Progression of multiple sclerosis is associated with gender differences in glutathione S-transferase P1 detoxification pathway

Bačić Baronica, Koraljka; Mlinac, Kristina; Petlevski, Roberta; Ozretić, David; Vladić, Anton; Kalanj-Bognar, Svjetlana; Žuntar, Irena

ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research paper Acta Neurobiol Exp 2014, 74: 257–265

Progression of multiple sclerosis is associated with gender differences in glutathione S-transferase P1 detoxification pathway

Koraljka Bačić Baronica[1], Kristina Mlinac[2], Roberta Petlevski[3], David Ozretić[4], Anton Vladić[1], Svjetlana Kalanj Bognar[2&]*, and Irena Žuntar[5&]

[&]Authors contributed equally to this work

[1]Department of Neurology, Clinical Hospital "Sveti Duh", Zagreb, Croatia; [2] Department for Chemistry and Biochemistry and Croatian Inst. for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia, *Email: svjetla@mef.hr; [3]Department for Medical Biochemistry and Hematology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia; [4]Department of Diagnostic and Interventional Radiology, University Hospital Centre Zagreb, Zagreb, Croatia; [5]Department of Analytical Toxicology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

The impact of glutathione S-transferases (GSTs) detoxification pathway on complex pathogenesis and heterogeneity of clinical findings in multiple sclerosis (MS), particularly the exact correlation between indicators of clinical severity and different GST genotypes, has not yet been fully elucidated. The aim of the study was to assess the relationship between disability level in multiple sclerosis (estimated by Kurtzke Expanded Disability Status Scale), disease progression (estimated by Multiple Sclerosis Severity Score), the level of brain atrophy and lesion load (determined by MRI) and detoxification status (analyzing glutathione S-transferase P1, GSTP1, genotype profile), in a group of 58 MS patients and 68 age/gender-matched controls. The results present the first evidence on significantly higher frequency of GSTP1 C341T polymorphism (C-T transition) in healthy subjects compared to MS patients, suggesting it may act as a moderating factor in developing MS clinical phenotype. Gender-dependent distribution of the C341T polymorphism was found in both MS patients and controls, with higher frequency of C-T transition in females. In addition, preliminary data showed higher proportion of male MS patients with higher median MSSS scores, as well as lower brain atrophy level and lesion load in MS patients carrying the C341T mutation. Observed gender difference in distribution of the C341T polymorphism in MS patients, as well as in disease progression, suggests that GSTP1 detoxification pathway occurs in a gender-dependent manner and could therefore add to clinical severity in male MS patients.

Key words: multiple sclerosis, clinical severity, glutathione S-transferase P1 detoxification pathway, gender differences

INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory demyelinating disease of the central nervous system with complex etiopathogenesis. It has been suggested that altered oxidative stress pathway including changed regulation of detoxifying enzymes like superoxide dismutase, catalase, glutathione peroxidase, paraoxonase and glutathione S-transferases (GSTs) which represent a defense system against oxidative reactions (Ortiz et al. 2013) may greatly contribute to the pathogenesis of multiple sclerosis (Korpela et al. 1989, Powell et al. 1992, Calabrese et al. 1994, Migliore and Coppede 2002, Dhib-Jalbut et al. 2006).

Glutathione (GSH) metabolism is involved in antioxidative protection by affecting individually different responses, susceptibility and coping with oxidative stress, which is particularly important for mammalian brain tissue. It has been documented that alterations of GSH metabolism lead to different levels of nerve cell loss and

Correspondence should be addressed to S. Kalanj Bognar Email svjetla@mef.hr

Received 17 January 2014, accepted 16 May 2014

neurodegeneration (Ballatori et al. 2009, Al et al. 2013). GSTs are supergene family of enzymes which participate in GSH metabolism; beside their role in protecting the cells from damage caused by oxidized metabolites, GSTs are involved in cellular processes such as biotransformation, detoxification, steroidogenesis, and cell signaling (Armstrong 1997, Henderson et al. 1998, Watson et al. 1998). The pi class of GSTs is most ubiquitous and prevalent GST isoenzyme in nonhepatic tissues (Watson et al. 1998, Migliore and Coppede 2002), appearing also as an oligodendrocytic marker (Tamura et al. 2007). The role of GSTs gene polymorphisms in the pathogenesis of multiple sclerosis has been supported by several studies mostly focused on the analysis of GSTM or GSTT allelic variants (Mann et al. 2000, Weatherby et al. 2000, Kantarci et al. 2002, Stavropoulou et al. 2007, Živković et al. 2013).

In this study, we investigated the influence of glutathione S-transferase P1 (GSTP1) detoxification pathway on the clinical phenotype in patients with multiple sclerosis. For that purpose, we analyzed the relationship between two estimated GSTP1 genotypes (A313G and C341T) and disability level, disease progression, level of brain atrophy and lesion load in MS patients in comparison with age- and gender-matched healthy controls.

