DNA Length Polymorphism of Tetranucleotide Repeat at the 5' Side of the Myelin Basic Protein Gene in Russian Multiple Sclerosis Patients

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Abstract—The myelin basic protein gene (MBP) can confer the susceptibility to multiple sclerosis, because its protein product is the main protein component of myelin of the central nervous system and a potential autoimmun e antigen in the disease. A possible association of multiple sclerosis with alleles and genotypes of a microsatellite repeat (TGGAG)n, located to the 5’ side from the first exon of MBP in ethnic Russians (126 patients with definite multiple sclerosis and 142 healthy controls from Central Russia) was analyzed in a case–control study. Upon separation of the tetranucleotide repeat amplification products in 1.5% agarose gel, one can see two distinct bands that can be analyzed as two allele groups (A and B). The distribution of allele A and B group frequencies as well as phenotype frequency of alleles B and genotype frequency of A/A differs significantly in multiple sclerosis patients and healthy controls. Alleles A and genotype A/A are associated with multiple sclerosis. We also analyzed the association of multiple sclerosis with combined bearing of alleles and genotypes A and B of MBP and groups of alleles of the DRB1 gene of the major histocompatibility complex that correspond to serological specificities DR1-DR18. The comparison of subgroups of multiple sclerosis patients and healthy individuals, stratified according to HLA-DRB1 phenotypes, has shown a reliable increase in the phenotype frequency of allele B in healthy individuals and the genotype A/A frequency in patients, only among DR4- and DR5-positive individuals. No significant difference was found in the MBP allele and genotype distribution between multiple sclerosis patients and healthy individuals in combined groups of (DR4,DR5)-negative individuals, i.e., in the group of carriers of any phenotype except DR4 and DR5. Thus, MBP or some other nearby gene is involved in the multiple sclerosis development in Russians, predominantly (or exclusively) among DR4 and DR5 carriers. In this case, without stratification of analyzed individuals by the MBP alleles, multiple sclerosis is associated only with DR2(15), but not DR4 and DR5 alleles of DRB1. The results obtained are in favor of the genetic heterogeneity of multiple sclerosis, and suggest the possibility of epistatic interactions between the MBP and DRB1 genes.

Key words: man, multiple sclerosis, DNA, genotyping, polymerase chain reaction, microsatellite repeat, myelin basic protein gene, DRB1 gene, major histocompatibility complex, allele polymorphism, functional genomics

INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune inflammatory disease, characterized by destruction of the myelin shell of axons in the central nervous system (CNS), and belonging to typical complex (multifactorial) genetic diseases. Studies of familial forms of MS and its occurrence among twins and adopted children point to the main role of genetic factors in susceptibility to MS [1]. Results of full genome search, carried out in multiplex families from Canada, USA, Great Britain, and Finland [2–6] and of global meta-analysis [7] correlate with the concept that MS is the result of epistatic interaction of several genes. The only genome region whose linkage to MS was revealed in all works is located on the short arm of chromosome 6 (6p21) and includes HLA genes of class II of the major histocompatibility complex. As in the case of other complex diseases, results of genomic search for linkage are indicative of insignificant contribution of each individual gene to the MS susceptibility. Therefore, studies based on a genetic association approach are preferable [6]. Association testing is extensively employed in candidate gene studies. Up to now, the DR2-haplotype of HLA class II (DR15, DQ6, Dw2) is the only region whose association with MS was repeatedly confirmed in Caucasians [8]. Genes of T cell receptors, heavy chains of immuno-
globulins, components of the complement system, cytokines, and potential CNS autoantigens are also considered as candidates genes [9, 10].

Among the latter, the gene encoding the main protein component of myelin, myelin basic protein (MBP), deserves most attention. The MBP gene is located on the long arm of chromosome 18 in the q22 region. The region flanking the 5' end of its first exon varies in length owing to the presence of a polymorphic site that contains tetranucleotide repeats (TGGA)_n and is represented by more than 10 alleles [11, 12]. Studying this repeat with a fluorescence semiautomatic polymerase chain reaction (PCR) technique revealed two allele clusters, one of 1218 ± 8 bp and the other of 1292 ± 12 bp [12]. This polymorphism in MS was also analyzed by other methods. First, using the restriction fragment length polymorphism (RFLP) technique, later by PCR with different sets of primers (as a result, reaction products of different length were obtained) [12–20]. In most cases the MBP alleles were combined in two groups and this polymorphism was analyzed as biallelic. Some authors observed the association of these allele groups with MS [13–16]. However, their data were not confirmed by other researchers [12, 17–20]. Data concerning the linkage of this microsatellite-containing region with familial MS are also contradictory. Such discrepancies can be caused by different factors, for example, by genetic heterogeneity of MS forms, genetic peculiarities of studied populations, differences in boundaries between chosen sites and repeats, in genetic typing techniques, as well as in the selection range of both patients and healthy controls.

