Cellular & Molecular Immunology (2015) 12, 777–779

www.nature.com/cmi

SHORT COMMUNICATION

The *GSTP1* gene variant rs1695 is not associated with an increased risk of multiple sclerosis

José AG Agúndez[1], Elena García-Martín[2], Carmen Martínez[1], Julián Benito-León[3,4,5], Jorge Millán-Pascual[6], María Díaz-Sánchez[4,5], Patricia Calleja[4,5], Diana Pisa[7], Laura Turpín-Fenoll[6], Hortensia Alonso-Navarro[6,8,9], Lucía Ayuso-Peralta[8], Dolores Torrecillas[8], Esteban García-Albea[8], José Francisco Plaza-Nieto[9] and Félix Javier Jiménez-Jiménez[8,9]

Cellular & Molecular Immunology (2015) **12,** 777–779; doi:10.1038/cmi.2014.121; published online 22 December 2014

We analyzed the allelic and genotypic frequencies of the *glutathione-S-transferase P1* (*GSTP1*) rs1695 single nucleotide polymorphism (SNP) in 290 patients with multiple sclerosis (MS) and in 310 healthy controls. We found no significant association between the rs1695 variant and MS. Among MS patients, there was no relationship between the rs1695 variant and either gender, clinical type of MS or the age of onset of MS. These results suggest that the *GSTP1* rs1695 polymorphism is not a risk factor for MS.

Genome-wide association studies in samples from MS patients have identified more than 100 loci with genome-wide significance, but most of these loci had a modest odds ratio (OR) in the range of 1.1–1.3; only HLA (especially the *HLA-DRB1*15:01* haplotype) had a strong association with MS risk.[1] A possible role for oxidative stress and lipid peroxidation in the pathogenesis of MS has been suggested by the presence of oxidative stress markers in the spinal cord, brain, and cerebrospinal fluid of MS patients and in experimental autoimmune encephalomyelitis (reviewed in Ref. 1).

Glutathione *S*-transferases (GSTs) are a superfamily of dimeric phase 2 metabolic enzymes that catalyze the conjugation of reduced glutathione with electrophilic groups of carcinogens, herbicides/pesticides, and other compounds. GSTP1 also plays a role in inflammatory processes (http://www.ncbi.nlm.nih.gov/pubmed/23596995). In humans, the GSTs are divided into a number of major classes that have distinct substrate specificities and tissue distributions. Polymorphisms in the *GSTM1*, *GSTP1* and *GSTT1* genes are known to alter gene function. The rs1695 variant of the *GSTP1* gene (chromosome 11q13; Gene identity 2950, MIM 134660; http://www.ncbi.nlm.nih.gov/gene/2950) causes an amino-acid substitution and reduces the catalytic activity of the enzyme (http://www.ncbi.nlm.nih.gov/pubmed/9600848, http://www.ncbi.nlm.nih.gov/pubmed/16488119 and http://www.ncbi.nlm.nih.gov/pubmed/22401947). The rs1695 variant is the only non-synonymous polymorphism of the *GSTP1* gene with a significant allele frequency in human populations and minor allele frequencies ranging from 17% to 44% (http://browser.1000genomes.org/Homo_sapiens/Variation/Population?db=core;r=11:67352189-67353189;v=rs1695;vdb=variation;vf=21985). The frequency of the *GSTP1* rs1695 variant is nearly 35% in the Spanish population.[2]

Although *GSTP1* polymorphisms were not identified as possible susceptibility genes by genome-wide association studies, we explored a possible relationship between *GSTP1* polymorphisms and allelic gene variants and the risk of MS due to the possible role of oxidative stress and lipid peroxidation in the pathogenesis of MS[1] and the upregulation of *GSTP1* gene expression found in active demyelinating MS lesions.[3] Although two preliminary studies did not find a relationship between polymorphisms in either *GSTM1*[4,5] or *GSTT1*[5] and the risk for MS, another recent study showed an association between *GSTT1* deletion and MS susceptibility[6]. Another study reported a relationship between *GSTM1*, *GSTM3* and *GSTP1*, but not *GSTT1*, and the degree of disability in MS.[7]

Alexoudi *et al.*[8] reported a similar distribution of *GSTP1* genotypes in MS patients and controls but a higher frequency