METHODS

Subjects

The study group consisted of 126 subjects. The case group consisted of 58 patients with diagnosis of the relapsing-remitting multiple sclerosis (RRMS; 41 females, age range: 22–57 years, mean age: 43.1; 17 males, age range: 18–68 years, mean age: 37.5) hospitalized at the Department of Neurology, Clinical Hospital "Sveti Duh", Zagreb, Croatia. All subjects were treatment naïve except standard corticosteroid treatment for relapses. No drugs were administered prior to blood samples collection. The control group included 68 age- and gender-matched healthy subjects (42 females, age range: 20–58 years; mean age: 37; 26 males, age range: 21–71 years; mean age: 35.6) with negative history of neuropsychiatric and neurodegenerative disorders. Individuals suffering from renal, cardiac, gastrointestinal, endocrine and liver diseases, as well as those suffering from hypertension and asthma, were excluded from the study, as were the subjects on any drug/vitamin/mineral therapy and alcoholics (consuming >50 g of alcohol/day). Patients and healthy volunteers which participated in the study were informed in details on the purpose and methodology of the study and gave their informed consent for the involvement in the project. The study was approved by the Research Ethics Committee of "Sveti Duh" Clinical Hospital, Zagreb, Croatia and Research Ethics Committees of the School of Medicine and Faculty of Pharmacy and Biochemistry, University of Zagreb.

Estimation of disability level and disease progression in patients with multiple sclerosis

The clinical diagnosis of RRMS was established according to revised McDonald's criteria (McDonald et al. 2001, Polman et al. 2005) and supported by neurophysiologic tests, laboratory diagnostics and magnetic resonance imaging (MRI). Disability status was estimated by Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke 1983). According to this method, three stages of disability are classified as mild (EDSS=0–4.0), moderate (EDSS=4.5–5.5) and severe disability (EDSS=6.0–9.5). Disease progression was determined using Multiple Sclerosis Severity Score (MSSS) which takes into account EDSS scores and duration of disease, giving information on disease progression (Roxburgh et al. 2005). Assigned MS Severity Score of 5.0 means disease progressing at the median rate, while MSSS>5.0 indicates fast progression and MSSS<5.0 slower progression of disease. In addition, the correlation between disease severity and genotype was analyzed, by comparing median MSSS scores in different genotype groups of patients (Roxburgh et al. 2005, online software – University of Cambridge NUMSGG 2013).

Determination of glutathione S-transferase P1 gene polymorphisms

For the purposes of genotype analysis, genomic DNA was isolated from whole blood samples, collected by venipuncture during morning hours and prior to administration of any medication (Miller et al. 1988). Two glutathione S-transferase P1 (GSTP1) polymorphisms – A313G (105 Ile/Val, A-G transition in GSTP1 exon 5) and C341T (114 Ala/Val, C-T transition in GSTP1 exon 6) – were analyzed by PCR-RFLP method, according to previously described procedure (Žuntar et al. 2004).

Table 1

Relationship between GSTP1 genotypes and gender in patients with multiple sclerosis and age/gender matched controls

	MS (*n*=58)			Controls (*n*=68)		
GSTP1 genotypes	Female (*n*=41) *n* (%)	Male (*n*=17) *n* (%)	*P*-value	Female (*n*=42) *n* (%)	Male (*n*=26) *n* (%)	*P*-value
GSTP1 exon 5						
AA	19 (46)	7 (41)		21 (50)	16 (62)	
AG	21 (51)	9 (53)		20 (48)	8 (30)	
GG	1 (3)[a,e]	1 (6)[i,m]	0.782	1 (2)	2 (8)	0.283
AG+GG	22 (54)[b,f]	10 (59)[j,n]	0.944	21 (50)	10 (38)	0.498
A/G frequency	72/28	68/32	0.643	74/26	77/23	0.742
HWE	0.467	0.723		0.464	0.745	
GSTP1 exon 6						
CC	30 (73)	16 (94)		23 (55)	11 (42)	
CT	10 (24)	1 (6)		18 (43)	12 (46)	
TT	1 (3)[c,g]	0 (0)[k,o]	0.197	1 (2)	3 (12)	0.243
CT+TT	11 (27)[d,h]	1 (6)[l,p]	0.151	19 (45)	15 (58)	0.454
C/T frequency	85/15	97/3	0.007	76/24	65/35	0.121
HWE	1.000	1.000		0.790	1.000	

