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Lack of Correlation between *CCL5* -28C/G Functional Polymorphism and Multiple Sclerosis in Tunisian Patients

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Abstract

Multiple sclerosis is a chronic demyelinating disease of the human central nervous system (CNS) of a still unknown etiology. CCL5 is localized in white matter tracts undergoing demyelination, suggesting that this chemokine participates in the pathogenesis of disease by attracting inflammatory cells into the CNS. The *CCL5* -28C/G functional polymorphism have been reported to be associated with multiple sclerosis, however, evidence remains conflicting. In the current study, we investigated distibution of the *CCL5* -28C/G in 51 patients with multiple sclerosis in comparison to 162 healthy blood donors. The data revealed no significant differences in the distribution of the *CCL5*-28C/G polymorphism in multiple sclerosis patients compared with the control group. To conclude, our study showed no association between *CCL5* -28C/G polymorphism and risk development of multiple sclerosis in Tunisian patients.

Keywords: Multiple sclerosis; Polymorphism; CCL5

Introduction

Multiple sclerosis is the most common chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) [1]. MS usually affects young adults and women more frequently than men and is clinically characterized by subsequently appearing neurologic deficits (relapses) and complete or incomplete recoveries (remissions) [2]. About 80% of multiple sclerosis patients start with a relapsing–remitting course that, over time, transforms into a secondary progressive course. In a smaller group of patients (20%), multiple sclerosis begins with a primary progressive [3].

The immunopathogenesis of multiple sclerosis is not completely understood, both polygenic and environmental factors contribute to disease onset and/or clinical exacerbation [2,4,5]. Viral pathogens have been implicated in the etiology and pathogenesis of multiple sclerosis [6]. Among those, strong data implicates Epstein-Barr Virus (EBV) a human DNA virus, as we [7] and others investigators have recently reported [8-12]. In addition to infection, genetic influence on multiple sclerosis is substantial, as evidenced by the 20-fold risk increase for siblings of multiple sclerosis patients [13]. Part of the genetic risk is explained by the MHC class II locus (HLA-DR15) [14]. In 2007 several novel risk alleles for multiple sclerosis were identified by a genome-wide association (GWA) study [4] and others confirmed the susceptibility loci by meta-analyses and replication [15]. These findings indicate that analyzing candidate genes could be an interesting approach in the search for multiple sclerosis susceptibility genes, but that other genetic risk factors such chemokines and their receptors [16] still need to be studied in different ethnic human groups.

CCL5 belongs to the family of CC chemokines, which are involved in immunoregulatory and inflammatory processes owing to their ability to recruit, activate and co-stimulate T cells and monocytes [17,18]. In addition to the trafficking effect, CCL5, like other CC chemokines, plays an important role in co-stimulation of T-cell proliferation [19,20] and activation of the T cells localized in the inflammatory lesion [21]. This chemokine have been detected within the CNS of multiple sclerosis patients as well as within active plaque lesions, suggesting that these molecules contribute to demyelination by attracting targeted populations of leukocytes into the CNS [22-24].

Several functional polymorphisms in the *CCL5* gene have been described [25,26]. Of these SNP, the -28C/G (rs2280788) occurring in proximity to the promoter region was associated with increased transcriptional activity and subsequent CCL5 expression in human cell lines [26], This polymorphism was associated with an increased risk to several inflammatory diseases such as asthma [25,27], sarcoidosis [28], rheumatoid arthritis [29], atopic dermatitis [30], diabetes type 1 [31] and infectious diseases, including HIV [32] and active tuberculosis as we have recently reported [33]. However, association between *CCL5* polymorphism and multiple sclerosis remain controversial. Some studies in the literature revealed an increased risk to multiple sclerosis [34,35].

Tunisia is considered as a low zone of prevalence for multiple sclerosis according to the data obtained from the Atlas of multiple sclerosis resources in the world 2008 reported by WHO [36]. In the present study, we investigated association between *CCL5* -28C/G functional polymorphism and multiple sclerosis.