[1]Department of Pharmacology, University of Extremadura, Cáceres, Spain; [2]Department of Biochemistry and Molecular Biology, University of Extremadura, Cáceres, Spain; [3]CIBERNED, Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, Instituto de Salud Carlos III, Spain; [4]Service of Neurology, Hospital Universitario Doce de Octubre, Madrid, Spain; [5]Department of Medicine, University Complutense, Madrid, Spain; [6]Section of Neurology, Hospital La Mancha-Centro, Alcázar de San Juan (Ciudad Real), Spain; [7]Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Facultad de Ciencias, Universidad Autónoma, Cantoblanco, 28049 Madrid, Spain; [8]Department of Medicine-Neurology, Hospital 'Príncipe de Asturias', Universidad de Alcalá, Alcalá de Henares (Madrid), Spain and [9]Section of Neurology, Hospital Universitario del Sureste, Arganda del Rey (Madrid), Spain
Correspondence: Dr FJ Jiménez-Jiménez, C/ Marroquina 14, 3° B, E-28030 Madrid, Spain.
E-mail: fjavier.jimenez@salud.madrid.org;
E-mail: felix.jimenez@sen.es
Received: 13 November 2014; Accepted: 13 November 2014

of GSTP1 heterozygotes in patients with relapsing–remitting MS. This was particularly evident for the benign forms of relapsing–remitting MS. These authors also found a significantly higher frequency of *GSTP1* heterozygotes and *NAD(P)H dehydrogenase, quinone 1 (NQO1)* variant genotypes in MS patients compared with controls, suggesting that an interaction between these two genes might contribute to the risk of MS.

We examined the frequency of the rs1695 SNP in the *GSTP1* gene of 290 unselected and unrelated Caucasian Spanish patients with no other previous neurological diseases who fulfilled the McDonald's criteria for definite MS[9] (90 men and 200 women, mean age: 43.76±11.32 years, mean age at onset 32.64±10.57 years; mean Expanded Disability Score Scale=3.27±2.44; 155 relapsing–remitting MS, 92 secondary progressive MS and 43 primary progressive MS), and in 310 healthy unrelated Caucasian Spanish individuals who were gender- and age-matched with the MS cases (97 men and 213 women; mean age: 43.74±12.2 years). The subject recruitment details are described elsewhere[1].

All of the participants who were included in the study provided written informed consent. The study protocol was approved by the Ethics Committees of the University Hospitals 'Príncipe de Asturias' and 'Infanta Cristina' (Badajoz). The study was conducted according to the principles expressed in the declaration of Helsinki. Most of the patients in this study had participated in previous genetic association studies for the risk of MS.[1]

Table 1 *GSTP1 rs1695* genotype and allelic variants of patients with multiple sclerosis (MS) and healthy volunteers