The analyzed genotypes were in Hardy-Weinberg equilibrium (HWE) for the MS patients (*n*=58) and control group (*n*=68)(*P*>0.05). (MS), Multiple sclerosis; (*P*) χ^2 test; [a] *P*=0.945, female MS vs. female controls; [b] *P*=0.909, female MS vs. female controls; [c] *P*=0.202, female MS vs. female controls; [d] *P*=0.129, female MS vs. female controls; [e] *P*=0.200, female MS vs. male controls; [f] *P*=0.336, female MS vs. male controls; [g] *P*=0.031, female MS vs. male controls; [h] *P*=0.023, female MS vs. male controls; [i] *P*=0.346, male MS vs. male controls; [j] *P*=0.336, male MS vs. male controls; [k] *P*=0.003, male MS vs. male controls; [l] *P*=0.002, male MS vs. male controls; [m] *P*=0.703, male MS vs. female controls; [n] *P*=0.744, male MS vs. female controls; [o] *P*=0.015, male MS vs. female controls; [p] *P*=0.010, male MS vs. female controls.

The relationship between GSTP1 gene polymorphisms and serum glutathione S-transferase activity was also analyzed in a group of MS patients and controls. Serum GST activity was determined using spectrophotometric method on a Trace spectrophotometer (Trace Scientific Ltd, Australia) utilising 1-chloro--2,4-dinitrobenzene (CDNB, Sigma) as a substrate (Habdous et al. 2002).

Neuroimaging techniques

Additional qualitative and semiquantitative interpretation of MRI findings of MS patients was performed in order to estimate whether brain pathomorphological changes are associated with the analyzed GSTP1 polymorphisms. MRI findings were compared between MS patients/GSTP1 mutation carriers and MS patients/mutation non-carriers, carefully selected and matched according to gender, age and disease duration. Neuroimaging was done by conventional MR techniques (T1-, T2-weighted and FLAIR sequences in multiple planes, with gadolinium enhancement, using 1.5 T MR device). Independent neuroradiological assessment included grading of disease severity imaging markers – atrophy level, number of hypointense lesions on T1-weighted images, number of hyperintense lesions on T2-weighted images, and number of gadolinium enhanced lesions. Grading of disease

severity imaging markers was done as follows: atrophy level was attributed with grade 0 – absent, 1 – mild, 2 – moderate, 3 – severe; T1- and T2-weighted lesions as well as gadolinium enhanced lesions were graded with 0 – absent, 1 – 1 to 9 lesions, 2 – 10 to 20 lesions, 3 – more than 20 lesions.

Statistical analysis

The frequency of each genotype and association between cases and controls were estimated by χ^2-test using the SigmaStat program (version 3.5, Jandel Corporation, Chicago, Illinois, USA). Odds ratios (OR) and 95% confidence interval (CI) were calculated by the MedCalc (version 7.0.0.2., MedCalc Software, B-9030 Mariakerke, Belgium) and were used to describe the strength of association. Analysis of MSSS scores in different genotype groups of MS patients was performed by Kruskal-Wallis test (Roxburgh et al. 2005; online software University of Cambridge NUMSGG). Comparison of disease severity neuroimaging markers in MS patients-mutation carriers vs. non-carriers was done by non-parametric Wilcoxon test. Statistical significance was set at 5% for all performed statistical analyses.

RESULTS

In this study the influence of GSTP1 detoxification pathway on clinical phenotype was analyzed in 58 MS patients. For this purpose, the relationship between estimated GSTP1 genotypes (A313G and C341T) and disability level, disease progression, serum glutathione S-transferase activity and neuroimaging markers of disease severity in MS patients was studied. Significant differences in analyzed genotype frequencies and distribution between MS patients and controls were found only in the case of C341T polymorphism. This polymorphism was detected in 12 MS patients (11 heterozygotes and 1 homozygote) and in 34 controls (30 heterozygotes and 4 homozygotes). Surprisingly, significantly higher frequency of combined CT+TT genotype and of the mutated allele (C-T transition) was found in control population as compared to MS group (50% vs. 21%; 28% vs. 11%; P=0.003 for genotypes; P=0.004 for alleles). The frequency of the A–G transition, another GSTP1 polymorphism analyzed in this study, was determined at 29% in MS patients and 25% in control subjects; no statistically significant difference was found in the distribution of the GSTP1 A313G genotypes (P=0.800) and alleles (P=0.633) between MS patients and controls.

Gender distribution of A313G and C341T polymorphism was additionally analyzed in both MS patients and control group (Table I). C341T polymorphism (C–T transition) was found to be significantly more frequent in healthy men in comparison with male MS patients while no such difference in frequency of C341T mutation was observed in female MS patients vs. healthy women (Table I). The study showed statistically significant difference between MS females and MS males in the distribution of GSTP1 C341T alleles and significantly higher frequency of T allele in female than in male MS patients (15% vs. 3%; P=0.007) (Table I).

