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Phenotype Determination of a Common Pro-Leu Polymorphism in Human Glutathione Peroxidase 1

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ABSTRACT: Oxidative stress has been implicated in human illness such as cardiovascular and neurodegenerative disease. The genetic mechanisms involved are only poorly understood. Here we describe the determination of the allelic frequency and phenotype of a common polymorphism in Se-dependent glutathione peroxidase 1 (GPX1) in Finnish/Swedish populations. A proline/leucine variant occurs at position 197 close to the C-terminus of the protein. The more common allele encoding the Pro variant is present at 59% in a Finnish/Swedish population ($n = 66$) and at 73% in a Swedish population ($n = 315$). The genotypes encoding Pro/Pro, Pro/Leu, and Leu/Leu are distributed according to the Hardy–Weinberg relationship. The Swedish population consisted of 101 stroke cases and 214 controls. No significant association between allele frequency and risk to suffer from stroke was evident. Erythrocyte GPX activity was determined in the Finnish/Swedish population and no significant differences were obtained between the genotypes. It can be concluded that the Pro/Leu genetic variation does not appear to compromise the defense against oxidative stress in red blood cells nor to be associated with stroke.

Key Words: human GPX1; polymorphism; erythrocyte activity; stroke.

INTRODUCTION

Human glutathione peroxidase 1 is an abundant and widely distributed seleno-protein (1). Mouse knock-out models have revealed that loss of the enzyme confers no obvious phenotype but that the animals become more sensitive to oxidative stress (2). Oxidative stress has been implicated in human illness such as cardiovascular and neurodegenerative disease (3, 4) as well as aging (5). To determine the actual impact of oxidative stress, tissue and plasma antioxidant levels are often determined. In addition, various antioxidant enzyme activities have been determined in blood. Although considerable variation in antioxidant capacity does occur in humans, the variation does not account for disease susceptibility in the majority of cases (6–9). It must either be concluded that oxidative stress is of limited importance or that current measurements of antioxidant capacity is a blunt instrument. As intraindividual variation in antioxidant capacity is well documented and influenced by nutritional status (10–12), other measurements of antioxidant capacity involving determination of genetic profile is highly desirable. Association studies of functional or linked surrogate marker polymorphic sites in genes offer a way to probe variant function (13). In an effort to survey polymorphisms in genes related to oxidative stress we recently determined a common allelic variant in human glutathione peroxidase 1 (14). Here we describe the genotyping of 66 individuals and comparison to the corresponding

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Abbreviations used: GPX1, Se-dependent glutathione peroxidase 1; SOD, superoxide dismutase.
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blood glutathione peroxidase activity levels and 315 individuals where 101 had suffered from stroke.

MATERIALS AND METHODS

Assay of Glutathione Peroxidase Activity

With informed consent blood was collected from all individuals. The erythrocyte GPX activity was determined in hemolysates with a coupled spectrophotometric assay (15). Hemoglobin was determined with a standard cyanomethemoglobin assay. Patients were not taking antioxidants including selenium and the study was approved by the research ethical committee of Umeå University.

Study Populations

The Finnish/Swedish population has previously been described (16). The MONICA/CASTRO study population (17, 18) was recruited in two surveys conducted in northern Sweden. First-ever stroke individuals were compared in a nested case-control design to age matched participants remaining free of cardiovascular disease, for methodology see (19, 20).

Isolation of Genomic DNA

Genomic DNA was prepared from whole blood using the Nucleon DNA extraction kit, Amersham Pharmacia.

PCR and Restriction Analysis

Human genomic DNA (0.05 μg) was amplified with the following primers: upper, 5′-GCCTGGTGGTGGGTTCGAGCC-3′; lower, 5′-GACAGCAGCACTGCAACTGCC-3′.

Amplifications were all carried out with 0.15 mM dNTP, 20 pmol of respective primer and 0.5 U *Taq* Polymerase (SIGMA) in the supplied buffer. 27 cycles were used, each involving denaturation at 94°C for 45 s, annealing at 57°C for 45 s, and extension at 72°C for 1 min. The amplifications included 10% DMSO. The amplified PCR-product was cleaved with the restriction enzyme *Dde*I (New England BioLabs, Beverly, MA) in the supplied buffer and analyzed in 1% agarose gels.

