THE PROMOTOR POLYMORPHISM OF MACROPHAGE METALLOELASTASE IN MULTIPLE SCLEROSIS

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ABSTRACT

Multiple sclerosis is a chronic demyelinating disease of the central nervous system in which the genetic background plays an important role. Its pathophysiology is characterised by two major processes: neuroinflammation and neurodegeneration. Matrix metalloproteinases are involved in both of them. Macrophage metalloelastase is one of the three matrix metalloproteinases the common elevation of which has been confirmed in multiple sclerosis and also in animal models with experimental allergic encephalomyelitis. To assess the association between its promotor polymorphism and demyelinating disease we genotyped a total of 92 patients (23 men, 69 women, mean age 37 years) with definite multiple sclerosis (according to the McDonald criteria) and 51 healthy controls (17 men, 34 women) matched for age and sex. Genotyping was performed by means of polymerase chain reaction with restriction analysis. We observed no statistically significant differences in genotype or allele distribution of –82 A/G polymorphism between the groups examined (OR=2.6, p=0.026, pcorr=0.078). Due to insufficient numbers of patients with the progressive form [9], no statistically significant differences in genotype or allele frequencies emerged among the patients with variant forms of multiple sclerosis. Nevertheless, all patients with the progressive form (which is associated with a more severe course and higher disability) were of the same genotype: homozygotes AA. This genotype is connected with higher promotor activity and a higher expression of the final protein. It may represent a variant genotype base for a different course of multiple sclerosis in the polymorphism under investigation.

ABBREVIATIONS USED

MS – multiple sclerosis
MMP – matrix metalloproteinase
CNS – central nervous system
EAE – experimental allergic encephalomyelitis

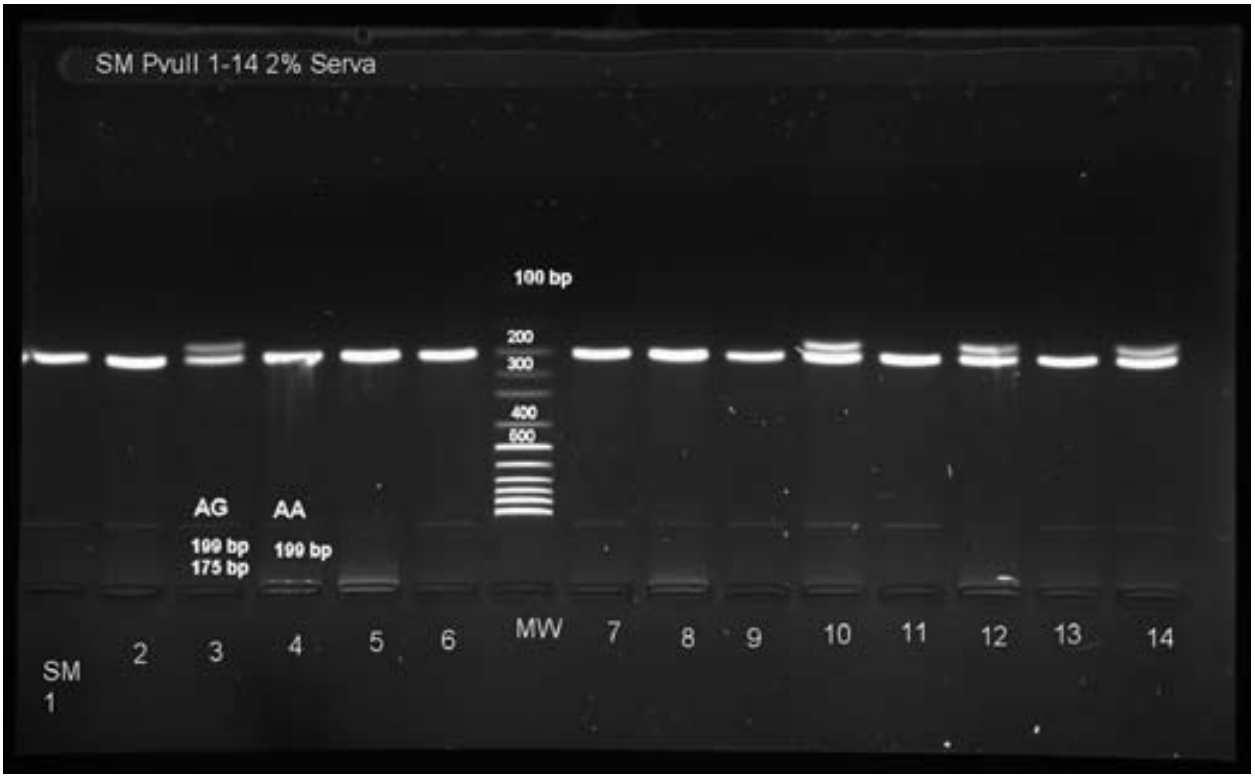

Figure 1
Final genotypes visualised in UV light after ELFO on 2% Serva agarose gel with ethidium bromide

