Four novel mutations in the mitochondrial ND4 gene of complex I in patients with multiple sclerosis

MARAM ATALLAH ALHARBI1*, GHADA AL-KAFAJI2*, NOUREDDINE BEN KHALAF3, SAFIA ABDULSALAM MESSAOUDI1, SAFA TAHA2, ABDULQADER DAIF4 and MOIZ BAKHIET2

1College of Forensic Sciences, Naif Arab University for Security Sciences, Riyadh 14812, Kingdom of Saudi Arabia; 2Department of Molecular Medicine, Al-Jawhara Centre for Genetics and Inherited Disorders, College of Medicine and Medical Sciences, Arabian Gulf University; 3Department of Life Sciences, College of Graduate Studies, Arabian Gulf University, Block 329, Manama, Kingdom of Bahrain; 4King Saud University Medical City, Riyadh 12372, Kingdom of Saudi Arabia

Received June 26, 2019; Accepted October 16, 2019

DOI: 10.3892/br.2019.1250

Abstract. Multiple sclerosis (MS) is an immune-mediated neurological, inflammatory disease of the central nervous system. Recent studies have suggested that genetic variants in mitochondrial DNA (mtDNA)-encoded complexes of respiratory chain, particularly, complex I (NADH dehydrogenase), contribute to the pathogenicity of MS among different ethnicities, and targeting mitochondrial function may represent a novel approach for MS therapy. In this study, we sequenced ND genes (ND1, ND2, ND3, ND4, ND4L, ND5 and ND6) encoding subunits of complex I in 124 subjects, 60 patients with relapsing-remitting MS and 64 healthy individuals, in order to identify potential novel mutations in these patients. We found several variants in ND genes in both the patients and controls, and specific variants only in patients with MS. While the majority of these variants were synonymous, 4 variants in the ND4 gene were identified as missense mutations in patients with MS. Of these, m.11150G>A was observed in one patient, whereas m.11519A>C, m.11523A>C and m.11527C>T were observed in another patient. Functional analysis predicted the mutations, m.11519A>C, m.11523A>C and m.11150G>A, as deleterious with a direct impact on ND4 protein stability and complex I function, whereas m.11527C>T mutation had no effect on ND4 protein stability. However, the 3 mutations, m.11519A>C, m.11523A>C and m.11527C>T, which were observed in the same patient, were predicted to cause a cumulative destabilizing effect on ND4 protein, and could thus disrupt complex I function. On the whole, this study identified 4 novel mutations in the mtDNA-encoded ND4 gene in patients with MS, which could lead to complex I dysfunction, and further confirmed the implication of mtDNA mutations in the pathogenicity of MS. The identified novel mutations in patients with MS may be ethnic-related and may prove to be significant in personalized treatment.

Introduction

Multiple sclerosis (MS) is a neurological chronic inflammatory disorder affecting the white matter of the central nervous system (CNS) that involves immune-mediated mechanisms. The disease is characterized by demyelination and axonal loss as a result of myelin sheath damage by the body’s own immune system, which affects the ability of nerve cells in the brain and spinal cord to effectively communicate with each other (1,2). However, the exact immunopathogenic mechanisms responsible for the initiation and progression of the disease remain unknown. Clinically, MS presents with a range of signs and symptoms that appear as episodic or progressive neurological impairments, such as numbness and tingling, blurry vision, mobility and balance issues, muscle weakness and tightness, bladder and bowel dysfunction, decreased memory and fatigue, as well as other conditions, such as depression (3,4). These symptoms present in each of the 4 types of MS that are as follows: i) Relapsing-remitting MS (RRMS), which is the most common type and occurs in 85-90% of patients; ii) secondary progressive MS (SPMS), which occurs in 70-80% of patients with RRMS within 10-15 years; iii) primary progressive MS (PPMS), which occurs in 15% of cases; and iv) progressive relapsing MS (PRMS), the least common form which occurs in 5% of patients (5). MS affects approximately 2.5 million individuals worldwide and commonly appears in young adults with a mean age of 32 years (6). Moreover, MS usually occurs 2-3 times more frequently in females than in males (7). Higher rates of MS have been reported in Europe, Southern Canada, Northern United States, New Zealand.
and Southeast Asia (8,9). Studies from the Arab Gulf countries, including Kuwait, Saudi Arabia and United Arab Emirates have also demonstrated noticeable increases in the incidence and prevalence of MS (10,11).

Although the cause of MS remains unclear, combinations of genetic and environmental factors may contribute to the etiopathogenicity of the disease (12-14). Recently, MS has become increasingly viewed as a neurodegenerative disorder, in which mitochondrial dysfunction occurs early in the pathogenic process and plays an important role in axonal degeneration and demyelination, as well as in disease progression (15-17). Therefore, it has been suggested that targeting mitochondrial pathways along with neuroprotection and immunomodulation may provide a novel approach for the treatment of MS (16-18).

The mitochondria are the main site of energy production in the cell and are also the major source of reactive oxygen species (ROS). Mammalian mitochondria have their own genome [mitochondrial DNA (mtDNA)], a single, circular double-stranded molecule of 16,569 base pairs. mtDNA encodes 2 rRNAs and 22 tRNAs, as well as 13 polypeptides that are all subunits of complexes of the respiratory chain, located in the inner mitochondrial membrane, that drives oxidative energy metabolism. While subunits of complex II are entirely encoded by mtDNA, subunits of complexes I, III, IV and V are encoded by either mtDNA or nuclear DNA (nDNA) (19). Specifically, mtDNA encodes 7 subunits of complex I (NADH dehydrogenase), 1 subunit of complex III (ubiquinol-cytochrome c oxidoreductase), 3 subunits of complex IV (cytochrome c oxidase) and 2 subunits of complex V (20). It has been demonstrated that mtDNA has a higher mutation rate than the nuclear genome. A number of factors contribute to the increased rate of mutations in mtDNA, such as the close proximity of mtDNA to the site of ROS production, the lack of protective histones and the low efficiency of DNA repair pathways (21,22). The loss of mitochondrial genomic integrity can lead to a progressive decline in mitochondrial function (23,24) and, eventually, to a reduction in energy within the cell, which has been implicated in a number of neuroinflammatory and neurodegenerative diseases (25). The role of defects in mtDNA in MS stems from the observation that a number of patients with Leber hereditary optic neuropathy (LHON), a maternally inherited mitochondrial disease caused by mutations in the ND1, ND4, ND4L and ND6 genes of complex I, develop neurological features, including inflammatory demyelinating disorders compatible with a diagnosis of MS (26,27). Studies on mtDNA in patients with MS from different ethnic backgrounds have identified variations within complex I genes to be associated with the pathogenicity of MS. A specific variant in the ND2 gene of complex I has been reported to play a role in the susceptibility to MS in Caucasians (28). Other variants in different genes of complex I have been shown to be associated with the risk of developing MS in a Filipino population (29). These observations suggest that although the disease etiology is common between populations, genetic variants can be population-specific. Therefore, in the current study, we carried out genetic analysis of mtDNA-encoded complex I genes to identify specific mutations in Saudi patients with MS. The identification of ethnicity-related mtDNA variations in MS may lead to the development of novel approaches for personalized treatment.

