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Analysis of CYP27B1 in multiple sclerosis

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Abstract

The analysis of genetic variability in *CYP27B1* and its effect on risk of multiple sclerosis (MS) has yielded conflicting results. Here we describe a study to genetically characterize *CYP27B1* and elucidate its role on MS risk in the Canadian population. Sequencing *CYP27B1* failed to identify mutations known to cause loss of enzymatic activity, however genotyping of p.R389H in cases and controls identified the mutation in one multi-incident family (allele frequency = 0.03%) in which the p.R389H mutation segregates with disease in five family members diagnosed with MS, thus providing additional support for CYP27B1 p.R389H in the pathogenicity of MS.

Keywords

Multiple sclerosis; CYP27B1; mutation; linkage

1 Introduction

Although several genes and loci have been associated with an increased risk of developing multiple sclerosis (MS), the identification of specific genetic variants within these loci which could account for the observed increased susceptibility has been challenging. Cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1) p.R389H is one of a few variants with a known biological outcome located within a locus associated with increased risk of MS (ANZgene, 2009). The original description of this variant in MS patients arose from an exome sequencing study characterizing disease associated loci in 43 MS probands from Canada (Ramagopalan et al., 2011). The identification of the p.R389H mutation (rs118204009) in one sample led to the evaluation of 10 nonsynonymous variants known to cause *CYP27B1* loss of function, and one novel variants in 3,046 parents-affected child trios, 422 parent-affected sib pairs and 1,873 ethnically matched healthy individuals. In total, five *CYP27B1* variants were identified in MS patients, the most prevalent being p.R389H with a carrier frequency of 0.67%. After performing segregation analysis, the authors concluded that these functional variants were overtransmitted in affected family members and overrepresented in MS patients (odds ratio = 4.7), suggesting that a loss of functional activity in *CYP27B1* confers an increased risk of MS.

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In contrast to this initial report, replication studies found no evidence of an increased frequency of *CYP27B1* p.R389H in large cohorts of MS patients of European descent (Ban et al., 2013, Barizzzone et al., 2013, Cortes et al., 2013, Reinthaler et al., 2013). Ban et al. genotyped rs118204009 in 495 multi-incident families, 2,092 single affected families, 4,594 additional cases, and 3,583 controls from Norway, the United Kingdom, and the United States. This analysis identified the mutant allele of p.R389H at a frequency of 0.07% and 0.06% in patients and controls, respectively (Ban et al., 2013). Barizzzone et al. identified p.R389H at a frequency of 0.05% in 2,608 MS patients and 1,987 controls from Italy and Belgium (Barizzzone et al., 2013). In addition to the lack of association, sequencing *CYP27B1* in 134 probands from multi-incident families failed to identify any of the previously reported variants resulting in a loss of enzymatic activity or any novel risk variants. Similarly, the p.R389H mutation was not identified in 999 MS patients and 397 controls from Austria (Reinthaler et al., 2013) or 3,269 MS patients and 3,577 healthy controls of European descent (Cortes et al., 2013). Thus these studies did not support a role for *CYP27B1* in MS susceptibility. To determine whether the association observed in the initial study is limited to the Canadian population we assessed the frequency of p.R389H and evaluated the presence of loss of function mutations in *CYP27B1* in MS patients and controls from Canada.

2 Materials and Methods

All samples were collected through the longitudinal Canadian Collaborative Project on the Genetic Susceptibility to Multiple Sclerosis (CCPGSMS) between 1997 and 2008 (Sadovnick et al., 1998). The ethical review boards at the University of British Columbia approved the study, and all participants provided informed consent. All patients were diagnosed with MS according to Poser criteria (Poser et al., 1983) prior to 2001, or McDonald criteria thereafter (McDonald et al., 2001, Polman et al., 2005). A total of 1,647 MS probands and 849 controls were characterized in this study. The mean age at collection was 66.9 years ($SD \pm 9.9$) for controls and 47.1 years ($SD \pm 11.6$) for MS patients, with a male to female ratio of 1:0.97 and 1:2.92 respectively. The mean age at onset of disease for MS patients was 31.0 years ($SD \pm 9.7$), with a median expanded disability status scale (EDSS) score of 3.5 and an average of 4.1 ($SD \pm 2.6$) (Kurtzke, 1983). Of note, samples characterized by Ramagopalan et al were part of the CCPGSMS and therefore an overlap may exist; however, genotype information from that study is not available to exclude duplicates.