Materials and Methods

Patients and controls

This study included 51 defined multiple sclerosis patients, enrolled at the department of Neurology, Fattouma Bourguiba Hospital, Monastir, Tunisia, and 162 healthy controls who had donated blood

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at the Regional Center for Blood Transfusion, CHU Farhat Hached, Sousse, Tunisia (Table 1). In the patient group, there were 18 men (mean age, 43.31 years; age range, 24-64; SD 16.970) and 33 women (mean age, 35.81 years; age range, 19-54; SD 6.363). The mean age at disease onset was 32.81 years (SD 14.849, range 16–58), the mean disease duration was 5.46 years (SD 8.485, range 1-15), and mean Extended Disability Status Scale (EDSS) score was 2.921 (SD 1.414, range 1–8). Diagnostic criteria incorporate magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) and evoked potentials testing. 25 of multiple sclerosis patients were receiving interferon β treatment, but none received steroid treatment prior to blood sampling.

In the control group of healthy blood donors, there were 140 men (mean age, 37 years; age range, 20-52, SD 9.36) and 22 women (mean age, 33 years; age range, 22-49; SD 7.47).

This study was approved by the local ethics committee and all of the participants gave informed consent before the experimental procedures.

Genotyping

Genomic DNA was extracted from peripheral blood samples collected on EDTA anticoagulant using spin column technique of QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions, eluted in 100 µL of water and subsequently quantified using Nanodrop spectrophotometer (UV-Visible NanoDrop 1000; Thermo Fisher Scientific Inc.) and standardized to 100 ng/mL.

The polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) method was used as described previously [37] for genotyping the *CCL5*-28C/G SNP.

Genomic DNA (100 ng) was amplified in a 25 mL PCR reaction under the following cycling conditions: denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 55 s, with a final extension at 72°C for 10 min. Amplifications were performed in a MyCycler thermal cycler (Bio-Rad).

The presence of wild type 'C' allele introduces a restriction enzyme site for HincII enzyme and yields 152 and 23 bp fragments. Five microliters of the PCR products were digested with 5U of HincII (Promega) at 37°C for 3 h. The digestion products were analyzed on a 4% agarose gels (Sigma) containing ethidium bromide (0.5 mg/mL) (Sigma) and visualized under UV illumination using the Gel Doc XR (Bio-Rad).

	Controls (n=162) (%)	Multiple sclerosis (n=51) (%)	P	OR (CI 95%)
Alleles				
G	65 (20)	19 (19)	0.1	0.91 (0.5-1.66)
C	259 (80)	83 (81)		
Genotypes				
GG	10 (6)	3 (6)	0.6*	0.92 (0.19-3.91)
CG	45 (30)	13 (25)	0.11	0.88 (0.4-1.93)
CC	107 (64)	35 (69)		

The CC genotype served as the reference category, GG or CG *vs*. CC

*: Fisher exact test

Table 1: RANTES -28C/G allele and genotype frequencies (n, %) in multiple sclerosis cases and controls.

Statistical analysis

The genotype distributions of each polymorphism were tested for Hardy–Weinberg equilibrium using the χ^2-test in multiple sclerosis patients and controls [38].

Statistical analyses were performed using statistical software (Epi Info software version 3.2.2). The distribution of *CCL5* polymorphism between multiple sclerosis patients and healthy controls were compared by χ^2 or Fischer's exact test. $p<0.05$ was considered significant. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated whenever χ^2 or Fischer's exact test was significant.

Results

Hardy-Weinberg equilibrium

In this study, evaluation of Hardy–Weinberg equilibrium showed that genotype frequencies of *CCL5*-28C/G polymorphism was in Hardy–Weinberg equilibrium in multiple sclerosis group and healthy blood donors ($P<0.05$).

No association of the *CCL5* -28C/G polymorphism with multiple sclerosis

The 28G allele frequencies were approximately similar in multiple sclerosis patients and control groups (Table 1), with no significant statistical difference. No difference was found in the distribution of genotype frequencies between multiple sclerosis cases and controls (Table 1).

Discussion

Multiple sclerosis is a chronic demylinating disease of the human central nervous system of a still unknown etiology; however, it has been suggested to be affected by an interaction between genetic, environmental and geographical factors [39,40]. Strong evidence from genetic epidemiologic studies over the past 3 decades suggests that environmental factors affect susceptibility to multiple sclerosis at a broad population level [14]. Even exposed to the same environment, individual susceptibility to multiple sclerosis may be different, which indicates that genetic factors are important in the pathogenesis of multiple sclerosis. Therefore, understanding the genetic basis of multiple sclerosis is essential for the development of therapeutic strategies. Linkage and association studies have found that chemokines and chemokines receptor are involved in the pathgenesis of multiple sclerosis [16]. To the best of our knowledge, this is the first study investigating the association between *CCL5*-28C/G genetic polymorphism and susceptibility to multiple sclerosis Tunisian populations. The present study showed that neither alleles nor genotypes of this SNP were associated with increased risk development of multiple sclerosis.