Subjects and methods

Subjects. Between October, 2016 and June, 2017, a total of 124 Saudi subjects were enrolled in this study, 60 patients with RRMS and 64 healthy control subjects. Patients diagnosed with RRMS at the Neurology Outpatient Clinic at King Khalid Hospital, King Saud University were selected for this study. The diagnosis of RRMS was performed according to the McDonald Criteria (30) with at least 2 previous relapses in different CNS regions, as confirmed by a neurological examination, medical history, clinical examination, magnetic resonance imaging (MRI) and electrophysiological analyses. The inclusion criteria for the patients were the following: Either sex, a relapsing-remitting course with at least 1 documented relapse during the previous year or 2 documented relapses during the previous 2 years, and a Kurtzke Expanded Disability Status Scale (EDSS) score of 0-5.5 inclusive. The exclusion criteria for the patients were the following: A documented history of infections, chronic diseases, such as diabetes, chronic diseases of the immune system other than MS, immunodeficiency syndrome, malignancy and pregnancy.

The healthy control subjects were recruited from King Khalid hospital Blood Bank, Kingdom of Saudi Arabia. Their medical history confirmed the absence of any neurological disorders, active infections or other medical conditions. The inclusion criteria for the controls were as follows: Either sex, and the absence of any neurological disorders, active infections or other medical conditions. Individuals with a documented history of chronic diseases, such as diabetes, immune system or inflammation disorders, immunodeficiency syndrome and malignancy were excluded from the study. Age, body mass index (BMI) and hypertension data were collected from the medical records of the patients and healthy controls. Medication, disease duration and disability status, evaluated using the Kurtzke EDSS were collected from the patients.

Ethics statement. Ethics approval was obtained from the Scientific and Ethics Committee in King Saud University, College of Medicine, Kingdom of Saudi Arabia and from the Medical Research and Ethics Committee in the College of Medicine and Medical Sciences, Arabian Gulf University, Kingdom of Bahrain. The participants were given a complete description of the study. Informed consent was obtained from all individual participants included in the study.

Extraction of genomic DNA. Venous blood samples were collected from the participants into ethylenediminetetraacetic acid (EDTA) tubes. Genomic DNA was extracted from peripheral blood samples using the QIAMP DSP DNA kit (Qiagen) as previously described (31). In brief, 20 µl protease were mixed with 200 µl EDTA-blood followed by the addition of lysis buffer (200 µl). The mixture was incubated at 56°C for 10 min, and then centrifuged at 20,000 x g for 1 min at 4°C. Absolute ethanol (200 µl) was then added to the mixture followed by centrifugation at 6,000 x g for 1 min at room temperature. Washing steps were carried out using 500 µl washing buffer followed by centrifugation at 6,000 x g for
1 min and then at 20,000 x g for 3 min (at room temperature). To elute the genomic DNA, elution buffer (200 µl) was added, incubated at room temperature for 1 min and then centrifuged at 6,000 x g for 1 min (at room temperature). The concentration of DNA was determined using a NanoDrop ND-1000 ultraviolet-visible light spectrophotometer (Thermo Fisher Scientific, Inc.). All DNA samples were stored at -20˚C until further analysis.

**Polymerase chain reaction (PCR) and DNA sequencing.** mtDNA-encoded ND1, ND2, ND3, ND4, ND4L, ND5 and ND6 genes of complex I were amplified by PCR. The sequences of the primers of the 7 subunits (Table I) were as previously described by Zonouzi et al (29). Each PCR reaction contained 50 ng of DNA, 0.4 µl of each primer, 0.2 µl Super Taq polymerase (Invitrogen; Thermo Fisher Scientific, Inc.), 0.8 µl dNTPs master mix (10 mM of each), and 2.5 µl 10X PCR buffer to a final volume of 25 µl. PCR was performed using an automated thermal cycler (Perkin-Elmer 2400) with the following cycling program: Denaturation at 95˚C for 1 min, 35 cycles of denaturation at 95˚C for 40 sec, annealing for 40 sec and extension at 72˚C for 90 sec and a final extension at 72˚C for 10 min. The PCR products of the ND1, ND2, ND3, ND4L, ND4, ND5 and ND6 genes were purified using a QIAquick PCR Purification kit (Qiagen). The PCR products were then directly sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Sequencing products were run on 3130XL Genetic Analyzer (Applied Biosystems) and analyzed using SeqScape Software v2.5 (Life Technologies; Thermo Fisher Scientific, Inc.).

**Bioinformatics analysis.** *Homo sapiens* mitochondrion complete genome, the Revised Cambridge Reference Sequence (GenBank no. NC_012920.1) was used as a reference sequence. Raw sequences obtained from DNA sequencing were first examined using Chromas software, version 2.6.6 (Technelysium Ltd.). Variations were identified and listed according to their corresponding positions on the reference genome. Artemis was used to annotate base variations and locate their positions on the annotated reference sequence (32). The variant sequence of each corresponding coding sequence was translated into amino acid sequence by Translate tool (EXPasy.org) using the vertebrate mitochondrial genetic code. ClustalOmega (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used to align the obtained sequence to the annotated one. To predict the effects of mutations on protein function, Site Directed Mutator (SDM) server was used (33). SDM is a computational method that analyses the variation of amino acid substitutions occurring at specific structural environment that are tolerated within the family of homologous proteins of known 3-D structures, and convert them into substitution probability tables. SMD is considered more effective than other published methods in the task of classifying mutations as stabilizing or destabilizing (33); mutations can be analyzed, either separately or in a cumulative manner, by incorporating all mutations in one single file. The results are expressed in Del Del G (Enthalpy).

### Results

**Characteristics of the study subjects.** In this study, 60 patients with RRMS and 64 healthy control individuals were included. The baseline characteristics of the participants are presented in Table II. The MS group included 14 males and 46 females, and the group of healthy subjects included 22 males and 42 females. No significant differences were observed in sex distribution between the 2 groups (P=0.679). The mean age in the MS group was 30±8.9 years and ranged between 18 to 50 years. The mean age in the control group was 36.7±12.2 years and ranged between 19 to 60 years. The mean age differed significantly between the patient group and the control group (P<0.005). However, no significant differences between the 2 groups were found as regards BMI (P=0.140) or mean blood pressure (P=0.089). The degree of disability of the patients as evaluated by the

| Target genes | Forward sequence (5‘-3’) | Reverse sequence (5‘-3’) | Annealing temperature (˚C) | PCR product (bp) |
|--------------|---------------------------|--------------------------|---------------------------|-----------------|
| **ND1**     | CTCAACTTAGTATTATACCC      | GAGCTTACGCCGCTGTGATGAG    | 59                        | 1,249           |
| **ND2**     | GTCATCCTACTCTACCTACCTAC   | GGCCTTAGAAGTGTAGATGAA     | 52                        | 689             |
| **ND3**     | CACTATCTGCTTCTCATCCGCC    | GAGCGATATACCTGATATCC      | 54                        | 1065            |
| **ND4**     | GCGCAGTCTCTTCTCATAATC     | TTTGTTAGGGTTAAACGAGGG     | 54                        | 729             |
| **ND4L**    | TCTGGGCCATATGAGTGACTAC    | ACTGGTGATGGCGTTCGTGGTA    | 54                        | 1,415           |
| **ND5**     | TTTTTGGTCCAACCTCCAAA      | GGTGGACCTGTGATGTTGGAG     | 50                        | 1,369           |
| **ND6**     | CTCCAAAGACCCACATCATCGAAAC | TTCATCATGCGGAGATGTTGG     | 52                        | 1,334           |

Table I. Primers of mtDNA-encoded complex I genes.
Variants in mtDNA-encoded complex I genes identified only in patients with MS. Sequence analysis revealed a number of variants only in patients with MS, while none of the healthy subjects carried these variants. These variants were found in the ND2, ND3, ND4L, ND4, ND5 and ND6 genes. Of these, 22 were observed in the ND1 gene, 2 in the ND3 gene, 7 in the ND4L gene, 27 in the ND4 gene, 31 in the ND5 gene and 19 in the ND6 gene (Table IV). Using bioinformatics analysis, it was revealed that the majority of the variants were synonymous and did not cause any amino acid changes. Therefore, they were not considered to be significant, but rather simple polymorphisms and were excluded from further analysis.

