The combined effect of nuclear and mitochondrial genomes on the risk of developing multiple sclerosis

Kozin M.S.[1–3], Kiselev I.S.[1,3], Boyko A.N.[1,2], Kulakova O.G.[1,3], Favorova O.O.[1,3]

[1]Department of Neurology, Neurosurgery, and Medical Genetics, N.I. Pirogov Russian National Research Medical University, Ministry of Health of Russia, Moscow; [2]Department of Neuroimmunology, Federal Center for the Brain and Neurotechnologies, Federal Biomedical Agency of Russia, Moscow;

[3]National Medical Research Center for Cardiology, Ministry of Health of Russia, Moscow

[1]1, Ostrovityanov St, Moscow 117997, Russia; [2]1, Ostrovityanov St, Build. 10, Moscow 117997, Russia;

[3]15A, Third Cherepkovskaya St., Moscow 121552, Russia

Multiple sclerosis (MS) is a severe chronic CNS disease characterized by autoimmune inflammation, demyelination, and neurodegeneration. The interaction of mitochondrial and nuclear genomes is shown to be important in the formation of a predisposition to many diseases.

Objective: *to analyze the association of MS with the carriage of biallelic combinations, including as components the polymorphisms of three genes of mitochondrial DNA (mtDNA) and those of 16 nuclear genes, the products of which are involved in the functioning of the immune system and may participate in the development of autoimmune inflammation in MS; and, if these combinations are identified, to determine the nature of an interaction between their components.*

Patients and methods. *The investigation enrolled 540 MS patients and 406 control group individuals; all were Russians. The mitochondrial genome was genotyped by polymerase chain reaction-restriction fragment length polymorphism analysis. APSampler software was used for multilocus association analysis.*

Results and discussion. *The investigators identified five biallelic combinations that were associated with MS (p=0.0036–0.022) and possessed protective properties (odds ratio (OR) 0.67–0.75). The mitochondrial component of the identified combinations was the polymorphisms m.4580 (rs28357975), m.13368 (rs3899498), and m.13708 (rs28359178) mtDNA; the nuclear component was CXCR5 (rs523604), TNFRSF1A (rs1800693), and CD86 (rs2255214) gene polymorphisms. The interaction between the components of the identified combinations was additive.*

Conclusion. *The data obtained in the Russian population suggest that the combined contribution of the mitochondrial and nuclear genomes may affect the risk of developing MS.*

Keywords: *multiple sclerosis; genetic polymorphism; mitochondrial genome; nuclear genome; association analysis; multilocus analysis.*

Contact: *Maksim Sergeevich Kozin;* ***kozinmax1992@gmail.com***

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Multiple sclerosis – (MS) is a chronic disease of the central nervous system (CNS) characterized by the processes of autoimmune inflammation, demyelination, and neurodegeneration leading to the CNS damage and progressive neurological dysfunction [1]. MS is an important social problem; according to the World Health Organization (WHO), there are about 2.5 million MS patients in the world. MS is a complex polygenic disease that develops in genetically predisposed individuals exposed to adverse environmental factors. The polygenic type of inheritance implies that there are many independent or interacting polymorphic gene variants each of which can only slightly affect the predisposition to MS.

In the course of genome-wide association studies, more than 200 independent nuclear loci associated with the development of MS were identified by means of the genome-wide association study (GWAS) [2]. At the same time, the aggregate variability of all loci found cannot explain more than 48% of the observed heritability of MS [2]. This discrepancy is called "missing heritability" [3].

One possible explanation for the phenomenon of "missing heritability" may be an unrecorded contribution of mitochondrial genome variability to MS predisposition. Indeed, although the importance of mitochondrial dysfunction in the development of neurodegeneration in MS has been reliably established [4], the association of mitochondrial DNA (mtDNA) variability with MS requires further clarification. To date, about 20 studies have been conducted to analyze the association of MS with variants of the mitochondrial genome – both individual polymorphisms and haplogroups, but the results are contradictory [5].

Another explanation for the phenomenon of "missing heritability" may be the existence of nonlinear (epistatic) interactions between the components of the genome, in which the effect of carrying one genetic variant depends on the carriage of one or several other non-allelic variants [6], since such interactions are not detected when analyzing the association of genes with the disease separately.

