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SHORT CONTRIBUTION

Association between *GPx1* Pro198Leu polymorphism, GPx1 activity and plasma selenium concentration in humans

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Abstract
Background Glutathione peroxidase 1 (GPx1) is an antioxidant selenoenzyme that protects the cells against reactive oxygen species. Its activity depends on the concentration of selenium (Se) which is present in the active centre of the enzyme. The genetic polymorphism of GPx1 encoding gene (*GPx1*) associated with the proline (Pro) to leucine (Leu) change at codon 198 is supposed to be functional. An in vitro study performed on human breast carcinoma cell line showed that *GPx1*Leu allele was associated with a lower responsiveness of the enzyme to Se added to the culture medium. Some authors observed a decrease in GPx1 activity associated with *GPx1* Leu allele in humans; however, there were no findings on how GPx1 activity changes with Se concentration in individuals with different *GPx1* genotypes.
Aim of the study To assess whether GPx1 activity that depends on the Se status may be influenced by *GPx1* polymorphism through studying this relationship in the blood of healthy individuals.
Methods The association between the Se status, GPx1 activity and *GPx1* genotype was assessed in 405 individuals of Polish origin. GPx1 activity in red blood cells was measured by the spectrophotometric method by Paglia and Valentine, using *t*-butylhydroperoxide as the substrate. Plasma Se concentration was measured using graphite furnace atomic absorption spectrometry. *GPx1* Pro198Leu polymorphism was determined with the Molecular Beacon Real-Time PCR assay.
Results In the subjects examined, the mean plasma Se concentration was 54.4 ± 14.2 mcg/L. The mean GPx1 activity was 15.1 ± 4.7 U/g Hb. No difference regarding both the parameters was found between individuals with different *GPx1* genotype. However, the association between GPx1 activity and Se concentration, analyzed separately for each genotype group, was not the same. The correlation coefficients amounted to $r = 0.44$ ($p < 0.001$) for Pro/Pro, $r = 0.35$ ($p < 0.001$) for Pro/Leu and $r = 0.25$ ($p = 0.45$) for Leu/Leu group, which indicates that the correlation strength was as follows: Pro/Pro > Pro/Leu > Leu/Leu. Notably, statistically significant difference in this relationship (analyzed as difference between correlation coefficients for linear trends) was found between genotypes Pro/Pro and Leu/Leu ($p = 0.034$).
Conclusions The findings of the present study provide evidence for the hypothesis based on in vitro studies which assumes that *GPx1* Pro198Leu polymorphism has a functional significance for the human organism and that this functionality is associated with a different response of GPx1 activity to Se. They also point to the importance of the genetic background in the assessment of the Se status with the use of selenoprotein biomarkers such as GPx1 activity.

Keywords Glutathione peroxidase · Selenium · Genetic polymorphism · Humans

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Introduction

The cytosolic glutathione peroxidase (GPx1), a protein described first in erythrocytes by Mills, is an antioxidant enzyme that catalyses the reduction of hydrogen peroxide and organic peroxides using glutathione as a reducing substrate [1, 11]. The studies by Rotruck and Flohe revealed that the enzyme is a selenoprotein and it consists of four 22 kDa subunits, each of which contains one atom of selenium (Se) [2, 18]. As in all mammals, Se is present in the GPx1 structure in the form of selenocysteine (Sec), an amino acid encoded by TGA "termination" codon [6]. Sec is an active site of the enzyme and a Se-deficient diet results in a decrease of GPx1 activity [9].

Human *GPx1* gene was found to possess several polymorphisms, one of which, *GPx1* Pro198Leu, is supposed to be functional. This polymorphism is associated with C to T substitution in the exon 2 of *GPx1*, which results in the amino acid change from proline (Pro) to leucine (Leu) at codon 198 [16]. The authors suggest that this change may cause some important conformational changes of GPx1, because Pro is the only amino acid without a free unsubstituted amino group on the alfa carbon atom [16]. Notably, in vitro studies conducted on human breast carcinoma cell line revealed that *GPx1* Leu allele was associated with a lower responsiveness of the enzyme activity to Se added to the culture medium [7]. GPx1 activity was also shown to be significantly lower in the transfected bovine aortic endothelial cells (BAECs) expressing 198Leu variant, compared to those expressing 198Pro variant [5]. The studies conducted on humans do not explicitly support the functional link between *GPx1* genotype and GPx1 enzyme. Ravn-Haren et al. [17] who studied *GPx1* Pro198Leu polymorphism among women with breast cancer, found that GPx1 activity was significantly lowered for the Leu allele compared to the Pro allele. This correlation was observed in women diagnosed with breast cancer as well as in the control group. However, the results of this study are not consistent with those by Forsberg et al. [3] who did not find any difference in the activity of GPx1 between individuals with either Pro/Pro, Pro/Leu or Leu/Leu genotype, suggesting no correlation between GPx1 activity and *GPx1* genotype.

