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Research paper

Multilocus evaluation of genetic predictors of multiple sclerosis

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ABSTRACT

Background: Genome-wide association studies identified numerous susceptibility loci for multiple sclerosis in populations of European ancestry, but the associations are not always reproducible in other populations due to admixture and different linkage disequilibrium patterns obscuring true association signals.
Objective: Our aim was to identify genetic predictors of multiple sclerosis in three ethnically homogenous populations from the Volga-Ural region of Russian Federation.
Methods: In the largest to date study of multiple sclerosis in Russian population, involving 2048 participants from the Republic of Bashkortostan, Russian Federation (641 patients with multiple sclerosis and 1407 unaffected individuals), we performed replication analysis of previously identified genome-wide signals for multiple sclerosis. Associations were tested using logistic regression analysis under additive genetic model adjusted for sex. Meta-analysis of the study results in three populations was performed under fixed effects and random effects models.
Results: We demonstrate the association with multiple sclerosis of the five variants (*INAVA* rs7522462, *EOMES* rs11129295, *C6orf10* rs3129934, *CD86* rs9282641, and *GPR65* rs2119704). The strongest association (OR = 2.16, CI:1.85–2.74, P = $2.53x10^{-13}$) was detected for rs3129934 polymorphism in the major histocompatibility region. Multilocus analysis has revealed 322 and 27 allelic patterns associated with multiple sclerosis in women and men, respectively. In women, the highest risk of MS was conferred by *C6orf10* rs3129934*T/T + *STAT3* rs744166*T combination (OR = 11.87), in men – by *C6orf10* rs3129934*T + *EOMES* rs11129295*C + *RPS6KB1* rs180515*C combination (OR = 3.25).
Conclusion: We confirm five associations with multiple sclerosis previously reported in genome-wide scans in Europeans in three ethnic groups from the Volga-Ural region of Russia.

1. Introduction

Multiple sclerosis (MS) is a complex disease with well-established genetic component. Genome-wide association studies (GWAS) have identified over 700 genetic variants associated with MS (https://www.ebi.ac.uk/gwas/efotraits/EFO_0003885), predominantly in populations of European ancestry. The earliest MS GWAS employing family-based approach (1003 trios in the discovery set, 931 in the replication sample), reported associations with MS reaching the GWAS significance level for two single nucleotide polymorphisms (SNPs), rs3135388 at *HLA-DRA* and rs12722489 at *IL12RA* (Hafler et al., 2007). The recent study totaling 47,429 MS patients and 68,374 control subjects has identified 26,395 SNPs significantly associated with MS (Patsopoulos et al., 2019).

Abbreviations: CI, confidence interval; dNTP, deoxynucleoside triphosphate; eQTL, expression quantitative trait locus; FDR, false discovery rate; GWAS, genome-wide association study; IMSGC, International Multiple Sclerosis Genetics Consortium; LD, linkage disequilibrium; MAF, minor allele frequency; MHC, major histocompatibility complex; MMP, matrix metallopeptidase; MS, multiple sclerosis; OR, odds ratio; PCR, polymerase chain reaction; RF, Russian Federation; SNP, single nucleotide polymorphism; WTCCC2, Wellcome Trust Case Control Consortium 2.

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It has been reported that the clinical course of MS can vary among different ethnic groups (Cree et al., 2004). The disease prevalence also shows distinct ethno-geographic patterns with the highest estimates per 100,000 population registered in North America (average 164.6, in Canada 291.0), Western Europe (average 127.0, in Denmark 227.0), and Australasia (91.1), and the lowest in eastern sub-Saharan Africa (3.3), central sub-Saharan Africa (2.8), and Oceania (2.0) (G. B. D. Multiple Sclerosis Collaborators, 2016; Browne et al., 2014). The evidence from GWAS supports the existence of population-specific patterns of MS heritability. For example, the major histocompatibility complex (MHC) region on chromosome 6 has been strongly and consistently associated with MS across ethnicities (Hafler et al., 2007; Comabella et al., 2008; De Jager et al., 2009; Bahlo et al., 2009; Jakkula et al., 2010; Sanna et al., 2010; Nischwitz et al., 2010; Patsopoulos et al., 2011; Martinelli-Boneschi et al., 2012; Andlauer et al., 2016). However, fine-mapping of the GWAS signals reveals a linkage disequilibrium (LD) block (r^2 = 0.9–1.0) containing SNPs (rs3135388, rs3129889, rs9271366, and rs3104373) associated with MS in Caucasian populations from the United Kingdom (UK), United States (US), Australia, New Zealand, Germany, Switzerland, and the Netherlands, (Hafler et al., 2007; De Jager et al., 2009; Bahlo et al., 2009; Nischwitz et al., 2010; Patsopoulos et al., 2011; Andlauer et al., 2016) and highlights distinct variants associated with MS exclusively in Spanish and Italian populations (rs3129934), (Comabella et al., 2008; Martinelli-Boneschi et al., 2012) in Finns (rs3135338), (Jakkula et al., 2010) and Sardinians (rs2040406) (Sanna et al., 2010). Furthermore, populations of non-European ancestry are currently underrepresented in GWAS, and limited data exist on the reproducibility of the GWAS findings. Additional challenge in interpreting GWAS results lies in detecting interactions between the established loci that may lead to interindividual differences in susceptibility to MS.