CCL5, a member of the beta (C-C) chemokine family, this chemokine plays an important role in the development of inflammation via its ability to chemoattract leukocytes and modulate their functions. CCL5 is a chemoattractant for many cell types, including unstimulated $CD4^+/CD45RO^+$ T cells, stimulated $CD4^+$ and $CD8^+$ lymphocytes, natural killer (NK) cells, basophiles, dendritic cells, monocytes and microglia. It can also be an activator for T cells, monocytes and NK cells [41,42]. Several studies have implicated CCL5 in multiple sclerosis pathogenesis and the expression of CCL5 was observed in

perivascular inflammatory foci in multiple sclerosis brain [43]. In actively demyelinating multiple sclerosis plaques CCL5 expression was restricted to the blood vessel endothelium, perivascular cells and astrocytes [44]. CCL5 level in the cerebrospinal fluid was increased during active multiple sclerosis [24]. Additionally, Glass et al. showed that antibody targeting of the CC chemokine ligand 5 results in diminished leukocyte infiltration into the central nervous system and reduced neurologic disease in a viral model of multiple sclerosis [45].

Genetic associations of *CCL5* SNPs with asthma, atopic dermatitis, sarcoidosis, multiple sclerosis and other inflammatory and autoimmune diseases have been reported [25, 27-31]. Therefore, *CCL5* could be considered a gene generally predisposing to autoimmune disease. To test this hypothesis, we investigated *CCL5*-28 C/G functional SNP in 51 multiple sclerosis patients in comparison to control blood donors. We did not find any association between this SNP and multiple sclerosis. However, our study did not support the recently published data in which authors reported that the 28C/G SNP in the promoter region was associated with multiple sclerosis susceptibility in USA and Netherland [34,35]. These findings could be related to genetic heterogeneity or population stratification within each ethnicity. In addition, interactions with other polymorphisms may be of great importance to understand the genetic mechanisms controlling development of multiple sclerosis. In fact, analysis of polymorphisms in candidate genes has established that, at least in some populations, variations in for example, nucleotide variation—in the HLADRB1, interleukin 7 receptor (IL7RA), the interleukin 2 receptor (IL2RA), the CD58 and the c-type lectin domain family 16 member A (CLEC16A) genes [46-48].

In summary, this is the first study demonstrating the no association of *CCL5*-28C/G functional SNP with multiple sclerosis in Tunisian patients. Our results suggest that the *CCL5*-28GG genotype is not a risk factor for development of this disease. As the genetic control of the autoimmune disorders seems to be polygenic [46], it is interesting to study other functional polymorphisms affecting other genes, including *CCR5* receptor and *CXCL10* chemokine that may ameliorate the comprehension of multiple sclerosis development in our country.

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Disclosure and Conflict of Interests

The authors state they have no conflict of interest.