Of note, bioinformatics analysis indicated 4 variants as missense mutations which were found in the coding sequence of the ND4 gene (Tables IV and V). These included m.11150G>A, which exhibited an alanine to threonine alteration (p.A131T), m.11519A>C which exhibited a threonine to proline alteration (p.T254P), m.11523A>C, which exhibited a lysine to threonine alteration (p.K255T) and m.11527C>T, which exhibited a histidine to leucine alteration (p.H256L). Notably, the m.11150G>A variant was observed in 1 patient (no. 48), whereas the other 3 variants, m.11519A>C, m.11523A>C and m.11527C>T, were all observed in another patient (no. 44). The clinical characteristics of these patients, including age, sex, years since diagnosis, number of relapses per year, main neurological dysfunction and the severity of disease, MRI and lumbar puncture (LP) findings, as well as previous and current medications are presented in Table VI. Patient no. 44, who carried the mutations, m.11519A>C, m.11523A>C and m.11527C>T, was a 31-year-old female and presented with blurry vision, pain on ocular movement, sensory ataxia, weakness, numbness and imbalance. Patient no. 48, who carried the mutation, m.11150G>A, was a 25-year-old female and presented with imbalance, incoordination of movement, weakness on lower limbs, numbness and imbalance.

Predicting the functional impact of mutations. The functional context of the 4 missense mutations identified in the patients with MS was predicted using the SDM server (33). As indicated by their scores (Table V), m.11150G>A, m.11519A>C and m.11523A were revealed to be destabilizing mutations in terms of protein stability with Del Del G of -2.05, 1.54 and -0.95, respectively. However, the m.11527C>T mutation exhibited no effect on protein stability (Del Del G of 0.99). Since the mutations, m.11519A>C, m.11523A>C and m.11527C>T, were observed in 1 patient (Table V), they were further analyzed for their cumulative functional effect on ND4 protein. The results predicted a cumulative effect of the 3 mutations together, causing an overall destabilizing effect on ND4 protein.

Discussion

In the current study, we carried out sequence analysis for the mtDNA-encoded complex I (NADH dehydrogenase) genes in 164 Saudi subjects, 60 patients with RRMS and 64 healthy controls, in order to identify mutations relevant to MS. We found several variants in different positions of the ND1, ND3, ND4L, ND4, ND5 and ND6 genes both in the patients with MS and the healthy individuals. However, no variants were found in the ND2 gene in the 2 groups. Some of the identified
Table III. Variations in mtDNA-encoded complex I identified in patients with multiple sclerosis and healthy controls.