Populations of the Volga-Ural region of Russia are an interesting object for genetic study due to the unique combination of European and Asian genetic ancestry. The aim of our study was to perform the replication analysis of previously identified GWAS signals for MS and to identify complex genetic markers of MS in a group of patients with MS and non-affected individuals from three ethnic groups originating from the Republic of Bashkortostan (Russian Federation).

2. Materials and methods

2.1. Study group

The study was conducted in accordance with the principles for medical research involving human subjects outlined in the Declaration of Helsinki of the World Medical Association (2000). Written informed consent was provided by all participants. The protocol of the study was approved by the ethics committee of the Institute of Biochemistry and Genetics of Ufa Federal Research Centre of Russian Academy of Sciences (No.1/28.10.2007).

The group of patients was composed of persons registered at the Republic Multiple Sclerosis Centre (RMSC). RMSC has recorded data on 1145 patients with MS; 641 were enrolled in the study (427 women, mean age 40.04 ± 9.6 yrs, and 214 men, mean age 41.28 ± 10.69 yrs). Control group was comprised of 1407 healthy subjects (708 women, mean age 38.08 ± 11.06 yrs; 699 men, mean age 37.48 ± 10.71 yrs) without neurodegenerative or other chronic diseases. Ethnic origin was established according to the data from questionnaire containing questions about ethnicity and the place of birth of the ancestors in three generations. All individuals included in study permanently resided in the Republic of Bashkortostan and belonged to Bashkir (n = 325), Russian (n = 772), or Tatar (n = 951) ethnic group. Persons of mixed ancestry were excluded from genotyping.

MS was diagnosed according to the McDonald criteria (2010). Expanded Disability Status Scale was used to assess the severity of MS. The disease progression rate was calculated by dividing the EDSS score by the duration of MS in years. Clinical characteristics of the group of patients are provided in Table 1. Healthy controls were recruited at the Republic Centre of Blood Transfusion (Ufa, Republic of Bashkortostan, Russian Federation).

2.2. Genotyping

Whole venous blood was collected from each participant and stored at −4°C. Total DNA was isolated from 6 ml of whole venous blood using standard extraction protocol. The DNA quality was assessed by electrophoresis in 0.8% agarose gel and quantified by ultraviolet absorbance spectrophotometry analysis. Genotyping was performed using PCR with subsequent restriction endonuclease digestion (rs3129934, rs4410871) or PCR with allele-specific assays (all other SNPs). Primers were designed using PrimerSelect 5.05 software (DNAStar Inc., Madison, WI, USA) (Supplementary Table S1). SNPs for the analysis were selected according to the the following criteria: previously reported GWAS associations with MS or other autoimmune traits, known or suggested functional significance, minor allele frequency > 0.05 in European populations according to the Ensembl database (Yates et al., 2019).

DNA sequences for the primer design were retrieved from NCBI (National Center for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov/SNP). The concentrations of primers and probe were optimised leading to the following PCR conditions: 0.2 mM of both primers, 20 ng of DNA template, 30 mM Tris-HCl (pH 8.6/25C), 16.6 mM $(NH_4)_2SO_4$, 2.5$MgCl_2$ 0.2 mM of each dNTPs (Thermo Fisher Scientific, Lithuania), and 0,5 U of Taq-polymerase enzyme. The amplification was performed in a T100^{TM} thermal cycler (BioRad, USA) programmed for initial denaturation step (95 °C for 1 min) followed by 28 cycles of amplification (denaturation at 95 °C for 20 s, primer annealing at specific temperature for 30 s, elongation at 72 °C for 30 s) and a final extension (72 °C for 4 min). Restriction analysis for rs3129934 and rs4410871 polymorphisms was performed by incubating 10 μl of PCR product in a 20 μl reaction volume at 37 °C for 16 h with 10 U of restriction endonuclease (*Rsr*2I and *Hind*III, respectively). PCR and restriction products were separated by electrophoresis in 2% agarose gel and identified using Mega-Bioprint 1100 gel documentation system (Vilber Lourmat, Collégien, France). As quality control, 5% of the

Table 1
Clinical characteristics of the group of patients with multiple sclerosis.

