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Short communication

No association of *IFI16* (interferon-inducible protein 16) variants with susceptibility to multiple sclerosis

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ABSTRACT

IFI16 encodes a nucleic acid-sensor which detects latent EBV and triggers inflammasome activation. We analysed *IFI16* variants in two multiple sclerosis (MS) case–control cohorts from Italy and Spain; results were combined with a previous study. A risk variant for celiac disease/rheumatoid arthritis, a polymorphic exon 7 duplication, and a copy number variant (CNV) in the 5′ region were genotyped. No significant association was detected, although heterogeneity was noted for the 5′ CNV in the Italian plus GeneMSA cohorts and the Spanish sample. Thus, *IFI16* variants do not contribute to MS susceptibility, although some heterogeneity may exist for the 5′ CNV.

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system with an established genetic component. Large-scale efforts including genome-wide association studies (GWAS) and meta-analyses have identified 110 non-MHC (major histocompatibility complex) susceptibility variants for MS that, together with the MHC effects, explain 28% of the sibling recurrence risk (International Multiple Sclerosis Genetics Consortium (IMSGC) et al., 2013). Thus, a large part of the genetic risk factors for MS remains to be identified. Common variants with small effect, which have been treated as false-negatives in GWAS, are likely to account for a portion of the still undefined MS risk loci. Indeed, recent evidence has indicated a polygenic model of disease susceptibility with multiple markers (that fail to reach even nominal significance in GWAS) contributing to disease risk with very small effects (International Multiple Sclerosis Genetics Consortium (IMSGC) et al., 2010). Rare polymorphisms, copy number variants (CNVs), and genetic interactions might help as well to explain a portion of the missing heritability of MS. Overall, these observations suggest that approaches distinct from large-scale genotyping might complement GWAS results.

The overwhelming majority of identified susceptibility alleles for MS indicated that immune and inflammatory response genes are major contributors to disease pathogenesis and suggest a substantial overlap with risk loci for other autoimmune diseases (International Multiple Sclerosis Genetics Consortium (IMSGC) et al., 2013). The *IFI16* (interferon-inducible protein 16) gene encodes a nucleic acid-sensing receptor involved in the nuclear and cytoplasmic detection of double-strand DNA (Unterholzner et al., 2010). *IFI16* has been involved in the pathogenesis of several autoimmune diseases (Mondini et al., 2010) and a susceptibility variant for rheumatoid arthritis (RA) and celiac disease has been identified in intron 7 (Zhernakova et al., 2011). Interestingly, *IFI16* is up-regulated in peripheral blood cells of MS patients during the disease relapses (Arthur et al., 2008). Thus, we assessed the possible contribution of *IFI16* single nucleotide polymorphisms (SNPs) and CNVs to MS genetic susceptibility.

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2. Materials and methods

2.1. HapMap samples, patients, and controls

Human genomic DNA from HapMap subjects was obtained from the Coriell Institute for Medical Research.

For the Italian MS case/control association study, we enrolled 849 MS patients (561 females, 288 males) and 516 age-matched healthy controls (HC; 278 females, 238 males). The Spanish cohort comprised 571 MS cases (370 females, 201 males) and 534 HC (283 females, 251 males). All patients and controls were Italian or Spanish of European origin and were recruited at the MS Centre of the Don Gnocchi Foundation in Milan and at Department of Neurological Sciences, University of Milan, and at the Centre d'Esclerosi Múltiple de Catalunya (Cemcat), in Barcelona. All patients satisfied McDonald's criteria for clinically definite MS (McDonald et al., 2001). All subjects gave informed consent according to protocols approved by the corresponding local Ethics Committees.

2.2. Genotyping

The insertion/deletion polymorphism upstream the transcription start site of *IFI16* was initially analysed using primer pairs that flank the predicted location of the CNV; these analyses revealed the presence of a 4.85 kb (NCBI/hg19, chr1: 158961352–158966201) insertion/deletion polymorphism ~13.5 kb upstream the transcription start site of *IFI16*. The CNV was genotyped in the case–control cohorts by PCR amplification using a fluorescently labelled forward primer (F: GCAGAGAGAGTTGCCTGGATG) and two unlabelled reverse primers (R-ins: CAGGCTGGTCTCAAACTCCTG, R-del: GACATGGGTG TAGACAACTGTG) that specifically amplify the inserted or deleted alleles. PCR-amplified fluorescently tagged samples were run on 3500xL Genetic Analyzer (Life Technologies) using the GeneScanTM 600 LIZ® size standard (Life Technologies). The PCR amplicons were separated by size electrophoresis and the dye labelled products were identified by fluorescence detection. GeneMapper® Software Version 4.0 was applied to size and genotype the alleles.

The segmental duplication of exon 7 in *IFI16* was analysed using a PCR-based method. In particular, PCR amplifications were performed with JumpStart AccuTaq LA DNA Polymerase (Sigma-Aldrich) and two sets of primers: one that amplifies only the duplicated form (F: GTCC TGTGCACCTTGTGTCA; R: CTGATGTATGGTGAGAGAGC), and one that flanks the segmental duplication (F: GTCCATTTCTGTAGCCATAGG; R: TCTGAGTTGTAGGAGAGCACT). The PCR products were electrophoretically separated on agarose gels.

