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SHORT REPORT

Hexose-6-phosphate dehydrogenase: a new risk gene for multiple sclerosis

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A recent genome-wide association study (GWAS) performed by the The Wellcome Trust Case–Control Consortium based on 12 374 nonsynonymous single-nucleotide polymorphisms (SNPs) provided evidence for several genes involved in multiple sclerosis (MS) susceptibility. In this study, we aimed at verifying the association of 19 SNPs with MS, with *P*-values ≤0.005, in an independent cohort of 732 patients and 974 controls, all Caucasian from the South of Spain. We observed an association of the rs17368528 polymorphism with MS (*P*=0.04, odds ratio (OR)=0.801, 95% confidence interval (CI)=0.648–0.990). The association of this polymorphism with MS was further validated in an independent set of 1318 patients from the Canadian Collaborative Project (*P*=0.04, OR=0.838, 95% CI=0.716–0.964). This marker is located on chromosome 1p36.22, which is 1 Mb away from the MS-associated kinesin motor protein *KIF1B*, although linkage disequilibrium was not observed between these two markers. The rs17368528 SNP results in an amino-acid substitution (proline to leucine) in the fifth exon of the hexose-6-phosphate dehydrogenase (*H6PD*) gene, in which some variants have been reported to attenuate or abolish H6PD activity, in individuals with cortisone reductase deficiency. This study corroborates the association of one locus determined by GWAS and points to *H6PD* as a new candidate gene for MS.
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INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune disease with a complex pathogenesis, in which demyelination and neurodegeneration are the main contributors to disability.[1] Susceptibility to MS is thought to be conferred by a combination of genetic and environmental factors.[2] The most established region in predisposition to MS is the major histocompatibility complex on chromosome 6p21, specifically, the *HLA-DRB1*1501* class II allele.[3] Currently, other loci have been identified that have convincing evidence for an association with MS, such as the *IL2RA*,[4,5] *IL7RA*,[6,7] *KIF1B*,[8] *IRF5*,[9] *EVI5*,[4,10] *CD226*[11,12] and *CLEC16A*[12] genes. Some of these genes were identified through a classical candidate gene approach; however, many new candidate risk factors arise from the hypothesis-free approach provided by genome-wide association studies (GWAS). The Wellcome Trust Case–Control Consortium (WTCCC) carried out a GWAS based on 12 374 nonsynonymous single-nucleotide polymorphisms (SNPs) typed in 975 patients and 1466 controls.[13] No SNP outside the HLA region provided a significant association with MS at the genome-wide level in this study. However, the initial screen yielded promising signals. One of the signals located at the tyrosine kinase 2 (*TYK2*) gene has since been confirmed with an independent cohort.[14]

In this study, we assessed the risk contribution of 19 SNPs that showed some degree of association in the GWAS performed by the WTCCC using MS patients and controls from the south of Spain. We identified hexose-6-phosphate dehydrogenase (*H6PD*, glucose-1-dehydrogenase) as a novel risk gene for MS and validated this finding in an independent cohort of Canadian MS patients.

MATERIALS AND METHODS

Subjects

Case samples comprised 732 patients with clinically definite MS, according to Poser's criteria.[15] They were obtained from four public hospitals: the Hospital Clínico in Granada (n=126), the Hospital Virgen de las Nieves in Granada (n=165), the Hospital Carlos Haya in Málaga (n=365) and the Hospital Virgen de la Macarena in Seville (n=76). All three cities are within a 200 km radius in the South of Spain. The mean age (±SD) of cases at the time of sample collection was 29.84 ± 10.66 years and that of controls was 33.43 ± 12.19 years. The percentage of females was 68% for cases and 68% for controls. All patients were classified as RR (relapsing remitting) or SP (secondary progressive) MS cases. Controls were 974 blood donors with no history of inflammatory disease attending the blood banks of Granada (n=823), Seville (n=71) and Málaga (n=80). Both cases and controls were Caucasians. The study was approved by the Ethics Committees of each of the hospitals participating in the study and a written informed consent was obtained from all participants.

Genotyping of individuals from the Spanish population

In this analysis, we included the 19 SNPs identified in the WTCCC GWAS with a *P*-value ≤0.005 from all chromosomes, except chromosome 6. We typed all 19 SNPs in 732 patients and 974 controls. Genotyping was performed using

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the MassARRAY SNP genotyping system (Sequenom, San Diego, CA, USA) according to the manufacturer's instructions.

