Author’s response to reviews

Title: Polymorphisms of superoxid dismutases and catalase and diabetes mellitus

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Author’s response to reviews: see over
Reviewer's report

Title: Polymorphisms of superoxid dismutases and catalase and diabetes mellitus

Version: 2  Date: 30 October 2007

Reviewer: Dimitry Chistiakov

Reviewer's report:

General

In their report, Flekac and coauthors studied association between polymorphisms within the CAT, Mn-SOD and CuZn-SOD genes and two commonest types of diabetes (type 1 and type 2). They also reported a correlation between functional polymorphisms in SOD1 and SOD2 genes and serum SOD activity. The authors showed a clear association between the SOD polymorphisms and the development of both micro- and macroangiopathy in diabetes. The authors well designed and performed this study. The paper is relatively well written. The results are interesting and adequate. Conclusions are clear. Finally, the authors showed an important role of oxidative stress in the pathogenesis of diabetic vascular complications as well a significant contribution of genetic varuations within the antioxidant enzyme genes to conferring susceptibility to diabetic angiopathy.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

In discussion, the authors should referer to the following articles.

.... Chistiakov et al. (Diabetes Metab Res Rev. 2004 20(3):219-24) found a new type 1 diabetes susceptibility locus near the CAT gene in a Russian population that have not been strongly confirmed by Pask et al (Diabetes Metab Res Rev. 2006 22(5):356-60) in the UK and US population datasets. For the SOD polymorphisms, the authors are encouraged to discuss papers by Ukkola et al. (J Intern Med. 2001 249(5):451-9), Nomiyama et al. (J Hum Genet. 2003;48(3):138-41), Lee et al. (Metabolism. 2006 55(1):1-7), Lee & Choi (Metabolism. 2006 55(12):1681-8) and Nemoto et al. (Cardiovasc Diabetol. 2007 6:23). This should significantly enrich and improve the discussion.

Our findings in SOD2 gene are in agreement with previous observation of other authors [24]. We also confirmed known fact that serum SOD activity is significantly reduced in diabetic patients [25]. The presence of TT (Val/Val) genotype in SOD2 gene was associated with poorer diabetes control than CT (Ala/Val) and CC (Ala/Ala) genotypes. Macroangiopathy was associated with significantly lower frequency of C (Ala) allele of Ala16Val SNP of SOD2 gene. This has not been confirmed by another study focused on the role of antioxidative enzymes (including SOD2) in determining genetic susceptibility to macroangiopathy (coronary artery disease) in T2DM [26]. Other studies suggest that Ala16Val SNP of SOD2 gene is not related to pathogenesis of diabetes but is associated with microangiopathy expressed as albuminuria [27] or macular edema in T2DM [28]. No such distrubution was found in
microangiopathy in our study. Finally, we found negative correlation between SOD activity in both types of DM and level.....

...... SNP in the signal sequence of SOD2 (Ala16Val) appears to be a minor determinant of carotid atherosclerosis [19]. The Ala type of SOD2 might have an common alfa-helical structure, while the Val type might change its conformation to beta-sheet [29]. The Val variant of the SOD2 might be present at a lower concentration in mitochondria.

...... catalase promoter have been identified in a Swedish population [33], but their relationship to vascular disease risk has not been determined. A variant within the catalase promoter region has been associated with essential hypertension in an isolated Chinese population [34]. Susceptibility locus on chromosome 11p13 near to the catalase gene supports that CAT gene may play a role in DM [35]. On the other side another authors found no evidence for a major effect of CAT SNPs on T1DM susceptibility in two large sample collections [36]. The study inconsistency in the association between.........


Reviewer's report

Title: Polymorphisms of superoxid dismutases and catalase and diabetes mellitus

Version: 2 Date: 26 November 2007

Reviewer: Su-Chi Lim

Reviewer's report:

General

The authors need to be applauded for attempting to investigate the relationship between three promising candidate SNPs involved in oxidative stress and diabetes.

1. Is the question posed by the authors new and well defined?
   Well defined but probably not new.

2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work?
   Satisfactory. Some deficiencies (described below)

3. Are the data sound and well controlled?
   Some major concerns (details below)

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
   Adequate

5. Are the discussion and conclusions well balanced and adequately supported by the data?
   Some major deficiencies (described below)

6. Do the title and abstract accurately convey what has been found?
   Adequate

7. Is the writing acceptable?
   Satisfactory.

1. The paper is too long.
   It has been shortened, especially in background and discussion. On the other hand some parts have been extended due to other requirements of referees.

2. Description of study subjects (i.e. phenotype) needs to be improved.
Diagnosis of T1 and T2DM was based on WHO/ADA definition of diabetes (1999), healthy subject didn’t fulfill the criteria for this diagnosis. They were in good health and namely free of any co-morbidities often associated with diabetes, especially with T2DM (hypertension, obesity, hyperlipoproteinemia) and other endocrine disorders.

3. Therefore, the conclusions drawn have to be a lot more tentative and the authors are encouraged to discuss this as part of the limitation of the study.

