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Association of Common Mitochondrial DNA Variants with Multiple Sclerosis and Systemic Lupus Erythematosus

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Abstract

Mitochondrial dysfunction has been implicated in the pathogenesis of multiple sclerosis (MS) and systemic lupus erythematosus (SLE). This study re-investigates the roles of previously suggested candidate genes of energy metabolism (Complex I genes located in the nucleus and in the mitochondria) in patients with MS relative to ethnically matched SLE patients and healthy controls. After stringent correction for multiple testing, we reproduce the association of the mitochondrial (mt) DNA haplotype K* with MS, but reject the importance of previously suggested borderline associations with nuclear genes of Complex I. In addition, we detect the association of common variants of the mitochondrial ND2 and ATP6 genes with both MS and SLE, which raises the possibility of a shared mitochondrial genetic background of these two autoimmune diseases.

Keywords

Multiple Sclerosis; Systemic Lupus Erythematosus; Candidate genes; mitochondrial genes

1. INTRODUCTION

Mitochondrial genes and proteins have been investigated in both MS and SLE. Mitochondrial dysfunction resulting in elevation of the mitochondrial trans-membrane potential and depletion of ATP was found to predispose to pro-inflammatory necrosis rather than physiological apoptosis in circulating T lymphocytes of patients with SLE [1,2]. Genes involved in mitochondrial electron transport are over-expressed in lupus T cells [3]. In MS, mitochondria have been implicated in neurodegeneration, and mtDNA markers consistently showed association with the disease [4–8]. Complex I is transcribed from 7 mitochondrial (mt)DNA and 38 nuclear (n)DNA encoded genes. By complete sequencing and high resolution Single

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Nucleotide Polymorphism (SNP) scans, we identified MS-associated missense polymorphic mutations (i.e. Leber's Hereditary Optic Atrophy - LHON mutations) within the mtDNA-encoded ND1 (nt4216), ND3 (nt10,398) and ND5 (nt13,708) subunits of Complex I, and in the cytochrome b (nt14,798) gene. These variants align in haplotypes J* and K* in the Caucasian haplogroups J and K [5,6]. In addition, our previous data suggested that MS families may express susceptibility-defining nDNA and mtDNA SNP combinations in Complex I [9].

In the present study, we reinvestigated if the associations of Complex I nuclear and mitochondrial SNPs can be reproduced in a large case-control MS cohort and if these markers also show association with SLE.

2. SUBJECTS AND METHODS

Subjects

All samples used in this study were collected according to previous and signed consent of patients and control subjects. This study was approved by the Institutional Review Boards (IRB) of Upstate Medical University and of the Syracuse Veterans Administration Medical Center and the Multiple Sclerosis Research Center of New York (MSRCNY). Collections of the specimens by the repositories were approved by the local IRB committees.

MS patients—We obtained genomic DNA, blood or frozen tissue specimens of 523 MS patients from the Multiple Sclerosis DNA Bank (MSDB), University of California San Francisco, San Francisco, CA, Accelerated Cure Project for Multiple Sclerosis (ACPMS, Boston, MA), Multiple Sclerosis Research Center of New York (MSRCNY, New York, NY), and the Human Brain and Spinal Fluid Resource Center (HBSFRC, Los Angeles, CA). All patients were Caucasians and had definite MS [10]. Sixty-one percent of patients had relapsing-remitting (RR)-MS, 10% primary-progressive (PP)-MS, 23% secondary progressive (SP)-MS, and 1% progressive-relapsing (PR)-MS course. The course of MS, age of onset (defined by the physician), age at diagnosis of MS, EDSS at the time of specimen collection, time to reach maximum EDSS (including time to death EDSS=10) from disease onset, and medications during the disease course were defined for each MS patient.

SLE patients—Genomic DNA of 97 patients with SLE that satisfied the established diagnostic criteria [11] was investigated. All patients were Caucasians. Normal controls: Buccal swab specimens of 466 Caucasian healthy controls were obtained from staff members, spouses of MS patients and constructors working at the MSRCNY, New York. Specimens from these patients and controls were included in a parallel study [12].