RR – relapsing remitting
SP – secondary progressive
PP – primary progressive
DNA – deoxyribonucleic acid
PCR – polymerase chain reaction
ELFO – electrophoresis
AP–1 – activator protein–1
HW – Hardy-Weinberg equilibrium

INTRODUCTION

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS). It is one of the most frequent causes of young adult disability in our geographical zone [1]. MS is a multifactorial disease in which the genetic background plays an important role. Its pathophysiology is characterised by neuroinflammation and neurodegeneration. Matrix metalloproteinases (MMPs) – a family of Zn-dependent endopeptidases [2] – are involved in both these processes. Some 26 MMPs are known to date, of which 23 may be present in humans. The expression of MMPs is usually low in the adult body and is typically observed in a local manner in the course of specific processes [2–4]. In the pathogenesis of multiple sclerosis, MMPs participate in blood-brain barrier disruption, leukocyte perivascular infiltration, myelin destruction, and conversion of the proforms of inflammatory molecules; they may also be responsible for neurotoxicity [4]. Because of their heavy destructive effect, their activity is under very tight regulation [2, 3]. The first step in this path is regulation through the gene polymorphisms.

Elevated levels of nine MMPs have been found in spinal cord samples taken at the peak of disease in experimental allergic encephalomyelitis (EAE), the accepted animal model of MS [5–7]. One of them was macrophage metalloelastase (MMP12), which up-regulated by a factor of more than three in one of these studies [8]. Increased expression of MMP12 was also confirmed in vitro in cultured rat astrocytes [9]. It has been found that the macrophages of MM12-deficient mice have a markedly diminished capacity to degrade extracellular matrix components. In addition, these macrophages were essentially unable to penetrate reconstituted basement membranes both in vitro and in vivo. MMP12 is therefore required for macrophage-mediated extracellular matrix proteolysis and tissue invasion [10]. Elevated MMPs have also been

Table 1
Primers and PCR conditions

Left primer (5´-3´) **Right primer (5´-3´)**	**PCR** *(temperature °C, time)*
GAGATAGTCAAGGGATGATATCAGC AAGAGCTCCAGAAGCAGTGG	95˚C/5´ 95˚/45´´-55˚/30´´-72˚/45´´ (30x) 72˚/7´ 10˚/10

Table 2
Restriction analysis

Restriction enzyme	**Conditions**	**Fragment length (bp)**
Pvu II, 5´CAG↓CTG 3´GTC↓GAC	37°C /4h: PCR product 15µl, H2O 2.7µl, buffer NEB 2 2µl, Pvu II 3U	AA 199 / 199 bp GG 24,175 / 24,175 bp AG 199 / 24,175 bp

Table 3
Genotypes and alleles

MMP12 -82 A/G	**PATIENTS** *number (frequence %)*			**CONTROLS** *number (frequence %)*		
Genotypes	**All (n=92)**	**Men (n=23)**	**Women (n=69)**	**All (n=51)**	**Men (n=17)**	**Women (n=34)**
AA	64 (69.57)	16	48	42 (82.35)	13	29
AG	27 (29.35)	7	20	7 (13.73)	3	4
GG	1 (1.09)	0	1	2 (3.92)	1	1
Alleles						
A	155 (0.84)	39	116	91 (0.89)	29	62
G	29 (0.16)	7	22	11 (0.11)	5	6

detected in serum and cerebrospinal fluid and the brains of MS patients on autopsy. Prominent MMP12 staining has been reported in macrophages found in active demyelinating MS lesions [11]. A lower proportion of phagocytes was positive for MMP12 in chronic active and inactive plaques [11]. Thus, MMP12 is one of the three MMPs (together with MMP9 and 3) the common elevation of which has been confirmed in MS and also in mouse and rat EAE models [4,12].

The MMP12 gene is located on the eleventh chromosome (11q22.2-q22.3) [13]. By single-strand polymorphism confirmation analysis of deoxyribonucleic acid (DNA) from healthy individuals a common polymorphism within the MMP12 gene promotor has been detected. This –82 A/G polymorphism of MMP12 presents a functional polymorphism in which allele A shows a higher affinity for the transcription factor activator protein–1 (AP–1) and is thus associated with a higher promotor activity and a higher MMP12 expression in assays [14].

In the present study we examined the relationship between –82 A/G MMP12 polymorphism and MS.