Both causes of "missing heritability" described above can have a cumulative effect. Indeed, the development of MS or other polygenic disease may be associated with the carriage of a specific variant of mtDNA against the background of a specific nuclear genome. The effect of such mitochondrial-nuclear interactions on the phenotypic characteristics of organisms has been shown in many studies. Adverse combinations of mitochondrial and nuclear genomes have been described for both invertebrates and vertebrates, leading to a decrease in the rate of development, viability and fertility [7]. Data on the importance of combinations of mitochondrial and nuclear genomes were also obtained in the studies of human genetics. It has been established that in the human genome there is linkage disequilibrium between certain variants of mitochondrial and nuclear DNA; since the mitochon-

drial and nuclear genomes are not physically linked, this can be explained by selection of favorable combinations [8]. In the study of mixed human populations, it was found that the number of mtDNA copies in cells decreases with increasing differences between the origin of nuclei and mtDNA [9]; the authors believe that this indicates insufficient regulation of mtDNA replication processes if the mitochondrial and nuclear genomes come from different populations.

The importance of interactions between individual mitochondrial and nuclear loci for phenotype manifestation is shown for some mitochondrial [10], neurodegenerative [11], and mental [12] human diseases. The only published work that examined the effect of mitochondrial-nuclear interactions on the risk of MS was conducted by our team on a relatively small sample (283 MS patients and 290 healthy individuals in the control group) and included five mtDNA polymorphisms [13]. This study is a continuation of this work. We used an extended sample of ethnic Russians to study MS associations with combinations that include as components three previously unknown mtDNA variants: m.4580 (rs28357975), m.13368 (rs3899498), m.13708 (rs28359178) and 16 variants of nuclear genes involved in the functioning of the immune system: CD58 (rs2300747), VCAM1 (rs7552544), EVI5 (rs11804321), CTLA4 (rs231775), CCR5 (rs333), cd86 (rs2255214), tcf7 (rs756699), il17a (rs2275913), il2ra (rs2104286), cxcr5 (rs523604), tnfrsf1a (rs1800693), irf8 (rs17445836), ccl5 (RS2107538), STAT3 (rs744166), tnfsf14 (rs1077667), TGFB1 (rs1800469). These nuclear genes were selected as candidates based on the idea that their protein products may be involved in the development of autoimmune inflammation in MS. The frequency of carriage of alleles and genotypes of nuclear genes in the study sample were described earlier [14]. For the identified combinations associated with the development of MS, we evaluated the possibility of epistatic interaction between their components.

Patients and methods. The study involved 540 patients with remitting MS (375 women and 165 men, mean age 38.5±10.6 years) and 406 healthy individuals (266 women and 140 men, mean age 43.5±16.2 years) without signs of neurological disorders (control group). All patients were treated at the Moscow Center for Multiple Sclerosis or the Moscow Inter-District Department of Multiple Sclerosis in Moscow State Medical Center No. 24. MS was diagnosed in accordance with the McDonald criteria (2010) [15]. The study included participants of Russian ethnicity (according to the survey, their parents were Russian) living in the Moscow region. Demographic and clinical characteristics of MS patients and healthy individuals are presented in Table 1.

DNA isolation and genotyping. Genomic DNA was isolated from peripheral blood using commercial QIAamp DNA blood Mini Kit (QIAGEN, Germany). Polymerase chain reaction with subsequent analysis of restriction fragment length polymorphism (PCR-pdrf) was used for genotyping of mitochondrial genome polymorphisms m.4580 (rs28357975), m.13368 (rs3899498), m.13708 (rs28359178). PCR was performed in T100 amplifiers (BioRad, USA) or Tertsik (DNA-Technology, LLC, Russia). We used components of PCR mixtures and oligonucleotide primers (Eurogen, Russia), and restriction endonucleases (SibEnzim, Russia). The conditions for PCR-pdrf are presented in Table. 2. The methods of genotyping for 16 variants of nuclear genes on the basis of PCR were described earlier [14].

Statistical analysis. To search for polymorphic variants of the mitochondrial genome and biallelic combinations associated

Table 1. Demographic and clinical characteristics of MS patients and healthy individuals

Parameters	MS (n=540)	Controls (n=406)
Females/Males	2.3/1	1.9/1
Mean age, years, M±SD	38.5±10.6	43.5±16.2
Mean age of MS onset, years, M±SD	27.3±9.2	–
Mean MS duration, years, M±SD	11.2±7.4	–
Mean EDSS, M±SD	2.8±1.4	–
Mean MSSS, points, M±SD	3.9±1.9	–

MSSS - Multiple Sclerosis Severity Scale, EDSS - Expanded Disability Status Scale.