	Genotype A/A	*Genotype A/G*	*Genotype G/G*	*Allele A*	*Allele G*
All MS patients (*N*=290), no. (%) (95% CI)	140 (48.3; 42.5–54.0)	122 (42.1; 36.4–47.8)	28 (9.7; 6.3–13.1)	402 (69.3; 65.6–73.1)	178 (30.7; 26.9–34.4)
Controls (*N*=310), no. (%) (95% CI)	151 (48.7; 43.1–54.3)	127 (41.0; 35.5–46.4)	32 (10.3; 6.9–13.7)	429 (69.2; 65.6–72.8)	191 (30.8; 27.2–34.4)
Intergroup comparison values, OR (95% CI); *P*	0.98 (0.70–1.37); 0.915	1.05 (0.75–1.47); 0.785	0.93 (0.53–1.64); 0.786	1.00 (0.78–1.30); 0.965	1.00 (0.77–1.29); 0.965
Negative predictive value (95% CI)	0.52 (0.47–0.56)	0.52 (0.49–0.56)	0.52 (0.50–0.53)	0.52 (0.47–0.56)	0.52 (0.50–0.54)
Men MS patients (*N*=90), no. (%) (95% CI)	38 (42.2; 32.0–52.4)	42 (46.7; 36.4–57.0)	10 (11.1; 4.6–17.6)	118 (65.6; 58.6–72.5)	62 (34.4; 27.5–41.4)
Men controls (*N*=97), no. (%) (95% CI)	47 (48.5; 38.5–58.4)	39 (40.2; 30.4–50.0)	11 (11.3; 5.0–17.7)	133 (68.6; 62.0–75.1)	61 (31.4; 24.9–38.0)
Intergroup comparison values, OR (95% CI); *P*	0.78 (0.42–1.44); 0.394	1.30 (0.70–2.42); 0.374	0.98 (0.36–2.64); 0.961	0.87 (0.55–1.38); 0.538	1.15 (0.73–1.81); 0.538
Negative predictive value (95% CI)	0.49 (0.42–0.56)	0.55 (0.48–0.61)	0.52 (0.49–0.55)	0.50 (0.42–0.57)	0.53 (0.49–0.57)
Women MS patients (*N*=200), no. (%) (95% CI)	102 (51.0; 44.1–57.9)	80 (40.0; 33.2–46.8)	18 (9.0; 5.0–13.0)	284 (71.0; 66.6–75.4)	116 (29.0; 24.6–33.4)
Women controls (*N*=213), no. (%) (95% CI)	104 (48.8; 42.1–55.5)	88 (41.3; 34.7–47.9)	21 (9.9; 5.9–13.9)	296 (69.5; 65.1–73.9)	130 (30.5; 26.1–34.9)
Intergroup comparison values, OR (95% CI); *P*	1.09 (0.73–1.64); 0.659	0.95 (0.63–1.43); 0.786	0.90 (0.44–1.84); 0.766	1.08 (0.79–1.47); 0.634	0.93 (0.68–1.27); 0.634
Negative predictive value (95% CI)	0.53 (0.48–0.58)	0.51 (0.47–0.55)	0.51 (0.50–0.53)	0.53 (0.47–0.58)	0.51 (0.49–0.53)
Relapsing–remitting MS (*N*=155), no. (%; 95% CI)	75 (48.4; 40.5–56.3)	64 (41.3; 33.5–49.0)	16 (10.3; 5.5–15.1)	214 (69.0; 63.9–74.2)	96 (31.0; 25.8–36.1)
Comparison values with controls, OR (95% CI); *P*	0.99 (0.66–1.48); 0.948	0.85 (0.57–1.28); 0.417	1.00 (0.51–1.96); 1.00	0.99 (0.73–1.35); 0.960	1.01 (0.74–1.37); 0.960
Negative predictive value (95% CI)	0.66 (0.62–0.71)	0.67 (0.63–0.71)	0.67 (0.65–0.68)	0.67 (0.62–0.71)	0.67 (0.65–0.69)
Secondary progressive MS (*N*=92), no. (%; 95% CI)	48 (52.2; 42.0–62.4)	34 (37.0; 27.1–46.8)	10 (10.9; 4.5–17.2)	130 (70.7; 64.1–77.2)	54 (29.3; 22.8–35.9)
Comparison values with controls, OR (95% CI); *P*	1.15 (0.70–1.88); 0.560	0.71 (0.43–1.17); 0.158	1.06 (0.47–2.36); 0.880	1.07 (0.74–1.56); 0.706	0.93 (0.64–1.36); 0.706
Negative predictive value (95% CI)	0.78 (0.74–0.83)	0.76 (0.73–0.80)	0.77 (0.76–0.79)	0.78 (0.73–0.82)	0.77 (0.75–0.79)
Primary progressive MS (*N*=43), no. (%; 95% CI)	17 (39.5; 24.9–54.1)	24 (55.8; 41.0–70.7)	2 (4.7; −1.6–10.9)	58 (67.4; 57.5–77.3)	28 (32.6; 22.7–42.5)
Comparison values with controls, OR (95% CI); *P*	0.69 (0.34–1.38); 0.260	1.53 (0.77–3.04); 0.190	0.42 (0.07–1.92); 0.238	0.99 (0.56–1.54); 0.742	1.08 (0.65–1.80); 0.742
Negative predictive value (95% CI)	0.86 (0.83–0.90)127	0.91 (0.87–0.94)	0.87 (0.87–0.89)	0.87 (0.83–0.91)	0.88 (0.87–0.90)

Abbreviations: CI, confidence interval; MS, multiple sclerosis; OR, odds ratio.

The detection of the *rs1695* allelic variant was performed on genomic DNA isolated from venous blood samples from the participants using *TaqMan* assays (C___3237198_20; Life Technologies, Alcobendas, Madrid, Spain) designed to detect the *rs1695* SNP. The methodology is similar to that used to detect other SNP allelic variants.[1]

Hardy–Weinberg equilibrium was analyzed using DeFinetti software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The allelic and genotypic frequency analyses were performed using SPSS software, ver. 17. International Business Machines España, Santa Hortensia 26–28, 28002 Madrid, SPAIN. The intergroup comparison values were calculated using either Chi-squared or Fisher's exact tests where appropriate. The 95% confidence intervals were also calculated. The sample size was determined using the allelic frequencies reported for South-European Caucasian individuals as described elsewhere[10] and a genetic model analyzing the disease gene frequency of risk alleles with an OR=1.5 (P=0.05) for bilateral and unilateral associations of the risk with a variant allele of 94.48% and 97.21%, respectively. The negative predictive value was calculated as d/r^2 (d, the number of control individuals with the risk factor absent; r^2, the sum of the patients and controls with the risk factor absent). The comparisons between the ages of onset for the different possible genotypes were performed using a Newman–Keuls test.

The frequencies of the *GSTP1* rs1695 genotypic and allelic variants in patients diagnosed with MS did not differ from those of the controls, were in Hardy–Weinberg equilibrium (Table 1) and were not influenced by gender (Table 1). The mean age of onset of MS did not differ significantly between the patients who were either homozygous for *GSTP1* rs1695 (A/A, mean±s.d.=31.76±10.54 years), heterozygous for *GSTP1* rs1695 (A/G, mean±s.d.=33.65±11.23 years) or lacked *GSTP1* rs1695 (G/G, mean±s.d.=32.30±8.67 years).

