MS

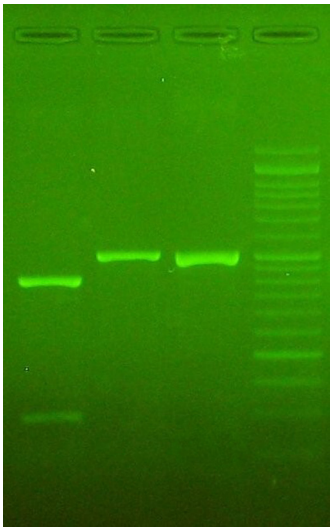

Fig. 1 – Electrophoresis of polymerase chain reaction (PCR) and restriction products of the mitochondrial DNA (mtDNA) G13708A variation: (from left to right) homoplasmic wild-type mtDNA fragments; homoplasmic G13708A mtDNA fragment; undigested PCR product; and DNA ladder.

diagnosis and the mtDNA G13708A variation (*P* = 0.7, OR: 0.8, 95% CI: 0.3–1.9).

These results corroborate the findings of Mayr-Wohlfart et al. [35], who assessed several mtDNA variations, including G13708A, in a German population (100 MS cases and 100 controls) using PCR–RFLP (restriction enzyme Mva I) analysis, but could find no association between the variation and MS. In their study, the variation was detected in 7% of MS cases *vs.* 4% in the healthy controls. Similarly, Hanefeld et al. [36] also demonstrated a lack of association between the variation and MS in a German population, with similar frequencies (6.7% in MS adults, 4.2% in controls). In addition, a PCR–RFLP (BstN I) study in Bulgaria could find the mtDNA G13708A variation in only four out of 80 MS cases (5%) and in three out of 58 healthy controls (5.2%), with no association between MS and the mtDNA variation (*P* = 1.04, OR: 1) [40].

Our present findings are inconsistent with those of Yu et al. [34], based on six European case–control cohorts and a total of approximately 5000 participants. Three well-matched cohorts were genotyped and had seven mtDNA variations, and G13708A was found to be significantly associated with susceptibility to MS (OR: 1.71, *P* = 0.0002). However, subsequent mtDNA sequencing of 50 subjects revealed that the association depended on a nucleotide at position 13708 rather than the variations. Although the cohorts were not well

Table 1 – Primers and restriction enzyme used for PCR–RFLP analysis, including the length of the PCR product.

MtDNA variation	Forward primer (5′–3′)	Reverse primer (3′–5′)	Total length of PCR product	Restriction enzyme
G13708A	CACACCTAGCATTCCTGCAC	GAGTTTTAGGTAGAGGGGGA	500 bp	Mva I

PCR–RFLP: polymerase chain reaction–restriction fragment length polymorphism; MtDNA: mitochondrial DNA.

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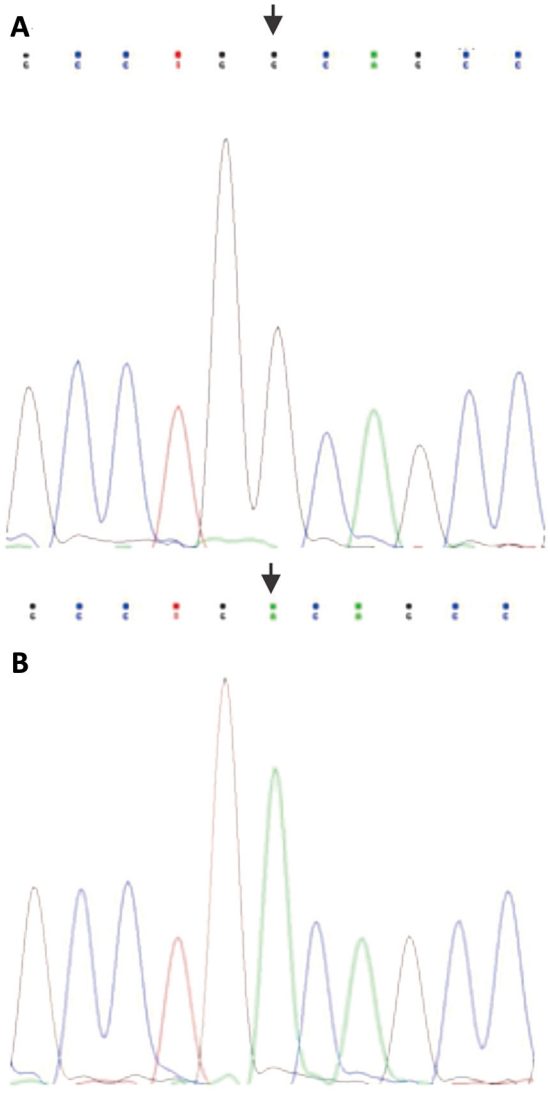

Fig. 2 – Sequencing results for the mitochondrial DNA (mtDNA) G13708A variation (A: wild-type allele; B: variant allele).

matched, the association of G13708A with the diagnosis was significant, suggesting that the population genetic admixture among healthy subjects had an impact on the significance of the association.

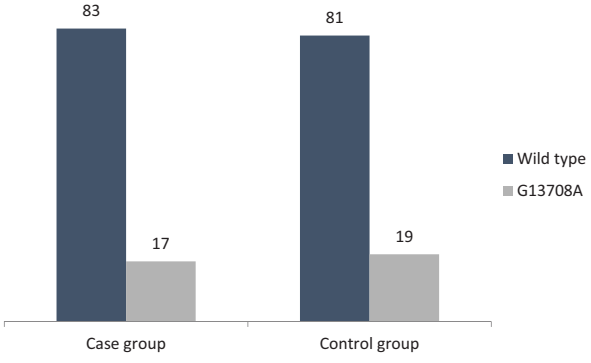

Fig. 3 – Allelic frequencies in the case and control groups for the mitochondrial DNA (mtDNA) G13708A variation.

5. Conclusion

Although the evidence from our present study showed that the mtDNA G13708A variation was not associated with MS in a selected group of Iranians, further research is nevertheless required to resolve the contradictory findings of previous studies in multiple countries.

Disclosure of interest

The authors declare that they have no competing interest.

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