Author's response to reviews

Title: Polymorphisms of superoxide dismutases and catalase and diabetes mellitus

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Author's response to reviews: see over
The authors need to be commended for the excellent effort in attempting to improve the paper.

Having said to, there are still a few important concerns:

1. In respond to point 5 of issue previously raised, the author wrote: Higher activities were found in AA than in CC genotypes of diabetic patients (Tab. 3). In the study of association between SOD activity and genotypes diabetic and healthy subjects have been pooled together as one group to improve statistical power of analysis. No statistics (e.g. ANOVA P value for trend) was described in either results section nor table 3.

.....Statistical analysis (analysis of variance) showed significant trend towards possible association of AA genotype with higher activity (P (trend) = 0.029). Diabetic and healthy subjects have been pooled together as one group in the study of association between SOD activity and genotypes to improve statistical power of analysis.......

2. Table 4
Column 2: the genotype frequency adds up to only 99%. Columns 3 & 4 numeric figures are identical (unlikely, please check).

Table 4 Genotype frequencies in CAT gene according to presence of vascular complications and values of glycated haemoglobin according to the genotype.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MA+</th>
<th>MI+</th>
<th>MA-MI-</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>0,28</td>
<td>0,32</td>
<td>0,34</td>
<td>6,28±1,25</td>
</tr>
<tr>
<td>AT</td>
<td>0,50</td>
<td>0,49</td>
<td>0,49</td>
<td>6,35±1,10</td>
</tr>
<tr>
<td>TT</td>
<td>0,22</td>
<td>0,19</td>
<td>0,17</td>
<td>6,32±1,22</td>
</tr>
</tbody>
</table>

Genotype frequencies of CAT gene according to the presence of vascular complications in patients with diabetes mellitus. MA+ means presence of macroangiopathy, MI+ means presence of microangiopathy, MA-MI- group involves patients without vascular complications. HbA1c is glycated haemoglobin (%) marked as mean±SD.

Given the RFLP fragment size (e.g. CAT -21A/T, A allele 203 & 47 bp), it is highly likely that the smaller fragment may not be clearly observed in agarose gel accompanied by molecular markers designed for bigger fragments. The authors are encouraged to provide some details on the RFLP assay procedure (e.g. gel concentration, size of molecular marker) to give the reader an idea of the accuracy of genotyping using RFLP.

...... 3% agarose gel including 0,5 µg/ml ethidium bromide, 10 µl of molecular markers (two different types used simultaneously) and 20 µl of amplicon for the other wells were applied for electrophoresis. 0,5xTBE buffer (pH 8) including 0,5 µl/ml ethidium bromide was used. Running conditions were 100 V, 40 mA and 140 min. Informations about all SNPs and SNP ID were obtained from the NCBI homepage......

4. Hardy-Weinberg equilibrium
Usually only need to be calculated for controls as one of the means to estimate likelihood of genotyping error.

...... Testing for deviation from Hardy-Weinberg equilibrium (HWE) was performed and all the observed genotype frequencies were in agreement with HWE......