| Gene | Patients with multiple sclerosis | Healthy controls |
|------|---------------------------------|------------------|
|      | Nucleotide change | No. of nucleotide changes | Frequency (%) | Nucleotide change | No. of nucleotide changes | Frequency (%) |
|      |                   |                       |              |                   |                       |              |
| ND1  | m.3316G>A         | 1                      | 1.66         | m.3316G>A         | 1                      | 1.56         |
|      | m.3438G>A         | 4                      | 6.66         | m.3438G>A         | 2                      | 3.13         |
|      | m.3531G>A         | 3                      | 5            | m.3531G>A         | 1                      | 1.56         |
|      | m.3834G>A         | 4                      | 6.66         | m.3531G>A         | 1                      | 1.56         |
|      | m.3915G>A         | 1                      | 1.66         | m.3666G>A         | 1                      | 1.56         |
|      | m.3480A>G         | 1                      | 1.66         | m.3693G>A         | 1                      | 1.56         |
|      | m.3720A>G         | 3                      | 5            | m.3705G>A         | 1                      | 1.56         |
|      | m.3537A>G         | 1                      | 1.66         | m.3834G>A         | 1                      | 1.56         |
|      | m.579A>G          | 3                      | 5            | m.3915G>A         | 1                      | 1.56         |
|      | m.3584A>G         | 1                      | 1.66         | m.4048G>A         | 1                      | 1.56         |
|      | m.3865A>G         | 2                      | 3.33         | m.3384A>G         | 1                      | 1.56         |
|      | m.3948A>G         | 1                      | 1.66         | m.3480A>G         | 5                      | 7.813        |
|      | m.4104A>G         | 5                      | 8.33         | m.3505A>G         | 1                      | 1.56         |
|      | m.4188A>G         | 1                      | 1.66         | m.3537A>G         | 3                      | 4.69         |
|      | m.14340A>G        | 1                      | 1.66         | m.3768A>G         | 1                      | 1.56         |
|      | m.3513C>T         | 1                      | 1.66         | m.4093A>G         | 1                      | 1.56         |
|      | m.3533C>T         | 6                      | 10           | m.4104A>G         | 3                      | 4.69         |
|      | m.3594C>T         | 1                      | 1.66         | m.4188A>G         | 2                      | 3.13         |
|      | m.4059C>T         | 1                      | 1.66         | m.4225A>G         | 1                      | 1.56         |
|      | m.4312C>T         | 3                      | 5            | m.4231A>G         | 1                      | 1.56         |
|      | m.3516C>T         | 4                      | 6.66         | m.4340A>G         | 1                      | 1.56         |
|      | m.3847T>C         | 10                     | 16.66        | m.4316A>G         | 1                      | 1.56         |
|      | m.3866T>C         | 4                      | 6.66         | m.3336T>C         | 1                      | 1.56         |
|      | m.3944T>C         | 1                      | 1.66         | m.3350T>C         | 1                      | 1.56         |
|      | m.4216T>C         | 16                     | 26.66        | m.3423T>C         | 1                      | 1.56         |
|      |                   |                       |              | m.3847T>C         | 7                      | 10.94        |
|      |                   |                       |              | m.4216T>C         | 13                     | 20.31        |
|      |                   |                       |              | m.4232T>C         | 1                      | 1.56         |
|      |                   |                       |              | m.4336T>C         | 1                      | 1.56         |
|      |                   |                       |              | m.3429T>C         | 1                      | 1.56         |
|      |                   |                       |              | m.3594T>C         | 4                      | 6.25         |
|      |                   |                       |              | m.4312T>C         | 1                      | 1.56         |
|      |                   |                       |              | m.3516C>A         | 1                      | 1.56         |
|      |                   |                       |              | m.3546C>A         | 1                      | 1.56         |
| ND3  | m.10373T>C        | 1                      | 1.66         | m.10172T>C        | 1                      | 1.56         |
|      | m.10289A>G        | 1                      | 1.66         | m.10325T>C        | 1                      | 1.56         |
|      | m.10115T>C        | 2                      | 3.33         | m.10217A>G        | 1                      | 1.56         |
|      | m.10238T>C        | 1                      | 1.66         | m.10289A>G        | 1                      | 1.56         |
|      | m.10355C>T        | 1                      | 1.66         | m.10295A>G        | 1                      | 1.56         |
|      |                   |                       |              | m.10398A>G        | 20                     | 31.25        |
|      |                   |                       |              | m.10238T>C        | 2                      | 3.13         |
|      |                   |                       |              | m.10410T>C        | 1                      | 1.56         |
|      |                   |                       |              | m.10115T>C        | 1                      | 1.56         |
|      |                   |                       |              | m.10343C>T        | 1                      | 1.56         |
|      |                   |                       |              | m.10400C>T        | 1                      | 1.56         |
|      |                   |                       |              | m.10410T>A        | 1                      | 1.56         |
| NDL4 | m.10586G>A        | 1                      | 1.66         | m.10589G>A        | 1                      | 1.56         |
|      | m.10589G>A        | 2                      | 3.33         | m.10685G>A        | 1                      | 1.56         |
|      | m.10688G>A        | 2                      | 3.33         | m.10688G>A        | 2                      | 3.13         |
| Gene | Patients with multiple sclerosis | Healthy controls |
|------|---------------------------------|------------------|
|      | Nucleotide change | No. of nucleotide changes | Frequency (%) | Nucleotide change | No. of nucleotide changes | Frequency (%) |
| m.10499A>G | 4 | 6.66 | m.10550A>G | 5 | 7.813 |
| m.10550A>G | 1 | 1.66 | m.10463T>C | 7 | 10.94 |
| m.10643C>T | 2 | 3.33 | m.11150G>A | 1 | 1.66 |
| m.10463T>C | 3 | 5 | m.10876A>G | 1 | 1.66 |
| ND4 | m.11150G>A | 1 | 1.66 | m.10810T>C | 10 | 15.63 |
|      | m.11176G>A | 3 | 5 | m.10810T>C | 1 | 1.56 |
|      | m.11377G>A | 3 | 5 | m.10915T>C | 1 | 1.56 |
|      | m.11440G>A | 4 | 6.66 | m.11025T>C | 1 | 1.56 |
|      | m.11719G>A | 32 | 53.33 | m.11299T>C | 6 | 9.38 |
|      | m.10819A>G | 2 | 3.33 | m.10822C>T | 1 | 1.56 |
|      | m.10876A>G | 3 | 5 | m.11332C>T | 1 | 1.56 |
|      | m.10895A>G | 1 | 1.66 | m.11674C>T | 1 | 1.56 |
|      | m.11002A>G | 4 | 6.66 | m.10876A>G | 1 | 1.56 |
|      | m.11172A>G | 2 | 3.33 | m.11251A>G | 16 | 25 |
|      | m.11251A>G | 17 | 28.33 | m.11467A>G | 10 | 15.63 |
|      | m.11337A>G | 1 | 1.66 | m.11530A>G | 1 | 1.56 |
|      | m.11380A>G | 1 | 1.66 | m.11641A>G | 1 | 1.56 |
|      | m.11467A>G | 14 | 23.33 | m.11671A>G | 1 | 1.56 |
|      | m.11530A>G | 1 | 1.66 | m.11708A>G | 1 | 1.56 |
|      | m.11641A>G | 3 | 5 | m.11719G>A | 29 | 45.31 |
|      | m.10822C>T | 4 | 6.66 | m.10984C>A | 1 | 1.56 |
|      | m.11332C>T | 1 | 1.66 | m.11260T>G | 1 | 1.56 |
|      | m.11527C>T | 1 | 1.66 | m.10822C>T | 3 | 5 |
|      | m.10810T>C | 1 | 1.66 | m.10810T>C | 2 | 3.33 |
|      | m.10810T>C | 2 | 3.33 | m.10810T>C | 1 | 1.66 |
|      | m.10873T>C | 11 | 18.33 | m.10873T>C | 11 | 18.33 |
|      | m.10915T>C | 3 | 5 | m.10873T>C | 1 | 1.66 |
|      | m.11299T>C | 1 | 1.66 | m.11299T>C | 3 | 5 |
|      | m.11518G>T | 1 | 1.66 | m.11518G>T | 1 | 1.66 |
|      | m.11519A>C | 1 | 1.66 | m.11519A>C | 1 | 1.66 |
|      | m.11523A>C | 1 | 1.66 | m.11523A>C | 1 | 1.66 |
| ND5 | m.12372G>A | 13 | 21.66 | m.12372G>A | 1 | 1.56 |
|      | m.12771G>A | 2 | 3.33 | m.12771G>A | 11 | 17.19 |
|      | m.13316G>A | 1 | 1.66 | m.12501G>A | 2 | 3.13 |
|      | m.13368G>A | 3 | 5 | m.12771G>A | 1 | 1.56 |
|      | m.1356G>A | 2 | 3.33 | m.13368G>A | 7 | 10.94 |
|      | m.13708G>A | 13 | 21.66 | m.13194G>A | 1 | 1.56 |
|      | m.13813G>A | 4 | 6.66 | m.13708G>A | 9 | 14.06 |
|      | m.13803G>A | 2 | 3.33 | m.12397G>A | 1 | 1.56 |
|      | m.12530G>A | 4 | 6.66 | m.12530G>A | 2 | 3.13 |
|      | m.12612G>A | 15 | 25 | m.12612G>A | 8 | 12.5 |
|      | m.12693G>A | 2 | 3.33 | m.12654G>A | 2 | 3.13 |
|      | m.12720G>A | 1 | 1.66 | m.12693G>A | 1 | 1.56 |
|      | m.12753G>A | 1 | 1.66 | m.12720G>A | 1 | 1.56 |
|      | m.12720G>A | 2 | 3.33 | m.12810G>A | 1 | 1.56 |
|      | m.12720G>A | 1 | 1.66 | m.12937G>A | 1 | 1.56 |
|      | m.13104G>A | 1 | 1.66 | m.12950G>A | 1 | 1.56 |
|      | m.13105G>A | 3 | 5 | m.13105G>A | 6 | 9.38 |
Table III. Continued.