Table 2. Conditions for PCR-pdrf for mtDNA polymorphism

mtDNA polymorphism	Direct and reverse PCR primer	t, °C	Restriction endonuclease	Size of fragments after restriction
m.4580	ACCTATCACACCCCATCCTAAA/ AGGATTATGGATGCGGTT	60	BmtI	G: 200+100 A: 300
m.13368	TAGCCTTCTCCACTTCAAGTC/ AGAAACCTGTAGGAAAGGTATT	58	AspS9I	G: 137+127 A: 264
m.13708	CCTCACAGGTTTCTACTCCAAA/ CCTCACAGGTTTCTACTCCAAA	60	Fsp4HI	G: 197+121 A: 318

Table 3. Allele frequencies of mtDNA in patients with MS and healthy individuals

Allele	Carriers, n (%)		p	OR [95% CI]
	MS (n=540)	Controls (n=406)		
m.4580*A	23 (4.3)	10 (2.4)	0.094	1.76 [0.84–3.60]
m.4580*G	517 (95.7)	396 (97.6)	0.094	0.57 [0.28–1.18]
m.13368*A	57 (10.6)	37 (9.1)	0.27	1.18 [0.76–1.83]
m.13368*G	483 (89.4)	369 (90.1)	0.27	0.85 [0.55–1.31]
m.13708*A	61 (11.3)	35 (8.6)	0.11	1.35 [0.88–2.08]
m.13708*G	479 (88.7)	371 (91.4)	0.11	0.74 [0.48–1.14]

Table 4. Allelic combinations associated with MS that include variants of mtDNA and nuclear genes (according to multilocus analysis)

Allelic combinations	Carriers, n (%)		p	OR [95% CI]
	MS (n=540)	Controls (n=406)		
m.4580 (rs28357975)*G + *CXCR5* (rs523604)*G	335 (62.0)	283 (69.7)	0.0084	0.71 [0.54–0.93]
m.13708 (rs28359178)*G + *TNFRSF1A* (rs1800693)*T	354 (65.6)	300 (73.9)	0.0036	0.67 [0.51–0.89]
m.13368 (rs3899498)*G + *CD86* (rs2255214)*T	311 (57.6)	261 (64.3)	0.022	0.75 [0.58–0.98]
m.13368 (rs3899498)*G + *TNFRSF1A* (rs1800693)*T	355 (65.7)	300 (73.9)	0.0043	0.68 [0.51–0.90]
m.4580 (rs28357975)*G + *TNFRSF1A* (rs1800693)*T	378 (70.0)	315 (77.6)	0.0054	0.67 [0.50–0.90]

with the risk of MS development, which, in addition to variants of the mitochondrial genome, include variants of nuclear genes, a multilocus analysis of the association was performed using APSampler software. The association was considered reliable if the p value for the allele carriage or combination was >0.05, and the 95% confidence interval (CI) for the odds ratio (OR) did not cross 1. The nature of interaction (epistatic or additive) between the components of the combinations was evaluated using an approach based on calculating p in the exact three-way Fisher-like interaction numeric test (FLINT) and the synergy factor (SF) included in the APSampler tools [16]. The interaction was considered epistatic at pFLINT<0.05, and a value of 95% CI for the SF did not cross 1.

Results. The frequencies of alleles of the studied mtDNA polymorphic variants are presented in Table. 3. Minor alleles had frequencies from 4.3% (for m. 4580*A) to 11.3% (for m. 13708*A) in MS patients and from 2.4 to 8.6% in the control group, while major alleles were more common in healthy individuals. However, a comparison of mtDNA allele frequencies in MS patients and in the control group did not reveal significant associations with MS.

At the same time, mtDNA alleles were significantly associated with MS in combinations with nuclear genome variants. Five biallelic combinations were identified (Table 4), including major mtDNA variants-m. 4580*G, m. 13368*G or m. 13708*G and nuclear variants-CXCR5*G, TNFRSF1A*T or CD86*T. All combinations have protective properties (OR 0.67–0.75) at p=0.0036–0.022.

The increase in the level of significance of associations with MS observed in the joint carriage of mtDNA variants and alleles of nuclear genes can occur due to the summation of their independent contributions (additive nature) or as a result of positive epistatic (synergistic) interaction between them. The value of pFLINT in the exact three-factor test for all detected combinations is >0.05, and the CI for SF crosses 1 (Table 5), which indicates the absence of epistatic interactions between the components of the combinations.