Demographics—The average ages of patients and controls were similar (MS patients: 46.5; SLE: 44.5; controls: 43.2). The mean age for disease onset was 35.2 years in MS and 33.8 years in SLE. The female to male ratio was 2.35 (367/156) in the MS cohort; 12.9 (90/7) in the SLE cohort and 2.61 (313/120) in controls.

Mild and severe subgroups of MS

We investigated the distributions of patients based on EDSS over time, and identified two subgroups of patients: 1. Mild with an EDSS \leq 4 at 10 years after onset and 2. Severe with an EDSS \geq 6 at 10 years after onset. The mild subgroup included 105 and the severe subgroup included 75 patients.

DNA extraction

The Qiagen Blood DNA Mini Kit (Qiagen, Valencia, CA) was used for DNA extraction according to the manufacturer's instruction.

Single Nucleotide Polymorphism (SNP) genotyping—Most SNPs have been previously tested using various techniques and showed association with MS in our previous studies [5,6,9]. Presently, 9 markers within mtDNA (SNPs at nt1719, nt4216, nt4529, nt4917, nt9055, nt10398, nt13708, 14798 and nt16391) and 7 markers within nDNA encoded genes of Complex I were validated and tested (2 SNPs in NDUFS5 - 1p34.2-p33; 3 SNPs in NDUFS7 - 19p13; 2 SNPs in NDUFA7 - 19p13) [6,9].

In this study, SNPs were identified by using the Sequenom MassARRAY™ System through a Cogenics division of Clinical Data Inc. contract.

Analyses—For associations of marker alleles and haplotypes, the χ^2 -test was used. Bonferroni correction was performed for multiple testing. The Complex I nDNA markers are encoded on different chromosomes, thus LD did not affect the analysis.

3. Results

Association of mtDNA markers and haplotypes with MS and SLE

Table I shows associations of mtDNA markers with MS and SLE. All markers with $p < 0.05$ are indicated. A total of 9 mtDNA markers were tested in two groups, requiring an uncorrected α -value of 0.0056 for a significant observation. The nt4917 and nt9055 variants in MS, and the nt9055 variant in SLE remain significant after Bonferroni correction.

Table II indicates association of mtDNA haplotypes J* and K* with MS and SLE. Only two haplotypes were tested, requiring an α -value of 0.025. The K* haplotype shows association with MS after correction for multiple testing.

Association of marker alleles and haplotypes with severity of MS

None of the investigated nDNA and mtDNA markers, or mtDNA haplotypes segregated differentially with severity subgroups of MS. Comparisons of marker alleles and haplotypes in the PP-MS/PR-MS vs. RR-MS/SP-MS subgroups were also unrevealing.

4. DISCUSSION

Multiple sclerosis is characterized by significant heterogeneity involving inflammation and neurodegeneration [13,14]. Neurodegeneration is the best correlate of clinical disability [15], nevertheless, only a few studies investigated genetic determinants of it. We have been studying mitochondrial mechanisms underlying cell and tissue integrity, and also reported genetic variants of Complex I likely involved in neurodegeneration in MS [5,6,8,9]. The previous mtDNA studies in several Caucasian case-control cohorts consistently revealed polymorphic markers (including LHON polymorphisms and a cytochrome b variant) and haplotypes (J*, K*) in association with MS [5,6,9], and established that primary pathogenic LHON mutations occur, but rarely in MS [16,17]. Here we aimed to conclude our analyses concerning these candidate markers in a larger cohort of MS, and to generate comparative data in another autoimmune disease, SLE.

Present findings in MS

1. Nuclear markers of Complex I genes tested in MS—In the present cohort of 523 MS patients and 466 controls, we only detected weak p-values for associations with previously highlighted nuclear Complex I SNP markers and haplotypes, which all became non-significant after Bonferroni correction. If we assume a genotypic relative risk of 1.3 for heterozygous and 1.8 for homozygous state of the high risk allele with 0.20 frequency, the power to detect $p = 0.05$ as 73%, while for risk alleles with 0.25 frequency we had 80% power and for risk alleles with

0.05 or lower frequency we had only 30% power [18]. As most of the markers had an allele frequency of 0.2 or higher, we had sufficient power to exclude any associations between SNPs in nuclear Complex I genes and MS. This conclusion is also in agreement with the outcome of the recent WGA study that revealed no significant p-values in the proximity of our Complex I nDNA markers [19].

