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Clinical trial

TREM2 R47H (rs75932628) variant is unlikely to contribute to Multiple Sclerosis susceptibility and severity in a large Greek MS cohort

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ABSTRACT

Background: Multiple Sclerosis is a multifactorial autoimmune disease of the central nervous system, characterized by focal inflammation, demyelination and secondary axonal injury. TREM2 is a signaling protein which participates in the innate immune system by implication to inflammation, proliferation and phagocytosis. The R47H (rs75392628) rare variant of the TREM2 gene has been related to various neurological diseases and leads to impaired signaling, lipoprotein binding, lipoprotein uptake and surface uptake.
Aim: To assess the role of TREM2 rs75932628 on MS risk through a genetic candidate gene association case-control study in a Greek population.
Methods: 1246 MS cases and 398 controls were genotyped for this variant.
Results: No MS or healthy subjects carried the variant.
Conclusion: This variant does not seem to play a determining role in the pathogenesis of MS, although further studies examining the presence of TREM2 mutations in other, phylogenetically different populations and the epigenetic regulation of this gene are needed in order to thoroughly investigate its role in MS.

1. Introduction

Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS), characterized by focal inflammation, demyelination and secondary axonal injury (Goldenberg, 2012). The definite etiology of MS is yet to be discovered but its pathogenesis is considered to be multifactorial, including genetic predisposition along with environmental factors (Dardiotis et al., 2019a; Hadjigeorgiou et al., 2019).

The inflammation of white and grey matter tissues in the CNS is the pathologic hallmark of MS. This inflammation is mediated by T helper cells via the production of inflammatory cytokines and the suppression of their anti-inflammatory counterparts (Dardiotis et al., 2017a). B lymphocytes also seem to have both a positive and a negative effect in the development of MS (Ghasemi et al., 2017).

It is evident that MS has a genetic component, which interplays with environmental factors to lead to the disease (Dardiotis et al., 2018). Genetic studies found more than 200 single nucleotide polymorphisms (SNP) and risk variants, and a recent meta-analysis of twins with MS showed that genetic variability may explain about half of the differences in MS susceptibility (International Multiple Sclerosis Genetics Consortium, 2018; Fagnani et al., 2015; Parnell and Booth, 2017). Most of these genetic risk loci lie in proximity with genes predominantly expressed by both acquired and innate immune cells (Booth, 2014).

The Triggering Receptor Expressed on Myeloid cells 2, known as TREM2, is a transmembrane protein coded by the TREM2 gene, located

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on human chromosome 6p21 (Dardiotis et al., 2017b). Interestingly, on this chromosome, the Major Histocompatibility Complex (MHC) and its products, with the various HLA alleles are encoded and specifically the HLADRB1×1501 allele, which is the most robust genetic risk factor for MS Caucasian populations, worldwide (Stamatelos and Anagnostouli, 2017). TREM2 is preferentially expressed on a subgroup of myeloid cells like dendritic cells, osteoclasts and macrophages (Gratuze et al., 2018), while in the CNS it is exclusively expressed by microglia (Sessa et al., 2004). TREM2 is a signaling protein which pairs up with Tyrosine Kinase-binding protein (TYROBP/DP12) and participates in the innate immune system by implication to inflammation, proliferation and phagocytosis (Hsieh et al., 2009). In a study levels of TREM2 were found increased in experimental autoimmune encephalomyelitis (EAE), an experimental model of MS in mice, than in normal controls . This increase led to an increase in lysosomal and phagocytic activity (Takahashi et al., 2007). Furthermore, disease-modifying drugs in MS, specifically interferon β−1a, were shown to upregulate the expression of TREM2 (Dalla Costa et al., 2018), while significantly higher cerebrospinal fluid levels of soluble TREM2 have been found in MS patients, which were normalized after treatment with natalizumab, a monoclonal antibody against a4-integrin (Ohrfelt et al., 2016).

R47H (rs75392628) is the most studied variant of the TREM2 gene. The substitution of guanine with adenine at exon 2 of the TREM2 gene on Chr6: 41161514 position (c.140G>A) results in the missense substitution of arginine with histidine (R47H). This genetic variant leads to impaired signaling, lipoprotein binding, lipoprotein uptake and surface uptake (Yeh et al., 2017). It has also been related to various neurological diseases, including Alzheimer's disease, frontotemporal dementia-behavioral variant, logopenic variant of primary progressive aphasia, sporadic amyotrophic lateral sclerosis and essential tremor (Borroni et al., 2014; Cady et al., 2014; Dardiotis et al., 2017b; Guerreiro et al., 2013; Ortega-Cubero et al., 2015; Rayaprolu et al., 2013; Thelen et al., 2014).