Parameters	Bashkirs	Russians	Tatars
Age (M ± SD), years	40.28 ± 9.86	40.6 ± 9.77	40.89 ± 9.75
Sex (% women)	66.0	66.8	66.7
Age of disease onset (M ± SD), years	27.93 ± 7.76	27.64 ± 8.9	27.53 ± 8.89
Disease duration (M ± SD), years	12.4 ± 8.65	13.17 ± 9.53	13.36 ± 9.93
Types of multiple sclerosis (%):			
relapsing-remitting	30.1	36.9	43.7
primary-progressive	15.1	10.2	15.6
secondary-progressive	54.8	52.9	40.7
Clinical symptoms (%):			
sensory impairments	16.5	17.1	12
oculomotor dysfunction	6.5	4.9	4.4
movement dysfunction	23.1	35	29.8
coordination loss	22	18.6	18.7
combined movement and coordination difficulties	12.1	4.2	6.4
symptoms of cranial nerve lesions	4.4	2.7	2.8
retrobulbar neuritis	11	14.4	19.9
others	4.4	3.1	6
EDSS (M ± SD)	4.9 ± 2.39	4.41 ± 1.56	4.46 ± 1.77
Progression rate (M ± SD), step/year	0.74 ± 1.05	0.73 ± 1.09	0.69 ± 0.89

M – mean value, SD – standard deviation, EDSS – Expanded Disability Status Scale.

genotyped samples were randomly selected for re-genotyping, and all newly obtained results were identical to the previously determined genotyping data.

2.3. Statistical analysis

The study data were stored and managed using IBM SPSS Statistics V22.0 (Chicago, IL, USA). All SNPs were tested for compliance with Hardy-Weinberg equilibrium in the control group (Table 2).

Associations between the studied SNP and MS were analysed separately in each group using logistic regression analysis under additive genetic model adjusted for sex implemented in PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell et al., 2007). Additive model assumes that there is a linear gradient in risk between the homozygotes for the non-effect allele, heterozygotes, and homozygotes for the effect allele. The effect allele was determined according to the GWAS results in European populations (De Jager et al., 2009; Bahlo et al., 2009; Jakkula et al., 2010; Sawcer et al., 2010)****. Meta-analysis of the study results was performed under fixed effects and random effects models. P values of <0.05 were considered statistically significant. Correction for multiple testing was performed using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995).

Multilocus analysis of association between allelic combinations of the studied loci and MS was performed using APSampler 3.6.0/ (the program and its description can be accessed at http://apsampler.sourceforge.net/). APSampler (Allelic Pattern Sampler) applies a Markov chain Monte Carlo method based on Bayesian approaches to identify combinations of allelic variants of multiple loci associated with the studied trait (Favorov et al., 2005). Since APSampler program does not allow for adjustment for potential confounders, including sex, the analysis was performed separately for men and women.

3. Results

3.1. Single locus analysis

The observed genotype frequencies of all studied SNPs among the control subjects were in agreement with Hardy-Weinberg equilibrium (Table 2). Testing the associations between the studied loci and MS in the three populations separately, we detected the association of *C6orf10* rs3129934*T and *EOMES* rs11129295*T alleles with the disease in the Russian and Tatar ethnic groups (Table 3).

3.2. Meta-analysis in the three ethnic groups

Meta-analysis of the study results in all three groups under the random effects model revealed the associations of the *INAVA* rs7522462*G, *EOMES* rs11129295*T, *CD86* rs9282641*G, *C6orf10* rs3129934*T, and *GPR65* rs2119704*C alleles with MS (Table 4). Comparing the results of our replication *meta*-analysis with the data obtained in the initial GWAS, we found that the directions of the observed associations were consistent with the previously identified for all loci, except for rs7522462 at *INAVA* (Table 4). Our data shows that *INAVA* rs7522462*G allele confers decreased risk of MS (OR = 0.74, $P_{FDR} = 1.25x10^{-3}$), while the findings of the International Multiple Sclerosis Genetics Consortium (IMSGC) indicate that in Europeans, this allele is associated with the increased risk of MS (Sawcer et al., 2011).

The effect allele frequencies in the three Russian populations and in five global super populations (according to the 1000 Genomes Project Phase 3 (http://www.ensembl.org/Homo_sapiens/Variation/Population) are shown in Table 4.

4. Multilocus analysis

Using APSampler program, we identified 322 allelic patterns significantly associated with MS in women, and 27 – men (Supplementary Tables S2 and S3). The allele/genotype combinations conferring the most potent risk of MS ($0.3 \geq OR \geq 3.0$) are presented in Table 5. Among them, most commonly featured were *C6orf10* rs3129934 and *SOX8* rs2744148 alleles/genotypes (11 combinations), followed by *IL7RA* rs6897932 (8), and *INAVA* rs7522462 (6). *EOMES* rs11129295 and *GPR65* rs2119704 were present in 5 patterns each, *RGS1* rs1323292 and *CD58* rs2300747 in 3, *PVT1* rs4410871 and *RPS6KB1* rs180515 – in 3, *STAT3* rs744166 and *ZBTB46* rs6062314 – in 2, while *CD86* rs9282641, *MANBA* rs228614, *TNFRSF1A* rs1800693, and *CD40* rs6074022 appeared only once.

Notably, the majority of the studied loci demonstrated the change in the direction of the observed associations (Supplementary Tables S2 and S3), with the only exception being *C6orf10* rs3129934 – its T allele invariably conferred the increased risk of MS, while C allele and C/C genotype was present in the combinations with beneficial effect towards MS. The highest risk of MS in women was conferred by *C6orf10* rs3129934*T/T genotype in combination with *STAT3* rs744166*T (OR = 11.87) and *CD58* rs2300747*A allele (OR = 10.98), in men – by *C6orf10* rs3129934*T allele together with *EOMES* rs11129295*C and *RPS6KB1* rs180515*C alleles (OR = 3.25).

