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SHORT REPORT

IFIH1-GCA-KCNH7 locus is not associated with genetic susceptibility to multiple sclerosis in French patients

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A recent investigation reported, for the first time, an association between variants in the IFIH1-GCA-KCNH7 locus and multiple sclerosis (MS). We sought to replicate this genetic association in MS with a new independent MS cohort composed of French Caucasian MS trio families. The two most significant IFIH1 single nucleotide polymorphisms, rs1990760 and rs2068330, reported as involved in MS susceptibility, were genotyped in 591 French Caucasian MS trio families, and analyzed using the transmission/disequilibrium test. No association with MS was found (rs1990760, $P = 0.45$ and rs2068330, $P = 0.27$). Similarly, no significant association was detected after stratification for HLA-DRB1*1501 carriers. Reasons that may explain this discrepancy between the original report and our study are discussed.
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Introduction

Multiple sclerosis (MS) is a major cause of disability in young adults. MS pathogenesis is not well understood but there is a large body of evidence for an autoimmune process triggered by environmental factors in susceptible individuals.[1] Genetic factors in MS have been suggested by epidemiological studies[2] and a strong genetic effect was pointed out more than 30 years ago with the HLA-DR2 locus. It is only recently that non-HLA variants (IL7R and IL2R,[3] and IL7R variants[4,5]) have been implicated with strong confidence in a genuine but small effect (odds ratio = 1.2 with $P = 5.84 \times 10^{-12}$) in a combined approach for IL7R.[6] These findings have already been replicated in populations with other ethnic backgrounds.[7,8] Unfortunately, before the IL7R reports, almost 30 years passed with a putative locus pinpointed by one study but not replicated by others. The reasons why it is difficult to confirm a positive genotype–phenotype association result are now well established, and recent outstanding guidelines for replication have been proposed by an NCI-NHGRI working group.[9] An alternative to replication, the so-called combination approach, has been proposed.[6,10,11] Both the replication and combination methods need to take positive and negative studies into account to overcome a well-known bias toward positive results. Here, we failed

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to replicate a Spanish, case–control, positive association between the IFNIH1-GCA-KCNH7 locus and MS[12] in an independent French cohort with a family-based approach.

Subjects and methods

Patients

In total 591 French Caucasian trio families composed of one affected subject and the two parents (with four European Caucasian grandparents) were recruited through a national media campaign followed by the selection of individuals who satisfied the criteria for MS.[13–15] All the subjects gave their informed consent, and the 'Comité consultatif de protection des personnes dans la recherche biomédicale Paris-Pitié-Salpêtrière' approved the study. For affected individuals, the F/M sex ratio was 2.3:1 with 412 women and 176 men. For three patients, no gender information was available. HLA-DRB1*1501 genotype was available from 368 MS patients. In these MS patients, 52% carried at least one HLA-DRB1*1501 allele (DRB1*1501$^{+/+}$ or DRB1*1501$^{+/-}$).

Molecular genotyping method

Genomic DNA was purified from fresh peripheral blood leukocytes by standard methods. We genotyped genetic variants, using a TaqMan 5′ allele-discrimination assay (Applied Biosystems, Foster City, CA, USA). The PCR was performed in a total reaction volume of 5 μl, with the following amplification protocol: denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s and annealing and extension at 60°C for 1 min. After the PCR procedure, the genotype of each sample was attributed automatically by measuring the allele-specific fluorescence. An ABI Prism 7900 Sequence detection system and the SDS 2.2.2 software (both from Applied Biosystems) were used for allele discrimination.

The percentages of nonmissing genotypes were 99.4 and 99.3% for rs1990670 and rs2068330, respectively.

SNP characteristics

Two polymorphisms in the IFIH1-GCA-KCNH7 locus, present on chromosome 2q24.3, were genotyped in 591 French Caucasian MS patients and in the two parents of each patient. The first selected polymorphism, rs1990670 (ID no. C___2780299_30) is located in exon 15 of the IFIH1 gene (leading the A946T substitution). The second one, rs2068330 (ID no. C___15862240_10), in the 3′ boundary of this locus, is located in intron 14 of the KCNH7 locus. Minor allele frequency is 39.6 and 36.4% for rs1990670 and rs2068330, respectively. These two polymorphisms were chosen with regard to previous studies where they have been shown to be associated with T1D, Graves' disease and MS.

Statistical analysis

In accordance with the recommendations of the NCI-NHGRI workgroup, the association analysis of the IFIH1-GCA-KCNH7 locus was performed by means of the transmission/disequilibrium test (TDT), using the Haploview program.[16] TDT compares the transmission of single nucleotide polymorphism (SNP) alleles from heterozygous parents to affected offspring with Mendel's expectation (50%), using a bilateral χ^2-test with 1 d.f.. *P*-values under 0.05 were considered as statistically significant. The genetic power of our population for detecting a genotype relative risk of 1.73 (homozygotes) and 1.16 (heterozygotes) was calculated with the genetic power calculation test (http://pngu.mgh.harvard.edu/~purcell/gpc/dtdt.html). The expected power for a type I error of 5% is 79.5% for our samples of 591 trios. As we performed a replication study, a type I error of 10% is acceptable (one way study). In this case, the power of our population is 87.3%.