The distribution of the *GSTP1* rs1695 genotypes and the allelic frequencies did not differ among the 'relapsing–remitting', 'primary progressive' and 'secondary progressive' phenotypes of MS or between each type and the controls (Table 1).

There were no significant differences between the MS patients and the controls in the genotype distribution analysis of combined *GSTP1* rs1695 and *NQO1* rs1800566 polymorphisms (data not shown).

The limitations of this study include the size of the cohorts analyzed, which may not have been sufficient to confirm or exclude a role for *GSTP1* in MS. Although the sample size is adequate to detect an OR as small as 1.5, more modest associations would not be detected. In addition, because this study included patients with different severities of MS disease, it does not allow for the investigation of the influence of the *GSTP1* genotypes on the disability or severity of the disease. The optimum design for this study would be a prospective design, including genotyping patients with a recent diagnosis of MS and re-examining the same patient cohort after long-term follow-up had established the final disease type.

Taking into account the limitations of the present study, the results suggest that the *GSTP1* rs1695 genotypes and allelic variants are not related to the risk for MS in Caucasian Spanish people, the age of onset of MS or the clinical type of MS. In addition, we found no evidence of an interaction between the *GSTP1* rs1695 and *NQO1* rs1800566 variant genotypes. The lack of an association between the *GSTP1* rs1695 SNP and MS risk in this study does not exclude the possibility that other SNPs in the *GSTP1* gene could contribute to the risk of developing MS.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICS APPROVAL

Ethics Committees of the University Hospitals 'Príncipe de Asturias' and 'Infanta Cristina' (Badajoz).

ACKNOWLEDGEMENTS/FUNDING

This work was supported in part by Grants PI12/00241, PI12/00324 and RETICS RD12/0013/0002 from Fondo de Investigación Sanitaria, Instituto de Salud Carlos III, Spain and GR10068 from Junta de Extremadura, Spain. Financed in part with FEDER funds from the European Union.

1 Agúndez JA, García-Martín E, Martínez C, Benito-León J, Millán-Pascual J, Calleja P *et al*. NQO1 gene rs1800566 variant is not associated with risk for multiple sclerosis. *BMC Neurol* 2014; **14**: 87.
2 Martínez C, Martín F, Fernández JM, García-Martín E, Sastre J, Díaz-Rubio M *et al*. Glutathione *S*-transferases mu 1, theta 1, pi 1, alpha 1 and mu 3 genetic polymorphisms and the risk of colorectal and gastric cancers in humans. *Pharmacogenomics* 2006; **7**: 711–718.
3 Tajouri L, Mellick AS, Ashton KJ, Tannenberg AE, Nagra RM, Tourtellotte WW *et al*. Quantitative and qualitative changes in gene expression patterns characterize the activity of plaques in multiple sclerosis. *Mol Brain Res* 2003; **119**: 170–183.
4 Landtblom AM, Wastenson M, Ahmadi A, Söderkvist P. Multiple sclerosis and exposure to organic solvents, investigated by genetic polymorphisms of the GSTM1 and CYP2D6 enzyme systems. *Neurol Sci* 2003; **24**: 248–251.
5 Stavropoulou C, Korakaki D, Rigana H, Voutsinas G, Polyzoi M, Georgakakos VN *et al*. Glutathione-*S*-transferase T1 and M1 gene polymorphisms in Greek patients with multiple sclerosis: a pilot study. *Eur J Neurol* 2007; **14**: 572–574.
6 Zivković M, Zivotić I, Dinčić E, Stojković L, Vojinović S, Stanković A. The glutathione S-transferase T1 deletion is associated with susceptibility to multiple sclerosis. *J Neurol Sci* 2013; **334**: 6–9.
7 Mann CL, Davies MB, Boggild MD, Alldersea J, Fryer AA, Jones PW *et al*. Glutathione *S*-transferase polymorphisms in MS: their relationship to disability. *Neurology* 2000; **54**: 552–557.
8 Alexoudi A, Zachaki S, Stavropoulou C, Chatzi I, Koumbi D, Stavropoulou K *et al*. Combined GSTP1 and NQO1 germline polymorphisms in the susceptibility to Multiple Sclerosis. *Int J Neurosci* 2015; **125**: 32–37.
9 McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD *et al*. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001; **50**: 121–127.
10 Pértegas Díaz S, Pita Fernández S. Cálculo del poder estadístico de un estudio. *Cad Atención Primaria* 2003; **10**: 59–63.