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Mitochondrial complex I gene variations; as a potential genetic risk factor in pathogenesis of multiple sclerosis

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ABSTRACT

Background and purpose: Multiple sclerosis (MS) is an autoimmune-mediated inflammatory and debilitating disease of the central nervous system. Several investigations have suggested that the mitochondrial DNA encoded subunits of complex I gene variations are involved in the progression of MS. In this study, we investigated the possible association between mitochondrial complex I gene variations and MS in a Filipino population.
Material and methods: A total of 300 individuals were included in the present study, two-hundred patients with MS clinical symptoms, and one-hundred healthy subjects without MS clinical features. We amplified target genes of mtDNA using polymerase chain reaction technique (PCR), and sequenced these to evaluate mitochondrial complex I gene variations.
Results: We found nine variations (Nt 4216 T > C, Nt 5153 A > G, Nt 10142 C > T, Nt 11353 T > C, Nt 11935 T > C, Nt 12062 C > T, Nt 13042 G > A, Nt 13708 G > A and Nt 14179 G > A) in mtDNA-encoded complex I subunit genes. Our results showed that the prevalence of ND1, ND2, ND3, ND4 and ND5 gene variations was significantly higher in patients than in healthy controls ($P < 0.0001$). Whereas, the frequency of Nt 14179 G > A variation in ND6 gene was significantly higher in the control group compared with the patients ($P < 0.0001$).
Conclusion: Taken together our data supports a strongly positive association between mitochondrial complex I gene variations and MS pathogenesis in a Filipino population.

1. Introduction

Multiple sclerosis (MS) is considered as a chronic inflammatory disease of the central nervous system (CNS) which is accompanied by demyelization and degeneration of axons [1]. MS is a multifactorial disorder caused by genetic and environmental factors. It seems that the role of genetic factors in the pathogenesis of MS far outweighs the environmental parameters [2]. The genetic background of MS is complicated, and both nuclear and mitochondrial genes are involved in MS development [3]. However, the precise pathophysiological mechanism of MS is still an unanswered enigma.

It has been established that the mitochondria can play fundamental roles in the pathogenesis of MS [4,5]. Mitochondria are present in all eukaryotic cells and are involved in various vital cellular processes including supplying cellular energy, programmed cell death, fatty acid oxidation, and calcium homeostasis [4]. They are consisted of extra-chromosomal genomes which are separate and distinct from the nuclear genome [6]. Mitochondrial DNA (mtDNA) consists of a circular double stranded DNA molecule and carries 37 genes, which are encoding 22 transfer RNAs (tRNAs), two ribosomal RNAs (rRNAs) and 13 polypeptides [7]. These polypeptides are part of the mitochondrial respiratory chain complexes. The respiratory chains are located in the inner mitochondrial membrane, and are consisted of five complexes (I–V) [8]. These complexes are made up of multiple subunits which are the majority of the subunits encoded by nuclear genome. Nicotinamide adenine dinucleotide (NADH)-ubiquinone oxidoreductase (complex I) is the largest of the respiratory chain complexes and is composed of at least 46 subunits [9,10]. Surprisingly, mtDNA encodes only seven of the 46 subunits in complex I (NADH dehydrogenase (ND)) [ND1, ND2, ND3, ND4, ND4L, ND5 and ND6]. Kinetic analysis of the mitochondrial respiratory chain complex I enzyme has suggested that the catalytic activity of complex I is significantly decreased in MS patients compared with healthy controls [10,11]. This finding revealed that complex I deficiency may contribute to disease susceptibility in MS. The mechanisms that cause the decrease of the catalytic activity of complex I are not very well understood. However, it has been suggested that low activity in complex I may result in mtDNA gene variations, nuclear gene mutations and acquired defect with toxins and free radicals [10].

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In this study, we addressed the question "Is there any association between mitochondrial complex I gene variations and pathogenesis of MS?" in Filipino MS patients.

2. Material and methods

2.1. Subjects

A total of two-hundred Filipino individuals (100 males; mean age, 34.03 ± 2.17 years and 100 females; mean age, 35.85 ± 3.39 years) with MS clinical symptoms were recruited from the MS Society of the Philippines and De La Salle University Medical Center (Cavite, Philippines), from 2011 to 2013. All patients were assessed according to the Poser et al. criteria for the diagnosis of MS [12]. MS clinical symptoms in the case group onset with vision problem or muscle weakness. One hundred of the MS patients had vision problem and others suffer from muscle weakness. In addition, one hundred healthy subjects (50 males; mean age, 34.48 ± 2.61 years and 50 females; mean age, 34.84 ± 2.88 years) without MS clinical features were recruited as the control group. The mean age of the subjects in the case and control groups was 34.94 ± 2.98 years (ranged between 30 and 40 years), and 34.66 ± 2.74 years (ranged between 31 and 42 years), respectively. This study was approved by the Ethics Committee of the De La Salle University of Medical Sciences and informed consent was obtained from all participants before enrolment.

