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GWAS-identified multiple sclerosis risk loci involved in immune response: Validation in Russians

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ABSTRACT

Multiple sclerosis (MS) is a chronic neuro-inflammatory disease of complex etiology. The results of GWAS, a high-throughput method to discover genetic architectureof MS, require replication in independent ethnic groups. We performed a replication study of nine GWAS-identified SNPs in immune response in Russians. Associations of *CLEC16A* and *IL2RA* with MS were validated. Besides, we observed the associations of *CLEC16A* and *IRF8* in women, and *IL7RA* and *CD58* in men. With multi-locus association analysis two protective biallelic combinations: (*TNFRSF1A**T + *CLEC16A**A) and (*TNFRSF1A**T + *IRF8**A) were identified in women. Associations of *CLEC16A**G/G and both biallelic combinations in women with MS survived the permutation test.

1. Introduction

Multiple sclerosis (MS, OMIM 126200) is a chronic neuro-inflammatory autoimmune disease characterized by myelin loss, axonal and oligodendrocyte pathology, and progressive neurological dysfunction. MS is believed to arise from complex interactions of allelic variations in the genome, epigenetic modifications, environmental factors, etc. (Hoffjan and Akkad, 2010; Lvovs et al., 2012).

Traditionally, two common approaches are used for searching allelic variants associated with MS susceptibility: candidate gene studies and genome-wide association studies (GWAS). The main locus, which is associated with MS in all genome-wide and candidate gene studies, is 6p21.3, where HLA class II genes are located. Among them HLA-*DRB1**1501 is the most widely recognized MS risk variant though it can explain only part of disease susceptibility (Oksenberg, 2013). In 14 GWASs of MS susceptibility conducted to date (National Institute of Health, 2014, http://www.genome.gov/gwastudies/), more than 110 non-HLA risk loci were found conferring moderate effect sizes (Sawcer et al., 2014). The level of reproducibility of the results from study to study is relatively low. Overall, approximately 40 MS risk non-HLA loci were found in at least two GWASs and met genome-wide significance threshold ($p \leq 5 \times 10^{-8}$) in at least one GWAS (Favorova et al., 2014). Of note, the majority of genes associated with MS according to genome-wide or candidate gene studies are immune response genes (Nylander and Hafler, 2012). This fact is in line with modern conceptions of the leading role of autoimmunity in disease etiology.

Genetic architecture of complex diseases may differ in people of different ancestry (Ntzani et al., 2012). In the case of MS the classic example of ethno-specificity is the association with MS of HLA-*DRB1* haplotypes DR3 and DR4 in Sardinians instead of DR15, as in other Caucasians (Sotgiu et al., 1998). Considering this, validation of GWAS results is required in different ethnic groups, either with GWASs or with candidate gene studies.

Genetic associations with MS are less investigated in individuals of Slavic ethnicity. The lack of associations with MS of some previously GWAS-identified SNPs, such as *IL7RA* rs6897932 in Southern Slavs (Stankovic et al., 2010) and *KIF1B* rs10492972 in Russians (Kudryavtseva et al., 2011) points out that further studies of MS genetic susceptibility in Slavs are required. Up to date, *CD40* rs6074022 is the

Abbreviations: GWAS, genome-wide association study; *CLEC16A*, C-type lectin domain family 16, member A; *IL2RA*, interleukin 2 receptor alpha chain; *IL7RA*, interleukin 7 receptor alpha chain; *IRF8*, interferon regulatory factor 8; LD, linkage disequilibrium; *TNFRSF1A*, tumor necrosis factor receptor superfamily, member 1A; TRAPS, tumor necrosis factor receptor-associated periodic syndrome; Th1/17, T-helper cell type 1/17; Treg, regulatory T cell.

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only one genome-wide identified non-HLA locus, which is confirmed as MS-associated in Russians (Sokolova et al., 2013).

Russia includes areas of high and medium MS prevalence (Boiko et al., 2000). Here we aimed to validate GWAS-identified associations of nine SNPs in eight non-HLA immune response genes, with MS in Russians. The genes *CD58*, *IL7RA*, *IL2RA*, *CD6*, *TNFRSF1A*, *CLEC16A*, *IRF8*, and *STAT3* were selected based on the following criteria: association with MS for them was found in at least two GWASs and *p*-value of $<5 \times 10^{-8}$ was observed in at least one of them (Table 1). Association study for these SNPs in Russian patients could clarify the genetic contribution to MS susceptibility in this population and elucidate the common features of MS etiology.

2. Subjects and methods

2.1. Patients and controls

Five hundred and nine unrelated MS patients from the Moscow City Multiple Sclerosis Center diagnosed according to the McDonald Criteria (McDonald et al., 2001) were enrolled in the study (351 women, 158 men; mean age 38.8 ± 10.6 years). Two hundred seventy-six volunteers without neurological disorders were included in the control group (168 women, 108 men; mean age 48.2 ± 20.9 years). All MS patients and healthy individuals described themselves as Russians and lived in Moscow region. The clinical characteristics of all MS patients, men and women separately are shown in Table 2. The study was approved by the local ethics committee and written informed consent had been obtained.