Table 2
The list of the studied loci, minor allele frequencies, and the results of testing for Hardy-Weinberg equilibrium in the three ethnic groups.

Chr	Locus	SNP	MA	Bashkirs		Russians		Tatars	
				MAF	P-value	MAF	P-value	MAF	P-value
1	*RGS1*	rs1323292	G	0.16	0.127	0.18	0.594	0.17	0.135
1	*CD58*	rs2300747	G	0.31	1	0.16	0.418	0.22	0.737
1	*INAVA*	rs7522462	A	0.18	0.114	0.23	0.121	0.24	0.340
3	*EOMES*	rs11129295	T	0.43	0.161	0.37	0.710	0.41	0.138
3	*CD86*	rs9282641	A	0.04	1	0.05	1	0.05	0.307
4	*MANBA*	rs228614	G	0.49	1	0.47	0.642	0.49	0.716
5	*IL7RA*	rs6897932	T	0.23	0.788	0.25	0.884	0.25	1
6	*C6orf10*	rs3129934	T	0.13	1	0.16	0.714	014	1
8	*PVT1*	rs4410871	T	0.31	0.207	0.25	0.396	0.26	1
12	*TNFRSF1A*	rs1800693	G	0.45	0.714	0.50	0.914	0.48	1
14	*GPR65*	rs2119704	A	0.06	1	0.07	1	0.07	0.704
16	*SOX8*	rs2744148	G	0.14	0.692	0.17	1	0.14	0.404
17	*STAT3*	rs744166	C	0.38	0.707	0.41	0.122	0.39	0.309
17	*RPS6KB1*	rs180515	C	0.37	0.436	0.37	0.127	0.41	0.619
20	*CD40*	rs6074022	C	0.27	0.0942	0.25	0.776	0.29	0.884
20	*ZBTB46*	rs6062314	C	0.04	0.141	0.10	0.771	0.06	1

Chr – chromosome, SNP – single nucleotide polymorphism, MA – minor allele, N – number of individuals with non-missing genotyping data, MAF – minor allele frequency in the control group, P-value – P-value for Hardy-Weinberg equilibrium in controls

Table 3
Results of the analysis of association of the studied loci with the risk of multiple sclerosis.

Locus	SNP	EA	Bashkirs			Russians			Tatars		
			OR (CI)	P	P_{FDR}	OR (CI)	P	P_{FDR}	OR (CI)	P	P_{FDR}
RGS1	rs1323292	A	1.06 (0.65–1.76)	0.808	0.864	1.21 (0.89–1.64)	0.226	0.452	0.85 (0.62–1.18)	0.338	0.499
CD58	rs2300747	G	0.84 (0.54–1.32)	0.457	0.732	0.97 (0.70–1.34)	0.854	0.945	0.72 (0.52–1.01)	0.054	0.172
INAVA	rs7522462	G	0.69 (0.43–1.12)	0.136	0.545	0.70 (0.53–0.92)	0.010	0.053	0.82 (0.61–1.08)	0.159	0.318
EOMES	rs11129295	T	1.22 (0.82–1.80)	0.330	0.677	1.56 (1.21–2.00)	$6.03x10^{-4}$	$4.83x10^{-3}$	1.48 (1.14–1.92)	0.003	0.026
CD86	rs9282641	G	0.36 (0.13–1.04)	0.059	0.531	1.94 (1.06–3.55)	0.032	0.128	2.04 (1.10–3.80)	0.024	0.096
MANBA	rs228614	G	1.38 (0.92–2.05)	0.115	0.545	0.91 (0.71–1.17)	0.453	0.652	1.02 (0.78–1.33)	0.886	0.945
IL7RA	rs6897932	C	0.77 (0.49–1.22)	0.264	0.677	1.00 (0.75–1.34)	0.977	0.977	1.12 (0.82–1.52)	0.485	0.647
C6orf10	rs3129934	T	1.41 (0.15–12.93)	0.764	0.864	2.00 (1.51–2.65)	$1.58x10^{-6}$	$2.53x10^{-5}$	2.38 (1.75–3.22)	$2.33x10^{-8}$	$3.72x10^{-7}$
PVT1	rs4410871	C	1.05 (0.69–1.60)	0.810	0.864	1.29 (0.97–1.72)	0.076	0.244	1.05 (0.78–1.41)	0.762	0.897
TNFRSF1A	rs1800693	G	1.11 (0.76–1.63)	0.580	0.843	1.02 (0.80–1.29)	0.886	0.945	1.22 (0.95–1.58)	0.116	0.266
GPR65	rs2119704	C	2.66 (0.94–7.57)	0.066	0.531	1.47 (0.91–2.38)	0.118	0.314	1.53 (0.92–2.56)	0.103	0.266
SOX8	rs2744148	G	1.39 (0.81–2.39)	0.234	0.677	1.12 (0.82–1.52)	0.489	0.652	1.20 (0.84–1.73)	0.313	0.499
STAT3	rs744166	T	0.92 (0.61–1.39)	0.685	0.864	1.04 (0.81–1.34)	0.760	0.936	0.96 (0.74–1.25)	0.785	0.897
RPS6KB1	rs180515	C	0.98 (0.64–1.50)	0.917	0.917	1.19 (0.92–1.52)	0.179	0.409	1.01 (0.78–1.31)	0.959	0.959
CD40	rs6074022	C	1.20 (0.77–1.87)	0.428	0.732	0.86 (0.66–1.12)	0.267	0.474	0.88 (0.67–1.15)	0.343	0.499
ZBTB46	rs6062314	T	0.64 (0.25–1.60)	0.339	0.677	1.23 (0.82–1.86)	0.319	0.510	2.20 (1.19–4.09)	0.012	0.065