2.2. DNA extraction, amplification and sequencing

Peripheral blood samples were taken from all participants and DNA extraction was carried out using a QIAamp DNA Micro Kit (Qiagen, USA), according to the manufacturer's instruction. The mtDNA-encoded complex I subunit genes were amplified in nine separate polymerase chain reactions (PCRs) using primers that are shown in Table 1 [11]. The PCR amplifications were carried out on a total volume of 25 μl solution containing 100 ng of genomic DNA, 10 pmol of each primers, 10 nmol of each deoxyribonucleotide triphosphates, 1.5 mmol of Mg^{2+}, 1× PCR buffer and 1 U of *Taq* polymerase. Initial denaturation at 94 °C for 5 min was followed by 35 cycles of denaturation at 95 °C for 40 s and annealing at temperatures that are mentioned in Table 1 for each primer pairs for 40 s, an extension at 72 °C for 90 s and a final extension at 72 °C for 7 min. The amplified products were separated by gel electrophoresis in 2% agarose. The amplified products including the mtDNA-complex I genes were analyzed using a direct sequencing method. All amplified fragments were sequenced in both forward and reverse directions for confirmation of detected variations. We used Chromas software to analyze the chromatograms and then the sequencing results were compared with the MITOMAP database, the Human Mitochondrial Genome Database (http://www.genpat.uu.se/mtDB/UppsalaUniversity) and GenBank (http://www.ncbi.nlm.nih.gov/GenBank/index.html/NIH, Bethesda, MD).

2.3. Statistical analysis

Statistical analysis was performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). The chi-squared (χ^2) and Fisher's exact tests were used for comparison of gene variation frequencies among the case and control groups. *P* values less than 0.05 were statistically considered as significant.

3. Results

We analyzed mtDNA-encoded complex I subunit gene variations using direct sequencing technique. In general, we found nine variations in mitochondrial complex I genes [ND1 (Nt 4216 T > C), ND2 (Nt 5153 A > G), ND3 (Nt 10142 C > T), ND4 (Nt 11353 T > C, Nt 11935 T > C and Nt 12062 C > T), ND5 (Nt 13042 G > A and Nt 13708 G > A), and ND6 (Nt 14179 G > A)].

The compared frequencies of the mitochondrial complex I gene variations between the case and control groups are displayed in Table 2. Our results indicated that the prevalence of ND1, ND2, ND3, ND4 and ND5 gene variations was significantly higher in patients than in healthy controls ($P < 0.0001$) (Table 2). Surprisingly, we failed to detect Nt 4216 T > C, Nt 10142 C > T, Nt 11353 T > C, Nt 11935 T > C and Nt 13708 G > A variations in the control group. Our findings revealed that the frequency of the Nt 14179 G > A variation in the ND6 gene was 13% in the control group, whereas none of the two-hundred MS patients carried this variation. Therefore, this difference was statistically significant ($P < 0.0001$) (Table 2).

When the frequencies of the mtDNA-encoded complex I subunit gene variations were compared between the males and females of the case group, we observed that the Nt 12062 C > T variation in the females of the case group was significantly higher than that in males ($P < 0.0001$) whereas, there is no significant difference in the prevalence of remnant variations between males and females of the case group (Table 3).

We detected only four variations (Nt 5153 A > G, Nt 12062 C > T, Nt 13042 G > A and Nt 14179 G > A) in the control group. Our results showed that the prevalence of these variations between males and females of the control group has no statistically significant differences ($P > 0.05$) (Table 4).

Table 1
Nucleotide sequence of primers used for amplification of mitochondrial complex I genes.