2.2. Genotyping

Genomic DNA was extracted from whole or frozen blood samples with modified phenol-chloroform method (Sambrook et al., 1989). The description of PCR-based methods applied to genotyping of nine SNPs in eight genes is presented in Supplementary Table A1. Briefly, *IL7RA* rs6897932 and *TNFRSF1A* rs4149584 were genotyped as described earlier (Marek-Yagel et al., 2010; Tsareva et al., 2012). *TNFRSF1A* rs1800693 was genotyped with PCR-RFLP method using custom-made primers and *PspEI* restriction endonuclease (all from "Sybenzyme", Novosibirsk, Russia). The rest of the SNPs were genotyped using real-time PCR assays with either pre-designed TaqMan kits from Applied Biosystems (Foster City, CA, US) (*CD58* rs2300747, *IRF8* rs17445836, and *STAT3* rs744166), or custom-made primers and fluorescent allele-specific probes ("Synthol", Moscow, Russia) (*IL2RA* rs2104286, *CD6* rs17824933, and *CLEC16A* rs6498169). No less than 20% of samples were genotyped in duplicates and no inconsistencies were observed.

2.3. Statistical analysis

Comparisons of genotype frequencies between MS patients and controls were performed using two-sided Fisher's exact test with GraphPad InStat software. The genotype was considered to be associated with MS susceptibility if its uncorrected *p*-value was <0.05 and 95% confidence

Table 1
GWAS data for MS susceptibility genes *CD58*, *IL7RA*, *IL2RA*, *CD6*, *TNFRSF1A*, *CLEC16A*, *IRF8*, and *STAT3* and their genetic polymorphisms selected for this study.

Gene	Locus	Data from original GWASs			SNP selected in this study		
		SNP ID	*p*-Value	References	ID, substitution, location in gene	Risk allele	Known or putative substitution effects (reference)
CD58	1p13	rs2300747	3.1×10^{-10}[a] 6.0×10^{-9}	De Jager et al. (2009b) Patsopoulos et al. (2011)	rs2300747; A>G, intron 1	A	Carriage of genotype AA is associated with decreased mRNA expression in peripheral blood mononuclear cells from MS subjects (De Jager et al., 2009a).
IL7RA	5p13	rs6897932	2.94×10^{-7} 1.67×10^{-6} 1.7×10^{-8}	Hafler et al. (2007) De Jager et al. (2009b) Sawcer et al. (2011)	rs6897932; C>T, exon 6	C	Carriage of allele C increases production of soluble IL7Rα (Gregory et al., 2007).
IL2RA	10p15-p14	rs2104286	2.16×10^{-7} 7.4×10^{-6} 9.33×10^{-8} 7.6×10^{-23}	Hafler et al. (2007) Bahlo et al. (2009) De Jager et al. (2009b) Beecham et al. (2013)	rs2104286; A>G, intron 1	A	Carriage of allele A is associated with increased soluble IL2RA levels in serum (Maier et al., 2009).
CD6	11q13	rs17824933 rs650258 rs34383631	3.79×10^{-9} 1.7×10^{-9} 3.7×10^{-23}	De Jager et al. (2009b) Sawcer et al. (2011) Beecham et al. (2013)	rs17824933; C>G, intron 1	G	Carriage of allele G is associated with diminished CD4 + T-cell proliferation capacity after non-specific stimulation (Kofler et al., 2011).
TNFRSF1A	12p13	rs1800693	1.59×10^{-11} 1.8×10^{-10} 6.92×10^{-16}	De Jager et al. (2009b) Sawcer et al. (2011) Beecham et al. (2013)	rs1800693; T>C, intron 6	C	Carriage of allele C correlates with increased level of sTNFR1 (Gregory et al., 2012).
		rs4149584	5.25×10^{-6} 2.75×10^{-5}	De Jager et al. (2009b) Beecham et al. (2013)	rs4149584; G>A (R92Q), exon 4	A	sTNFR1 levels failed to increase with the TRAPS[b] attack and were lower out of attacks in 92Q positive patients (Aksentijevich et al., 2001).
CLEC16A	16p13	rs6498169 rs11865121 rs7200786 rs12927355	4.0×10^{-6} 2.0×10^{-7} 6.3×10^{-14} 6.4×10^{-46}	Hafler et al. (2007) De Jager et al. (2009b) Sawcer et al. (2011) Beecham et al. (2013)	rs6498169; G>A, intron 22	G	Carriage of allele G correlates with higher expression of DEX1 and SOCS1 in thymus (Leikfoss et al., 2013).
IRF8	16q24	rs17445836 rs13333054 rs35929052	3.73×10^{-9} 1.0×10^{-8} 5.9×10^{-12}	De Jager et al. (2009b) Sawcer et al. (2011) Beecham et al. (2013)	rs17445836; G>A, 61.5 kb downstream	G	Several interferon-response pathway genes expression is upregulated in MS carriers of allele G (De Jager et al., 2009b).
STAT3	17q21	rs744166	2.75×10^{-10}	Jakkula et al. (2010)	rs744166; C>T, intron 1	C	Allele C is positively associated with some other Th1/Th17-mediated diseases (Cenit et al., 2013; Wang et al., 2014).

Note: All selected genes correspond to our inclusion criteria: association with MS was found in at least two GWASs and *p*-value of $<5 \times 10^{-8}$ was observed in at least one of them. For the genes *CD58*, *IL7RA*, *IL2RA*, *TNFRSF1A*, and *STAT3* only SNPs corresponding to these criteria were included in the study and are shown. In *TNFRSF1A* in addition to rs1800693, rs4149584 was tested in the study. In *CD6*, *CLEC16A*, and *IRF8* there were no distinct SNPs tested in several GWASs and corresponding to the abovementioned criteria. For these genes, several SNPs are presented in the Table including those selected for our study.

[a] Data in the original study were given for the alternative (protective) allele G.

[b] TRAPS — tumor necrosis factor receptor-associated periodic syndrome.