References

1. Sospedra M, Martin M (2005) Immunology of multiple sclerosis. Annu Rev Immunol 23: 683-747.
2. Compston A, Coles A (2002) Multiple Sclerosis. Lancet 359: 1221-1231.
3. Compston A, Coles A (2008) Multiple sclerosis. Lancet 372: 1502-1517.
4. International Multiple Sclerosis Genetics Consortium, Hafler DA, Compston A, Sawcer S, Lander ES, et al. (2007) Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med 357: 851-862.
5. Noseworthy J, Lucchinetti C, Rodriguez M, Weinshenker B (2000) Multiple sclerosis. N Engl J Med 343: 938-952.
6. Giovannoni G, Ebers G (2007) Multiple sclerosis: the environment and causation. Curr Opin Neurol 20: 261-268.
7. Ben Fredj N, Rotola A, Nefzi F, Chebel S, Rizzo R, et al. (2012) Identification of human herpesviruses 1 to 8 in Tunisian multiple sclerosis patients and healthy blood donors. J Neurovirol 18: 12-19.
8. Giovannoni G, Cutter GR, Lunemann J, Martin R, Munz C, et al. (2006) Infectious causes of multiple sclerosis. Lancet Neurol 5: 887-894.
9. Haahr S, Hollsberg P (2006) Multiple sclerosis is linked to Epstein-Barr virus infection. Rev Med Virol 16: 297-310.
10. Levin LI, Munger KL, Rubertone MV, Peck CA, Lennette ET, et al. (2003) Multiple sclerosis and Epstein-Barr virus. JAMA 289: 1533-1536.
11. Lindsey JW, Hatfield LM, Crawford MP, Patel S (2009) Quantitative PCR for Epstein–Barr virus DNA and RNA in multiple sclerosis. Mult Scler 15: 153-158.
12. Lunemann JD, Edwards N, Muraro PA, Hayashi S, Cohen J, et al. (2006) Increased frequency and broadened specificity of latent EBV nuclear antigen-1-specific T cells in multiple sclerosis. Brain 129: 1493-1506.
13. Sadovnick AD, Baird PA, Ward RH (1988) Multiple sclerosis: updated risks for relatives. Am J Med Genet 29: 533-541.
14. Ebers GC (2008) Environmental factors and multiple sclerosis. Lancet Neurol 7: 268-277.
15. Hoppenbrouwers IA, Aulchenko YS, Janssens AC, Ramagopalan SV, Broer L, et al. (2009) Replication of CD58 and CLEC16A as genome-wide significant risk genes for multiple sclerosis. J Hum Genet 54: 676-680.
16. Szczuciński A, Losy J (2007) Chemokines and chemokine receptors in multiple sclerosis. Potential targets for new therapies. Acta Neurol Scand 115: 137-146.
17. Gerard C, Rollins BJ (2001) Chemokines and disease. Nat Immunol 2: 108-115.
18. Luster AD (1998) Chemokines: chemotactic cytokines that mediate inflammation. N Engl J Med 338: 436-445.
19. Bacon KB, Premack BA, Gardner P, Schall TJ (1995) Activation of dual T cell signaling pathways by the chemokine RANTES. Science 269: 1727-1730.
20. Taub DD, Turcovski-Corrales SM, Key ML, Longo DL, Murphy WJ (1996) Chemokines and T lymphocytes activation: I. beta-chemokines costimulate human T lymphocyte activation in vitro. J Immunol 156: 2095-2103.
21. Wong MM, Fish EN (2003) Chemokines: attractive mediators of the immune response. Semin Immunol 15: 5-14.
22. Balashov KE, Rottman JB, Weiner HL, Hancock WW (1999) CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. Proc Natl Acad Sci USA 96: 6873-6878.
23. Boven LA, Montagne L, Nottet HS, De Groot CJ (2000) Macrophage inflammatory protein-1alpha (MIP-1alpha), MIP-1beta, and RANTES mRNA semiquantification and protein expression in active demyelinating multiple sclerosis (MS) lesions. Clin Exp Immunol 122: 257-263.
24. Sorensen TL, Tani M, Jensen J, Pierce V, Lucchinetti C, et al. (1999) Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. J Clin Invest 103: 807-815.
25. Fryer AA, Spiteri MA, Bianco A, Hepple M, Jones PW, et al. (2000) The–403 G/A promoter polymorphism in the RANTES gene is associated with atopy and asthma. Genes Immun 1: 509-514.
26. Liu H, Chao D, Nakayama EE, Taguchi H, Goto M, et al. (1999) Polymorphism in RANTES chemokine promoter affects HIV-1 disease progression. Proc Natl Acad Sci USA 96: 4581-4585.
27. Hizawa N, Yamaguchi E, Konno S, Tanino Y, Jinushi E, et al. (2002) A functional polymorphism in the RANTES gene promoter is associated with the development of late-onset asthma. Am J Respir Crit Care Med 166: 686-690.
28. Takada T, Suzuki E, Ishida T, Moriyama H, Ooi H, et al. (2001) Polymorphism in RANTES chemokine promoter affects extent of sarcoidosis in a Japanese population. Tissue Antigenes 58: 293-298.
29. Makki RF, al Sharif F, Gonzalez-Gay MA, Garcia-Porrua C, Ollier WE, et al. (2000) RANTES gene polymorphism in polymyalgia rheumatica, giant cell arteritis and rheumatoid arthritis. Clin Exp Rheumatol 18: 391-393.
30. Nickel RG, Casolaro V, Wahn U, Beyer K, Barnes KC, et al. (2000) Atopic dermatitis is associated with a functional mutation in the promoter of the C–C chemokine RANTES. J Immunol 164: 1612-1616.
31. Zhernakova A, Alizadeh BZ, Eerligh P, Hanifi-Moghaddam P, Schloot NC, et al. (2006) Genetic variants of RANTES are associated with serum RANTES level and protection for type 1 diabetes. Genes Immun 7: 544-549.
32. McDermott DH, Beecroft MJ, Kleeberger CA, Al-Sharif FM, Ollier WE, et al. (2000) Chemokine RANTES promoter polymorphism affects risk of both HIV

