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GSTA1, *GSTM1*, *GSTP1* and *GSTT1* polymorphisms in progressive myoclonus epilepsy: A Serbian case–control study

Marko Ercegovac [a,f], Nebojsa Jovic [b,f], Dragoslav Sokic [a,f], Ana Savic-Radojevic [c,f], Vesna Coric [c,f], Tanja Radic [c], Dimitrije Nikolic [d,f], Miljana Kecmanovic [e], Marija Matic [c,f], Tatjana Simic [c,f], Marija Pljesa-Ercegovac [c,f,*]

[a] *Clinic of Neurology, Clinical Centre of Serbia, Dr Subotica 2, 11000 Belgrade, Serbia*
[b] *Clinic of Neurology and Psychiatry for Children and Youth, Clinical Centre of Serbia, Dr Subotica 6a, 11000 Belgrade, Serbia*
[c] *Institute of Medical and Clinical Biochemistry, Pasterova 2, 11000 Belgrade, Serbia*
[d] *University Children's Hospital, Tirsova 10, 11000 Belgrade, Serbia*
[e] *Faculty of Biology, University in Belgrade, Studentski trg 3, 11000 Belgrade, Serbia*
[f] *Faculty of Medicine, University in Belgrade, Belgrade, Serbia*

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ABSTRACT

Purpose: Oxidative stress is recognized as an important factor in progressive myoclonus epilepsy (PME). Genetic polymorphism of glutathione *S*-transferases (GSTs), which are involved in both protection from oxidative damage and detoxification, might alter the capacity for protecting tissues from exogenous and endogenous oxidants. We aimed to assess a possible association between GST polymorphism and PME, as well as, correlation between GST genotypes and oxidative phenotype in PME patients.
Methods: *GSTA1*, *GSTM1*, *GSTP1* and *GSTT1* genotypes were determined in 26 patients with PME and 66 controls. Byproducts of protein oxidative damage (thiol groups (P-SH) and nitrotyrosine), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities were determined.
Results: The frequency of *GSTA1*, *GSTM1* and *GSTP1* genotypes was not significantly different between PME patients and controls, while individuals with *GSTT1-null* genotype were at 5.44-fold higher risk of PME than carriers of *GSTT1-active* genotype. Moreover, significant risk of PME was obtained in carriers of both *GSTT1-null* and *GSTM1-null* genotypes. Carriers of combined *GSTA1- active* and *GSTT1-null* genotype were at highest, 7.55-fold increased risk of PME. Byproducts of protein damage did not reach statistical significance, while SOD and GPX activities were significantly higher in PME patients then in controls. When stratified according to GST genotype, P-SH groups were significantly lower only in patients with *GSTT1-null* genotype in comparison to carriers of *active* genotype. Only SOD activity was increased in *GSTT1-null* when compared to corresponding *active* genotype.
Conclusions: *GSTT1-null* genotype might be associated with the increased risk and enhanced susceptibility to oxidative stress in PME patients.

1. Introduction

Progressive myoclonus epilepsies (PMEs) are a group of rare inherited disorders characterized by the association of epilepsy, myoclonus and progressive neurological deterioration [1]. Oxidative stress, recognized as one of the predisposing factors in various neurological disorders, is suggested as an important factor in this process [2–5]. It has long been known that oxidative stress may contribute to neuronal hyperexcitability, while antioxidants may alleviate the progression of PME [2,3,6]. Furthermore, an improvement in the general condition and even reduction of seizures and myoclonus by antioxidants has been reported in both familial and

* Corresponding author at: Faculty of Medicine University in Belgrade, Institute of Medical and Clinical Biochemistry, Pasterova 2, 11000 Belgrade, Serbia. Tel.: +381 11 3643249; fax: +381 11 3643270.
E-mail addresses: ercegovacmarko@gmail.com (M. Ercegovac), njjovic@eunet.rs (N. Jovic), dragoslav.sokic@kcs.ac.rs (D. Sokic), anasavicradojevic@med.bg.ac.rs (A. Savic-Radojevic), coricmvesna@gmail.com (V. Coric), tanjajevtic@gmail.com (T. Radic), dimitrije.nikolic@gmail.com (D. Nikolic), miljana@bio.bg.ac.rs (M. Kecmanovic), marija_opacic@yahoo.com (M. Matic), tatjanasimic@med.bg.ac.rs (T. Simic), marijaercegovac@med.bg.ac.rs (M. Pljesa-Ercegovac).

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non-related patients with the same PME syndrome [2,3,6]. Still, the molecular mechanisms underlying oxidative stress and its role in progressive myoclonus epilepsy remain elusive.

Oxidative reactions occurring in mitochondria produce oxygen radicals physiologically in nervous system cells. If a strong antioxidant defense system is present in the cells, they will be protected from the damaging effects of reactive oxygen species [7,8]. Glutathione *S*-transferases (GSTs) are a superfamily of enzymes involved in both protection from oxidative damage and detoxification [9]. Furthermore, they play an important role in metabolizing antiepileptic drugs (AEDs) in liver [10,11]. Namely, many conventional AEDs undergo metabolism generating active metabolites, including epoxides, which might have effect on suppression of epileptic spike, but unfortunately, also in systemic toxicity via epoxide-induced covalent binding to proteins and lipids [10,12]. Glutathione *S*-transferases play an important role in catalyzing the conjugation of these metabolites to glutathione, especially in the removal of epoxide metabolites that are generated during the metabolism of AEDs. Apart from decreasing their toxicity and facilitating their excretion from the body, it is assumed that this way GSTs may also affect the response to anticonvulsant therapy [10].