Genotypes for rs1772408 and rs62621173 were obtained by allelic discrimination real-time PCR, using predesigned TaqMan probe assays (Applied Biosystems, Foster City, CA). Reactions were performed using TaqMan Genotyping Master Mix in an ABI 9700 analyser (Applied Biosystems). Genotyping rate was >0.98 for all variants. Analysed polymorphisms were in Hardy–Weinberg equilibrium in all cohorts, as assessed by the application of Exact Tests (Wigginton et al., 2005).

2.3. GWAS data and statistical analysis

GeneMSA data (International Multiple Sclerosis Genetics Consortium et al., 2007) were retrieved through dbGAP (http://www.ncbi.nlm.nih.gov/). Association p values for the International Multiple Sclerosis Genetics Consortium (IMSGC) ImmunoChip study (International Multiple Sclerosis Genetics Consortium (IMSGC) et al., 2013) were retrieved from ImmunoBase website (https://www.immunobase.org/). Genetic association was investigated by logistic regression using genotypes/haplotypes as the independent predictor variables with sex as a covariate. Results from different cohorts/studies were combined using a random-effect meta-analysis; all analyses were performed using PLINK (Purcell et al., 2007).

3. Results

3.1. Genetic diversity at IFI16

As mentioned above, a common variant in *IFI16* (rs1772408, intronic) has previously been associated with autoimmune diseases (Zhernakova et al., 2011). Because CNVs are thought to contribute significantly to human phenotypic diversity (Gamazon et al., 2011) but are often not surveyed in GWASs, we analysed CNVs in *IFI16*. Specifically, the human *IFI16* gene carries a polymorphic segmental duplication of exon 7 (the duplicated exons are identical in sequence) (Fig. 1). Also, a CNV located upstream the gene transcription start site has been repeatedly described (database of Genomic Variants, http://dgv.tcag.ca/dgv/app/home) (Fig. 1). Thus, we applied a PCR-based approach to determine the allelic status of these CNVs in 20 HapMap subjects of European ancestry (CEU). Results showed that the minor alleles (deletion in both cases) for the 5′ CNV and the exon 7 segmental duplication had a frequency of 17.5% and 7.5%, respectively in CEU. Analysis of SNP genotype data (as derived from HapMap) indicated that the 5′ CNV is tagged by rs9887904 ($r^2 = 1$), whereas several SNPs (the closest being rs62621173) are in full linkage disequilibrium (LD, $r^2 = 1$) with the exon 7 duplication polymorphism; none of the variants tagging the exon 7 duplication is included in GWAS platforms (http://www.broadinstitute.org/mpg/snap). LD analyses were replicated for 50 Italian and 50 Spanish healthy controls with the same results ($r^2 = 1$ in all instances).

3.2. Association with MS susceptibility

To determine whether *IFI16* variants/haplotypes modulate the risk to develop MS, we recruited two case–control cohorts, one from Italy (MS = 849, HC = 516) and one from Spain (MS = 571, HC = 534). Genotypes for the 5′ CNV, rs1772408, and rs62621173 (for the exon 7 CNV) were obtained for all subjects. Logistic regression (additive model) indicated a significant association between the 5′ CNV polymorphism and MS susceptibility in the Italian cohort; this finding was not observed in the Spanish sample (Table 1). Overall, a meta-analysis of the two samples plus data from the GeneMSA study (with rs9887904 used as a proxy for the 5′ CNV) (International Multiple Sclerosis Genetics Consortium et al., 2007) indicated that, after multiple test correction, none of the variants we analysed was significantly associated with the risk of developing MS (Table 1). Results from the IMSGC ImmunoChip study (International Multiple Sclerosis Genetics Consortium (IMSGC) et al., 2013) indicated a very weak association with MS susceptibility for the 5′ CNV and for rs1772408 (Table 1).

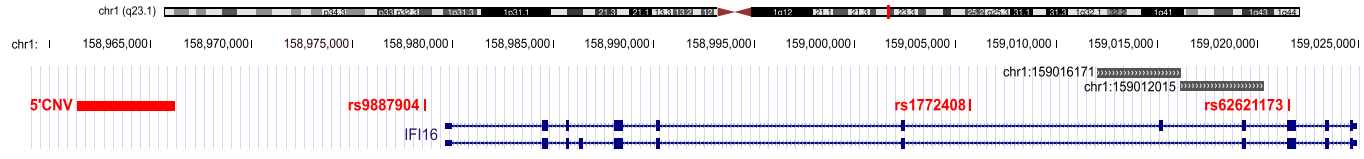

Fig. 1. Analysed variants. Representation of the *IFI16* gene region within the UCSC Genome Browser view. The exon 7 segmental duplication is shown (grey bars), as well as the 5′ CNV and the SNPs we analysed.