EA – effect allele, OR – odds ratio, CI – 95% confidence interval, P – P-value, P_{FDR} – significance level corrected for multiple testing using Benjamini–Hochberg procedure. The OR is aligned to the effect allele as reported in the discovery study. Statistically significant associations are shown in bold

5. Discussion

We performed the replication analysis of the 16 GWAS-derived risk loci for MS in three ethnic populations from the Republic of Bashkortostan (Russian Federation), and confirmed the association of five SNPs with the disease (Table 4). Four of these loci (*INAVA* rs7522462, *EOMES* rs11129295, *CD86* rs9282641, and *GPR65* rs2119704) were initially identified in GWAS performed as part of the Wellcome Trust Case Control Consortium 2 (WTCCC2) project, with European ancestry cases recruited through IMSGC in 15 countries (Finland, Sweden, Norway, Denmark, Australia, New Zealand, United Kingdom, Germany, Belgium, Poland, Ireland, USA, France, Spain, and Italy), and WTCCC2 common control set (Sawcer et al., 2011). The effect allele frequencies observed in the studied ethnic groups were largely similar to European populations (Table 4).

C6orf10 rs3129934 polymorphism is located in near the *HLA-DRB/DQA* region, and its association with MS was first reported in the GWAS conducted in Spanish population (242 cases, 242 controls) with subsequent replication in 1586 individuals from Spain and the U.S (Comabella et al., 2008). This association was confirmed in a later GWAS in Italian population with replication in Australian and Northern Europe collections (Martinelli-Boneschi et al., 2012). In our study, rs3129934*T allele was consistently associated with higher MS risk in the Russian and Tatar ethnic groups when analysed separately, in all three populations according to the *meta*-analysis results, and as part of the genotype/allele combinations conferring increased risk of MS.

The rs11129295 polymorphism is located in the intergenic region near *EOMES* gene encoding for eomesodermin, a transcription factor from the T-box family. Eomesodermin activates interleukin 10 expression, driving the differentiation of type 1 regulatory T cells that are critical for the prevention of graft-versus-host disease, and thus plays an immunoregulatory role (Gruarin et al., 2019). It also promotes the differentiation of CD8 + memory T cells responsible for the development of immunological memory, providing protection against subsequent infection with the same pathogen following the initial immune response (Reiser et al., 2019). Transcriptional activity of *EOMES* gene was downregulated in untreated MS patients, and rs11129295 was an eQTL for EOMES in the peripheral blood (Parnell et al., 2014). Another study established heritability of *EOMES* expression in twins, and observed lower levels of *EOMES* expression in patients on fingolimod and interferon beta compared to untreated patients (McKay et al., 2016). T allele of the rs11129295 was associated with increased risk of relapsing-remitting MS in the Chinese population (Chen et al., 2018). *EOMES* rs11129295 is also in linkage disequilibrium with SNPs associated with MS (rs2371108, $r^2 = 0.86$) and other autoimmune diseases, such as chronic lymphocytic leukemia (rs9880772, $r^2 = 0.72$), rheumatoid arthritis and Hodgkin's lymphoma (rs3806624, $r^2 = 0.7$), ankylosing spondylitis (rs13093489, $r^2 = 0.91$) (Beecham et al., 2013; Cortes et al., 2013).

Polymorphism rs7522462 is located in the innate immunity activator (*INAVA*) locus on chromosome 1, also known as open reading frame 106 (*C1orf106*). This SNP is in tight linkage disequilibrium with two other MS markers: rs55838263 ($r^2 = 0.98$) and rs59655222 ($r^2 = 0.995$), also associated with celiac disease, (Patsopoulos et al., 2019; Beecham et al., 2013) and is also linked with SNPs associated with various autoimmune disorders: Crohn's disease (rs7554511, $r^2 = 0.995$; rs11584383, $r^2 = 0.8$), ulcerative colitis (rs7554511, $r^2 = 0.95$), inflammatory bowel disease (rs7554511, $r^2 = 0.95$; rs35730213, $r^2 = 0.99$); ankylosing spondylitis (rs41299637, $r^2 = 0.995$; rs2297909, $r^2 = 0.78$), celiac disease (rs10800746, $r^2 = 0.91$) (Cortes et al., 2013). Notably, rs7554511*C allele was correlated with rs7522462*G allele ($r^2 = 0.995$), and rs7554511*C allele carriers demonstrated lower *INAVA* expression and decreased cytokine production in macrophages (Yan et al., 2017).