infection and disease progression in the Multicenter AIDS Cohort Study. AIDS 14: 2671-2678.

33. Ben-Selma W, Harizi H, Bougmiza I, Ben Kahla I, Letaief M, et al. (2011) Polymorphisms in the RANTES gene increase susceptibility to active tuberculosis in Tunisia. DNA Cell Biol 30: 789-800.
34. Gade-Andavolu R, Comings DE, MacMurray J, Vuthoori RK, Tourtellotte WW, et al. (2004) RANTES: a genetic risk marker for multiple sclerosis. Mult Scler 10: 536-539.
35. van Veen T, Nielsen J, Berkhof J, Barkhof F, Kamphorst W, et al. (2007) CCL5 and CCR5 genotypes modify clinical, radiological and pathological features of multiple sclerosis. J Neuroimmunol 190: 157-164.
36. World Health Organization (WHO) Report (2008) Atlas Multiple sclerosis ressources in the world 2008. WHO Press.
37. Yao TC, Kuo ML, See LC, Chen LC, Yan DC, et al. (2003) The RANTES promoter polymorphism: a genetic risk factor for near-fatal asthma in Chinese children. J Allergy Clin Immunol 111: 1285-1292.
38. Rodriguez S, Gaunt TR, Day IN (2009) Hardy–Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol 169: 505-514.
39. Bahreini SA, Jabalameli MR, Saadatnia M, Zahednasab H (2010) The role of non-HLA single nucleotide polymorphisms in multiple sclerosis susceptibility. J Neuroimmunol 229: 5-15.
40. Oksenberg JR, Baranzini SE, Sawcer S, Hauser SL (2008) The genetics of multiple sclerosis: SNPs to pathways to pathogenesis. Nat Rev Genet 9: 516-526.
41. Bischoff SC, Krieger M, Brunner T, Rot A, Tschamer VV, et al. (1993) RANTES and related chemokines activate human basophil granulocytes through different G protein-coupled receptors. Eur J Immunol 23: 761-767.
42. Schall TJ, Bacon K, Toy KJ, Goeddel DV (1990) Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. Nature 347: 669-671.
43. Hvas J, Bernard CC (1998) Molecular detection and quantitation of the chemokine RANTES mRNA in neurological brain. Apmis 106: 598-604.
44. Simpson JE, Newcombe J, Cuzner ML, Woodroofe MN (1998) Expression of monocyte chemoattractant protein-1 and other beta-chemokines by resident glia and inflammatory cells in multiple sclerosis lesions. J Neuroimmunol 84: 238-249.
45. Glass WG, Hickey MJ, Hardison JL, Liu MT, Manning JE, et al. (2004) Antibody targeting of the CC chemokine ligand 5 results in diminished leukocyte infiltration into the central nervous system and reduced neurologic disease in a viral model of multiple sclerosis. J Immunol 172: 4018-4025.
46. Hoffjan S, Akkad DA (2010) The genetics of multiple sclerosis: An update 2010. Mol Cell Probes 24: 237-243.
47. Hoppenbrouwers IA, Hintzen RQ (2011) Genetics of multiple sclerosis. Biochim Biophys Acta 1812: 194-201.
48. Lincoln MR, Montpetit A, Cader MZ, Saarela J, Dyment DA, et al. (2005) A predominant role for the HLA class II region in the association of the MHC region with multiple sclerosis. Nat Genet 37: 1108-1112.