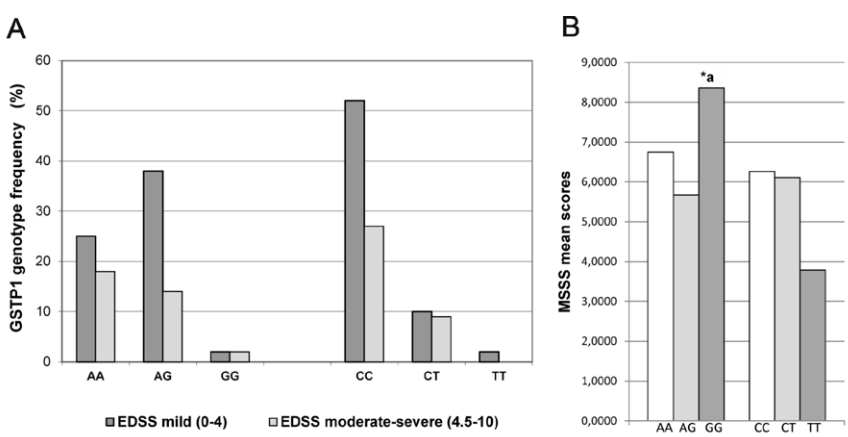

Fig. 1. Correlation between GSTP1 genotypes and (a) disability status as measured by EDSS, and (b) disease severity status as measured by MSSS, in a group of patients with multiple sclerosis. (EDSS) Expanded Disability Status Scale; (MSSS) Multiple Sclerosis Severity Score; *[a] P=0.033, Kruskal-Wallis test

In this study, total serum GST activity of MS patients and controls (103.96±16.88 U/L vs. 102.91±26.03 U/L, P=0.823, t-test) was analyzed and the relationship between GSTP1 gene polymorphisms and enzyme activity in a group of MS patients was additionally estimated. Comparison of total serum GST activities in MS patients carrying AA vs. combined AG+GG genotype (108.42±20.55 U/L vs. 106.29±14.86 U/L), as well as CC vs. CT+TT genotype (107.99±18.91 U/L vs. 104.47±11.09 U/L) did not reveal statistical difference, and no connection was found between enzyme activity and analyzed GSTP1 genotypes.

In order to determine the possible effects of different GSTP1 genotypes on the clinical phenotype of multiple sclerosis, the disability level and disease progression were estimated by EDSS scores and MSSS testing in all MS patients. It was observed that majority of male and female MS patients/mutation carriers presented with mild disability (7 of 10 male, and 15 of 22 female MS patients/mutation carriers had EDSS score =0–4.0). There was no correlation between GSTP1 A313G and C341T genotypes and disability level of MS patients, estimated by EDSS scores (P=0.504; P=0.588) (Fig. 1). Additional determination of disease progression by MSSS testing showed high proportion of MS patients/mutations carriers with MSSS>5.000, indicating so-called fast disease progressors, in both male and female MS patients (8 of 10 male, and 11 of 22 female MS patients/mutation carriers). Also, higher median MSSS score was determined in male (median =6.1, range =4.568–9.496) vs. female (median =5.382, range =1.889–9.453) MS patients/mutation carriers. MSSS

