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Mitochondrial and nuclear genes as the cause of complex I deficiency

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ABSTRACT. Multiple sclerosis (MS) is an immunological inflammatory disease of the central nervous system. The pathogenesis of MS is incompletely understood, but various studies have suggested that mitochondrial dysfunction is associated with the disease. Mitochondria are among the main cellular sources of reactive oxygen and nitrogen species, and they play a pivotal role in many neuro-pathological conditions. The mitochondrial nuclear subunit of complex I gene in mitochondria may play a role in MS, and understanding this role may provide rationale for novel approaches to treatment of the disease and the development of novel therapies. We designed a molecular study to

demonstrate biochemical defects in complex I activity and found some novel nucleotide substitutions in mitochondrial DNA that might be involved in the pathogenesis of MS. The mitochondrial complex subunit I sequence was amplified and sequenced in MS patients. Although no reported pathogenic mutations were found in these patients, other studies have clearly indicated that the mitochondrial nuclear complex subunit I gene plays a significant role in MS pathogenesis.

Key words: Multiple sclerosis; Mitochondrial nuclear gene complex I; Pathogenic mutation

INTRODUCTION

Multiple sclerosis (MS) is a chronic disease of the central nervous system (Ghabaee et al., 2009) characterized by demyelization and devastation of axons (Mao and Reddy, 2010). The mitochondrion is an organelle that is present in all human cells (DiMauro and Schon, 2003; Kumleh et al., 2006). Mitochondria have an inner and an outer membrane. The respiratory chain, located in the inner membrane, is composed of 5 enzyme complexes (DiMauro and Schon, 2001; Zeviani and Di Donato, 2004). The respiratory chain is controlled by 2 separate genetic systems in the genomic and mitochondrial DNA (mtDNA). Respiratory complexes I, II, III, and IV contain both nuclear and mtDNA-encoded polypeptides. mtDNA is a small, closed circular and double-stranded molecule containing 37 genes, of which 24 are needed for translation (including 2 ribosomal RNAs and 22 transfer RNAs) and 13 encode subunits of the respiratory chain (DiMauro and Schon, 2001). Complex I, or nicotinamide adenine dinucleotide (NADH)-ubiquinone oxidoreductase, is composed of at least 46 subunits and its defects are the most frequent deficiencies of the respiratory chain (Smeitink et al., 2001). Lebers hereditary optic neuropathy is the mitochondrial disease in which mutations in mtDNA NADH genes were first detected (Fauser et al., 2002). Biochemical studies have established that catalytic activity of complex I in MS patients is significantly decreased compared with that in control subjects (Kumleh et al., 2006). Therefore, mutation of mitochondrial complex I subunit genes in MS patients may be an expected occurrence.

MATERIAL AND METHODS

We investigated 14 clinically diagnosed MS patients and 100 healthy normal controls. All of the patients were referred from the Iranian Center of Neurological Research and confirmed according to the McDonald criteria. The initial presenting symptom in the MS patients was optic neuritis. The Extended Disability Status Scale values of the patients ranged from 2.4 to 8. Patients ranged in age from 24 to 37 years, and the female/male ratio was 1.8:1.

Consent forms were signed by parents and probands, and then peripheral blood samples were obtained, DNA was purified using a DNA extraction kit (Diatom, Gene Fanavaran, Tehran, Iran), and mtDNA was amplified using the primers listed in Table 1. The polymerase chain reaction (PCR) solution contained 0.4 µL of each primer, 0.2 µL Super Taq polymerase (Invitrogen, USA), 0.8 µL $MgCl_2$, 0.4 µL master mix deoxyribonucleotide triphosphate, 10 mM of each deoxyribonucleotide triphosphate, and 2.5 µL 10X PCR buffer in a final volume of 25 µL. The PCR amplifications were performed using a BioRad thermal cycler (USA). The

amplified fragments were separated with gel electrophoresis on 1% agarose. Amplified products, including the mtDNA-complex I gene, were analyzed using a direct sequencing method. The Finch TV software was used for analysis of chromatograms, and bioinformatical analysis was carried out to detect sequence variations (PerkinElmer Company, USA).

Table 1. Mitochondrial primers for PCR-sequencing.