INAVA expression was upregulated in tumor cells, and was correlated with fibroblast growth factor 1 (FGF1) and matrix metallopeptidase 9 (MMP9) levels (Guan et al., 2018). FGF1 is involved in a wide range of biological processes including proliferation, morphogenesis, cell growth, and cell survival. *FGF1* expression is reduced in active MS lesions and increased in remyelinated MS lesions in astrocytes, neurons, oligodendrocytes, microglia, and infiltrating T cells and B cells (Mohan et al., 2014). FGF1 accelerates myelination *in vitro* and might contribute to CNS repair by inducing lipid synthesis by astrocytes and microglia (Berghoff et al., 2017). MMPs are a family of enzymes that are responsible for degrading extracellular matrix proteins, and MMP9 levels were increased in cerebrospinal fluid (CSF) of MS patients, particularly during clinical relapse, presumably due to intrathecal synthesis (Liuzzi et al., 2002). Serum and CSF levels of MMP9 were higher in patients with relapsing-remitting MS than with primary progressive MS or healthy controls (Sastre-Garriga et al., 2004). MMP9 has been studied as a potential therapeutic target for MS treatment. Moreover, treatment by interferon beta reportedly reduced MMP9 activity, subsequently decreasing T-lymphocyte infiltration into central nervous system, while natalizumab infusions lead to the increase in MMP9 plasma levels (Iannetta et al., 2019).

The results of the *meta*-analysis revealed the association between the common allele rs9282641*G and increased risk of MS in the three studied populations. This SNP is mapped to *CD86* gene, and the carriers

Table 4
Results of the replication *meta*-analysis analysis of previously identified genetic markers of multiple sclerosis in three ethnic groups.

Locus	SNP	EA	GWAS		Replication *meta*-analysis in three Russian populations		EAF							
			OR (CI)	P-value	OR (CI)	P-value	BASH	RUS	TAT	AFR	AMR	EAS	EUR	SAS
RGS1	rs1323292	A	1.12 (1.1–1.14) (Sawcer et al., 2011)	$2.3x10^{-8}$	1.03 (0.85–1.27)	0.762	0.842	0.819	0.835	0.980	0.764	0.802	0.815	0.925
CD58	rs2300747	G	0.77 (0.68–0.88) (De Jager et al., 2009)	$3.10x10^{-10}$	0.84 (0.69–1.03)	0.097	0.310	0.160	0.220	0.266	0.334	0.594	0.142	0.385
INAVA	rs7522462	G	1.11 (1.1–1.13) (Sawcer et al., 2011)	$1.9x10^{-9}$	0.74 (0.62–0.89)	$1.43x10^{-3}$	0.817	0.769	0.765	0.888	0.844	0.997	0.726	0.874
EOMES	rs11129295	T	1.11 (1.09–1.12) (Sawcer et al., 2011)	$1.2x10^{-9}$	1.46 (1.24–1.71)	$6.72x10^{-6}$	0.430	0.370	0.410	0.335	0.566	0.776	0.386	0.687
CD86	rs9282641	G	1.21 (1.18–1.24) (Sawcer et al., 2011)	$1.2x10^{-11}$	1.56 (1.06–2.30)	0.030	0.960	0.949	0.948	0.977	0.916	0.999	0.908	0.908
MANBA	rs228614	G	1.09 (1.07–1.1) (Sawcer et al., 2011)	$1.40x10^{-7}$	1.02 (0.86–1.19)	0.808	0.490	0.470	0.490	0.432	0.659	0.499	0.508	0.430
IL7RA	rs6897932	C	1.11 (1.09–1.13) (Sawcer et al., 2011)	$1.7x10^{-8}$	1.00 (0.82–1.21)	0.986	0.770	0.752	0.750	0.934	0.817	0.831	0.729	0.788
C6orf10	rs3129934	T	3.30 (2.3–4.9) (Comabella et al., 2008)	$9.0x10^{-11}$	2.16 (1.85–2.74)	$2.53x10^{-13}$	0.126	0.162	0.136	0.222	0.121	0.096	0.142	0.081
PVT1	rs4410871	C	1.11 (1.09–1.12) (Sawcer et al., 2011)	$7.7x10^{-9}$	1.15 (0.96–1.39)	0.148	0.689	0.748	0.743	0.858	0.599	0.632	0.693	0.718
TNFRSF1A	rs1800693	G	1.12 (1.11–1.14) (Sawcer et al., 2011)	$4.1x10^{-14}$	1.11 (0.95–1.30)	0.194	0.450	0.500	0.480	0.361	0.281	0.123	0.425	0.283
GPR65	rs2119704	C	1.22 (1.19–1.25) (Sawcer et al., 2011)	$2.2x10^{-10}$	1.59 (1.13–2.19)	0.006	0.944	0.926	0.926	0.761	0.941	0.804	0.925	0.962
SOX8	rs2744148	G	1.12 (1.1–1.14) (Sawcer et al., 2011)	$8.4x10^{-8}$	1.19 (0.95–1.45)	0.120	0.140	0.170	0.140	0.149	0.091	0.013	0.163	0.305
STAT3	rs744166	T	0.87 (0.83–0.91) (Jakkula et al., 2010)	$2.75x10^{-10}$	0.99 (0.84–1.17)	0.894	0.622	0.585	0.612	0.281	0.713	0.602	0.585	0.489
RPS6KB1	rs180515	C	1.09 (1.08–1.11) (Sawcer et al., 2011)	$8.8x10^{-8}$	1.08 (0.92–1.28)	0.373	0.370	0.370	0.410	0.282	0.316	0.463	0.310	0.335
CD40	rs6074022	C	1.2 (NA) (Bahlo et al., 2009)	$1.30x10^{-7}$	0.91 (0.77–1.10)	0.303	0.270	0.250	0.290	0.023	0.212	0.440	0.257	0.268
ZBTB46	rs6062314	T	1.16 (1.14–1.19) (Sawcer et al., 2011)	$1.30x10^{-7}$	1.33 (0.98–1.84)	0.080	0.956	0.903	0.942	0.775	0.960	0.992	0.923	0.965