To facilitate comparison between our data and those of Martinez *et al*, we reformed our samples as case–controls using the parents as a control cohort (see Table 1).

Results

Both variants, rs1990670 and rs2068330, were in Hardy–Weinberg equilibrium in the control population derived from the nontransmitted parental chromosomes. Individually, neither of these two polymorphisms in the IFIH1-GCA-KCNH7 locus showed a significant association with MS susceptibility in the TDT test. The allele A of polymorphism rs19907660 was nonsignificantly overtransmitted (Table 2) and rs2068330 polymorphism did not show significant overtransmission of the allele C to MS patients even though it was the best marker from a statistical point of view (Table 3).

As reported in previous studies, we observed strong linkage disequilibrium between the SNP located in the IFH1 gene (rs1990760) and the SNP in the KCNH7 gene (rs2068330): $D' = 0.85$ and $r^2 = 0.63$. The analysis of haplotypes showed that three of the four possible haplotypes had a frequency higher than 5% in our population but no haplotype was significantly associated with MS susceptibility (Table 4).

HLA-DRB1*15 genotype was available from 368 MS patients. To assess the influence of the HLA locus, we subdivided the MS patients into two groups according to their HLA-DRB1*1501 positivity: the first group was composed of HLA-DR1*1501-negative MS patients and the second included MS patients who were HLA-DR1*1501 positive for at least one allele. One hundred and ninety-one patients were positive for the DRB1*1501 allele (24 homozygous and 167 heterozygous) and 177 were negative. After stratification of MS patients by carriage of HLA-DRB1*15 allele, no marker or haplotype

Table 1 Comparison of studies by Martinez *et al* and Couturier *et al*

	KCNH7, rs2068330		*IFIH1, rs1990760*	
	C	*G*	*A*	*G*
Martinez *et al*[a]				
Controls	660 (61.7%)	410 (38.3%)	630 (58.9%)	440 (41.1%)
MS patients	568 (68.9%)	256 (31.1%)	523 (63%)	307 (37%)
	G *vs* C, OR = 0.73 (0.6–0.88); $P = 0.001$		G *vs* A, OR = 0.84 (0.7–1.02); $P = 0.07$	
Couturier *et al*[b]				
Controls	1499 (63.6%)	859 (36.4%)	1427 (60.4%)	934 (39.6%)
MS patients	761 (64.6%)	417 (35.4%)	720 (61.1%)	458 (38.9%)
	G *vs* C, OR = 0.96 (0.83–1.11); $P = 0.5$		G *vs* A, OR = 0.97 (0.84–1.12); $P = 0.72$	

MS, multiple sclerosis; OR, odds ratio.
[a]Martinez *et al* is a case–control study.
[b]Couturier *et al* is a family-based study. Therefore, the control cohort is made up of the parents in trio families.

Table 2 Association analysis of the IFIH1 SNP rs1990760 (A946T) in 591 French MS trio families

	Transmitted, N *(%)*	*Untransmitted*[a], N *(%)*	P-*value*
Allele A	287 (51.6)	269 (48.4)	0.45

[a]Untransmitted (pseudocontrol) genotypes are estimated.

Table 3 Association analysis of the KCNH7 SNP rs2068330 in 591 French MS trio families

	Transmitted, N *(%)*	*Untransmitted*[a], N *(%)*	P-*value*
Allele C	283 (52.4)	257 (47.6)	0.27

[a]Untransmitted (pseudocontrol) genotypes are estimated.

Table 4 Analysis of rs1990760/rs2068330 haplotypes in 591 French MS trio families

			TDT		
	rs1990760	*rs2068330*	*Transmitted*, N *(%)*	*Untransmitted*[a], N *(%)*	P-*value*
Haplotype 1[b]	A	C	293 (51.5)	276 (48.5)	0.47
Haplotype 2[b]	G	G	249 (47.8)	272 (52.2)	0.32
Haplotype 3[b]	G	C	77 (52.4)	70 (47.6)	0.55

TDT, transmission/disequilibrium test.
[a]Untransmitted (pseudocontrol) genotypes are estimated.
[b]Haplotypes with a frequency >5%.

of the IFIH1-GCA-KCNH7 locus was significantly associated with disease susceptibility (data not shown).

Discussion

The human IFIH1-GCA-KCNH7 locus is located on chromosome 2q24-3 and encodes for a key protein in the type 1 interferon (IFN) pathway. Therefore, it is a potential key player in MS pathogenesis. The IFIH1 gene has been implicated as a susceptibility gene in other autoimmune diseases, such as type I diabetes[17] or Graves' disease.[18] Common genetic variants have been implicated in various autoimmune diseases or in families with several cases affected by different diseases.[19,20] It is of interest to note that the IFIH1-GCA-KCNH7 locus was not a region of interest in a recent first genome-wide association scan in MS. Nevertheless, the authors stated that it was 'likely that other loci with similar low-risk ratios exist'. That is why a consortium was formed to increase the number of patients available worldwide. Altogether, we believe that IFIH1-GCA-KCNH7 locus is a good susceptibility locus for genetic susceptibility to MS.