In this work we studied a possible MS association in Russians with alleles and genotypes of the region containing microsatellite repeat (TGGA)_n at the 5' side of the MBP first exon. To assess the possible epistatic interaction of MBP and DRB1 genes, patients and healthy controls were divided into subgroups by their DR specificity.

**EXPERIMENTAL**

**Formation of MS patient and healthy control groups.** The analyzed group of unrelated patients included 126 people (66 women and 60 men) with “definite MS” according to Poser's criteria [21]; the MS course was relapsing–remitting in 89, secondary progressive in 26, and primary progressive in 11. The average age of patients was 32 ± 11, the average age by the onset of disease was 23 ± 9. All patients were residents of the central region of Russia, with both parents Russian. The control group (142 people, 88 women and 54 men) consisted of healthy volunteers of the same ethnic group: students of the Russian State Medical University and donors of Moscow blood transfusion units. The average age of the control group was 28 ± 10.

**DNA isolation.** A 5% EDTA solution was used to preserve blood samples. Genomic DNA was isolated from peripheral blood mononuclears with phenol–chloroform according to a standard protocol.

**Length polymorphism of microsatellite repeat** was analyzed by PCR with a pair of primers 5'-ATATGTTGATGGATGACGAAT-3' and 5'-CAGGATCTCACTCATATTTCTCTG-3' [16]. The reaction mixture (25 μl) contained 50 mM KCl; 10 mM Tris-HCl, pH 8.4; 1.5 mM MgCl₂; 0.25 μM primers; four dNTP, 200 μM each (Takara Shuzo Co., LTD, Japan); 2.5 units of Taq polymerase (Takara Shuzo); and 250 ng genomic DNA. Samples were heated at 95°C for 5 min, cooled on ice, then amplification was carried out in a Gene Amp 2400 Thermal Cycler (Perkin Elmer Cetus) in 35 cycles of 1 min, 94°C; 1 min, 58°C; and 1 min, 72°C; the final step of elongation at 72°C lasted 7 min. PCR products were analyzed by electrophoresis in 1.5% agarose gel in Tris-borate buffer, pH 8.3 and stained with ethidium bromide.

**Genomic typing of HLA-DRB1 locus.** For total typing of the DRB1 gene, two-step PCR with a mixture of allele-specific primers was used [22]. At the first step, standard generic amplification of DRB1 was carried out with the pair of primers recommended by the Eleventh International Histocompatibility Workshop [23]. The reaction mixture (10 μl) contained 5 pmol of each primer, 20 ng genomic DNA; 50 mM KCl; 10 mM Tris-HCl, pH 8.4; 2.5 mM MgCl₂; 1 μg gelatin; 0.02% NP-40; four dNTP, 200 μM each (Silex M); and 0.5 units of Taq polymerase. Amplification was carried out under the following conditions: 5 cycles (1 min 45 s, 94°C; 1 min 45 s, 65°C; 1 min 45 s, 72°C), then 30 cycles (1 min, 92°C and 1 min, 72°C). The PCR products were analyzed by electrophoresis in 2% agarose gel.

At the second step, the product of the first PCR was amplified in parallel with two group-specific mixtures of primers, which allow one to obtain discrete products differing in length (no less than by 10%) corresponding to separate groups of DRB1 alleles. One mixture of primers was used to amplify the following specificities: DR1, DR2, DR4, DR7, DR9, and DR10, while the other was used for amplification of DR3(17), DR3(18), DR5(11), DR5(12), DR6(13), DR6(14), and DR8. One microliter of the first PCR product, diluted to DNA concentration ca. 100 ng/μl, was added to 10 μl of reaction mixture, and the other components were added as at the first step of PCR. Amplification was run in 12 cycles of 1 min, 92°C and 1 min, 60°C. PCR products were analyzed by electrophoresis in 12% PAG. As positive controls, DNA from panels of the Eleventh International Histocompatibility Workshop (1991) were used.