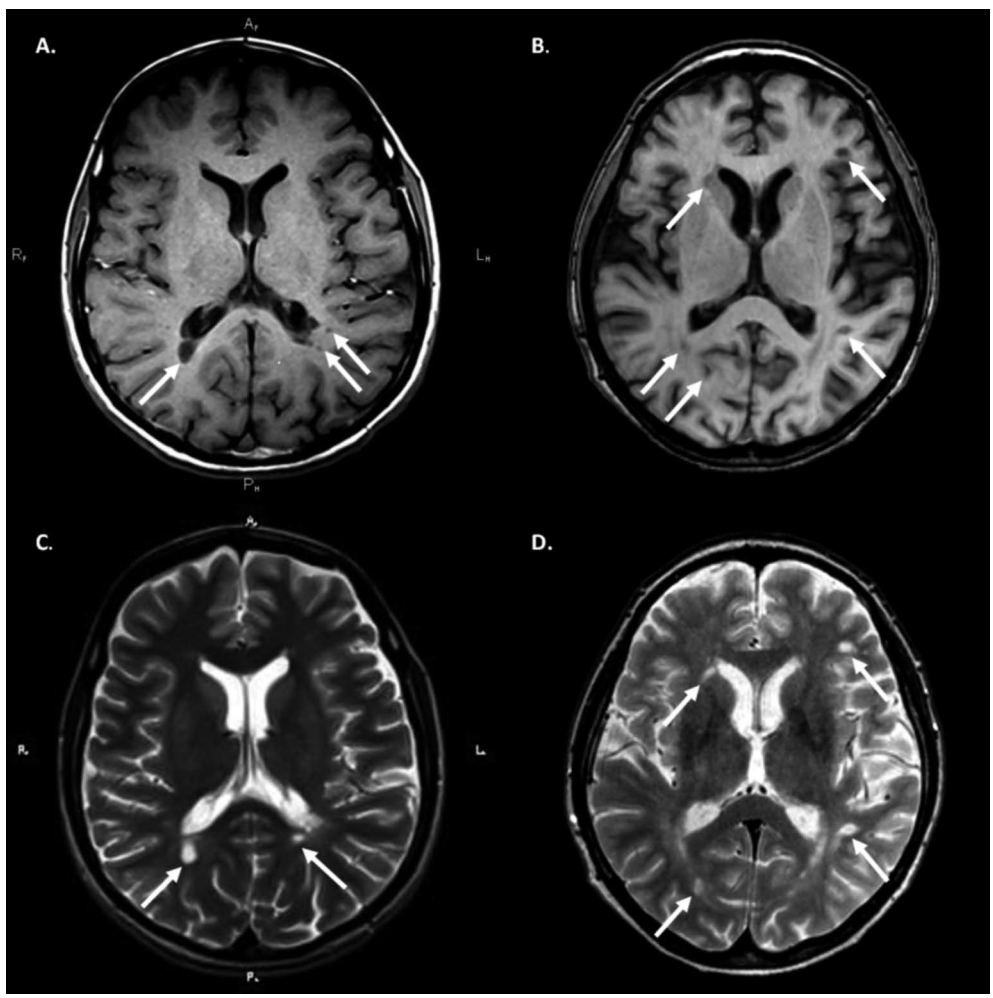

Fig. 2. Axial T1- weighted (A, B) and T2- weighted (C, D) MR images in two multiple sclerosis female patients, confirmed as C341T mutation carrier (A and C) and C341T mutation non-carrier (B and D) matched for age, gender and disease duration. T1 and T2 lesions are indicated by white arrows.

testing showed that patients carrying GG genotype (A313G polymorphism) have statistically significantly higher median MSSS score than patients with AA or AG genotype (P=0.032, Kruskal-Wallis test) while no such correlation was found for TT genotype (C341T polymorphism) (Fig. 1).

Qualitative and semiquantitative analysis of MRI findings showed significantly lower atrophy grade and smaller number of T2-weighted lesions in MS patients-carriers of C341T mutation when compared with mutation non-carriers matched for gender, age and disease duration (Wilcoxon test, P<0.05)(Fig. 2). A notable difference was also a smaller number of hypointense lesions on T1- weighted images in MS patients-carriers of C341T mutation. Figure 2 shows representative MR images, revealing described difference in lesion load in two female MS patients: C341T mutation carrier (age: 42, duration of disease: 6 years, MSSS 6.807, EDSS 4.0) vs. C341T mutation non-carrier (age: 47 years, duration of disease: 9 years, MSSS 3.448, EDSS 2.5).

DISCUSSION

Recent studies on glutathione metabolism showed that any alteration in this important antioxidative protective system affects the level of neurodegeneration and nerve cell loss (Al et al. 2013), and that decreased GSH content may be correlated with a clinical severity of multiple sclerosis in humans (Ljubisavljevic et al. 2014). In addition, there is evidence on significant reduction of GSH in grey matter and white matter lesions in multiple sclerosis patients, as revealed by MR spectroscopic imaging of glutathione (Srinivasan et al. 2010). Investigation of genes coding for GSTs, enzymes involved in the antioxidative GSH utilization, as well as on the role of GSTs gene polymorphisms in the pathogenesis of multiple sclerosis led to a conclusion that genetically determined vulnerability for oxidized metabolites may contribute to alteration of detoxification pathway *via* GSTs in multiple sclerosis (Mann et al. 2000, Weatherby et al. 2000, Kantarci et al. 2002, Stavropoulou et al. 2007, Živković et al. 2013). Majority of these studies focused on analysis of GSTM and GSTT allelic variants.

In our study, two polymorphisms in the GSTP1 gene were estimated – A313G and C341T. The A313G polymorphism was described previously in MS patients (Mann et al. 2000). Our results on the distribution of the A313G genotypes and alleles in MS patients are similar to those obtained by Mann and coworkers (2000) in the study which analyzed GSTP1 (A313G), GSTM1, GSTM3 and GSTT1 genotypes in a large group of MS patients. In Mann´s study, only combined GSTM1 null/GSTP1 AA genotype, as well as GSTT1 and GSTM1 null genotypes, correlated negatively to long-term prognosis of disease (Mann et al. 2000). Data on the C341T polymorphism analyzed in our study have not yet been reported in available literature. We observed significantly higher frequency of C–T transition and combined CT+TT genotype in control population vs. MS patients, as well as significantly higher frequency of that mutation in healthy male population in comparison with male MS patients. This finding is in line with the hypothesis that carriers of the C341T polymorphism might be less susceptible to developing MS clinical phenotype. In addition, observed gender-dependent distribution of C–T transition in both healthy women and MS female patients when compared to male controls and patients, indicates gender differences in GSTs oxidative detoxification actions. Stravropoulou and colleagues (2007) suggested a role of GSTs in a gender-dependent manner in the MS pathogenesis providing possible explanation for higher disease prevalence amongst females due to observed higher incidence of GSTM1 null genotypes in female MS patients. Gender-dependent distribution of GSTP1 C341T genotype in MS patients, found in our study, as well as hypothesized moderating role of C341T polymorphism may also contribute to generally less severe disease course and better prognosis observed in female than in male MS patients.

