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 nutriational 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.
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'-GCCTGGTGGGTGGTGTCGAGCC-3'; Reverse, 5'-GACAGCAGACACTGCAACTGCC-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 and controls along with the odds ratio of allelic frequency.

Table 1.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>n (%)</th>
<th>GPX activity (Units/g of Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro/Pro (n = 35)</td>
<td>23</td>
<td>1.28 - 0.23</td>
</tr>
<tr>
<td>Pro/Leu (n = 48)</td>
<td>32</td>
<td>1.29 - 0.23</td>
</tr>
<tr>
<td>Leu/Leu (n = 17)</td>
<td>11</td>
<td>1.28 - 0.33</td>
</tr>
</tbody>
</table>

Table 2.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (%) (n)</th>
<th>Controls (%) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro/Pro</td>
<td>55 (56)</td>
<td>53 (113)</td>
</tr>
<tr>
<td>Pro/Leu</td>
<td>38 (38)</td>
<td>40 (85)</td>
</tr>
<tr>
<td>Leu/Leu</td>
<td>6.9 (7)</td>
<td>7.4 (16)</td>
</tr>
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</table>

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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