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Multiple sclerosis: Association of gelatinase B/matrix metalloproteinase-9 with risk and clinical course the disease

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ABSTRACT

Background: Multiple sclerosis (MS) is an autoimmune disease characterized by inflammation and axonal degeneration of the central nervous system and a leading cause of disability in young adults. The matrix metalloproteinases in general and specially gelatinase B/metalloproteinase-9 (MMP-9) plays a role in the pathogenesis of multiple sclerosis.
Objective: To investigate the presence of the MMP-9 −1562 C/T polymorphism in a Portuguese population of MS patients and assess its impact in susceptibility and course of the disease. The relation of MMP-9 serum levels with the polymorphism and with clinical and therapeutic factors will also be assessed.
Methods: Our study included 355 Caucasian individuals distributed as MS patients (n=169) and controls (n=186). Samples were genotyped for −1562 C/T polymorphism by PCR-RFLP analysis. MMP-9 concentration in serum was analyzed using a commercially available enzyme-linked immunosorbent assay.
Results: A significant increase in T-allele frequency was found in female MS patients, but not in the total patient population. No association between the presence of the polymorphism and disease progression was found. MMP-9 serum concentrations were increased in patients, and although not influenced by the −1562 C/T polymorphism, were modified by INF-beta therapy.
Conclusion: Although we did not find an association of this polymorphism with disease susceptibility or prognosis, MMP-9 appears to be a good therapeutic response marker for multiple sclerosis.

1. Introduction

Multiple sclerosis (MS) is a progressive autoimmune disease characterized by inflammation, demyelination, and axonal degeneration, resulting in the interruption of myelinated tracts of the central nervous system (CNS) and a leading cause of disability in young adults. The heterogeneous clinical course of the disease and underlying pathophysiological mechanisms, make MS prognosis extremely challenging to define (Hauser and Oksenberg, 2006).

Although the etiology of the disease remains unclear, an autoimmune reaction directed against antigens of cerebral white matter has been proved (Hartung et al., 2004) and the migration of autoreactive immune cells through the blood-brain barrier (BBB) into the CNS seems crucial for the formation of inflammatory lesions (Martin et al., 2000). An important role in this process is played by matrix metalloproteinases (MMPs). In particular, gelatinase B/MMP-9, has been demonstrated to facilitate the influx of inflammatory cells into the CNS (Gijbels et al., 1993; Lee et al., 1999), as well as, the BBB breakdown (Chandler et al., 1995; Gijbels et al., 1993) and to cleave human myelin basic protein *in vitro* (Proost et al., 1993). MMPs are secreted by a wide range of cell types, capable of degrading all protein components of the extracellular matrix (Chandler et al., 1995; de Souza et al., 2005). The

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activity of the MMPs is regulated at different levels (Yong et al., 2001; Yushchenko et al., 2003), such as gene expression, proenzyme activation, and by the activity of the tissue inhibitors of matrix metalloproteinases (TIMPs). The transcriptional activity of the *MMP-9* gene (located in chromosome 20q13) is influenced by two polymorphisms identified in the promoter region. These are a CA_n microsatellite polymorphism from position −90 (St Jean et al., 1995) and a single nucleotide polymorphism at position -1562 caused by a C to T substitution (Zhang et al., 1999). *In vitro* studies have shown that this substitution prevents the binding of a nuclear transcription repressor protein to this region of the *MMP-9* gene promoter, being associated with increased MMP-9 expression (Zhang et al., 1999). On this basis, it is reasonable to hypothesize that both −1562T and the longest repeat alleles may be highly plausible genetic risk factors for MS and therefore, there has been considerable research interest in the possible association of variations in the *MMP-9* gene and MS susceptibility.

A few studies have addressed the involvement of the −1562 C/T polymorphism, alone or in combination with the CAn microsatellite polymorphism, with susceptibility to MS (Benesova et al., 2008; Fiotti et al., 2004; Nelissen et al., 2000; Zivkovic et al., 2007). Despite controversial results (La Russa et al., 2010; Mirowska-Guzel et al., 2009), it is generally accepted that the −1562 C/T polymorphism has no impact on susceptibility to MS (Nischwitz et al., 2015), but its possible influence on disease course is still largely unknown.

At protein level, both gelatinase B/MMP-9 increased concentration (Gijbels et al., 1992; Lee et al., 1999) and activity (Avolio et al., 2003; Liuzzi et al., 2002) in serum and in the cerebrospinal fluid (CSF) of MS patients, compared to controls, have been well established. In fact, this MMP has been suggested as a useful marker for the evaluation of the clinical type, disability and severity of MS (Benesova et al., 2009). However, the relation between MMP-9 levels in peripheral blood and the −1562 C/T and CA_n microsatellite polymorphisms has been addressed only in two small studies (Fernandes et al., 2012; Mirowska-Guzel et al., 2009).