This genotyping platform is at the Spanish National Genotyping Center's facilities in Santiago de Compostela University, Galicia, Spain. As quality control, they genotyped a trio of CEPH samples (CEPH pedigree 1340; samples NA07019, NA07029 and y NA07062). The genotypes of these samples resulted in no detection of Mendelian inconsistencies and corresponded with the ones deposited in HapMap. On the other hand, 4% of random samples were subjected to regenotyping. The resulting data were concordant with an average accuracy of $>99.9\%$.

Genotyping of participants from The Canadian Collaborative Project on the Genetic Susceptibility to MS

A total of 1318 individuals with definite MS and 1507 of their unaffected first-degree relatives were typed for rs17368528, rs10489990 and rs3746887 as part of the Canadian Collaborative Project on the Genetic Susceptibility to MS (CCPGSMS).[16] Genotyping was carried out using the MassARRAY system/Homogeneous MassExtend assay, following the protocol provided by Sequenom. All genotypes were generated blind to the pedigree structure and disease status of the individual.

Statistical analysis of data from the Spanish population

A χ^2-test ($P>0.05$) was used to assess whether genotypic distributions fulfilled the Hardy–Weinberg equilibrium. PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/) was used to compare the allele counts in cases and controls by Fisher's exact test and to calculate odds ratios (ORs) with 95% confidence intervals (CIs). Power was calculated for the size of effect detected in the WTCCC GWAS at an α-level of 0.05, assuming a multiplicative allelic effects model, using 732 cases and 974 controls.

Statistical analysis of data from the Canadian Collaborative Project on the Genetic Susceptibility to MS

Transmission disequilibrium testing was performed using the PLINK analysis package. For transmission disequilibrium tests, the χ^2 distribution was used to assess significance. The effects of rs10492972 and rs17368525 on the risk of MS were assessed by regression using the R statistical package (www.r-project.org).

RESULTS

A total of 19 MS-associated variants, with a *P*-value ≤ 0.005, reported by WTCCC GWAS were selected. The polymorphisms were first genotyped in a Spanish cohort of MS patients (n=732) and controls (n=974). No marker showed deviation from the Hardy–Weinberg equilibrium. Three of the 19 polymorphisms showed significant signals of association with MS ($P<0.05$) (Table 1). However, the association for rs10489990 and rs3746887 was with the opposite allele in the Spanish cohort as compared with the original study. To replicate this finding, we genotyped these three SNPs in an independent family cohort containing more than 1300 patients from the Canadian Collaborative Project on the Genetic Susceptibility to MS. Only the association for SNP, rs17368528, was replicated in this study (P=0.04, OR=0.83, 95% CI=0.72–0.96), as indicated in Table 2. The SNP rs17368528 is a nonsynonymous (leucine/proline) SNP localized in the fifth exon of *H6PD* (glucose 1-dehydrogenase) gene. This marker is located on chromosome 1p36.22, which is 1 Mb away from the MS-associated *KIF1B* gene. The linkage disequilibrium (LD) value between these two markers was r^2=0 for the Spanish cohort and r^2=0.21 for the Canadian cohort, and the association of *H6PD* was independent of the *KIF1B*-associated SNP (rs10492972), as assessed by logistic regression analysis ($P<0.05$ for both SNPs).

DISCUSSION

In MS, the current number of markers in GWAS with evidence for association exceeding a genome-wide significance threshold is small.