MATERIALS AND METHODS

Patients and control subjects

In a case-control study a total of 92 unrelated patients (23 men and 69 women, mean patient age 37.3 ± 9.0 years, mean EDSS score 3.71 ± 1.4, and mean disease duration 9.9 ± 5.1

years) with definite MS according to the McDonald criteria were recruited from the MS centre at the Department of Neurology, Faculty Hospital and Faculty of Medicine, Masaryk University, Brno. The cohort included 83 patients with the remitting relapsing form of MS – RRMS, 8 patients with the secondary progressive form – SPMS (5 women and 3 men, mean EDSS score 5.8 ± 1.4, mean disease duration 10.4 ± 4.3 years), and one patient with the primary progressive form – PPMS (a man, EDSS 4.0, disease duration 4 years). The control group consisted of 51 healthy controls (17 men and 34 women) with no history of MS or other autoimmune disease, and the subjects were matched for age and sex. All patients and controls were of Czech ancestry. Written informed consent was obtained from all the subjects examined. The study was approved by the Committee for the Ethics of Medical Experiments on Human Subjects, Faculty of Medicine, Masaryk University, Brno [ref. No. 48/ 2003].

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes by a standard technique using proteinase K digestion of cells. The resulting DNA was used as a template for polymerase chain reaction (PCR). The method used to type the MMP12 –82A/G promotor polymorphism has been described previously [14]. Primers and conditions for amplification are shown in Table 1. PCR was performed at a final volume of 25µl containing 3µl of genomic DNA, 0.5µl dNTP, 1.25µl of each primer, 2.5µl buffer, 0.14µl Taq polymerase, and 2µl 25mM MgCl2. Equal amounts of 15µl of the PCR products were digested with specific endonucleases to obtain final fragments (Table 2). The final genotypes were visualised in UV light after ELFO on 2% Serva agarose gel with ethidium bromide (Figure 1).

Statistical analysis

Hardy-Weinberg equilibria (HW) of the study polymorphism in all cases and controls were chi-square tested. Comparisons of the allele variant frequencies as well as comparisons of genotype incidence in the case-control study were calculated using the Fisher exact test. Statistical significance was considered as $p<0.05$. The Holm test for multiple comparisons was employed where appropriate.

RESULTS

Differences were evident in genotype distribution between our study groups, in which the AG heterozygotes were more frequent in MS patients (OR=2.6, p=0.026). Using the Holm test for multiple comparison, the results indicated only a trend, lacking statistical significance (pcorr=0.078). Even after the stratification of the subjects by sex, no allele or genotype differences were observed between the groups examined. The representation of the appropriate genotypes and alleles is shown in Table 3. The control group was not in HW equilibrium. No differences were found in genotypes or alleles among MS patients with variant forms of the disease. Nevertheless, all the patients with the progressive form (which is associated with a more severe course and higher disability) were of the same genotype: homozygotes AA (data not shown).

DISCUSSION

It is assumed that different genetic backgrounds exist for the various forms of MS and also that genetic backgrounds vary from population to population. The Czech Republic is a country with both a high genetic homogeneity and a high prevalence of MS. The group examined therefore constitutes a proper and representative sample. On the other hand, the study group is too small to assess susceptibility to the different forms of MS. Despite this, all the patients with the progressive form of MS were of the same genotype, homozygotes AA. This genotype is associated with a higher promotor activity, a higher expression, and a higher final MMP12 plasma level. This corresponds with the idea of different genetic backgrounds for the various courses of MS.

In animal models elevated MMP12 has repeatedly been observed not only at the peak disease activity but also in the late phase of infection [11, 15–17]. MMP12 protein has been localised by immunohistochemistry in intralesional microglia/macrophages and astrocytes and might account for ongoing demyelination [15]. One of these studies demonstrated that MMP12 was the most highly up-regulated MMP. However, in contrast to previously published findings, this increase was associated with protection, as MMP12-null mice had a significantly worse maximum severity and EAE disease burden compared with wild-type controls [17]. MMP12 can therefore play both negative and positive roles in the pathophysiology of MS. At the peak phase of the disease during relapses, it may participate in blood-brain barrier disruption and potentiation of leukocyte infiltration, but in the late phase of the neuroinflammation the increased MMP12 level may be connected, for example, with some remyelination. Elevated levels of MMP12 have been observed at the time of myelination in mouse brain maturation [18].

CONCLUSIONS

There can be no doubt about MMP participation in the pathophysiology of MS, but the exact function of specific MMPs is not sufficiently understood. This results from the high degree of interaction among most MMPs, not only at the gene level but

also at further stages, where they may activate or inhibit each other. For this reason, further and more extensive investigations into the genotypes, and especially the haplotypes of MMPs, are indicated. They may well disclose more profound effects on susceptibility to MS or on tendencies to different courses of MS.

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