MMP-9 has also been considered a therapeutical response biomarker for interferon-beta (IFN-beta), a common immunomodulatory first-line treatment employed in MS. In fact, serum MMP-9 concentration decrease with IFN-beta therapy (Alexander et al., 2010; Comabella et al., 2009; Yilmaz et al., 2012), correlating with the decrease of active lesions during treatment (Avolio et al., 2005; Trojano et al., 1999).

The aim of our study was to investigate the presence of the −1562 C/T polymorphism in the *MMP-9* gene in a Portuguese population of MS patients, and assess its impact in MS course. We also evaluated serum concentration of MMP-9, and their relation with the −1562 C/T polymorphism, as well as with clinical and therapeutical factors.

2. Materials and methods

2.1. Subjects

This study comprised 355 Caucasian individuals originated from Portugal that represent a genetically stable and homogenous population. MS patients (n=169) were recruited at the Neurology Department of the Centro Hospitalar e Universitário of Coimbra (CHUC)-Coimbra, Portugal and the Braga Hospital-Braga, Portugal. MS was diagnosed according to the McDonald and Polman criteria (McDonald et al., 2001; Polman et al., 2011) and all patients had a minimum clinical follow-up time, of two years. Information regarding patient gender, age at onset, disease duration, initial symptoms (optical *vs* other pathways), subtypes (relapsing-remitting-RR; secondary progressive-SP; primary progressive-PP), severity (estimated using the Expanded Disability Status Scale-EDSS), (Kurtzke, 1983) and treatment, were retrieved from local MS database or individual medical records and included in the analysis. All MS patients had been treated with first-line therapy only (59.7%; including IFN-beta and glatiramer acetate) or with second-line therapy (40.3%; including fingolimod and natalizumab) (Kerbrat et al., 2015). At time of blood collection, 60% of patients were undergoing therapy with IFN-beta, and all RR patients were in remission.

The control group included 186 unrelated healthy volunteers, matched for age, gender and ethnicity. The study protocol was approved by the Hospital (CHUC) Ethics Committee. All participants in this study provided written informed consent.

2.2. Genotyping

Blood samples were collected with EDTA tubes, and genomic DNA was extracted using a standard procedure (Spin Blood Mini Kit, Invisorb®, Stratec molecular - Berlin). The polymorphism at position −1562 C/T (rs3918242 deposited in the NCBI database) in *MMP-9* gene promoter was genotyped by PCR-restriction fragment length polymorphism (RFLP) analysis. PCR amplification, was performed using the primers 5'-ATGGCTCATGCCCGTAATCC-3' (forward) and 5'-GGGCAGGGTCTATATTCACC-3' (reverse), as previously described by (Fernandes et al., 2009; Zhang et al., 1999). The amplified products were digested with *Sph*I (Thermo Scientific, USA), overnight at 37 °C, producing fragments of 224 and 124 bp (T allele) or an undigested fragment of 348 bp (C allele). Fragments were then separated by electrophoresis in 2% agarose gel and visualized through ethidium bromide.

2.3. MMP-9 concentration in serum

MMP-9 concentration in serum (both 92 kDa Pro- and 82 kDa active forms) of 96 MS patients and 63 controls was measured using a commercially available enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (Quantikine Human MMP-9 Immunoassay, R & D Systems Europe, Ltd. UK).

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 21.0 (IBM). Differences in both allele and genotype frequencies distribution between study groups as well as deviation from Hardy-Weinberg equilibrium were estimated by χ^2 test. Hardy-Weinberg equilibrium was evaluated using a Hardy-Weinberg test Equilibrium Calculator for 2 Alleles (Emerson, 2010). Differences in demographic, clinical variables and MMP-9 serum concentration between groups were compared using the Mann-Whitney *U* test (two groups) or the Kruskal Wallis test (three groups) for continuous variables or the Pearson chi-square (χ^2) test for categorical variables. A two-way ANOVA was used for establishing interactions between diagnosis and MMP-9 genotype. Binary logistic regression models, controlled for age and gender were used to assess the contribution of clinical variables in predicting the −1562 C/T polymorphism carrier status. Survival analysis was used to assess the influence of the −1562 C/T polymorphism in the probability of reaching an EDSS ≥ 3, previously defined as indicative of moderate disability (Kerbrat et al., 2015; Leray et al., 2010). Kaplan-Meier survival curves were plotted and the survival distributions according to the presence or absence of the T allele were compared by the log-rank test. Survival time was calculated as the interval from the initial baseline evaluation to the time to reach an EDSS of 3. For patients who did not reach this score, survival time was censored at the date of the last clinical assessment. The results were presented as mean ± standard error (SEM) and $p \leq 0.05$ was considered statistically significant.