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No evidence of association of the rare nsSNP rs35667974 in *IFIH1* with Multiple Sclerosis

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Abstract

Studies suggest that different autoimmune diseases share a common genetic background, in particular, an overlap between multiple sclerosis (MS) and type 1 diabetes (T1D) susceptibility loci has been established. A recent study found that four rare SNPs in the *IFIH1* (*interferon induced with helicase C domain 1*) were significantly associated with T1D.

To establish if these SNPs were also involved in MS susceptibility, we chose to examine the nonsynonymous SNP rs35667974/Ile923Val which displayed the strongest effect in T1D and was also shown to lead to a loss of IFIH1 function in an in vitro study. We have performed the first association study to test if this rare variant is involved in MS susceptibility in a very large sample consisting of 3037 MS patients and 10657 healthy controls recruited from Italy and the UK. This study has 99% power to demonstrate an association at the 5% level with this rare variant. Our analysis shows that the nsSNP rs35667974/Ile923Val does not have a role in susceptibility to MS.

Keywords

Multiple Sclerosis; genetics; IFIH1

1 Introduction

Different autoimmune diseases can share a common genetic background (Zhernakova et al., 2009). This was originally hypothesised by epidemiological studies which observed an increased risk of a second autoimmune disorder in patients, and/or in the families of patients, affected by an autoimmune disease and confirmed by the recent identification of susceptibility genes shared by different autoimmune diseases (Zhernakova et al., 2009). In this respect, the relationship between type 1 diabetes (T1D) and multiple sclerosis (MS) is particularly

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intriguing, in view of recent studies showing that several genes associated with T1D (such as IL7R, IL2RA, CLEC16A, CD226 and SH2B3) are also robustly associated with MS (Zhernakova et al., 2009; MSGC, 2009). These observations further support the hypothesis that loci associated with one autoimmune disease are possible candidates for other autoimmune disorders.

Among the recently identified T1D genes, *IFIH1* (interferon induced with helicase C domain 1) also represents an interesting candidate for MS. *IFIH1*, mapping on chromosome 2q24, codes for a cytoplasmic protein mediating immune activation and induction of interferon production in response to the recognition of viral double strand RNA. Epidemiological and experimental data strongly suggest that infectious agents could be triggering elements in the development of MS, especially in genetically predisposed individuals (Ascherio and Munger, 2007; Serafini et al., 2007; Challoner et al., 1995; Sriram et al., 1999).

A first series of association studies in T1D suggest that the minor allele of a common nonsynonymous SNP (nsSNP) rs1990760/Thr946Ala in the *IFIH1* gene confers a protective effect (Smyth et al., 2006; Liu et al., 2009). This variant is not associated with MS as demonstrated by a recent large study of over 18000 individuals recruited from six countries (MSGC, 2009).

More recently, Nejentsev S. and colleagues (Nejentsev et al., 2009), using new high-throughput sequencing technology, identified 29 rare SNPs in *IFIH1* in UK T1D patients and/or matched controls, and found that four of these variants (two coding and two intronic mutations) were significantly associated with T1D. Moreover, the association of these four SNPs was independent of the previously identified rs1990760/Thr946Ala T1D associated variant. These independent effects prompted us to investigate the association of rare *IFIH1* variants in MS. Among the four rare *IFIH1* variants, we selected the nsSNP rs35667974/Ile923Val since it displayed the strongest effect in T1D (OR= 0.51 95% CI: 0.43–0.61, for the minor allele, $p=2.1 \times 10^{-16}$) (Nejentsev et al., 2009). In vitro studies have demonstrated that this variant, but not the common Thr946Ala, results in loss of IFIH1 function (Shigemoto et al., 2009).

2 Materials and Methods

2.1 Samples

We analyzed a large sample of MS patients and healthy matched controls recruited from the Italian (827 MS patients and 628 controls) and UK (2,210 MS patients and 10,029 controls) population. Italian patients were collected from Continental Italy and recruited from hospital based clinics. The majority were collected in North-West Italy. Patients with Sardinian ancestors were excluded to avoid the introduction of confounding source of heterogeneity. Italian controls included medical students, university and hospital staff, blood donors (female:male ratio 1:1.1) matched for age and regional origin with the MS patients.

The UK patients were recruited through the University of Cambridge from across the UK with a particular emphasis on the South East. All were identified and referred by Members of the Association of British Neurologists. Of the 10,029 UK controls, 467 samples were obtained from the European Collection of Cell Culture (ECACC) Human Random Control Panel composed of unrelated UK Caucasian blood donors. The remaining 9,562 were obtained from the work of Nejentsev et al. (Nejentsev et al., 2009).

Clinical features of the analyzed samples are shown in Table 1. All samples were collected with informed consent and appropriate ethical approvals. All the UK and Italian samples were of European ancestry.

2.2 rs35667974/Ile923Val genotype

All samples were typed using a pre-designed TaqMan® SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA). Reactions were performed according to the manufacturer's protocol using 25 ng of DNA and run on a 9700 Applied Biosystems Thermocycler. Genotypes were detected using HT 7900 Applied Biosystems instrument and SDS 2.1 software.

2.3 Statistical analysis

Power analysis was carried out with Power Program v.3.0.0 (<http://dceg.cancer.gov/tools/design/power>) based on Lubin et al. (Lubin and Gail, 1990) and Garcia-Closas et al (Garcia-Closas and Lubin, 1999).