EA – effect allele; OR – odds ratio; CI – 95% confidence interval; EAF – effect allele frequency; BASH – Bashkirs, RUS – Russians, TAT – Tatars, AFR – African, AMR – American, EAS – East Asian, EUR – European, SAS – South Asian; NA – not available. Statistically significant associations are shown in bold.

of rs9282641*G allele had higher expression of *CD86*, primarily in the naïve B-cells (CD19+/CD27 +) (Smets et al., 2018). Hi-C approach also identified association of the regulatory element containing rs9282641 with *CD86* promoter (Corradin et al., 2016). CD86 is a costimulatory molecule expressed on B-cells and other antigen-presenting cells, and the percentage of CD86 + B-cells was significantly increased in the peripheral blood of MS patients (Fraussen et al., 2016). In addition to rs9282641, CD86 locus harbours three other MS-associated SNPs: rs4308217, rs2255214, and rs2681424, none of which is linked with rs9282641, but rs2255214 and rs2681424, located in the upstream region of CD86, are in LD with each other ($r^2 = 1.0$).

Another polymorphism associated with MS according to the results of the *meta*-analysis was rs2119704, located in the *LINC01147* locus, in the downstream region of the *GPR65* gene. This polymorphism was an eQTL for *GPR65* expression in fibroblasts according to the Genotype-Tissue Expression (GTEx) Project data. This gene encodes a glycosphingolipid receptor and its altered expression may affect myelin integrity during the pathogenesis of MS.

The results of the multilocus analysis performed using APSampler show distinctive patterns of association with MS in women and men (Supplementary Tables S1 and S2). The number of allele/genotype combinations associated with MS in women was much larger than in men (322 and 27, respectively), which in part is due to predominance of women among study participants (female-to-male ratio in the group of patients was 2.00, overall – 1.24). In men, *C6orf10* rs3129934 was the core element of the majority of identified patterns (N = 20), while in women it was found only in 13 out of 322, and the most commonly featured were *IL7RA* rs6897932 (108 combinations), *SOX8* rs2744148 (88), *CD58* rs2300747 (83), *GPR65* rs2119704 (79), *INAVA* rs7522462 (67), *MANBA* rs228614 (67). Due to overrepresentation of loci with weaker effects, the same alleles and/or genotypes are often included both in predisposing and protective combinations.

6. Conclusion

The results of our study confirm the association with multiple

Table 5
The allele/genotype combinations most significantly associated with multiple sclerosis.