| Gene     | Nucleotide change | Patients with multiple sclerosis | Healthy controls |
|----------|-------------------|---------------------------------|-----------------|
|          | No. of nucleotide change | Frequency (%) | No. of nucleotide change | Frequency (%) |
| m.13276G>A | 3 | 5 | m.13276G>A | 1 | 1.56 |
| m.13542G>A | 2 | 3.33 | m.13542G>A | 2 | 3.13 |
| m.13966G>A | 2 | 3.33 | m.13542G>A | 2 | 3.13 |
| m.14007G>A | 2 | 3.33 | m.13780G>A | 2 | 3.13 |
| m.12615C>T | 1 | 1.66 | m.13803G>A | 2 | 3.13 |
| m.12705C>T | 13 | 21.66 | m.13927G>A | 1 | 1.56 |
| m.13188C>T | 12 | 20 | m.13966G>A | 5 | 7.813 |
| m.13188C>T | 2 | 3.33 | m.13980G>A | 1 | 1.56 |
| m.13506C>T | 3 | 5 | m.13986A>G | 1 | 1.56 |
| m.13650C>T | 4 | 6.66 | m.14013A>G | 1 | 1.56 |
| m.13695C>T | 1 | 1.66 | m.14028A>G | 1 | 1.56 |
| m.14100C>T | 1 | 1.66 | m.14053A>G | 1 | 1.56 |
| m.14110C>T | 2 | 3.33 | m.12633C>A | 2 | 3.13 |
| m.14155C>T | 1 | 1.66 | m.13880C>A | 1 | 1.56 |
| m.14167C>T | 2 | 3.33 | m.12633C>T | 1 | 1.56 |
| m.13111T>C | 1 | 1.66 | m.12705C>T | 14 | 21.88 |
| m.14178T>C | 1 | 1.66 | m.12741C>T | 1 | 1.56 |
| m.13392T>C | 3 | 5 | m.13188C>T | 7 | 10.94 |
| m.14094T>C | 3 | 5 | m.13506C>T | 1 | 1.56 |
| m.14139A>G | 2 | 3.33 | m.13547C>T | 1 | 1.56 |
| m.14233A>G | 2 | 3.33 | m.13650C>T | 5 | 7.813 |
| m.14308T>C | 2 | 3.33 | m.14109C>T | 1 | 1.56 |
| m.14212T>C | 1 | 1.66 | m.14167C>T | 4 | 6.25 |
| m.14305G>A | 1 | 1.66 | m.12705C>G | 1 | 1.56 |
| ND6      | m.14139A>G | 2 | 3.33 | m.14139A>G | 2 | 3.13 |
|          | m.14233A>G | 2 | 3.33 | m.14203A>G | 1 | 1.56 |
|          | m.14308T>C | 2 | 3.33 | m.14233A>G | 5 | 7.813 |
|          | m.14212T>C | 1 | 1.66 | m.14323G>A | 1 | 1.56 |
|          | m.14305G>A | 1 | 1.66 | m.14239C>T | 1 | 1.56 |
|          | m.14153T>C | 1 | 1.56 | m.14153T>C | 1 | 1.56 |
|          | m.14178T>C | 3 | 4.69 | m.14178T>C | 3 | 4.69 |
|          | m.13743T>C | 2 | 3.13 | m.13743T>C | 2 | 3.13 |
|          | m.13752T>C | 3 | 4.69 | m.13752T>C | 3 | 4.69 |
|          | m.13789T>C | 1 | 1.56 | m.13789T>C | 1 | 1.56 |
|          | m.13965T>C | 1 | 1.56 | m.13965T>C | 1 | 1.56 |
|          | m.14094T>C | 1 | 1.56 | m.14094T>C | 1 | 1.56 |
|          | m.14110T>C | 2 | 3.13 | m.14110T>C | 2 | 3.13 |
Table IV. Variations in mtDNA-encoded complex I genes identified only in patients with multiple sclerosis.

| Gene | Nucleotide change | No. of nucleotide changes | Amino acid change |
|------|------------------|--------------------------|------------------|
| ND1  | m.3513C>T        | 1                        |                  |
|      | m.3533C>T        | 1                        |                  |
|      | m.3594C>T        | 5                        |                  |
|      | m.4059C>T        | 1                        |                  |
|      | m.4312C>T        | 3                        |                  |
|      | m.3944A>G        | 1                        |                  |
|      | m.3720A>G        | 3                        |                  |
|      | m.3948A>G        | 1                        |                  |
|      | m.3865A>G        | 3                        |                  |
|      | m.3866T>C        | 1                        |                  |
|      | m.3944T>C        | 2                        |                  |
| ND3  | m.10343C>T       | 1                        |                  |
|      | m.10355G>A       | 1                        |                  |
| NDL4 | m.10499A>G       | 4                        |                  |
|      | m.10586G>C       | 1                        |                  |
|      | m.10664C>T       | 2                        |                  |
| ND4  | m.10819A>G       | 2                        |                  |
|      | m.10895A>G       | 2                        |                  |
|      | m.11002A>G       | 1                        |                  |
|      | m.11172A>G       | 4                        |                  |
|      | m.11337A>G       | 2                        |                  |
|      | m.11380A>G       | 1                        |                  |
|      | m.1143C>T        | 2                        |                  |
|      | m.11150G>A       | 1                        | A131T            |
|      | m.11377G>A       | 4                        |                  |
|      | m.11440G>A       | 4                        |                  |
|      | m.11518G>T       | 1                        |                  |
|      | m.11519A>C       | 1                        | T254P            |
|      | m.11523A>C       | 1                        | K255T            |
|      | m.11527C>T       | 1                        | H256L            |
| ND5  | m.12570A>G       | 4                        |                  |
|      | m.12615A>G       | 1                        |                  |
|      | m.12753A>G       | 1                        |                  |
|      | m.13104A>G       | 1                        |                  |
|      | m.14007A>G       | 2                        |                  |
|      | m.14070A>G       | 1                        |                  |
|      | m.12843T>C       | 1                        |                  |
|      | m.12879T>C       | 1                        |                  |
|      | m.13020T>C       | 3                        |                  |
|      | m.13174T>C       | 2                        |                  |
|      | m.13373T>C       | 3                        |                  |
|      | m.13116C>T       | 2                        |                  |
|      | m.13695C>T       | 1                        |                  |
|      | m.13317G>A       | 1                        |                  |
|      | m.13590G>A       | 2                        |                  |
|      | m.13813G>A       | 4                        |                  |
|      | m.14100C>T       | 1                        |                  |
|      | m.14155C>T       | 2                        |                  |
|      | m.14305G>A       | 1                        |                  |
|      | m.14308T>C       | 1                        |                  |

variants in the ND1, ND3, NDL4, ND4, ND5 and ND6 genes were commonly observed at high frequencies in the patients with MS and the healthy individuals. These included variants in the ND4 gene, namely m.11150G>A and m.11251A>G, and variants in the ND5 gene, namely m.12372G>A, m.13708G>A, m.12612G>A and m.12705C>T. Moreover, numerous variants were identified which were observed only in patients with MS, whereas none of the healthy subjects carried any of these variants. These were found to be located in different positions of the ND1, ND3, ND4L, ND4, ND5 and ND6 genes. Bioinformatics analysis revealed that the majority of these variants were synonymous and did not cause any amino acid changes, and were thus considered as simple polymorphisms. Importantly, the significant finding in the group of MS patients was the identification of 4 mutations in the ND4 gene that were revealed to encode missense mutations. Of these, m.11150G>A exhibited an alanine to threonine alteration, m.11519A>C a threonine to proline alteration, m.11523A>C a lysine to threonine alteration and m.11527C>T a histidine to leucine alteration. It was also noted that 1 patient (patient no. 48, Table V) carried the m.11150G>A mutation, whereas another patient (patient no. 44, Table V) carried the m.11519A>C, m.11523A>C and m.11527C>T mutations.