Genotype distributions of the SOD1 and SOD2 in patients with both types of diabetes mellitus may be different from nondiabetic individuals. We are conscious of limitation of this study with relatively small sample size in comparison with wide epidemiological studies, especially by providing subgroup analysis within the group with diabetes mellitus. But results of these types of small studies with similar conclusions may spark off subsequent research. Genetic background may be at least partly associated with diabetes control.

4. Did the authors attempt statistical adjustment for the difference in age?

No gender or age influence on its activity was found in diabetic or healthy subjects. Difference in SOD activity between diabetic and healthy subjects is probably accountable not only by genotype background but also by various effects in terms of diabetes, e.g. enzyme glycation. The lower serum SOD.

5. In addition, it appears somewhat surprising that the authors reported a positive association between SOD activity and SOD1 & 2 genotypes. The numeric figures in table 3 appear non-convincing and unlikely to be statistically significant (within the stratum of T1DM, T2DM or healthy), especially given the small sample size (e.g. only 5 T1DM subjects with SOD2 CC genotype).

was related to SOD serum activity. 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. Differences between these subjects in age, duration of diabetes, presence of other co-morbidities were included.

6. Numeric figures in table 4 are also difficult to understand. Within the group MA+, the genotype frequency adds-up to be greater than 100% (0.32+0.54+0.28=1.04). Similarly, MI+ genotype distribution adds-up to be 113%. These seem improbable.
Table 4  Genotype frequencies in CAT gene according to presence of vascular complications and values of glycated haemoglobin according to genotype in CAT gene.

<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>0,29</td>
<td>0,32</td>
<td>0,32</td>
<td>6,28±1,25</td>
</tr>
<tr>
<td>AT</td>
<td>0,49</td>
<td>0,49</td>
<td>0,49</td>
<td>6,35±1,10</td>
</tr>
<tr>
<td>TT</td>
<td>0,21</td>
<td>0,19</td>
<td>0,19</td>
<td>6,32±1,22</td>
</tr>
</tbody>
</table>

1. Genotyping methods

Information about all SNPs, SNP ID was obtained from the NCBI homepage and all SNPS have been validated by multiple, independent submissions to the refSNP cluster. The genotyping success rate was 95.0% (range 91.1 to 98.4%). Water control, internal controls and previously genotyped samples were included in each plate to ensure accuracy of genotyping. Positive and negative controls were used in each genotyping assay. To ensure quality control, the genotyping analysis was performed "blind" with respect to case/control status. About 10% of the samples were randomly selected to be genotyped again by a different investigator, who was also unaware of the patients’ status. The results were concordant. The polymorphisms were also examined by PCR and RFLP analysis described previously [21,22,23].

<table>
<thead>
<tr>
<th>SNP</th>
<th>sequence of used primers</th>
<th>restriction endonuclease</th>
<th>restriction fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD1</td>
<td>5´CTATCCAGAAAACACGGTG GCCC 3´</td>
<td>Hhal</td>
<td>C allele 71bp and 207 bp</td>
</tr>
<tr>
<td></td>
<td>5´CTATATTTCAATCAAATGCT ACAAAAC3´</td>
<td></td>
<td>A allele 278 bp</td>
</tr>
<tr>
<td>SOD2 A16V (C/T)</td>
<td>5´GCTGTGCTTTTCTGCTTCA G 3´</td>
<td>BsaWI</td>
<td>C allele 267 bp</td>
</tr>
<tr>
<td></td>
<td>5´TGTTACTTCTCTCCTGACGAC 3´</td>
<td></td>
<td>T allele 183bp and 84bp</td>
</tr>
<tr>
<td>CAT</td>
<td>5´-AATCAGAAGGACGTCTCC-3´</td>
<td>HinfI</td>
<td>A allele 203 bp and 47bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T allele 250bp</td>
</tr>
</tbody>
</table>

2. Consistency of genotype distribution with Hardy-Weinberg Equilibrium among controls. For negative observation e.g. CAT SNP, the estimated power of the
Testing for deviation from Hardy-Weinberg equilibrium was performed by using genetic statistics, separately in cases and controls.

Differences between T1 and T2 in SOD activity were not found statistically significant (p=0.14).

Differences between frequencies in alleles of CAT SNP between DM patients and healthy subjects (p=0.294).

No effect of SNP in SOD1 gene on diabetes control was found. Glycated haemoglobin was in AA genotype of SOD1, p=0.124.

Similar findings were made in CAT gene, glycated haemoglobin in AA genotype of CAT, p=0.249.

When compared AA genotype vs. AT and TT genotypes of CAT, OR was 1.05; 95%CI 0.78-1.13, p=0.851.

Macroangiopathy was associated whereas no such distribution was found in CAT gene, p=0.594.

No statistically significant distribution in allele frequencies was found in SNPs of all studied genes in microangiopathy (C allele in SOD1 was 0.47 in MI group vs. 0.42 in DM group without complications with p=0.118, T allele in SOD2 was 0.35 in MI group vs. 0.39 in DM group without complications with p=0.242).

3. Typographical error-

level, type of diabetes, duration of diabetes, SOD activity and genotype. P values....