Pattern	MS	Control	OR	CI(95%)	P	P_{FDR}
Women						
C6orf10 rs3129934*T/T + *STAT3* rs744166*T	0.07	0.01	11.87	2.75–51.23	$1.67x10^{-5}$	$1.34x10^{-4}$
CD58 rs2300747*A + *C6orf10* rs3129934*T/T	0.07	0.01	10.98	2.53–47.61	$4.14x10^{-5}$	$2.66x10^{-4}$
C6orf10 rs3129934*T/T	0.06	0.004	5.11	2.14–12.23	$8.04x10^{-5}$	$4.41x10^{-4}$
CD58 rs2300747*A + *EOMES* rs11129295*T + *IL7RA* rs6897932*C + *C6orf10* rs3129934*T	0.32	0.09	4.87	2.93–8.10	$3.24x10^{-11}$	$1.32x10^{-7}$
INAVA rs7522462*A + *IL7RA* rs6897932*T + *SOX8* rs2744148*G + *ZBTB46* rs6062314*T	0.07	0.02	4.82	1.81–12.78	$3.56x10^{-4}$	$1.45x10^{-3}$
INAVA rs7522462*A + *IL7RA* rs6897932*T + *SOX8* rs2744148*G	0.07	0.02	4.80	1.81–12.74	$3.66x10^{-4}$	$1.48x10^{-3}$
RGS1 rs1323292*A/A + *IL7RA* rs6897932*T + *SOX8* rs2744148*G	0.12	0.03	3.76	1.90–7.46	$3.19x10^{-5}$	$2.17x10^{-3}$
EOMES rs11129295*T + *IL7RA* rs6897932*C + *GPR65* rs2119704*C/C + *SOX8* rs2744148*G	0.21	0.07	3.49	2.12–5.74	$1.45x10^{-7}$	$4.70x10^{-6}$
CD58 rs2300747*A + *C6orf10* rs3129934*T + *GPR65* rs2119704*C + *ZBTB46* rs6062314*T	0.47	0.20	3.46	2.37–5.05	$3.19x10^{-11}$	$2.60x10^{-7}$
CD58 rs2300747*A + *C6orf10* rs3129934*T + *GPR65* rs2119704*C	0.47	0.21	3.36	2.31–4.90	$6.32x10^{-11}$	$1.72x10^{-7}$
SOX8 rs2744148*G/G + *STAT3* rs744166*T	0.04	0.01	3.30	1.18–9.26	$1.46x10^{-2}$	$3.26x10^{-2}$
INAVA rs7522462*A/A + *PVT1* rs4410871*C + *TNFRSF1A* rs1800693*A	0.09	0.03	3.26	1.52–6.98	$9.48x10^{-4}$	$3.28x10^{-3}$
RGS1 rs1323292*A + *IL7RA* rs6897932*T + *PVT1* rs4410871*C + *SOX8* rs2744148*G	0.14	0.05	3.20	1.78–5.74	$2.88x10^{-5}$	$2.00x10^{-4}$
INAVA rs7522462*A + *RPS6KB1* rs180515*C/C	0.07	0.02	3.19	1.42–7.15	$2.25x10^{-3}$	$6.58x10^{-3}$
RGS1 rs1323292*A + *IL7RA* rs6897932*T/C + *SOX8* rs2744148*G	0.15	0.05	3.07	1.73–5.44	$3.85x10^{-5}$	$2.52x10^{-4}$
EOMES rs11129295*T/T + *SOX8* rs2744148*G	0.08	0.03	3.01	1.39–6.50	$2.48x10^{-3}$	$7.41x10^{-3}$
RGS1 rs1323292*A + *IL7RA* rs6897932*T + *GPR65* rs2119704*C + *SOX8* rs2744148*G	0.15	0.06	3.00	1.72–5.25	$3.71x10^{-5}$	$2.45x10^{-4}$
INAVA rs7522462*A + *MANBA* rs228614*A/G + *SOX8* rs2744148*G	0.08	0.03	3.00	1.39–6.45	$2.43x10^{-3}$	$7.28x10^{-3}$
INAVA rs7522462*G + *C6orf10* rs3129934*C + *SOX8* rs2744148*A	0.77	0.91	0.31	0.18–0.52	$1.98x10^{-6}$	$2.82x10^{-5}$
INAVA rs7522462*G + *C6orf10* rs3129934*C	0.81	0.93	0.30	0.17–0.54	$1.47x10^{-5}$	$1.21x10^{-4}$
CD86 rs9282641*G + *C6orf10* rs3129934*C + *SOX8* rs2744148*A	0.88	0.97	0.24	0.11–0.52	$6.94x10^{-5}$	$3.96x10^{-4}$
C6orf10 rs3129934*C	0.94	0.99	0.20	0.08–0.47	$8.04x10^{-5}$	$4.41x10^{-4}$
Men						
EOMES rs11129295*C + *C6orf10* rs3129934*T + *RPS6KB1* rs180515*C	0.27	0.10	3.25	1.92–5.48	$8.76x10^{-6}$	0.014
C6orf10 rs3129934*T + *RPS6KB1* rs180515*C + *CD40* rs6074022*T	0.31	0.13	3.00	1.83–4.90	$1.03x10^{-5}$	0.011
EOMES rs11129295*C + *PVT1* rs4410871*T + *GPR65* rs2119704*A	0.01	0.08	0.14	0.03–0.58	$6.630x10^{-4}$	0.013

sclerosis of the five variants (*INAVA* rs7522462, *EOMES* rs11129295, *C6orf10* rs3129934, *CD86* rs9282641, and *GPR65* rs2119704) identified earlier in genome-wide scans. The strongest association was detected for the rs3129934 polymorphism located in the MHC region. The lack of association for the rest of the tested SNPs may be due to the limited sample size and the population admixture. However, this is the largest study of multiple sclerosis in Russian population conducted to date (641 patients and 1407 unaffected individuals), and the study group was formed taking into account the ethnic origin of the participants. Using multilocus approach, we were able to identify complex markers of MS that may reflect the interindividual differences in predisposition to MS. Future endeavours implementing approaches such as whole genome-sequencing or exome-sequencing may help further elucidate mechanisms underlying the development of MS in these three ethnic populations.

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CRediT authorship contribution statement

YT: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **TRN:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Writing – review & editing. **IAT:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision. **VVE:** Data curation, Investigation. **TRG, OVZ:** Data curation, Investigation, Project administration. **KZB:** Investigation, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2021.146008.

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