MS has traditionally been considered as an immune-mediated neurological inflammatory demyelinating disease of the central nervous system with axonal degeneration (1,2). Recent studies have suggested that mitochondrial dysfunction occurs early in MS and plays an important role in disease development and progression (15-17). Targeting mitochondrial pathways along with neuroprotection and immunomodulation are a potential therapeutic target in MS (16-18). The mitochondria are the most efficient producers of cellular energy in the form of adenosine ATP and are also the major source of ROS. The mitochondrial respiratory chain is located in the inner mitochondrial membrane and consists of 5 multimeric protein complexes I-V (19). While complex II subunits are entirely encoded by nuclear DNA (nDNA), the subunits of complexes I, III, IV and V are encoded by both nDNA and mtDNA. Complex I is the largest enzyme complex of the mitochondrial respiratory chain, which is responsible for electron transport and the generation of protons across the mitochondrial inner membrane to drive energy production (20). It is a multi-subunit complex consisting of 44 subunits, of which 7 subunits, including ND1, ND2, ND3, NDL4, ND4, ND5 and ND6 are encoded by the mitochondrial genome (20).

In this study, numerous synonymous variants were found in different genes of mtDNA-encoded complex I in the patients with MS and the healthy individuals, as well in patients with MS only. Synonymous mutations, which do not result in changes in amino acid sequences and are considered biologically silent, have been shown to directly affect gene expression and function through diverse mechanisms, and may thus be implicated in human diseases (34-36). The presence of rare codons may lead to a premature arrest of the translation process, hence resulting in a truncated form of a protein. Although in the present study, we did not analyze the function of the identified polymorphisms, studies in our laboratory are ongoing to investigate the effects of these variant on gene expression, where silent mutations can lead to the generation of rare codons.
A deficiency in complex I is the most frequently enzyme deficit in mitochondrial diseases (37) and degenerative diseases (38), as well as in other pathological conditions (39), and contributes to neurodegeneration in MS (15,40). Moreover, ROS generated by mitochondrial complex I are considered the main source of cellular oxidative stress (41), and impaired complex I activity mediated by mtDNA oxidative damage has been shown in chronic active plaques in MS (42) and is also implicated in axonal degeneration (15).

It has been demonstrated that ND genes of complex I encoded by mtDNA are hotspots for pathological mutations (43). These mutations in complex I cause a deficiency in NADH ubiquinone oxidoreductase enzyme activity and can lead to mitochondrial dysfunction with increased ROS production (37). In particular, mutations in the ND4, ND5 and ND6 genes have been shown to affect complex I assembly and activity, leading to complex I dysfunction (44-47). Previous studies have confirmed a possible implication of several variations in mtDNA-encoded complex I genes in the pathogenicity of MS in different populations. For example, specific variants in the ND2 gene of complex I have been strongly linked to MS in Caucasians (28). Several other variants in different genes of complex I have been shown to be risk factors in the pathogenicity of MS in a Filipino population (29). The study by Yu et al (48) demonstrated an association between the m.13708G>A variant in the ND5 gene and an increased risk of MS in European cohorts. However, Kellar-Wood et al (49) demonstrated that this base change at position 13708 of the mtDNA-encoded ND5 gene does not contribute to genetically determined susceptibility in typical MS patients. Moreover, in LHON, a rare maternally inherited mitochondrial disease with clinical features associated with an MS-like illness (26,27), the m.13708G>A variant in the ND5 gene has been reported as a secondary mutation when occurring in patients with LHON (50-52) and does not functionally impair mitochondrial oxidative metabolism in vivo or determine the deficit of energy metabolism in LHON (52). In this study, the m.13708G>A variant in the ND5 gene was observed at high frequencies in both patients with MS and healthy individuals. These inconsistencies between studies suggest that the contribution of specific mtDNA variants to disease risk and susceptibility varies among different ancestral and ethnic groups.

In the current study, 4 missense mutations, including m.11150G>A, m.11519A>C, m.11523A>C and m.11527C>T in the ND4 gene of complex I were found in 2 Saudi patients with MS. As indicated by functional analysis, the identified mutations, m.11150G>A, m.11519A>C and m.11523A>C, in this study were predicted to be deleterious and to directly cause ND4 protein instability, which may render the protein non-functional. As mentioned above, 3 mutations (m.11519A>C, m.11523A>C and m.11527A>C) were observed in 1 patient (patient no. 44, Table V) and 2 of them (m.11519A>C and m.11523A>C)
caused protein instability, whereas the m.11150G>A mutation was found in another patient (patient no. 48, Table V). Although the m.11527C>T mutation in patient no. 44 had no effect on protein function, it may cause a cumulative destabilizing effect, and may thus disrupt ND4 protein function. Indeed, when these 3 mutations in patient 44 were analyzed for their cumulative destabilizing effect, they were found to affect ND4 protein stability. ND4 subunit is part of a large complex, and these mutations, if not affecting ND4 stability directly, may perturb the protein-protein interaction responsible for maintaining the complex and may consequently affect complex I function. The missense mutations in the ND4 gene observed in Saudi patients with MS in the present study have not been reported in previous studies, at least to the best of our knowledge, and may therefore be considered novel. Since all previous mtDNA studies have revealed a number of variations in MS patients of different populations, such as Caucasians and Filipinos (28,29,48), the identified novel mutations in Saudi patients with MS in the present study could be ethnic-related and may be important to personalized treatment. This study also confirmed the implication of mtDNA mutations in the pathogenicity of MS and the newly identified mutations may serve as a reference for future studies on the mitochondrial genome in MS.

The polyploid nature of the mitochondrial genome with the presence of up to several thousand copies per cell gives rise to an important feature of mitochondrial genetics, homoplasy and heteroplasy. Homoplasy is when all copies of mtDNA are identical, which may be normal or mutated. Heteroplasy is when there is a mixture of normal and mutated mtDNA (43). The level of heteroplasmatic mutations is important for both the clinical expression of the disease and for biochemical defects (53). In most mtDNA disorders, when the percentage of mutant mtDNA exceeds a certain threshold level, mitochondrial dysfunction becomes clinically apparent (54). Due to organ-specific energetic requirements, the proportion of mutant mtDNA in any cell or tissue may be extremely variable, giving rise to variable disease severity (54). It is generally accepted that high levels of heteroplasy are associated with severe clinical presentations (55). On the other hand, lower levels of heteroplasy have been commonly detected in maternally inherited diseases (56,57). Moreover, low-level heteroplasmatic sequence changes are important features of the pathology of several diseases, including neurodegenerative diseases, which can lead to mtDNA damage and consequently may induce alterations of OXPHOS enzymes (58).

For a DNA alteration to be classified as a deleterious mutation, it should not be present in healthy individuals, and must occur in a structurally and functionally important region (53). While in our results no heteroplasmatic mutations were indicated, the identified mutations in the ND4 gene of complex I, which is a key element in cellular energy production, were observed in only patients with MS and were not found in healthy individuals, suggesting that they are pathogenic mutations and may play an important role in the pathogenicity MS. Nevertheless, further studies are required to predict the energetic effects of amino acid changes caused by these mutations.

A limitation of the current study was the relatively low number of subjects, which affected the statistical differences in age and sex distribution between the patients and controls. Theoretically, the age and sex distribution between cases and controls in a high number of participants should be very similar (Gaussian distribution). Moreover, further studies are warranted with larger cohorts to analyze the possible association between these variants and the risk of and/or susceptibility to MS.