Genotyping was achieved using TaqMan Assays-by-Design (Applied Biosystems, Foster City, CA) probes with a positive reference sample. All patients considered positive were confirmed by Sanger sequencing. Sanger sequencing was also used to characterize all *CYP27B1* coding exons and exon-intron boundaries in 189 randomly selected MS probands from our case-control series. Primer sequences are available on request. Genotyping and sequencing protocols have been previously described (Keeling et al., 2009, Vilarino-Guell et al., 2009).

3 Results

Genotyping of rs118204009 in 1,647 MS probands and 849 controls identified the p.R389H mutation in no controls and only one proband (allele frequency = 0.03%). Twelve additional family members, including four diagnosed with MS, were available for segregation analysis in this family. This analysis identified four additional MS patients and two healthy family members harboring the CYP27B1 p.R389H mutation. Genotypes with corresponding age at onset of disease or age at last examination for healthy individuals is provided in Figure 1. To evaluate the co-segregation of this variant with disease we performed linkage analysis, as previously describe (Chartier-Harlin et al., 2011), which resulted in a LOD (logarithm of the odds) score of 2.28 ($p = 0.0006$).

Sequencing of *CYP27B1* in 189 probands failed to identify mutations known to cause loss of enzymatic activity. A rare missense mutation (rs372223837; c.41G>A; p.R14H) was identified. Genotyping this variant in 2,496 cases and controls failed to identify any additional carriers. Segregation analysis for the assessment of pathogenicity could not be performed as additional affected family members were not available for study. *In-silico* analysis for the effect of p.R14H on protein function and structure provided conflicting outcomes, as SIFT predicted the substitution as a benign polymorphism and Polyphen-2 probably damaging (Adzhubei et al., 2010, Kumar et al., 2009).

4 Discussion

Previous analysis of CYP27B1 p.R389H in the Canadian population resulted in a significantly increased carrier frequency in MS patients (0.67%) (Ramagopalan et al., 2011). Contrastingly, our replication study in a similar population yielded a drastically lower carrier frequency (0.06%), and a mutation allele frequency (0.03%) similar to that observed in recent replication studies (Ban et al., 2013, Barizzzone et al., 2013, Cortes et al., 2013, Reinthaler et al., 2013). Therefore our data suggests that the elevated frequency of rs118204009 previously reported in MS patients from Canada is likely a stochastic event. Similarly, sequencing of *CYP27B1* in MS patients did not provide additional support for pathogenicity as mutations resulting in loss of enzymatic activity were not identified.

However, in contrast to previous studies, we identified the p.R389H mutation in a probands from a multi-incident kindred, thus allowing for the first time assessment of segregation with disease in numerous family members. Interestingly, this analysis identified the p.R389H mutation in all family members diagnosed with MS, as well as two healthy individuals. Linkage analysis resulted in a LOD score of 2.28, highlighting the high level of co-segregation between CYP27B1 p.R389H and disease, thus corroborating the claim of overtransmission of *CYP27B1* mutations with disease within families (Ramagopalan et al., 2011), thus supporting a role for CYP27B1 p.R389H in the pathogenicity of MS, albeit with incomplete penetrance.

In conclusion, although the frequency of *CYP27B1* mutations in our replication study was not consistent with previous reports in the Canadian population, segregation analysis within a multi-incident kindred provides additional support for a role of p.R389H in the

pathogenicity of MS. However, further characterization of *CYP27B1* and particularly the p.R389H mutation in large number of MS patients and controls, but more importantly in multi-incident families, is paramount to further define the effect of *CYP27B1* loss of function mutations in the development of MS.

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Fig. 1.

Pedigree with *CYP27B1* p.R389H mutation. Males are represented by squares, females by circles, and diamonds indicate undefined gender to protect privacy. Patients affected by MS have filled symbols, the proband is arrowed and a diagonal line indicates deceased subjects. Heterozygote mutation carriers (M) and wild-type (wt) genotypes are indicated, with corresponding age at last examination (grey font) for unaffected family members or age at onset of disease (black font) for those diagnosed with MS