Interestingly, interpretation of neuroimaging markers of disease severity confirmed that MS patients carrying C341T mutation had lower atrophy grade and lesion load in comparison with MS patients/mutation non-carriers, even regardless of their EDSS and MSSS scores. A notable difference was also smaller number of hypointense lesions on T1-weighted images, seen as areas of low signal intensity compared to normal-appearing white matter. These so-called 'black holes' have various pathological substrates depending on the disease stage; it has been suggested that the number of chronic 'black holes' which are more frequent in patients with progressive disease could serve as surrogate markers of disability in MS (Truyen et al. 1996, Rovira and Leon 2008). Our observation on lower atrophy grade and lesion load in MS patients carrying C341T mutation is based on a small sample size, how-

ever it supports the idea on putative moderating role of this mutation in multiple sclerosis pathogenesis.

The effect of detected GSTP1 polymorphisms on the enzyme activity was assessed by determination of total GST activity in serum samples of MS patients and controls. It has been previously evidenced that A313G and C341T polymorphisms affect enzyme activity *in vitro* (Ali-Osman et al. 1997, Habdous et al. 2004). Also, a recent study, dealing with various peripheral markers of oxidative stress in relapsing-remitting multiple sclerosis, showed significantly lower total GST activity in erythrocytes derived from MS patients when compared with controls (Tasset et al. 2012). The results from that study are not fully comparable with our results, as we used a different method for measuring the enzyme activity in serum samples, not erythrocytes. We did not find a direct connection between serum GST activity and the GSTP1 genotypes when comparing MS patients/carriers and MS patients/non-carriers of either A313G or C341T mutation. This is not surprising, as many GST genes regulate the production of the enzyme and a single abnormality may not be adequate to reduce the total level of GST activity (Sheehan et al. 2001). Also, measured total serum GST activity is a sum of pi and alpha GST isoenzymes activity (Habdous et al. 2002, 2004), and thus may be related to different GSTP1 and GSTM polymorphisms. Difficulty in interpretation of our result might be related to recently reported limitations of the method used for GST activity determination as well (Fabrini et al. 2012). Final conclusion referring to exact relationship of GST enzyme activity and different GST genotypes in MS should be furthermore supported and clarified by studies using a larger sample size. In addition, keeping in mind a high expression of pi class of GSTs in brain tissue, it would be tempting to explore the effects of GSTP1 polymorphisms on brain GST activity and probable consequent alterations of brain GSH metabolism in multiple sclerosis.

In order to investigate the influence of analyzed GSTP1 gene polymorphisms on the clinical phenotype, we estimated disability level and disease progression in MS patients by EDSS scores and MSSS testing. EDSS is a standard clinical tool for estimation a disability level in multiple sclerosis but is limited to single assessment within the course of the disease, giving no insight into disease progression. This explains significant variations of individual EDSS results depending whether the estimation was performed during disease relapse or remission. We did not manage to find a correlation between GSTP1 A313G and C341T genotypes and disability level of MS patients, as estimated by EDSS scores. It is important to note that none of the MS patients was a carrier of independent C341T mutation (unlike control subjects), meaning that at least a part of analyzed MS phenotypic characteristics are a consequence of combined A–G and C–T genotypes. However, additional determination of disease progression by MSSS testing showed that patients bearing mutated A313G genotype (GG) are fast disease progressors; such a correlation was not found for C341T genotypes and MSSS. The advantage of the MSSS method is that it takes into account a disability level score as well as the duration of the disease. MSSS is thus an accurate method for correlation analysis of groups of patients with different genotypes and disease progression and for identifying factors influencing disease progression such as gene polymorphisms. Our observation on higher proportion of male MS patients/mutation carriers with MSSS scores >5.000 and higher median MSSS score in male than in female MS patients is in line with described gender-dependent clinical characteristics of multiple sclerosis – higher incidence, milder clinical features and better prognosis in majority of female than in male MS patients (Tomassini and Pozzilli 2009). Additional evidence on gender as one of determining factors for relapse incidence and disease progression is provided by a recent cohort study showing higher relapse frequency in females than in males while lower female to male sex ratio was observed in primary progressive multiple sclerosis (Kalincik et al. 2013). Finally, it can be concluded that both gender differences and antioxidative glutathione protection system are implicated in complex and multifactorial etiopathogenesis of multiple sclerosis.