In conclusion, in this study, we identified a number of variants as synonymous mutations in both patients with MS and healthy individuals in the mtDNA-encoded ND1, ND3, ND4L, ND4, ND5 and ND6 genes of complex I. Some of these variants in the ND4 and ND5 genes were commonly observed at high frequencies in the investigated groups. We also identified numerous variants in patients with MS only, which were absent in healthy individuals. While the majority of these variants in patients with MS were synonymous, 4 variants in the ND4 gene were missense mutations and could lead to complex I dysfunction. This study, to the best of our knowledge, is the first to report novel mutations in mtDNA-encoded ND4 gene of complex I in Saudi patients with MS, which may prove to be of importance in personalized treatment.

Acknowledgements

The authors wish to gratefully acknowledge the technical support with sample transportation in Saudi Arabia provided by Mrs. Maram Atallah Alharbi. The authors would also like to thank the technical staff of Al-Jawhara Centre for Molecular Medicine, Genetics, and Inherited Disorders at the College of Medicine and Medical Sciences, Arabian Gulf University, Kingdom of Bahrain.

Funding

This study was funded by a research grant (no. 37-PI-01/15) from the College of Medicine and Medical Sciences, Arabian Gulf University, Kingdom of Bahrain.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

MAA, GAK and MB conceived and designed the study and edited the manuscript; NBK, SAM, ST and AD collected the data; all authors performed data analysis, managed the data and wrote the manuscript. The final version of the manuscript has been read and approved by all authors, and each author believes that the manuscript represents honest work.

Ethics approval and consent to participate

The study received ethical approval from both the Scientific and Ethics Committee in King Saud University, College of Medicine, Kingdom of Saudi Arabia and from the Medical Research and Ethics Committee in the College of Medicine and Medical Sciences, Arabian Gulf University, Kingdom of Bahrain. The participants were given a complete description of
the study. Informed consent was obtained from all individual participants included in the study.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Hernández-Pedro NY, Espinosa-Ramírez G, de la Cruz VP, Pineda B and Sotelo J: Initial immunopathogenesis of multiple sclerosis: Innate immune response. Clin Dev Immunol 2013: 413463, 2013.

2. Frohm EM, Racie MK and Raine CS. Multiple sclerosis - the plaque and its pathogenesis. N Engl J Med 354: 942-955, 2006.

3. Goldenberg MM. Multiple sclerosis review. PT 37: 175-184, 2012.

4. Kister I, Bacon TE, Chamot E, Salter AR, Cutter GR, Kalina JT and Herbert J: Natural history of multiple sclerosis symptoms. Int J MS Care 15: 146-158, 2013.

5. Loma I and Heyman R: Multiple sclerosis: Pathogenesis and treatment. Curr Neuropharmacol 9: 409-416, 2011.

6. Kennedy J, O’Connor P, Sadovnick AD, Perera M, Yee I and Banwell B: Age at onset of multiple sclerosis may be influenced by place of residence during childhood rather than ancestry. Neuropediatrics 26: 162-167, 2006.

7. Harbo HF, Gold R and Tintore M: Sex and gender issues in multiple sclerosis. Therv Adv Neurol Disorder 6: 237-248, 2013.

8. Simpson S Jr, Blizzard L, Otahal P, Van der Me I and Taylor B: Latitude is significantly associated with the prevalence of multiple sclerosis: A meta-analysis. J Neurol Neurosurg Psychiatry 82: 1132-1141, 2011.

9. Ebers GC: Environmental factors and multiple sclerosis. Lancet Neurolog 7: 268-277, 2008.

10. Al-Afasy HH, Al-Obaidan MA, Al-Ansari YA, Al-Yatama SA, Al-Rukaibi MS, Makki NI, Suresh A and Akhtar S: Risk factors for multiple sclerosis in Kuwait: A population-based case-control study. Neuroepidemiology 40: 30-35, 2013.

11. Bohlela S, Inshasi J, Al Tahan AR, Madani AB, Qahtani H and Al-Rukaibi MS, Makki NI, Suresh A and Akhtar S: Risk factors for multiple sclerosis in Kuwait: A population-based case-control study. Neuroepidemiology 40: 30-35, 2013.

12. O’Gorman C, Lucas R and Taylor B: Environmental risk factors for multiple sclerosis: A review with a focus on molecular mechanisms. Int J Mol Sci 13: 11787-11752, 2012.

13. Mandia D, Ferraro OE, Nosari G, Montomoli C, Zardini E and Mandia D, Ferraro OE, Nosari G, Montomoli C, Zardini E and Bergamaschi R: Environmental factors and multiple sclerosis severity: A descriptive study. Int J Environ Res Public Health 11: 6417-6432, 2014.

14. Zuvich RL, McCauley JL, Pericak-Vance MA and Haines JL: Genetics and pathogenesis of multiple sclerosis. Semin Immunol 21: 328-333, 2009.

15. Dutta R, McDonough J, Yin X, Peterson J, Chang A, Torres T, Gudz T, Macklin WB, Lewis DA, Fox RJ, et al: Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. Ann Neurol 59: 478-489, 2006.

16. Mahad D, Lassmann H and Turnbull D: Review: Mitochondria and disease progression in multiple sclerosis. Neuropathol Appl Neurobiol 34: 577-589, 2008.

17. Mao P and Reddy PH: Is multiple sclerosis a mitochondrial disease? Biochim Biophys Acta 1802: 66-79, 2010.

18. Peruzzotti-Jametti L and Pluchino S: Targeting mitochondrial metabolism in neuroinflammation: Towards a therapy for progressive multiple sclerosis. Trends Mol Med 24: 838-855, 2018.

19. Taanman JW: The mitochondrial genome: Structure, transcription, translation and replication. Biochim Biophys Acta 1440: 103-123, 1999.

20. Wirth C, Brandt U, Hunte C and Zickermann V: Structure and function of mitochondrial complex I. Biochim Biophys Acta 1857: 894-916, 2016.

21. Bohr VA: Repair of oxidative DNA damage in nuclear and mitochondrial DNA, and some changes with aging in mammalian cells. Free Radic Biol Med 32: 804-812, 2002.

22. Santos JH, Hunakova L, Chen Y, Bortner C and Van Houten B: Cell sorting experiments link persistent mitochondrial DNA damage with loss of mitochondrial membrane potential and accelerated cell death. J Cell Sci 127: 1728-1734, 2004.

23. Dirks AJ, Hofer T, Marzetti E, Pahor M and Leeuw enburgh C: Mitochondrial DNA mutations, energy metabolism and apoptosis in aging muscle. Ageing Res Rev 5: 179-195, 2006.

24. Al-Kafaji G, Sabry MA and Skrypnyk C: Time-course effect of high-glucose-induced reactive oxygen species on mitochondrial biogenesis and function in human renal mesangial cells. Cell Biol Int 40: 36-48, 2016.

25. Cha MY, Kim DK and Mook-Jung I: The role of mitochondrial DNA mutation on neurodegenerative diseases. Exp Mol Med 47: e150, 2015.

26. Brannell AE, Riordan-Eva P and Gogvann GG: Mitochondrial DNA diseases: Genotype and phenotype in Leber's hereditary optic neuropathy. Muscle Nerve Suppl 3: S82-S84, 1995.

27. Bargiela D and Chinnery PF: Mitochondria in neuro inflammation - Multiple sclerosis (MS), leber hereditary optic neuropathy (LHON) and LHON-MS. Neurosci Lett 710: 132932, 2019.