Discussion. This study is a continuation of our previous work [13]. The study of three previously unknown mtDNA polymorphisms in an expanded sample of MS patients and healthy individuals revealed five combinations, including mitochondrial and nuclear genome polymorphisms, the carriage of which has an impact on the risk of MS development. All established combinations have protective properties. The mitochondrial components of the combinations are variants m. 4580*G, m.13368*G and m.13708*G, for which we have not observed significant associations with MS if they are taken separately.

Previously, the involvement of the m.13708 polymorphism, located in the gene of the fifth subunit of the first electron transport chain complex (MT-ND5) in the development of MS was studied. The association of MS with the variant m. 13708*A has been shown for three European populations – Spaniards, Norwegians and Germans [17]. Due to the lack of diploidy of the mitochondrial genome, with the exception of a separate phenomenon of heteroplasmy, mtDNA polymorphisms cannot exist in a heterozygous state, so the alternative allele of risk will be protective. Thus, the data of X. Yu et al. [17] are consistent with the results of this study, since the allele m.13708*G, alternative to the risk allele m. 13708*A, according to our data, is part of the protective combination.

Table 5. Analysis of the nature of interaction between the components of combinations associated with MS

Biallelic combinations	p_{FLINT}	SF [95% CI]
m.4580 (rs28357975)*G + *CXCR5* (rs523604)*G	0.67	0.65 [0.13–3.39]
m.13708 (rs28359178)*G + *TNFRSF1A* (rs1800693)*T	0.60	0.77 [0.28–2.16]
m.13368 (rs3899498)*G + *CD86* (rs2255214)*T	0.09	0.43 [0.17–1.08]
m.13368 (rs3899498)*G + *TNFRSF1A* (rs1800693)*T	0.10	0.41 [0.14–1.14]
m.4580 (rs28357975)*G + *TNFRSF1A* (rs1800693)*T	0.30	5.60 [0.28–112.0]

The relationship of MS with the m.13336 polymorphism located in the gene of the fifth subunit of the first electron transport chain complex (MT-ND5) has not been specially described in the literature. The specific features of mtDNA include maternal inheritance and lack of recombination. It is often possible to determine the set of mutations that occurred in an individual's mtDNA, in his/her maternal ancestors, or in his/her haplotype. Groups of related haplotypes present in individuals who share a common maternal ancestor are usually grouped into haplogroups. Variant m. 13368*A is a marker of haplogroup T, for which the study performed on the basis of previous GWAS [18] showed a nominal association with MS ($p<0.05$, but did not reach the significance level $p<5\times10-8$ required in full-genomic studies). This indirectly confirms the results of this work, since the two identified protective combinations included a variant opposite to the marker of the haplogroup T-m. 13368*G.

The relationship of the m.4580 polymorphism – the third identified mtDNA variant associated with MS as part of the combination – has not been described in the literature.

The nuclear components of the detected combinations were polymorphisms in the genes encoding the cxcr5 chemokine receptor, the CD86 membrane protein, and a receptor from the TNFRSF1A tumor necrosis factor receptor superfamily. Previously, we described the association of the CXCR5 and CD86 genes with MS at $p<0.05$, but we did not observe the association of the TNFRSF1A*T variant in this sample of patients and healthy individuals (controls) [14]. This study shows the effect of the TNFRSF1A*T variant on the risk of MS, specifically in combination with three different mtDNA variants. Thus, the use of multi-locus analysis increases the statistical power of the study, which allowed us to identify a variant of the nuclear genome associated with MS that was not identified individually.

Each of the identified combinations was characterized by a value of pFLINT >0.05 and a CI for SF crossing 1.Thus, we did not observe any mutual amplification of the effects of carrying the components of the combinations, or their mutual suppression, and the interaction of the components in the identified combinations, apparently, is additive.

Conclusion. This study, conducted on a representative sample of MS patients and healthy individuals (controls), shows the existence of five combinations of mitochondrial and nuclear genes with protective properties. However, for final conclusions about the contribution of the identified mitochondrial-nuclear combinations to the development of MS and the nature of the interaction of their components, the results of this work need to be reproduced in independent groups of patients with MS and healthy individuals as controls.

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Conflict of Interest Statement

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Kozin M.S. https://orcid.org/0000-0001-6587-1243
Kiselev I.S. http://orcid.org/0000-0003-3366-4113
Boyko A.N. http://orcid.org/0000-0002-2975-4151
Kulakova O.G. https://orcid.org/0000-0002-5321-3101
Favorova O.O. https://orcid.org/0000-0002-5271-6698