Reviewer's report

Title: Common Genetic Variants of the Ion Channel Transient Receptor Potential Membrane Melastatin 6 and 7 (TRPM6 and TRPM7), Magnesium Intake, and Risk of Type 2 Diabetes in Women

Version: 1 Date: 14 July 2008

Reviewer: Karl Schlingmann

Reviewer's report:

Song and co-workers report on genetic variants in two genes coding for subunits of magnesium permeable ion channels (TRPM6 and TRPM7) and their association with the risk of developing diabetes mellitus type II.

The presented work comprises molecular genetic analyses of polymorphisms in two genes involved in magnesium metabolism in participants of the Women’s Health Study. The study has a case-control design with approximately 350 incident diabetes cases and the same number of healthy controls. In these ~700 probands, 25 haplotype-tagging single nucleotide polymorphisms (SNPs) within the TRPM6 and TRPM7 genes are genotyped and analyzed for a potential association with type II diabetes.

The main conclusion of Song and co-workers is that they were unable to identify a significant association of any of the 25 investigated SNPs with the risk of developing diabetes. Therefore, the authors proceeded with a subgroup analysis by first applying a sliding window haplotype analysis (looking at haplotypes of two adjacent SNPs) and second dividing the probands groups by their daily magnesium intake (comparing the lowest quintile with the four other quintiles). By this strategy, the authors were able to identify a haplotype of two coding SNPs within the TRPM6 gene (V1393I and K1584E; the only two non-synonymous coding SNPs with significant minor allele frequencies within the TRPM6 gene) that showed a weak but significant association with diabetes in the subgroup of participants with low magnesium intake.

Major revisions:

Some important aspects of the authors’ conclusions concerning the described genetic association remain unclear to the reader: As stated in the article’s text, the authors conclude that homozygous carriers of the minor-allele haplotype (1393Ile+1584Glu) had an increased risk of type 2 diabetes when they had a low magnesium intake.

However, in my opinion, it remains unclear from table 3 if the authors really compared homozygous carriers of the minor allele with the remaining genotyped individuals (homozygous major allele + heterozygotes). Table 3 only lists numbers of individuals homozygous for the major allele versus heterozygotes and homozygotes of the minor allele together (i.e. GG vs. GA/AA for V1393I, AA vs. AG/GG for K1583E). For the haplotype combining both polymorphisms only
allele frequencies are presented (i.e. 82.4%+7.72% vs. 9.86% for patients). The reader cannot necessarily imply the number of homozygotes from these allele frequencies of haplotypes. Therefore, the absolute numbers of carriers of the minor allele haplotype are essential and should be presented.

Furthermore, assuming Hardy-Weinberg-Equilibrium, the percentage of homozygotes of the minor allele haplotype (1393Ile+1584Glu) is low (probably less than two percent). Therefore, the patients` and control groups could hardly bear more than half a dozen subjects with homozygocity for the minor allele making reliable chi square calculations a challenge. In addition, a subgroup analysis of women within the lowest quintile of magnesium intake further significantly decreases the number of individuals per subgroup (absolute number of women within the lowest quintile for both groups not given, genotype data for this subgroup not provided). Again, percentages of allele frequencies do not reflect the absolute size of the subgroups tested.

In my opinion, the reader should be able to perform chi square calculations from the numbers presented in table 3. As stated in the article`s text, the authors performed calculations under the assumption of an additive, recessive, and dominant genetic model. The results section (page 9-10) as well as table 3 only show comparisons of the major allele (wild type) with heterozygotes plus homozygotes for the minor allele (dominant model).

Minor revisions:
page 2 “the TRPM6 gene comprises 43 exons spanning 163kb ...”
according to GenBank and Ensembl the TRPM6 gene comprises 39 exons spanning 163kb
page 2 “the TRPM6 gene” - please mark gene symbols in italics
page 2 “TRPM6 is a magnesium-permeable channel protein” – TRPM6 is thought to be an ion channel subunit, if it forms homomeric channels or heteromeric channels with TRPM7 is still an unresolved issue
page 2 “TRPM7 ... encodes a protein of 1863 aa” – the TRPM7 protein has 1865 aa
page 6 “... to capture the common genetic variability across 167kb of the TRPM6 and 128kb of the TRPM7 gene, covering their corresponding 30kb 5´ upstream and 30kb 3´ downstream regions.” – did the SNPs cover 167/128kb or 167/128kb plus 60kb?
page 8 “the cutpoints for magnesium levels were prespecified by those for quintiles among controls” – what was the distribution of DMII patients in the different quintiles?
page 9 “---based on these 20 SNPs in TRPM6 ...” – did you genotype 30 or 20 SNPs in TRPM6 (compare page 6)?
Page 9-10 within the text and in Table 3 you use different nomenclatures for alleles and haplotypes of the two TRPM6 polymorphisms (major allele C-T and/or A-G).

Page 11 SIFT scores in Table 4 are all <0.05 indicating “intolerant” behaviour.

Page 11 “Given the large sizes of both genes, it seems plausible, ...” what impact has the size of the two genes?

Page 12 “There are several untranslated exons in proximity to the promotor region of TRPM6 ...” – is that true?

Page 13 “Given the limited number of SNPs across TRPM7 ...” – this argument is in contrast to citation 37 which you cite on page 12 - tSNPs should be provided by the HapMap project should be sufficient to detect common haplotypes.

Page 14 “we lacked stable and reliable measures of extracellular or intracellular magnesium status ...” – is there information on serum magnesium values or urinary excretion?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I do hold a patent relating to the content of the manuscript with the following title:

(WO/2006/128726) METHOD FOR DIAGNOSING A DEFECTIVE MAGNESIUM CHANNEL