

Proline-leucine polymorphism of human glutathione peroxidase 1 in Thalassemia major

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ABSTRACT

Thalassemia is associated with low antioxidant enzyme deficiency especially glutathione peroxidase. GPX exists in 6 isomeric forms out of which GPX1 Single Nucleotide Polymorphism is found to be associated with Thalassemia major. In our study, the determination of the allelic frequency and phenotype of a common polymorphism in Se-dependent glutathione peroxidase 1 (GPX1) was observed in Thalassemic populations. A proline/leucine variant occurs at position 197 close to the C-terminus of the protein. The genotypes encoding Pro/Pro, Pro/Leu, and Leu/Leu are distributed according to the Hardy-Weinberg relationship. The study has been carried out in 40 Thalassemic cases and 40 control subjects. No significant association between allele frequency and risk to get fatal was evident. Erythrocyte GPX activity was determined 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 or to be associated with significant pathology.

Keywords: GPX1; Thalassemia; SNP

1. 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 the animals become more sensitive to oxidative stress [2]. Oxidative stress often has been implicated in Thalassemia [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 intra individual variation in antioxidant capacity is well documented and influenced by nutritional status [10-12], other measurements of antioxidant capacity involving determination of genetic profile are 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]. We observed the genotyping of 30 subjects and comparison to the corresponding blood glutathione peroxidase activity levels and 80 subjects where 40 had suffered from Thalassemia.

2. MATERIALS AND METHODS

2.1. Assay of Glutathione Peroxidase Activity

With informed consent from patient 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 Gajra Raja Medical College Gwalior [16]. First ever thalassemic individuals were compared in a nested case-control design to age matched participants remaining free of oxidative stress.

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2.2. Isolation of Genomic DNA

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

2.3. PCR and Restriction Analysis

Human genomic DNA (0.05 mg) was amplified with the following primers: Forward, 5'-GCCTGGTGGTGGGTTCGAGCC-3'; Reverse, 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 sec annealing at 57°C for 45 sec, and extension at 72°C for 1 min. The amplifications included 10% DMSO. The amplified PCR-product was cleaved with the restriction enzyme DdeI (New England BioLabs, Beverly, MA) in the supplied buffer and analyzed in 1% agarose gels.

3. RESULTS

Above table shows GPX enzyme activity from groups of donors representing the frequency of Pro/Pro, Leu/Leu and Pro/Leu genetic variants.

3.1. Statistics

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

Above table shows the allele frequency of the more common Homozygous Pro encoding variant as compared to Heterozygous Pro/Leu and Homozygous Leu among Thalassemic cases and controls along with the odds ratio of allelic frequency.

2. DISCUSSION AND CONCLUSION

We have previously identified, by a bioinformatics 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]. Here in the study, we observed allelic frequency in 80 Thalassemic cases/controls 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 distributions 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, ac-

tivity levels are virtually identical. It is known that changes in erythrocyte activity do occur for other polymorphic enzymes (e.g., Cu-Zn-SOD) [16] 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 2592 and 12 [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 Thalassemia. As evident from our results, there was no difference between genotype frequencies, in Thalassemia 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

Table 1. Glutathione peroxidase 1 genotype frequencies in Thalassemia patient and corresponding GPX1 levels in erythrocytes.

Genotype	n (%)	GPX activity (Units/g of Hb)
Pro/Pro (n = 35)	23	1.28 - 0.23
Pro/Leu (n = 48)	32	1.29 - 0.23
Leu/Leu (n = 17)	11	1.28 - 0.33

Table 2. Glutathione peroxidase 1 genotype frequencies in Thalassemic 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)	

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 Thalassemia.

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