2. mtDNA variants in MS—The previously identified mtDNA K* haplotype defined by variants at nts 9055, 10,398 and 14,798 showed again strong association with MS in the present study [6]. Individual marker associations were detected at nt9055 of the ATP6 gene (Complex V) and at nt4917 of the ND2 gene (Complex I). In contrast to previous findings, only a trend for increase but not a significant association was found for haplotype J* in MS, which might be related to a different sub-ethnic composition of the present and past Caucasian cohorts. The nt4917 variant with the nt4216 variants (secondary LHON mutations) align in the Caucasian haplogroup T that shares a common ancestry with haplogroup J [20].

Among variants defining the K* haplotype, the A→G transition at nt9055 changes alanine to threonine in the ATP6 subunit of complex V or F₀F₁-ATPase, while the A→G transition at nt10,398 changes threonine to alanine in the ND3 subunit of Complex I [6]. Their functional significance is unknown. The T→C transition at nt14,798 changes phenylalanine to leucine in cytochrome b (subunit of Complex III). This variant is located at the inner CoQ-binding site of the enzyme and reduces proton pumping and coupling efficiency [21]. Therefore, it is tempting to postulate that variants (particularly nt14,798) in the K* haplotype cause an increased endogenous free radical production, which along with the inflammation-related free radical and nitric oxide release, synergistically contribute to the observed oxidative damage, impaired OXPHOS activity and neuroaxonal loss in MS lesions [8,21,22].

3. Candidate markers in subgroups of MS—Testing the distribution of all nuclear and mitochondrial Complex I markers in the PP-MS/PR-MS vs. RR-MS/SP-MS subgroups resulted in no significant observations. Likewise, no differences in marker distribution were noted when EDSS-based mild and severe subgroups of MS were compared.

Findings in SLE

None of the tested SNPs in nuclear genes of Complex I showed association with SLE. Similarly, no mtDNA haplotype association was detected in this patient cohort. The borderline association at nt4917 (secondary LHON mutation) requires testing in larger cohorts. However, the association of the variant at nt9055 in the ATP6 or F₀F₁-ATPase gene (Complex V) with SLE remains significant even after correction for multiple testing. Since inhibition of F₀F₁-ATPase by oligomycin elicits mitochondrial hyperpolarization and ATP depletion [1], and thus, mimics the changes persistently observed in lupus T cells [1,23,24], it is conceivable that the alanine→threonine change in this enzyme could influence the pathophysiology of SLE.

In summary, in the present follow up study, we confirmed the association of the mtDNA K* haplotype with MS and excluded the importance of nuclear Complex I variants. This study also suggests that the observed intra-familial recurrence of MS and SLE [25–27] may not only be related to common immune regulatory genes, but also to shared variants in mtDNA.

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Table I

Association of mtDNA markers with multiple sclerosis (MS) and systemic lupus erythematosus SLE. All $p < 0.05$ are indicated. After correction for multiple testing, significant associations are observed for the mtDNA nt9055 variant of the ATP6 gene (in haplogroup K) in both MS and SLE, and for the nt4719 variant of the ND2 gene (in haplogroup T) in MS.

	Gene	ND2	ATP6	CYTb
	Marker	Mt4917	mt9055	mt14798
MS-control	χ^2	7.56	34.7	4.76
	p	0.006	<0.0001	0.029
Associated variant		G(A/G)	A(G/A)	C(T/C)
Freq. in controls/MS		0.876/0.928	0.024/0.133	0.151/0.205
SLE-control	χ^2	11.8	7.45	0.31
	p	0.033	0.006	0.576
Associated	*	G(A/G)	A(G/A)	C(T/C)
Freq. in controls/SLE	*	0.876/0.970	0.025/0.089	0.849/0.872

Table II Association of mtDNA haplotypes with multiple sclerosis (MS) and systemic lupus erythematosus (SLE).

Haplo type	Markers	Variants	Haplotype frequency in controls/		MS-control		SLE-control	
			MS	SLE	χ^2	p	χ^2	p
K*	mt9055, mt10398, mt14798	AGC	0.018/0.085	0.0180/0.033	20	<0.0001	0.75	0.386
J*	mt10398, mt13708, mt14798	GAC	0.050/0.063	0.050/0.023	0.81	0.37	1.20	0.274