Gene	Sequence of primers	Annealing temperature (°C)	PCR product (bp)
ND1	F 5-CTCAACTTAGTATTATACCC-3 R 5-GAGCTTAGCGCTGTGATGAG-3	57	1353
ND2	F 5-GTCATCTACTCTACCTACTT-3 R 5-GGCGGGAGAAGTAGATTGAA-3	59	1249
ND3	F 5-CACTATCTGCTTCATCCGCC-3 R 5-GAGCGATATACTAGTATTCC-3	52	689
ND4	F 5-TCTCCAACACATATGGCCTA-3 R 5-ACTGTGAGTGCGTTCGTAGTTTGAG-3	54	1065
	F 5-GCGCAGTCATTCTCATAATC-3 R 5-TTTGTTAGGGTTAACGAGGG-3	54	729
ND4L	F 5-TCTGGCCTATGAGTGACTAC-3 R 5-ACTGTGAGTGCGTTCGTAGTTTGAG-3	54	1415
ND5	F 5-TTTTGGTGCAACTCCAAA-3 R 5-GGTTGACCTGTTAGGGTGAG-3	50	1369
	F 5-GCAGTCTGCGCCCTACA-3 R 5-TCAGGGTTCATTCGGGAGGA-3	54	1079
ND6	F 5-TTCATCATGCGGAGATGTTGGATGGGGTGG-3 R 5-CTCCAAAGACCACATCATCGAAAC-3	52	1334

Table 2
The frequencies of mitochondrial complex I gene variations in case and control groups.

Variation	Gene	Frequency of variation in the case group (%)	Frequency of variation in the control group (%)	*P*-value[a]	OR	95% CI
Nt 4216 T > C	ND1	34.5	0	0.000	0.655	0.592–0.724
Nt 5153 A > G	ND2	31.5	7	0.000	0.164	0.072–0.373
Nt 10142 C > T	ND3	10	0	0.000	0.900	0.859–0.943
Nt 11353 T > C	ND4	28.5	0	0.000	0.715	0.655–0.780
Nt 11935 T > C	ND4	7.5	0	0.003	0.925	0.889–0.962
Nt 12062 C > T	ND4	48.5	16	0.000	0.202	0.111–0.369
Nt 13042 G > A	ND5	44	2	0.000	0.026	0.006–0.108
Nt 13708 G > A	ND5	11.5	0	0.000	0.885	0.842–0.930
Nt 14179 G > A	ND6	0	13	0.000	1.149	1.066–1.240

Abbreviations: Nt, nucleotide; ND, NADH dehydrogenase; OR, odds ratio; CI, confidence intervals.
[a] Evaluated by Fisher's chi-squared test.

Finally, when the frequencies of complex I subunit gene variations were compared in males of both groups, we found that the Nt 5153 A > G and Nt 13042 G > A variations in males of the patient group were significantly higher than those in the males of the control group ($P < 0.0001$) (Table 5). In comparing the Nt 5153 A > G, Nt 12062 C > T, Nt 13042 G > A and Nt 14179 G > A variation frequencies between females of the case and control groups, we detected that the Nt 5153 A > G, Nt 12062 C > T and Nt 13042 G > A variations in the females of the patient group were significantly higher than those in the females of the control group ($P < 0.0001$) (Table 5).

4. Discussion

Increasing evidences suggest that the mitochondria may play a fundamental role in the pathogenesis of neurodegenerative diseases such as MS, and Alzheimer's and Parkinson's diseases [13–17]. The mtDNA variations which lead to impaired mitochondrial respiratory chain functioning could decrease ATP production, increase formation of toxic free radicals, and alter Ca^{+2} homeostasis [18]. The mitochondrial respiratory chain dysfunction may result in further mitochondrial damage, including oxidation of mitochondrial DNA, proteins, and lipids, and opening of the mitochondrial permeability transition pore, an event associated with cell degeneration and death [18–20]. Recently, several genetic association studies have suggested a significant association between MS and mtDNA variations, especially for respiratory chain complex I genes [3,11,21]. Additionally, biochemical analysis of mitochondrial respiratory chain complex I activity has indicated that the catalytic activity of this complex is reduced in MS patients [10]. Hence, we investigated the possible association between mtDNA-encoded complex I gene variations and MS. This is the first report to study the prevalence of mtDNA-encoded subunits of respiratory NADH dehydrogenase in a Filipino population with MS.

