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No evidence of association between mutant alleles of the *CYP27B1* gene and MS

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Abstract

An association has previously been reported between susceptibility to multiple sclerosis and the rare mutant alleles of the *CYP27B1* gene responsible for autosomal recessive Vitamin D Dependent Rickets type 1 (VDDR1). In an attempt to replicate this finding, we screened 495 multiplex families and 2092 single affected families, together with 4594 cases and 3583 controls (a total of 17073 individuals) but were unable to find any evidence supporting this putative association. Our data do not indicate that mutations responsible for VDDR1 influence the risk of developing multiple sclerosis.

Although Genome Wide Association Studies (GWAS) provide an efficient means for identifying common variants associated with complex traits, the functionally relevant genes implicated by these findings are not always immediately apparent. For many of the Single Nucleotide Polymorphisms (SNPs) identified by GWAS, the correlation with neighbouring genetic variations (linkage disequilibrium, LD) is extensive, thereby generating a long list of genes that potentially are implicated in conferring risk. The association of multiple sclerosis with SNPs from Chromosome 12q14.1¹ illustrates the point, identifying more than 30 putative susceptibility genes.² Finding mutant alleles from any one of these candidates that also associate with the disease can be extremely informative.³ In this context, by confirming the relevance of *CYP27B1* (a plausible candidate given the role of cytochrome P450 in synthesizing 1 α ,25-dihydroxyvitamin D₃ and the independent evidence suggesting vitamin D deficiency as an environmental risk factor for multiple sclerosis⁴), the study from Ramagopalan et al⁵ appears to remove the need for further fine mapping of this region.

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Ramagopalan et al.⁵ first searched for rare variants exerting large effects on the risk of multiple sclerosis by whole exome sequencing the affected index individual from 43 highly multiplex Canadian families. This approach failed to find any rare functionally relevant variants present in more than one of these individuals - a not altogether unexpected result given the absence of evidence for significant linkage in the region^{6, 7} and the relative rarity of multiplex families with the disease.⁷ However, after comparing their results with those from the latest multiple sclerosis GWAS,² Ramagopalan et al.⁵ noted that rare functionally relevant variants are present in three genes already implicated by GWAS (*CBLB*,⁸ *IL7R*⁹ and *CYP27B1*); and after typing 3046 trio families (an affected individual and both parents), 1873 ethnically matched controls and 422 affected sib pair families, they found evidence suggesting that the VDDR1 related SNP rs118204009 from the *CYP27B1* gene is associated with multiple sclerosis. Ramagopalan et al.⁵ then tested nine other SNPs known to cause VDDR1 and found examples of two present in their trios. Finally, by resequencing *CYP27B1* in 96 multiple sclerosis patients known to have a persistently low level of vitamin D, they identified two additional variants that were also over transmitted to cases in their families. Combining these data, Ramagopalan et al.⁵ conclude that variants responsible for VDDR1 also confer an increased risk for the development of multiple sclerosis, with an estimated odds ratio of 4.7 in heterozygous carriers.

CYP27B1 was first proposed as a possible susceptibility gene in multiple sclerosis based on the identification of three patients with VDDR1 who also had multiple sclerosis.¹⁰ However, to date, there are no further reports of multiple sclerosis occurring in patients with VDDR1, making it unlikely that there is any statistical significance to this co-occurrence. Nevertheless, the GWAS approach has added independent, but indirect, evidence suggesting a role for *CYP27B1* by demonstrating significant association with common variants from the linkage disequilibrium interval containing this gene (rs703842 in the ANZgene GWAS;¹ and rs12368653 in the IMSCG and WTCCC2 GWAS²).

Against this background, and in an effort to replicate the association reported by Ramagopalan et al.⁵ and thereby confirm the role of *CYP27B1* in the aetiology of multiple sclerosis, we genotyped rs118204009 (R389H) in 495 multiplex families, 2092 single affected families, 4594 cases and 3583 controls from Norway, the United Kingdom (UK) and the United States (see table for population specific breakdown). All genotyping was performed by standard Taqman methods, the details of which are provided in the supplementary file, together with raw cluster plots, quality control measures and basic demographics. Based on the carrier frequency in cases reported by Ramagopalan et al.⁵ (0.67%), we anticipated that approximately 48 of our index cases would be heterozygous carriers. In fact we did not observe the mutant allele in any of our multiplex or single case families; and found only 5 heterozygous carriers amongst the cases (2 from the UK and 3 from Norway) and 2 in the controls (1 from the UK and 1 from Norway). Considering only the index case from each family, this corresponds to a mutant allele frequency of 5/7181 (0.07%) in affected individuals and 2/3583 (0.06%) in controls ($p=0.79$; Chi-square test with Yates' correction). Even if we include the three original Norwegian VDDR1 cases who also have multiple sclerosis (two of whom are full siblings),¹⁰ the estimated frequency in unrelated cases is only 7/7183 (0.098%; $p=0.73$). We found no statistically significant evidence for any difference in the frequency of rs118204009 between the populations of the UK, Norway or Canada. To explore further the individual SNPs suggested by Ramagopalan et al.,⁵ we also genotyped rs118204011 (L343F), but found no copies of the mutant allele in any of our families or controls, and only one mutant allele carrier in the cases ($p=0.55$).

Based on the prevalence of VDDR1 (c1 per 500,000), approximately 3 in every 1000 individuals are likely to be heterozygous carriers of functionally relevant mutant alleles of *CYP27B1*. To date, 10 SNPs causing VDDR1 have been described (dbSNP build 135) but

only one of these (rs118204009_T) has been seen, thus far, in the exome sequencing project (<https://esp.gs.washington.edu/drupal/>). The frequency of these individual mutant alleles is likely to vary between populations with rs118204009_T generally being the most common, at least in populations of Northern European descent. Although our observations do not address the epidemiological evidence suggesting a role for vitamin D in the aetiology of this disease, the very low frequency of these alleles in our samples and the absence of any statistically significant difference in their distribution between cases and controls, does not support the claim that mutant *CYP27B1* alleles influence the risk of developing multiple sclerosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table

Families and Samples genotyped.

	Norway	UK	USA	Total
Cases	2495	2099		4594
Controls	1027	2556		3583
Single affected Families *	-	1092	1000	2092
Multiplex Families	-	255	240	495
- Typed Individuals		875	1617	2492
- Typed Affecteds		526	613	1139
Samples				
- Total Typed	3522	8806	4745	17073
- Independent Affected	2495	3446	1240	7181

* In the UK all the single affected families were full trios (an affected individual and both parents). In the USA these families included other structure such as discordant sib pairs and incomplete trios (a total of 3128 individuals made up these 1000 families)