TABLE 1

Glutathione Peroxidase 1 Genotype Frequencies in a Finnish/Swedish Population and Corresponding Erythrocyte Activity Levels

Genotype	GPX activity (μkat/g of Hb)	*n* (%)
Pro/Pro	1.28 ± 0.23	23 (35)
Pro/Leu	1.29 ± 0.23	32 (48)
Leu/Leu	1.28 ± 0.33	11 (17)

Statistics

Odds ratios (OR) were calculated as estimates of relative risks for the development of stroke in the MONICA/CASTRO study. The influence of age was adjusted for by using logistic regression when calculating ORs with 95% confidence intervals.

RESULTS AND DISCUSSION

We have previously identified, by a bioinformatic approach (21), a polymorphism in the human GPX1 gene where a C/T variation results in either a Pro or Leu at amino acid position 197. This polymorphism was recently confirmed by others (22) and had also been noted in a loss of heterozygosity study (23). In our previous study, DNA from 25 individuals showed 13 homozygotes for Pro, 3 homozygotes for Leu, and 9 heterozygotes. Here we have extended these studies to 315 Swedish stroke case/controls and 66 Finnish/Swedish individuals with known erythrocyte GPX activity. As shown in Tables 1 and 2 the allele frequency of the more common Pro encoding variant is 73 and 59%, respectively, in these populations. The distribution of Pro, Leu homozygotes and heterozygotes are in Hardy–Weinberg equilibrium in both populations.

When measured in erythrocytes, the GPX enzyme activity from groups of donors representing the Pro/Pro, Leu/Leu and Pro/Leu genetic variants was not significantly altered (Table 1). In fact, activity levels are virtually identical. It is known that changes in erythrocyte activity does occur for

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TABLE 2

Glutathione Peroxidase 1 Genotype Frequencies in a Swedish Stroke Case/Control Population

Genotype	Cases (%) (*n*)	Controls (%) (*n*)
Pro/Pro	55 (56)	53 (113)
Pro/Leu	38 (38)	40 (85)
Leu/Leu	6.9 (7)	7.4 (16)
Odds ratio (95% CI) (Leu/Leu vs Pro/Leu + Pro/Pro)	0.92 (0.37–2.3)	

other polymorphic enzymes (e.g., Cu-Zn-SOD (16) and references therein) where enzyme stability is implied. Erythrocytes do not replenish proteins and are on average 60 days old. The normal GPX activity in erythrocytes from carriers of the rare allele suggests that the variant enzyme has a high stability and that normal activity also should be found in other cells of the body with faster turnover. This suggestion of course needs to be investigated separately using nucleated cells. It is also known that erythrocyte GPX activity can be upregulated under conditions of oxidative stress (24). Therefore, if one of the variants had impaired activity, a compensatory up-regulation cannot be excluded.

The Pro encoding allele is linked to a polymorphic site containing GCG triplet nucleotide repeats coding for either 5 or 7 alanines whereas the Leu encoding variant is linked to a 6 alanine encoding nucleotide repeat and base changes at −592 and +2 (23). The alanine stretch commences at position 7 after the N terminus. Our analysis indicates that these variants do not differ dramatically in terms of activity and stability.

As an independent measure of a possible contribution of GPX1 variation to disease where oxidative stress is implicated in the etiology, we also examined whether GPX1 genotypes were associated with stroke. As evident from our results, there was no difference between genotype frequencies, in stroke cases (Table 2). The odds ratio of Leu/Leu vs the combined Pro/Pro and Pro/Leu genotypes indicated no risk (OR = 0.92), the 95% confidence interval was (0.37–2.3) (Table 2).

In conclusion, this is the first study showing that the Pro and Leu variants of human GPX1 do not differ in activity and stability. At least the erythrocyte GPX capacity clearly does not vary with genotype. Also, this genetic variation is not significantly associated with an increased risk for stroke.

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