CONCLUSION

This study presents significantly different distribution and higher frequency of GSTP1 C341T polymorphism in healthy individuals in comparison with MS patients, suggesting its possible role as a moderating factor in developing MS clinical phenotype. Observed gender difference in distribution of C341T polymorphisms between female and male MS patients indicates that GSTP1 detoxification pathway may occur in a gender-dependent manner. Our results showing associa-

tion of investigated GSTP1 gene polymorphisms with indicators of disability, disease progression and neuroimaging findings demonstrate a probable influence of GSTP1 on clinical course and disease severity, and its involvement in pathogenesis of multiple sclerosis.

ACKNOWLEDGEMENT

These materials are based on work financed by Croatian Ministry of Science, Education and Sports (projects SKB and IŽ).

REFERENCES

Al NF, Strom M, Lindblom R, Aeinehband S, Bellander BM, Nyengaard JR, Lidman O, Piehl F (2013) Naturally occurring variation in the glutathione-s-transferase 4 gene determines neurodegeneration after traumatic brain injury. Antioxid Redox Signal 18: 784–794.

Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J (1997) Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. J Biol Chem 272: 10004–10012.

Armstrong RN (1997) Structure, catalytic mechanism, and evolution of the glutathione transferases. Chem Res Toxicol 10: 2–18.

Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL (2009) Glutathione dysregulation and the etiology and progression of human diseases. Biol Chem 390: 191–214.

Calabrese V, Raffaele R, Cosentino E, Rizza V (1994) Changes in cerebrospinal fluid levels of malondialdehyde and glutathione reductase activity in multiple sclerosis. Int J Clin Pharmacol Res 14: 119–123.

Dhib-Jalbut S, Arnold DL, Cleveland DW, Fisher M, Friedlander RM, Mouradian MM, Przedborski S, Trapp BD, Wyss-Coray T, Yong VW (2006) Neurodegeneration and neuroprotection in multiple sclerosis and other neurodegenerative diseases. J Neuroimmunol 176: 198–215.

Fabrini R, Bocedi A, Massoud R, Federici G, Ricci G (2012) Spectrophotometric assay for serum glutathione transferase: a re-examination. Clin Biochem 45: 668–671.

Habdous M, Vincent-Viry M, Visvikis S, Siest G (2002) Rapid spectrophotometric method for serum glutathione S-transferases activity. Clin Chim Acta 326: 131–142.

Habdous M, Siest G, Herbeth B, Vincent-Viry M, Visvikis S (2004) Glutathione S-transferases genetic polymorphisms and human diseases: overview of epidemiological studies. Annales de Biologie Clinique 62: 15–24.

Henderson CJ, McLaren AW, Moffat GJ, Bacon EJ, Wolf CR (1998) Pi-class glutathione S-transferase: regulation and function. Chem Biol Interact 111–112: 69–82.

Kalincik T, Vivek V, Jokubaitis V, Lechner-Scott J, Trojano M, Izquierdo G, Lugaresi A, Grand'Maison F, Hupperts R, Oreja-Guevara C, Bergamaschi R, Iuliano G, Alroughani R, Van Pesch V, Amato MP, Slee M, Verheul F, Fernandez-Bolanos R, Fiol M, La Spitaleri D, Cristiano E, Gray O, Cabrera-Gomez JA, Shaygannejad V, Herbert J, Vucic S, Needham M, Petkovska-Boskova T, Sirbu CA, Duquette P, Girard M, Grammond P, Boz C, Giuliani G, Rio MA, Barnett M, Flechter S, Moore F, Singhal B, Bacile EA, Saladino ML, Shaw C, Skromne E, Poehlau D, Vella N, Spelman T, Liew D, Kilpatrick TJ, Butzkueven H (2013) Sex as a determinant of relapse incidence and progressive course of multiple sclerosis. Brain 136: 3609–3617.

Kantarci OH, de Andrade M, Weinshenker BG (2002) Identifying disease modifying genes in multiple sclerosis. J Neuroimmunol 123: 144–159.

Korpela H, Kinnunen E, Juntunen J, Kumpulainen J, Koskenvuo M (1989) Serum selenium concentration, glutathione peroxidase activity and lipid peroxides in a co-twin control study on multiple sclerosis. J Neurol Sci 91: 79–84.

Kurtzke JF (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33: 1444–1452.

Ljubisavljevic S, Stojanovic I, Cvetkovic T, Vojinovic S, Stojanov D, Stojanovic D, Bojanic V, Stokanovic D, Pavlovic D (2014) Glutathione homeostasis disruption of erythrocytes, but not glutathione peroxidase activity change, is closely accompanied with neurological and radiological scoring of acute CNS inflammation. Neuroimmunomodulation 21: 13–20.