ND1	ONP82[F] 5'-CTC AAC TTA GTA TTA TAC CC-3'
	ONP84[R] 5'-GAG CTT AGC GCT GTG ATG AG-3'
ND2	ONP64[F] 5'-GTC ATC TAC TCT ACC TAC TT-3'
	ONP89[R] 5'-GGC GGG AGA AGT AGA TTG AA-3'
ND3	ONP91[F] 5'-CAC TAT CTG CTT CAT CCG CC-3'
	ONP94[R] 5'-GAG CGA TAT ACT AGT ATT CC-3'
ND4	ONP9[F] 5'-TCT CCA ACA CAT ATG GCC TA-3'
	ONP203[R] 5'-ACT GTG AGT GCG TTC GTT CGT AGT TTG AG-3'
	ONP14[F] 5'-GCG CAG TCA TTC TCA TAA TC-3'
	ONP46[R] 5'-TTT GTT AGG GTT AAC GAG GG-3'
ND4L	ONP93[F] 5'-TCT GGC CTA TGA GTG ACT AC-3'
	ONP203[R] 5'-ACT GTG AGT GCG TTC GTT CGT AGT TTG AG-3'
ND5	ONP11[F] 5'-TTT TGG TGC AAC TCC AAA-3'
	ONP74[R] 5'-GGT TGA CCT GTT AGG GTG AG-3'
	ONP21[F] 5'-GCA GTC TGC GCC CTA CA-3'
	ONP12[R] 5'-TCA GGG TTC ATT CGG GAG GA-3'
ND6	ONP204[F] 5'-CTC CAA AGA CCA CAT CAT CGA AAC-3'
	ONP318[R] 5'-TTC ATC ATG CGG AGA TGT TGG ATG GGG TGG-3'

RESULTS

To identify genetic causes of complex I activity decrease and investigate the association of complex I mutations with the pathogenesis of MS, we analyzed mtDNA-encoded complex I subunit genes. Several sequence variations in NADH dehydrogenase (ND) subunit, ND2, ND3, ND4, and ND6, were found. One patient had a 4143A>G variation in ND2, and 2 patients had a 10142C>T variation in ND3. Eight patients displayed nucleotide changes in ND4, including 1 patient with 11214C>T, 2 patients with 11343T>C, and 3 patients with 11934T>C (Table 2). In 2 patients, a novel variation in the ND4 gene was detected as 12062C>T. The other variation in ND4 was detected in 11 patients and was located at position 12662A>G. Finally, in 2 patients, 2 sequence variations in ND6 were detected: 14179G>A and 14263T>C. No alterations were found in the ND1, ND4L, and ND5 genes.

Table 2. List of mtDNA sequence variations in the MS patients studied.

No.	Subunit	Nucleotide position	Nucleotide change	N (%)
1	ND1	-	-	0
2	ND2	4143	A>G	1
3	ND3	10142	C>T	2
4	ND4	11214	C>T	1
		11343	T>C	2
		11934	T>C	3
		12062	C>T	2
		12662	A>G	11
5	ND4L	-	-	0
6	ND5	-	-	0
7	ND6	14179	G>A	1
		14263	T>C	1

DISCUSSION

Several studies have confirmed the hypothesis that mitochondria are involved in MS pathogenesis. We know that mtDNA changes play a central role in neurodegenerative diseases, and abnormalities in complex I subunits can lead to neurodegeneration and demyelization (Kumleh et al., 2006). Leber's hereditary optic neuropathy, which is caused by mtDNA mutations, is characterized by symptoms of inflammatory demyelization similar to those in MS (Yu et al., 2008). Additional evidence supports predominantly maternal transmission and a maternally inherited genetic factor in MS (Kumleh et al., 2006). Furthermore, haplotype analysis and restriction site polymorphism have revealed that mtDNA haplogroups K and J are associated with MS (Houshmand et al., 2005; Kumleh et al., 2006). Biochemical studies have shown that catalytic activity of complex I is significantly decreased in MS patients compared with that in control subjects (Kumleh et al., 2006). Although some sequence alterations including reported and novel variations were detected in mtDNA-encoded complex I subunit genes in this study, we found no reported pathogenic mutations. The novel nucleotide changes in mtDNA found in this study were screened in healthy normal controls and these may offer new variations related to MS pathogenesis, but functional analysis is necessary to confirm this association.

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