The most characterized GST classes have been named alpha (GSTA), mu (GSTM), pi (GSTP) and theta (GSTT). Almost all members of GST family exhibit genetic polymorphism, resulting in complete lack or lowering of enzyme activity [9]. Consequently, individuals lacking certain GST isoenzyme activity may have altered capacity for protecting tissues from exogenous and endogenous oxidants, as well as, AEDs [13]. In agreement with this assumption are the results of Liu and Tsai on enhanced lipid peroxidation in patients with *GSTM1-null* genotype and intractable seizure [10]. Another member of GST family, GSTP1, has been associated with resistance to AEDs treatment. Namely, high levels of GSTP1 expression in endothelial cells and glial cells/astrocytes have been shown to correlate to medical intractable epilepsy [11]. Furthermore, the lack of GSTT1 has been associated with susceptibility to brain diseases, including neurodegenerative diseases [14]. Still, the potential role of genetic polymorphism of GSTs in both risk for development and resistance to AEDs therapy in patients with PME has to be established.

This has prompted us to assess a possible association between GST polymorphism and progressive myoclonus epilepsy. Whether the *null* or *low-activity GSTA1*, *GSTM1, GSTP1* and *GSTT1*genotypes, alone or in combination, correlate with biomarkers of oxidative stress in patients with progressive myoclonus epilepsy will be additionally evaluated.

2. Methods

2.1. Study participants

Genomic DNA was isolated from 26 patients (13 male and 13 female, mean age 20 ± 7.04 years) with progressive myoclonus epilepsy. Five patients had Unverricht–Lundborg disease, fourteen Lafora body disease, another five myoclonic epilepsy with ragged red fibers and 2 had late infantile neuronal ceroid lipofuscinosis. Patients were diagnosed based on the clinical history, electrophysiological findings and confirmed by molecular genetic studies.

The seizure types in these patients were generalized tonic–clonic seizures and myoclonus. All patients were on valproic acid with multiple other antiepileptic drug regimens. Control group consisted of 66 healthy, non-epileptic subjects, without any drug treatment, matched for sex, age, ethnicity and geographic origin.

All the participants and/or their parents provided written informed consent. This study protocol was approved by the Institutional Review Board, and the research was carried out in compliance with the Helsinki Declaration (as revised in 2000).

2.2. Molecular genetic studies

Using PCR protocol with betaine, we amplified promoter region of the CSTB gene. After separation of amplified products in 2% agarose gel along with DNA size standard, we detected a homozygous expansion of dodecamer repeats in four ULD patients [15]. Molecular diagnostics of LD, MERRF and NCL patients was performed by sequencing coding regions of appropriate genes.

2.3. GST genotyping

Genomic DNA was isolated from whole blood using the QIAGEN QIAmp kit (Qiagen, Inc., Chatsworth, CA). GSTA1 C-69T polymorphism was determined by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) by Ping et al. [16]. The presence of restriction site resulting in two fragments (481 and 385 bp) indicated mutant allele (*T*/*T*) and if *C*/*T* polymorphism incurred, it resulted in one more fragment of 96 bp (Fig. 1a).

GSTM1 genotyping was performed by multiplex PCR [17]. Exon 7 of the CYP1A1 gene was co-amplified and used as an internal control. The presence of the *GSTM1 active* genotype was detected by the band at 215 bp, since the assay does not distinguish heterozygous or homozygous wild-type genotypes (Fig. 1b).

GSTP1 Ile105Val polymorphism was analyzed using the PCR–RFLP method by Harries et al. [18]. The presence of restriction site resulting in two fragments (91 and 85 bp) indicated mutant allele (*Val*/*Val*), while if *Ile/Val* polymorphism incurred, it resulted in one more fragment of 176 bp (Fig. 1c).

GSTT1 genotyping was performed by multiplex PCR [17]. The assay does not distinguish between heterozygous or homozygous wild-type genotypes; therefore, the presence of 480 bp bands was indicative for the *GSTT1 active* genotype (Fig. 1d).

2.4. Biomarkers of oxidative stress in plasma

The amount of plasma thiol (P-SH) groups was determined according to the method of Jocelyn and expressed as mmol/g of proteins (mmol/g prot) [19]. Nitrotyrosine content was measured by enzyme immunoassay (OxiSelectTM ELISA kits, Cell Biolabs). Cu, Zn SOD activity in the plasma was measured by the method of Misra and Fridovich, based on the ability of SOD to inhibit autooxidation of epinephrine at alkaline pH (pH 10.2) [20]. GPX activity was determined by the coupled assay procedure of Gunzler et al. [21].

2.5. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software (IBM Statistics SPSS, version 20.0). In descriptive statistics, we summarized all continuous variables by means $\pm$ standard deviations (SD). The relative associations between the studied genotypes and PME risk were evaluated by multinomial logistic regression to calculate odds ratios (OR) and corresponding 95% confidence intervals (CI), adjusted according to age and gender as potential confounding factors. Differences in investigated biomarkers of oxidative stress were assessed by using χ^2 test. Statistical evaluation of relationships between biomarkers of oxidative stress (SH groups, nitrotyrosine, superoxide dismutase and glutathione peroxidase) and GST genotypes in PME patients was performed by using the Mann–Whitney rank-sum test (for between-two-group comparisons) and the Kruskal–Wallis non-parametric test that compared three unpaired groups. Two-tailed p-values of <0.05 were considered statistically significant.