Mann CL, Davies MB, Boggild MD, Alldersea J, Fryer AA, Jones PW, Ko KC, Young C, Strange RC, Hawkins CP (2000) Glutathione S-transferase polymorphisms in MS: their relationship to disability. Neurology 54: 552–557.

McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van den Noort S, Weinshenker BY, Wolinsky JS (2001) Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 50: 121–127.

Migliore L, Coppede F (2002) Genetic and environmental factors in cancer and neurodegenerative diseases. Mutat Res 512: 135–153.

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215.

Ortiz GG, Pacheco-Moisés FP, Bitzer-Quintero OK, Ramírez-Anguiano AC, Flores-Alvarado LJ, Ramírez-Ramírez V, Macias-Islas MA, Torres-Sánchez ED (2013) Immunology and oxidative stress in multiple sclerosis: clinical and basic approach. Clin Dev Immunol 2013:708659 [doi: 10.1155/2013/708659]

Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 58: 840–846.

Powell T, Sussman JG, Davies-Jones GA (1992) MR imaging in acute multiple sclerosis: ringlike appearance in plaques suggesting the presence of paramagnetic free radicals. AJNR Am J Neuroradiol 13: 1544–1546.

Rovira A, León A (2008) MR in the diagnosis and monitoring of multiple sclerosis: an overview. Eur J Radiol 67: 409–414.

Roxburgh RH, Seaman SR, Masterman T, Hensiek AE, Sawcer SJ, Vukusic S, Achiti I, Confavreux C, Coustans M, le PE, Edan G, McDonnell GV, Hawkins S, Trojano M, Liguori M, Cocco E, Marrosu MG, Tesser F, Leone MA, Weber A, Zipp F, Miterski B, Epplen JT, Oturai A, Sorensen PS, Celius EG, Lara NT, Montalban X, Villoslada P, Silva AM, Marta M, Leite I, Dubois B, Rubio J, Butzkueven H, Kilpatrick T, Mycko MP, Selmaj KW, Rio ME, Sa M, Salemi G, Savettieri G, Hillert J, Compston DA (2005) Multiple Sclerosis Severity Score: using disability and disease duration to rate disease severity. Neurology 64:1144–1151.

Sheehan D, Meade G, Foley GV, Dowd CA (2001) Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 360: 1–16.

Srinivasan R, Ratiney H, Hammond-Rosenbluth KE, Pelletier D, Nelson SJ (2010) MR spectroscopic imaging of glutathione in the white and gray matter at 7 T with an application to multiple sclerosis. Magn Reson Imaging 28: 163–170.

Stavropoulou C, Korakaki D, Rigana H, Voutsinas G, Polyzoi M, Georgakakos VN, Manola KN, Karageorgiou CE, Sambani C (2007) Glutathione-S-transferase T1 and M1 gene polymorphisms in Greek patients with multiple sclerosis: a pilot study. Eur J Neurol 14: 572–574.

Tamura Y, Kataoka Y, Cui Y, Takamori Y, Watanabe Y, Yamada H (2007) Intracellular translocation of glutathione S-transferase pi during oligodendrocyte differentiation in adult rat cerebral cortex in vivo. Neuroscience 148: 535–540.

Tasset I, Aguera E, Sanchez-Lopez F, Feijoo M, Giraldo AI, Cruz AH, Gascon F, Tunez I (2012) Peripheral oxidative stress in relapsing-remitting multiple sclerosis. Clin Biochem 45: 440–444

Tomassini V, Pozzilli C (2009) Sex hormones, brain damage and clinical course of Multiple Sclerosis. J Neurol Sci 286: 35–39

Truyen L, van Waesberghe JH, van Walderveen MA, van Oosten BW, Polman CH, Hommes OR, Adèr HJ, Barkhof F (1996) Accumulation of hypointense lesions ("black holes") on T1 spin-echo MRI correlates with disease progression in multiple sclerosis. Neurology 47: 1469–1476.

University of Cambridge NUMSGG (2013) http://www.gene.cimr.cam.ac.uk/MSgenetics/GAMES/MSSS/

Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA (1998) Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 19: 275–280.

Weatherby SJ, Mann CL, Davies MB, Fryer AA, Haq N, Strange RC, Hawkins CP (2000) A pilot study of the relationship between gadolinium-enhancing lesions, gender effect and polymorphisms of antioxidant enzymes in multiple sclerosis. J Neurol 247: 467–470.

Živković M, Životić I, Dinčić E, Stojković L, Vojinović S, Stanković A (2013) The glutathione S-transferase T1 deletion is associated with susceptibility to multiple sclerosis. J Neurol Sci 334: 6–9.

Žuntar I, Kalanj-Bognar S, Topic E, Petlevski R, Stefanovic M, Demarin V (2004) The glutathione S-transferase polymorphisms in a control population and in Alzheimer's disease patients. Clin Chem Lab Med 42:334-339