28. Vyskhina T, Sylvestre A, Saduj S, Bonilla E, Canter JA, Perl A and Kalman B: Association of common mitochondrial DNA variants with multiple sclerosis and systemic lupus erythematosus. Clin Immunol 129: 31-35, 2008.

29. Zonouzi PA, Ghorbani S, Abkar M, Zonouzi PA and Azadid A: Mitochondrial Complex I gene variations as a potential risk factor in the pathogenesis of multiple sclerosis. J Neurol Sci 345: 220-233, 2014.

30. Polan CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Fujihara K, Havrdova E, Hutchinson M, Kappos L, et al: Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 69: 292-302, 2011.

31. Al-Kafaji G, Aljadaa A, Kamal A and Bakhiet M: Peripheral blood mitochondrial DNA copy number as a novel potential biomarker for diabetic nephropathy in type 2 diabetes patients. Exp Ther Med 16: 1483-1492, 2018.

32. Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajendram MA and Barnwell J: Artemis: Sequence visualization and annotation. Bioinformatics 16: 944-945, 2000.

33. Worth CL, Preissner R and Blundell TL: SDM - a server for predicting effects of mutations on protein stability and malfunction. Nucleic Acids Res 39: W215-W222, 2011.

34. Buske OJ, Manickaraj A, Mital S, Ray PN and Brudno M: Identification of deleterious synonymous variants in human genomes. Bioinformatics 29: 1843-1850, 2013.

35. Supek F, Miñaña B, Valcárcel J, Gabaldón T and Lehner B: Synonymous mutations frequently act as driver mutations in human cancers. Cell 156: 1324-1335, 2014.

36. O’Gorman C, Gartner JJ, Qutob SN, Elkins L and Samuels Y: The functional relevance of somatic synonymous mutations in melanoma and other cancers. Pigment Cell Melanoma Res 28: 673-684, 2015.

37. Rodenburg RJ: Mitochondrial complex I-linked disease. Biochim Biophys Acta 1857: 938-945, 2016.

38. Winkhofer KF and Haass C: Mitochondrial dysfunction in Parkinson's disease. Biochim Biophys Acta 1802: 29-44, 2010.

39. Sharma LK, Lu J and Bai Y: Mitochondrial respiratory complex I: Structure, function and implication in human diseases. Curr Med Chem 16: 1266-1277, 2009.

40. Kumble H, Riuz GH, Housmand M, Sanati MH, Gharagozli K and Shafii M: Complex I deficiency in Persian multiple sclerosis patients. J Neurol Sci 243: 65-69, 2006.

41. Hirst J: Towards the molecular mechanism of respiratory complex I. Biochim Biophys Acta 1825: 39-55, 2016.

42. Santos JH, Hunakova L, Chen Y, Bortner C and Van Houten B: Cell sorting experiments link persistent mitochondrial DNA damage with loss of mitochondrial membrane potential and accelerated cell death. J Cell Sci 127: 1728-1734, 2004.

43. Dirks AJ, Hofer T, Marzetti E, Pahor M and Leeuwenburgh C: Mitochondrial DNA mutations, energy metabolism and apoptosis in aging muscle. Ageing Res Rev 5: 179-195, 2006.

44. Al-Kafaji G, Sabry MA and Skrypnyk C: Time-course effect of high-glucose-induced reactive oxygen species on mitochondrial biogenesis and function in human renal mesangial cells. Cell Biol Int 40: 36-48, 2016.

45. Cha MY, Kim DK and Mook-Jung I: The role of mitochondrial DNA mutation on neurodegenerative diseases. Exp Mol Med 47: e150, 2015.

46. Brannell AE, Riordan-Eva P and Gogvann GG: Mitochondrial DNA diseases: Genotype and phenotype in Leber's hereditary optic neuropathy. Muscle Nerve Suppl 3: S82-S84, 1995.
ALHARBI et al: ND4 GENE MUTATIONS IN PATIENTS WITH MS

46. Bai Y, Hu P, Park JS, Deng JH, Song X, Chomyn A, Yagi T and Attardi G: Genetic and functional analysis of mitochondrial DNA-encoded complex I genes. Ann NY Acad Sci 1011: 272-283, 2004.

47. Malfatti E, Bugiani M, Invernizzi F, de Souza CF, Farina L, Carrara F, Lamantea E, Antozzi C, Confalonieri P, Sanseverino MT, et al: Novel mutations of ND genes in complex I deficiency associated with mitochondrial encephalopathy. Brain 130: 1894-1904, 2007.

48. Yu X, Koczan D, Sulonen A-M, Akkad DA, Kroner A, Comabella M, Costa G, Corongiu D, Goertxhes R, Camina-Tato M, et al: mtDNA m13708A variant increases the risk of multiple sclerosis. PLoS One 3: e1530, 2008.

49. Kellar-Wood H, Robertson N, Govan GG, Compston DA and Harding AE: Leber's hereditary optic neuropathy mitochondrial DNA mutations in multiple sclerosis. Ann Neurol 36: 109-112, 1994.

50. Fauser S, Luberichs J, Besch D and Leo-Kottler B: Sequence analysis of the complete mitochondrial genome in patients with Leber's hereditary optic neuropathy lacking the three most common pathogenic DNA mutations. Biochim Biophys Res Commun 295: 342-347, 2002.

51. Dogulu CF, Kansu T, Seyran tepe V, Ozguc M, Topaloglu H and Johns DR: Mitochondrial DNA analysis in the Turkish Leber's hereditary optic neuropathy population. Eye (Lond) 15: 183-188, 2001.

52. Lodi R, Montagna P, Cortelli P, Iotti S, Cevoli S, Carelli V and Barbieri B: 'Secondary' 4216/ND1 and 13708/ND5 Leber's hereditary optic neuropathy mitochondrial DNA mutations do not further impair in vivo mitochondrial oxidative metabolism when associated with the 11778/ND4 mitochondrial DNA mutation. Brain 123: 1896-1902, 2000.

53. Wong LJ, Liang MH, Kwon H, Park J, Bai RK and Tan DJ: Comprehensive scanning of the entire mitochondrial genome for mutations. Clin Chem 48: 1901-1912, 2002.

54. Rossignol R, Faustin B, Rocher C, Malgat M, Mazat J and Letellier T and Biochem J: Mitochondrial threshold effects. Biochem J 370: 751-762, 2003.

55. Wallace DC and Chalkia D: Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. Cold Spring Harb Perpert Biol 5: a021220, 2013.

56. Li M, Schönberg A, Schaefer M, Schroeder R, Nasidze I and Stoneking M: Detecting heteroplasmy from high-throughput sequencing of complete human mitochondrial DNA genomes. Am J Hum Genet 87: 237-249, 2010.

57. Payne BA, Wilson JJ, Yu-Wai-Man P, Coxhead J, Deehan D, Horvath R, Taylor RW, Samuels DC, Santibanez-Koref M and Chinnery PF: Universal heteroplasmy of human mitochondrial DNA. Hum Mol Genet 22: 384-390, 2013.

58. Casoli T, Spazzafumo L, Stefano G and Conti F: Role of diffuse low-level heteroplasmy of mitochondrial DNA in Alzheimer's disease neurodegeneration. Front Aging Neurosci 7: 142, 2015.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.