Considering the size of the available samples, the minor allele frequencies and the OR reported by Nejentsev et al. (Nejentsev et al., 2009) for the four rare variants in T1D, we selected to study only the nsSNP rs35667974/Ile923Val which displayed the strongest effect in T1D. For the remaining three variants, the available sample size did not have sufficient power (power <80%) to detect the effect reported for T1D or an effect in the range of ORs so far reported for MS outside the MHC region.

All the statistical analyses were performed using PLINK program v 1.06 (Purcell et al., 2007). For the case/control association study in the cohorts taken separately, we performed a basic association study. In the combined analysis of the Italian and UK samples we used a Cochran-Mantel-Haenszel test and Breslow-Day statistic to test for heterogeneity between the two populations. The empirical p-value (E p-value) was calculated using an adaptive permutation test performed with 10000 permutations and standard values for the arguments.

3 Results and discussion

We have performed a case/control association study to test whether rs35667974 in *IFIH1* is associated with MS in a large dataset consisting of 3037 MS patients and 10657 healthy controls recruited from Italy and the UK. Based on the OR reported in T1D (OR=0.51) by Nejentsev et al. (Nejentsev et al., 2009), the combined cohort we studied had 99% power to demonstrate an association at the 5% level.

We analyzed the two populations separately, and in combination (Table 2). The distribution of genotypes did not deviate from Hardy-Weinberg equilibrium in either dataset. Allele and genotype frequencies among patients and controls were not significantly different (Table 2) in either the single populations or in the combined sample. In the Italian population, we observed a non-significant protective odds ratio (OR = 0.76, p-value= 0.58, Table 2) for the minor allele which conferred protection in T1D (Nejentsev et al., 2009). Conversely, in the UK samples the minor allele frequency (Table 2) in MS cases and healthy controls was very similar (2.2% vs 2.1%, respectively, OR = 1.04). In the combined cohort, the OR conferred by the minor allele was 1.03 (95% CI: 0.83–1.28, p-value of 0.79) (Table 2). The Breslow-Day test (Purcell et al., 2007) showed no evidence of heterogeneity (p-value=0.59) between the two tested populations.

In conclusion, this study has identified that the rare nsSNP rs35667974/Ile923Val does not have an effect size in MS as strong as that found in T1D. Based on the sample size screened, this study cannot exclude the possibility that this variant has a weak effect on susceptibility to MS ($0.60 < \text{OR} < 1.45$). Moreover, our work cannot exclude the possibility that other rare variations in *IFIH1* could have an effect on the development of MS. It is well recognized that different autoimmune diseases can share susceptibility genes, but not necessarily the same associated variants. This was recently demonstrated for the *IL2RA* (*interleukin 2 receptor alpha chain*) gene for which Maier et al. have identified three different groups of associated variants

that showed a remarkable degree of allelic heterogeneity between MS and T1D (Maier et al., 2009).

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Table 1

Description of the analyzed samples and clinical features of MS patients

	Controls		MS patients								
Samples	N	Age at Onset ^b	Female:Male Ratio		EDSS %			Disease Course %			
					<3	3 to <6	≥6	RR	SP	PP	PR
Italy	628	827	32.5±10.5	2:1	53	28	19	76	12	10	2
UK	10029 ^a	2210	29.8±9.4	2.7:1	30	26	44	59	31	10	0

Notes: MS: Multiple Sclerosis; EDSS: Expanded Disability Status Scale (Kurtzke, 1983); RR: Relapsing Remitting; SP: Secondary Progressive; PP: Primary Progressive; PR: Progressive Relapsing.

^a Controls included in the paper by Nejtenset et al. (Nejtenset et al., 2009), with the exception of 467 new samples.

^b Mean ± Standard Deviation.

Table 2

MS association analysis of rs35667974 SNP

Samples		Genotypes ^a			MAF %	OR (95% CI)	E p-value ^b
		AA (%)	AG (%)	GG (%)			
Italy	MS	821 (99.27)	6 (0.73)	0 (0.0)	0.36	0.76 (0.24–2.36)	0.58
	Controls	622 (99.04)	6 (0.96)	0 (0.0)	0.48		
UK	MS	2113 (95.61)	96 (4.34)	1 (0.05)	2.22	1.04 (0.83–1.30)	0.73
	Controls	9605 (95.77)	420 (4.19)	4 (0.04)	2.13		
Combined cohort	MS	2934 (96.61)	102 (3.36)	1 (0.03)	1.71	1.03 (0.83–1.28) ^c	0.79
	Controls	10227 (95.97)	426 (4.00)	4 (0.04)	2.04		

Notes: MS: Multiple Sclerosis; statistical analysis was carried out with PLINK program v 1.06 (Purcell et al., 2007). MAF: Minor Allele Frequency.

^a All genotypes are in Hardy-Weinberg equilibrium.

^b E p-value: empirical p-value, calculated using the adaptive permutation test option, with standard value arguments: minimum number of permutations per SNP=10000, maximum number of permutations per SNP=10000 alpha level threshold (alpha)=0, confidence interval on empirical p-value (beta)=0.0001, interval to prune list (intercept)=1, interval to prune list (slope)=0.001.

^c In the combined cohort the OR is calculated by Cochran-Mantel-Haenszel test that is based on an “average” odds ratio adjusted for the cluster variable.