Our results revealed that the prevalence of the ND1 (Nt 4216 T > C), ND2 (Nt 5153 A > G), ND3 (Nt 10142 C > T), ND4 (Nt 11353 T > C, Nt 11935 T > C and Nt 12062 C > T) and ND5 (Nt 13042 G > A and Nt 13708 G > A) variations were significantly higher in MS patients than in healthy controls ($P < 0.0001$). The Nt 14179 G > A variation in the ND6 gene was only detected in the control group. Some of these variations have been previously reported in several populations. A study performed by Mihailova et al. in a Bulgarian population clarified that there is an evidence to support the association of Nt 4216 T > C variation with MS [22]. This finding confirms our results. In contrast, in a study by Ban et al., the frequency of the 4216C allele was reported in 24.3% and 27.2% of cases and controls, respectively [3]. Kumleh et al., investigated the prevalence of mtDNA-encoded complex I gene variations in 14 patients with MS and 100 healthy controls and found that two patients have an Nt 10142 C > T variation in the ND3 gene. They also reported that two cases have an Nt 12062 C > T variation in the ND4 gene [11]. Similarly, our findings showed that the prevalence of these variations was higher in the case group. In accordance with our data, Yu et al., reported that the Nt 13708 G > A variation is more prevalent in patients than in healthy controls [21]. The Nt 14179 G > A variation in ND6 was detected in one patient in the Kumleh et al. study [11] whereas none of the two-hundred MS patients carried this variation.

With respect to the result of studies mentioned above, it seems that some of the mitochondrial complex I gene variations may be associated with the pathogenesis of MS. However, in this study, we detected novel variations in mtDNA-encoded complex I subunit genes and further studies are needed to confirm the association of these novel variations with MS. On the other hand, the frequencies and types of the mtDNA-encoded subunits of NADH dehydrogenase gene variations are different in various studies. This may be a consequence of racial differences of populations and/or differences in number of studied subjects.

Regarding to all of the above arguments about the relation between mitochondrial complex I gene variations and MS, our results confirm such association. These findings indicate that the variations may increase MS susceptibility. The importance of mtDNA variations as a potential molecular etiology for complex I deficiency was not in doubt. So, it seems that its screening is essential in the genetic diagnosis of complex I deficiency.

Conflict of interest

There are no potential conflicts of interest for each author concerning the submitted manuscript.

Table 3
Comparison of the frequencies of mitochondrial complex I gene variations between men and women of the case group.

Variation	Gene	Frequency of variation in case group males (%)	Frequency of variation in case group females (%)	*P*-value[a]	OR	95% CI
Nt 4216 T > C	ND1	33	36	0.766	1.142	0.637–2.047
Nt 5153 A > G	ND2	31	32	1.000	1.047	0.577–1.902
Nt 10142 C > T	ND3	13	7	0.238	0.504	0.192–1.321
Nt 11353 T > C	ND4	31	26	0.531	0.782	0.422–1.448
Nt 11935 T > C	ND4	4	11	0.105	2.966	0.911–9.655
Nt 12062 C > T	ND4	34	63	0.000	3.305	1.851–5.901
Nt 13042 G > A	ND5	36	52	0.032	1.926	1.093–3.393
Nt 13708 G > A	ND5	8	15	0.183	2.029	0.819–5.028

Abbreviations: Nt, nucleotide; ND, NADH dehydrogenase; OR, odds ratio; CI, confidence intervals.
[a] Evaluated by Fisher's chi-squared test.

Table 4
Comparison of the frequencies of mitochondrial complex I genes variations between men and women of control group.

Variation	Gene	Frequency of variation in control group males (%)	Frequency of variation in control group females (%)	*P*-value[a]	OR	95% CI
Nt 5153 A > G	ND2	4	10	0.436	2.667	0.492–14.445
Nt 12062 C > T	ND4	20	6	0.414	0.545	0.182–1.637
Nt 13042 G > A	ND5	4	0	0.495	0.960	0.907–1.016
Nt 14179 G > A	ND6	8	18	0.234	2.524	0.723–8.818

Abbreviations: Nt, nucleotide; ND, NADH dehydrogenase; OR, odds ratio; CI, confidence intervals.
[a] Evaluated by Fisher's chi-squared test.

Table 5
Comparison of the frequencies of mitochondrial complex I genes variations between men and women of both group.

Variation	Gene	Male			Female		
		Frequency in the case group	Frequency in the control group	*P*-value[a]	Frequency in the case group	Frequency in the control group	*P*-value[a]
Nt 5153 A > G	ND2	31	4	0.000	32	10	0.000
Nt 12062 C > T	ND4	34	20	0.038	63	6	0.000
Nt 13042 G > A	ND5	36	4	0.000	52	0	0.000
Nt 14179 G > A	ND6	0	8	0.007	0	18	0.000

Abbreviations: Nt, nucleotide; ND, NADH dehydrogenase; OR, odds ratio; CI, confidence intervals.
[a] Evaluated by Fisher's chi-squared test.

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