Table 1 Result of the WTCCC GWAS data and the replication analysis

			WTCCC data				*Spanish data*						
Gene	*Ch*	*SNP*	*Controls*	*MS*	*MAF WTCCC*	*P-value*	*OR*	*Power*	*Controls*	*MS*	*MAF Spain*	*P-value*	*OR*	*CI*
H6PD	1	rs17368528	22/330/1113	7/182/779	0.12	0.004	0.77	0.64	17/206/657	7/148/569	0.13	**0.04**	0.801	(0.648–0.990)
CD207	2	rs10489990	169/662/635	133/479/363	0.34	0.003	1.194	0.67	167/407/304	115/331/278	0.42	**0.04**	0.865	(0.751–0.997)
TMEM39A	3	rs1132200	61/432/969	27/229/717	0.19	6E−05	0.728	0.94	19/215/645	13/153/556	0.14	0.09		
C8orf32	8	rs6470147	150/631/685	124/460/391	0.32	0.0009	1.225	0.77	101/395/383	109/305/310	0.34	0.18		
INPP5A	10	rs3818511	52/468/946	44/374/556	0.2	0.0003	1.283	0.84	879/0/0	723/0/0	0	—		
CHUK	10	rs7903344	321/742/403	265/476/233	0.47	0.002	1.194	0.70	177/428/274	161/366/196	0.44	0.08		
SLC35C1	11	rs7130656	14/256/1168	14/212/738	0.1	0.005	1.130	0.2	9/198/672	10/1439/574	0.12	0.2		
STAB2	12	rs1609860	7/179/1279	5/164/806	0.07	0.002	1.389	0.74	6/115/755	6/109/608	0.07	0.2		
GARNL1	14	rs2274068	43/369/1054	32/304/639	0.16	0.002	1.266	0.72	26/309/544	25/237/462	0.21	0.58		
NF1	17	rs11080149	18/329/1089	30/242/688	0.13	0.003	1.282	0.71	5/126/748	2/100/624	0.08	0.5		
SP2	17	rs2229358	244/769/452	138/476/361	0.43	0.0012	0.835	0.72	204/427/248	167/349/207	0.47	0.8		
CEP192	18	rs2282542	23/343/1100	12/181/782	0.13	0.004	0.768	0.68	16/212/651	10/185/529	0.14	0.8		
GIPR	19	rs1800437	58/514/894	31/266/676	0.21	6E−05	0.741	0.92	28/277/573	21/228/476	0.19	0.8		
ZNF283	19	rs2195980	19/288/1130	17/245/706	0.11	0.002	1.316	0.73	21/220/637	14/171/538	0.15	0.3		
ZNF45	19	rs407731	323/728/374	213/482/266	0.48	0.00191	0.962	0.1	232/430/214	178/367/175	0.51	0.6		
C19orf48	19	rs4801853	144/576/738	61/378/532	0.3	0.004	0.824	0.70	60/316/503	47/280/397	0.25	0.5		
B3GALT5	21	rs3746887	37/415/1013	43/304/628	0.17	0.003	1.248	0.70	39/282/557	13/211/499	0.21	**0.003**	0.761	(0.635–0.912)
DLG3	23	rs2281868	148/361/223	187/347/170	0.45	0.0007	1.289	0.94	149/286/174	99/214/124	0.48	0.71		
DMD	23	rs228406	77/306/343	91/337/276	0.32	0.004	1.259	0.88	88/265/260	42/213/182	0.36	0.34		

Ch, chromosome; MAF, minor allele frequency.
Significant *P*-values ($P<0.05$) are given in bold. Power was calculated for the size of effect detected in the WTCCC GWAS at an α-level of 0.05, assuming a multiplicative allelic effects model, using 732 cases and 974 controls.

Table 2 Results of replication of the three SNPs associated with the Spanish population in the Canadian cohort

SNP	*MA*	*TR:NT*	*OR (95% CI)*	χ^2*-test*	P*-value*
rs17368528	T	239:285	0.838 (0.716–0.964)	4.038	0.0445
rs10489990	A	566:537	1.054 (0.897–1.232)	0.7623	0.3826
rs3746887	T	324:327	0.991 (0.847–1.161)	0.01382	0.9064

MA, minor allele; TR:NT, transmitted: not transmitted.

Markers that do not exceed this threshold are generally neglected, as they may represent false positives. However, some of the markers with a level of significance $\leq P=0.05$ may reflect true associations, given the current modest sample size used in initial MS GWA screens. In this study, we aimed to verify the association of 19 SNPs with MS, with *P*-values of association <0.005 reported in the WTCCC GWAS,[13] and found evidence of association for the rs17368528 SNP located on chromosome 1p36.22 in the Spanish and Canadian Cohorts.

The *KIF1B* gene, which is also located in the 1p36.22 locus, has been previously associated with MS susceptibility.[8] The *KIF1B* gene is an excellent candidate for MS, as it is required for the localization of MBP (myelin basic protein) mRNA to processes myelinating oligodendrocytes in zebrafish.[17] However, our data indicate that both markers are independently associated by regression analysis.

H6PD is a good candidate for MS, as it encodes an enzyme that catalyzes the initial steps of the pentose phosphate pathway (PPP) within the lumen of the endoplasmic reticulum, providing reducing equivalents such as NADPH(H^+). In neurons, most PPP-derived NADPH(H^+) is used for the regenerating pathway of the antioxidant glutathione (GSH).[18] The decrease in H6PD activity could alter the apoptotic processes in neurons affecting cytochrome C reduction by GSH. This could perhaps lead to neurodegeneration.[19] In fact, the missense polymorphism (Arg-Gln) rs6688832, also in the fifth exon of the *H6PD* gene and in LD ($D'=0.49$) with the SNP reported here, produces a reduction of 50% in enzyme activity in individuals with cortisone reductase deficiency.[20]

In summary, we report here an association with the *H6PD* gene, first identified by a GWAS and confirmed in two large cohorts of MS patients from Spain and Canada. The *H6PD* gene is an interesting candidate for MS for its possible role in neurodegeneration.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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