Interestingly, some studies indicate an association between *GPx1* Pro198Leu polymorphism and an increased risk of cancer [7, 15–17]. Thus, it is possible that this polymorphism may be functional and has influence on the phenotypic properties of the GPx1 enzyme in the human organism. The studies comparing GPx1 activity between individuals with different *GPx1* variants are not enough to clarify the genotype–phenotype interaction. A more objective way to assess the functionality of *GPx1* polymorphism in humans is to "apply" the experimental model on cells used by Hu and Diamond to an epidemiological study on humans, and analyze how the GPx1 activity changes in individuals with different *GPx1* genotype in response to Se supplementation. Alternatively, the association between the Se status and GPx1 activity in individuals with different *GPx1* genotype can be assessed when no Se supplementation is introduced. The aim of the present study, based on this alternative approach, was to assess the relationship between the following three elements: GPx1 enzymatic activity, its genetic polymorphism and Se status.

Materials and methods

Study group

The study population consisted of a group of 405 hospital patients (282 men and 123 women), mean age 57.7 ± 10.9 years, who were residents of the city of Lodz and were enrolled between 1998 and 2001 as controls in a multicenter case-control study of lung cancer [8]. They were admitted to hospitals for non-neoplastic diseases unrelated to tobacco smoking. After obtaining a written consent to participate from the subjects, their blood samples were collected as well as the data on age, gender and smoking status. The subjects comprised 304 current smokers and 98 nonsmokers (for 3 individuals the data could not be obtained). The complete data for the genotype and GPx1 activity were available for 371 subjects (174 Pro/Pro, 158 Pro/Leu and 39 Leu/Leu genotype group).

Glutathione peroxidase activity

The GPx1 activity of RBC hemolysate was assessed using the method of Paglia and Valentine [13], with *t*-butyl hydroperoxide as the substrate. 20 μL of hemolysate was transferred to a quartz cuvet containing the reaction mixture (Tris buffer, 50 mmol/L, pH 7.6, containing per liter: 1 mmol of Na_2EDTA, 2 mmol of reduced glutathione, 0.2 mmol of NADPH, 4 mmol of sodium azide, and 1,000 U of glutathione reductase). After 5 min of incubation at 37°C, the reaction was initiated by adding 0.74 μmol of *t*-butyl hydroperoxide. The enzyme activity was expressed in units per gram of Hb.

Selenium status determination

Plasma Se concentration was determined using graphite furnace atomic absorption spectrometry (GFAAS) [12]. The method was validated using Seronorm™ lyophilized human reference serum samples (Nycomed Pharma AS, Oslo, Norway) and through participation in an interlaboratory quality assurance program.

Genotype analysis

DNA was isolated from whole blood using QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's instructions. Genotype analysis was performed using Molecular Beacon Real-Time polymerase chain reaction assay (RT-PCR). Oligonucleotide sequences for PCR primers and fluorescent probes were designed using Molecular Beacon Software (Premier Biosoft International) and were as follows: forward primer 5′-TGA CGG TGT GCC CCT ACG-3′, and reverse primer 5′-CCG AGA CAG CAG CAC TGC-3′, probe C 5′-(6-Fam) CGC GAT CAG GCA CAG CTG GGC CCT TGA GAC GAT CGC G (Dabcyl)-3′, probe T 5′-(Hex) CGC GAT CAG GCA CAG CTG AGC CCT TGA GAC GAT CGC G (Dabcyl)-3′. RT-PCR was performed using the IQ™ 5 real-time PCR detection system (Bio-Rad) and JumpStart™ *Taq* Ready-Mix™ for Quantitative PCR (Sigma).

Statistical analysis

We used a general linear model with gamma distribution for error and identity link function. We have tested for a linear relationship between Se status and GPx1 activity in each genotype subgroup and we have compared trends between the groups. We considered gender, age and smoking status (expressed as cigarettes per day multiplied by the years of smoking) as the potential confounders. There were no uneven distributions of these factors in the genotype groups. In the final model we included only gender as a potential confounder. All the analyses were conducted using *R* statistical package [14].

