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MEDICAL GENETICS

Associations of Polymorphic DNA Markers and Their Combinations with Multiple Sclerosis

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Abstract—Multiple sclerosis (MS) is regarded as multifactorial, polygenic disease; its development is the result of autoimmune and neurodegenerative processes which lead to multifocal lesions of the central nervous system. The aim of the study was to analyze associations between MS and polymorphic markers rs3129934 (*C6orf10*), rs1109670 (*DDEF2/MBOAT2* gene), rs9523762 (*GPC5* gene), rs28362491 (*NFKB*1 gene), rs10974944 (*JAK2* gene), and rs2304256 (*TYK2* gene). The material for the study was DNA samples of unrelated MS patients ($N = 224$) aged 17 to 67 years and individuals of a control group ($N = 312$) aged 18 to 66 years. Both samples were formed from the ethnic group of Russians. The results of the investigation demonstrated that, for women, MS was associated with genotypes rs3129934*C/T ($p = 0.001$, OR = 2.23), rs3129934*T/T ($p = 0.028$, OR = 4.04), and rs2304256*C/C ($p = 0.049$, OR = 1.6); for men, with genotype rs1109670*C/A ($p = 0.017$, OR = 2.06). In addition, using the APSampler algorithm, we identified combinations of alleles associated with increased risk of MS separately for women and men, in which the most frequent alleles of polymorphic markers were rs3129934*T, rs1109670*C, rs10974944*G, and rs2304256*C.

Keywords: multiple sclerosis, candidate gene, APSampler

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INTRODUCTION

Multiple sclerosis (MS) is a severe demyelinating disease of the central nervous system disabling young people of working age. Epidemiologic data indicate a steady increase in incidence of MS both in European states and in some regions of Russia, including in the Republic of Bashkortostan [1, 2].

The etiology of MS has not been fully elucidated, thus impeding the elaboration of effective prophylaxis and treatment strategies. The results of familial and twin studies indicate a significant contribution of genetic susceptibility to the development of this disease. The risk of MS occurrence among the related patients is 20–40 times higher than in the general population [3], and concordance for MS among monozygotic twins is 25–30%, and among dizygotic twins it is 3–5% [4].

The molecular-genetic basis of genetic susceptibility for MS is currently actively being investigated. One of the approaches is an analysis of associations of the disease with polymorphic DNA markers located in the regions of the genes whose products have presumptive implication in the pathogenesis of MS. Since MS is an autoimmune disease, researchers pay particular attention, when studying the genetic nature of the disease, to the markers located in the region of genes responsible for immune response [5]. Another approach is genome-wide screening for markers distributed throughout the genome (GWAS—genome-wide association studies) using high density arrays allowing the detection of new genomic regions associated with the disease [6–10]. A mandatory condition of these studies is the validation of the results using independent samples.

The vast majority of studies focused on the search for associations of polymorphic DNA markers with MS assess the role of individual polymorphic markers in genetic susceptibility to this disease. According to current views, MS is regarded as a multifactorial polygenic disease caused by a complex interaction of multiple genes and external factors. Therefore, the influence of any one factor is most often insignificant and moreover its role can vary substantially and depend on the elements interacting with this factor. Hence, an

Table 1. List of amplified markers, their location, primer sequence, and sizes of DNA fragments

Gene, chromosome location	Polymorphism	Primers (restrictase)	Allele (size of DNA fragments, bp)
C6orf10 6p21.32	rs3129934 NC_000006.11:g.32336187T>C	F 5'-gcc tca tac caa tcc tgt gct-3' R 5'-ctg agg ctc cct tca gat gc-3' *Rsr*2 I	T (303) C (168, 135)
DDEF2/MBOAT2 2p25.1	rs1109670 NC_000002.11:g.9250038C>A	F 5'-agc aca gct agg gac gta gg-3' R 5'-gaa agc ata ggt ccc cct cg-3' A 5'-cgt ctg tta ggc ttt ttc cag ta-3' C 5'-cgt ctg tta ggc ttt ttc cag tc-3'	IC (264)* Allele (170)
GPC5 13q31.3	rs9523762 NC_000013.10:g.93331886G>A	F 5'-aca ctt tca gag agc aga cag a-3' R 5'-ttg gaa ggg aac agg aaa cac a-3' *Sse*9 I	G (121) A (56, 65)
NFKB1 4q24	rs28362491 NC_000004.11:g.103422155_ 103422158delATTG	F 5'-gga ccg cat gac tct atc agc-3' R 5'-ccg aat ccc aag ggc tgg ag-3'	D (127) I (131)
JAK2 9p24.1	rs10974944 NC_000009.11:g.5070831C>G	F 5'-ctt gca gat gca gaa ccc g-3' R 5'-tgc tct taa atc tta acc ccc tt-3' *Ksp*22I	G (202) C (162, 40)
TYK2 19p13.2	rs2304256 NP_003322.3:p.Val362Phe exon 8	C 5'-agg cca agg ctc aca agg cag-3' A 5'-agg cca agg ctc aca agg cat-3' F 5'-cca ggc act tgt tgt cct gc-3' R 5'-tgc agg agg tat aaa cgg gc-3'	IC (260)* Allele (147)

* IC—internal control, explained in the text.

analysis of MS associations with a combination of several factors is more informative.

Therefore, the aim of this study is to analyze the associations between MS and polymorphic markers of immune response genes (rs28362491, *NFKB*1 gene; rs10974944, *JAK2* gene; rs2304256, *TYK2* gene) and polymorphic markers that showed association with MS in GWAS (rs3129934, rs1109670 of the *DDEF2/MBOAT2* gene, rs9523762 of the *GPC5* gene), as well as to analyze the associations between MS and allele combinations of these DNA markers.

MATERIALS AND METHODS

The study material was DNA samples from unrelated MS patients (224 people: 148 females, 76 males) aged from 17 to 67 years (the mean age was 39.89 ± 9.34 years). All patients underwent complete clinical examination at the Republic Clinical Hospital in Ufa; the diagnosis of MS was established according to criteria by McDonald et al. [11]. The mean length of the disease was 12.3 ± 9.56 years; the mean age of disease manifestation was 28.83 ± 9.07 years. The control group included 312 people (158 men, 154 women) aged from 18 to 66 years (the mean age was 38.67 ± 10.72 years). All participants were Russians by ethnic origin, permanently living in the Republic of Bashkortostan. All subjects included in the study signed an informed voluntary consent on participation.

Samples of DNA were obtained from peripheral blood leukocytes by phenol-chloroform extraction. The polymorphic markers (except for rs2304256 and rs1109670) were genotyped by polymerase chain reaction (PCR) and subsequent treatment of the amplification products with a corresponding restriction endonuclease. The rs2304256 and rs1109670 markers were analyzed by site-specific PCR. Two PCR reactions were performed for each DNA sample; each reaction contained only one of the allele-specific primers and a pair of primers for elongation of a fragment with the substitution under study, which was used as a control for PCR. Primers and restrictases for each marker were selected using the DNAStar software package 5.05 and databases http://www.ncbi.nlm.nih.gov/snp. The sequences of primers, names of restrictases, and sizes of the amplified fragments are shown in Table 1. The amplicons were separated using 7% polyacrylamide or 2% agarose gel electrophoresis.

To compare the groups for distribution of genotype and allele frequencies, we used the two-tailed Fisher's exact test. The deviation of the observed genotype frequencies from the theoretical distribution according to the Hardy–Weinberg equilibrium was evaluated using an exact test implemented in the Arlequin 3.0 software