We failed to replicate the initial positive association between IFNIH1-GCA-KCNH7 locus and MS found by Martinez *et al*. It is obvious that a different ethnic background between French and Spanish patients could explain such a discrepancy. Nevertheless, alternative explanations may exist. First, we used a different, family-based approach. It is widely accepted that family studies

permit both linkage and association analyses and decrease the potential for false-positive findings because of population substructure. This raises the possibility that Martinez *et al*'s finding may be related to a type I error.[21] Second, the discrepancy may be related to our study's power to detect small genetic effects. We calculated the power to detect genotype relative risks of 1.73 (homozygotes) and 1.16 (heterozygotes) under two scenarios. First, with a type I error of 5% (for a two-tail test), the power was 79.5%. The second scenario was for a type I error of 10%, which is acceptable in a context of a replication study (one-way test). Here, the power becomes 87.3%. Although our cohort power may be considered as acceptable, we cannot exclude the possibility that our negative replication may be because of an underpowered study.

In conclusion, our negative replication is of interest in the framework of the effort that is necessary before it can be confirmed, or not, that the IFIH1-GCA-KCNH7 locus is a genuine locus for genetic susceptibility to MS.

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References

1 Brassat D, Oksenberg J: Genetics of multiple sclerosis; in Research signpost (eds):: *Molecular Bases of Neurodegeneration* 2005, pp 43–49.
2 Dyment DA, Ebers GC, Sadovnick AD: Genetics of multiple sclerosis. *Lancet Neurol* 2004; **3**: 104–110.
3 Hafler DA, Compston A, Sawcer S *et al*: Risk alleles for multiple sclerosis identified by a genomewide study. *N Engl J Med* 2007; **357**: 851–862.
4 Lundmark F, Duvefelt K, Iacobaeus E *et al*: Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. *Nat Genet* 2007; **39**: 1108–1113.
5 Gregory SG, Schmidt S, Seth P *et al*: Interleukin 7 receptor alpha chain (IL7R) shows allelic and functional association with multiple sclerosis. *Nat Genet* 2007; **39**: 1083–1091.
6 Harley JB: IL-7Ralpha and multiple sclerosis risk. *Nat Genet* 2007; **39**: 1053–1054.
7 Ramagopalan SV, Anderson C, Sadovnick AD, Ebers GC: Genome-wide study of multiple sclerosis. *N Engl J Med* 2007; **357**: 2199–2201.
8 Weber F, Fontaine B, Cournu-Rebeix I *et al*: IL2RA and IL7RA genes confer susceptibility for multiple sclerosis in two independent European populations. *Genes Immun* 2008; **9**: 259–263.
9 Chanock SJ, Manolio T, Boehnke M *et al*: Replicating genotype-phenotype associations. *Nature* 2007; **447**: 655–660.
10 Skol AD, Scott LJ, Abecasis GR, Boehnke M: Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* 2006; **38**: 209–213.
11 Barnetche T, Constantin A, Cantagrel A, Cambon-Thomsen A, Gourraud PA: New classification of HLA-DRB1 alleles in rheumatoid arthritis susceptibility: a combined analysis of worldwide samples. *Arthritis Res Ther* 2008; **10**: R26.
12 Martinez A, Santiago JL, Cenit MC *et al*: IFIH1-GCA-KCNH7 locus: influence on multiple sclerosis risk. *Eur J Hum Genet* 2008; advance online publication.
13 Goodkin DE, Doolittle TH, Hauser SS, Ransohoff RM, Roses AD, Rudick RA: Diagnostic criteria for multiple sclerosis research involving multiply affected families. *Arch Neurol* 1991; **48**: 805–807.
14 Poser CM, Paty DW, Scheinberg L *et al*: New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 1983; **13**: 227–231.
15 Polman CH, Reingold SC, Edan G *et al*: Diagnostic criteria for multiple sclerosis: revisions to the 'McDonald Criteria. *Ann Neurol* 2005; **58**: 840–846.
16 Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**: 263–265.
17 Smyth DJ, Cooper JD, Bailey R *et al*: A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. *Nat Genet* 2006; **38**: 617–619.
18 Sutherland A, Davies J, Owen CJ *et al*: Genomic polymorphism at the interferon-induced helicase (IFIH1) locus contributes to Graves' disease susceptibility. *J Clin Endocrinol Metab* 2007; **92**: 3338–3341.
19 Pearce SH, Merriman TR: Genetic progress towards the molecular basis of autoimmunity. *Trends Mol Med* 2006; **12**: 90–98.
20 Hauser S, Oksenberg J, Brassat D: Genetics of immune-mediated neurologic diseases. *Clinical Neuroimmunology*. Oxford University Press, 2005.
21 Clayton DG, Walker NM, Smyth DJ *et al*: Population structure, differential bias and genomic control in a large-scale, case–control association study. *Nat Genet* 2005; **37**: 1243–1246.