Results

In the group examined, the allele distribution, assessed using the chi square test, was in Hardy-Weinberg equilibrium. 194 (48%) individuals were Pro/Pro homozygotes, 170 (42%) were Pro/Leu heterozygotes and 41 (10%) were Leu/Leu homozygotes. The mean plasma Se concentration was 54.4 ± 14.2 mcg/L and the mean GPx1 activity was 15.1 ± 4.7 U/g Hb. No difference regarding both the parameters was found between individuals with different *GPx1* genotypes. However, the association between GPx1 activity and Se concentration, analyzed separately for each genotype group, was not the same. The correlation coefficients were $r = 0.44$ ($p < 0.001$) for Pro/Pro, $r = 0.35$ ($p < 0.001$) for Pro/Leu and $r = 0.25$ ($p = 0.45$) for Leu/Leu group (Fig. 1). Considering these values, the correlation strength was as follows: Pro/Pro > Pro/Leu > Leu/Leu. Notably, statistically significant difference in this relationship (analyzed as difference between correlation coefficients for linear trends) was found between genotypes Pro/Pro and Leu/Leu ($p = 0.034$).

Discussion

The results of the present study, conducted on 405 individuals, revealed that the effect of Se on the GPx1 activity may depend on the genotype. These findings indicate that the correlation noted in earlier in vitro studies may also occur in vivo in humans. Based on this observation, we may presume that in the response to dietary Se intake or Se supplementation, the GPx1 activity will change in a different way in individuals with different *GPx1* genotype. Namely, relative increase in the enzyme activity is expected to be higher in individuals having at least one *GPx1*198Pro allele than in those with both the *GPx1*198Leu alleles. However, this hypothesis is based on the preliminary results and has to be verified through a supplementation trial. It should be also noted that the GPx1 activity was correlated with the concentration of Se measured in plasma, not in erythrocytes. To support the presented hypothesis in the supplemented individuals, it would be relevant to correlate GPx1 activity with the concentration of Se in erythrocytes.

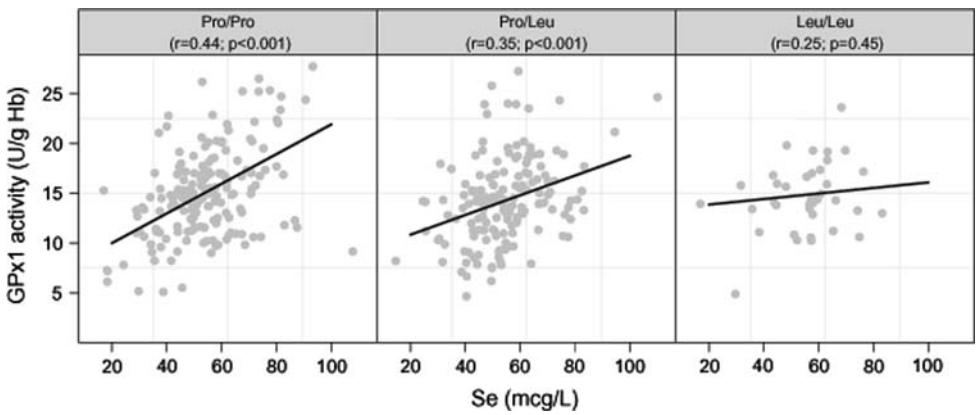

Fig. 1 Association between GPx1 activity and plasma Se concentration in healthy individuals with different *GPx1* genotypes (Pro/Pro, Pro/Leu and Leu/Leu)

The selenium status is assessed either by measuring Se concentration in the body (in whole blood, plasma, serum, erythrocytes, hair, nails or urine) or by measuring the activity/concentration of certain selenoproteins, including GPx1. Selenoproteins are not good indicators of a high Se status as they reach saturation above certain concentration of this element [4]. Supposing that genetic polymorphism affects GPx1 activity, this parameter should not be considered as a good biomarker also of low Se status, or a biomarker used in the supplementation trials, unless the *GPx1* genotype is analyzed. Recently, an interaction between the genetic polymorphism of SEPP (selenoprotein P) and the response of selenoprotein markers to Se supplementation has been reported (SELGEN study) [10]. Notably, those authors showed that two SNPs within *SEPP* gene had influence not only on the parameters measured during Se supplementation (the activity and concentration of certain selenoproteins in different blood fractions) but also on the baseline plasma Se concentration before supplementation (these findings were observed regardless of the BMI). These findings indicate that a better understanding of the functional polymorphism of genes encoding selenoproteins is necessary. Providing that a biological response to any factor (including dietary factors) may differ between humans due to the genetic variation, we may conclude that the use of certain biomarkers, especially in the supplementation trials, would be more valuable if data about the genetic background related to these biomarkers were available.

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