Taking into consideration the aforementioned information, the aim of this study is to hereby assess the role of TREM2 rs75932628 on MS risk through a genetic candidate gene association case-control study in a Greek population. To the best of our knowledge, this is the first study assessing rs75932628 in MS.

2. Methods

2.1. Participants

This case-control study consists of 1396 MS-affected cases and 400 controls from three MS centers in Greece: The University Hospital of Larissa in Central Greece, the Eginition Hospital of the University of Athens Medical School in Athens, and the AHEPA Hospital of Aristotle University in Thessaloniki in North Greece. The diagnosis of MS was made by a specialized neurologist according to the 2017 McDonald criteria (Thompson et al., 2018). All participants signed an informed consent form and the study was approved by the local Ethics committee.

2.2. DNA isolation and genotyping procedure

The salting out method was applied in order for DNA to be extracted from peripheral blood leucocytes (Dardiotis et al., 2019a; Siokas et al., 2019a, 2019b). The genotyping for rs75932628 was performed with the TaqMan allele specific discrimination assays on an ABI PRISM 7900 Sequence Detection System, with the SDS software (Applied Biosystems, Foster City, California, USA). The overall laboratory experimental procedure (DNA extraction and genotyping) was performed by personnel blinded to information regarding the participants.

3. Results

The genotype call rate was equal to 89.3% for cases and 99.5% for controls, resulting in 1246 MS cases and 398 controls for comparison. In the total of 1644 individuals genotyped, we did not find any allelic rs75932628-T variant. Consequently, our data in this particular population suggest that rs75932628 is not a major genetic risk contributor for MS.

4. Discussion

It is well known that MS is a multifactorial disease with genetic, epigenetic and environmental components (Dardiotis et al., 2019b; Sokratous et al., 2018, 2016). The environmental influence consists of geographical factors (latitude), infectious factors (Epstein-Barr virus), cigarette smoking and vitamin uptake (Vitamin D) (O'Gorman et al., 2012). The life-time risk of MS is 10- to 30- fold greater in the age-adjusted general population than in first-degree relatives, thus a genetic contribution to MS exists but has yet to be clarified (Patsopoulos, 2018). During the past years, genome-wide association studies (GWASs) have led to the identification of multiple SNPs contributing to the genetic risk, under the common variant- common disease hypothesis (Sawcer et al., 2011), yet candidate gene association studies (CGASs), based on pathogenetic hypothesis, such as the one presented here, still play a crucial role in the deciphering of the genetic component of MS.

A growing body of evidence has indicated autoimmunity as a major contributor to MS development, but the exact pathogenetic mechanisms have yet to be clearly identified. Traditionally, MS is considered a CD4 T-cell mediated disease, however studies of the recent decades showed that other pathways may also be involved, such as a primary degenerative process, gray matter pathology and the involvement of CD8 T cells, B cells and innate immunity (Rahmanzadeh et al., 2018). TREM2 is an important signaling protein expressed on innate immune cells of CNS, which was found to foster phagocytosis, regulate inflammation in vitro and affect the disease course of experimental encephalomyelitis (Takahashi et al., 2007); its soluble form in MS affected cases' CSF was also found to correlate to disease activity (Ohrfelt et al., 2016). Increased levels of sTREM2 were found in MS patients, which were normalized after treatment with natalizumab or mitoxandrone (Ohrfelt et al., 2016). This phenomenon is indicative of the microglial activation in the pathogenesis of MS (Takahashi et al., 2007). sTREM2 has been investigated in the pathogenesis of Alzheimer's disease where mutations of the TREM2 has been found to be associated with the disease (Carmona et al., 2018; Piccio et al., 2016; Suarez-Calvet et al., 2019). Despite these intriguing findings, it remains unclear what biological or pathological roles sTREM2 plays and how its pathogenic mutations impact these functions.

The clinically well-characterized group of MS patients is one of the strengths of our study. Moreover, in the current study is the selection of the polymorphism is based on biological bases and rational, which is strongly requested in candidate gene-association studies.

A major limitation of our study was the small sample size. Minor allele rs75932628-T frequency was reported between 0.0017 to 0.003 in European populations (Sherry et al., 2001). Hence a sample size of tens of thousands would be more appropriate to add adequate power for detection of an association in our study. Additionally, a tagging SNP selection approach could have possibly revealed the effect of the entire TREM2 gene and not only of rs75932628 proxy variants.

In this candidate gene, case-control association study, we found no MS cases nor controls carrying the R47H variable of TREM2 in the Greek population under examination. However, further studies examining the presence of TREM2 mutations in larger multicenter samples or phylogenetically different populations and in familial MS (as rare variants might have larger effects in multiplex MS families) and the epigenetic regulation of this gene are more than encouraged to

thoroughly investigate its role in MS pathogenesis.

Declarations of interest

None.

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