Author's response to reviews

Title: Association between variations in the TLR4 gene and incident type 2 diabetes is modified by the ratio of total cholesterol to HDL-cholesterol

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Author's response to reviews: see over
Response to the comments of referee #1

We would like to thank the reviewer for the favorable summary and the very helpful comments.

*General:* This is an interesting hypothesis generating exercise, which has explored the effect of haplotypic structure of TLR4 on type 2 diabetes incidence, with a view to an interaction with TC/HDL ratio. The authors report that the interaction term works between different SNPs/haplotypes in men and women.

Indeed many apparently significant observations are reported, far greater than would be expected by chance (as confirmed by their Bonferroni correction). However the picture is really muddled particularly by the gender issue. At no point in the manuscript is the idea of testing for a three way interaction between gender/genotype/ and TC/HDL. This would probably be the best way forward in a regression model with the different haplotypes. It is clear that there are similarities in the observations between men and women as well as dissimilarities in this analysis, and that many of the comparisons rely on incredibly small sample groups, which will provide very volatile results. It may be that the gender issue is a red herring.

*I would prefer to see the whole analysis run on the joint gender group, with gender in as an interacting factor and as a covariate. I also think that performing a step wise modelling of combinations of the variants in regression models may help refine the genetic model.*

The reviewer proposed repeating the whole analysis for men and women combined, which we considered a very valuable suggestion. We performed such analysis in the joint gender group including sex as covariate and two and three way interaction terms between sex, TC/HDL-C and genetic variants in the “interaction effect model” to assess whether the effect of TLR4 genotypes and haplotypes on the risk of incident type 2 diabetes was modified by sex and TC/HDL-C. The three way interaction term was significant for two out of seven SNPs and one of the haplotypes after correction for multiple testing (p<0.01), demonstrating that the relation of TLR variants and diabetes is substantially modified by sex and the level of TC/HDL-C.

The interaction terms of sex*TC/HDL-C were significant in all SNP and haplotype models with p-values < 0.016. Additionally, several studies (e.g. Meisinger et al., ref. 22) revealed substantial sex-specific differences of the effect of risk factors on type 2 diabetes. Therefore,
after careful discussions concerning presentation and interpretation of our results, we decided to keep the analysis separately for men and women, but additionally provide the results of the joint analysis as supplemental material (Additional file 1). We believe that the stratified analysis will be easier to interpret by the casual reader. We have also included the following section in the revised manuscript (see page 14, paragraph 2, lines 1-7):” In order to investigate the role of sex-specific differences and to verify that our findings are not artefacts resulting from stratified analysis, we also estimated models including three-way interaction terms between sex, TC/HDL-C, and the genetic variants. These additional analyses gave evidence not only for the presence of sex-specific differences of the influence of TC/HDL-C but also for modification of genetic effects by sex, TC/HDL-C as well as sex-specific TC/HDL-C. For detailed results of the three-way interaction haplotype model, please see additional file 1.”

We also considered modelling epistatic effects between SNPs through main effect and interaction models. The high LD between the analysed markers, however, allows us to replace this step through haplotype estimation and modelling, which we considered an elegant solution. This way, we circumvent the problem of high dimensional models with the danger of detection of artefacts due to overfitting.
Response to the comments of referee #2

We thank the reviewer for his/her favorable comments. We were very pleased to accommodate the suggestions.

**General:** This is an interesting study which prospectively investigates the association between genetic variants in the gene coding for the TLR4 receptor and type 2 diabetes. The authors report that minor alleles of several TLR4 variants might increase the risk for type 2 diabetes in male subjects with high TC/HDL-C level. However, no effect was seen in women and none of the investigated SNPs or haplotypes was associated with type 2 diabetes or TC/HDL-C alone.

The paper is original, the question analysed – of high interest. Data is reported for the first time. The study design is appropriate. Materials and methods are carefully described. Style is good. Conclusions drawn are justified by the methods used.

**Minor critics:**

1. Type 2 diabetes should be given as “type 2”, t.i. without a capital letter T.

   We have changed “Type 2 diabetes” to “type 2 diabetes” throughout the whole manuscript.

2. Correct is “atherosclerosis” or “arteriosclerosis”, but not “arteriosclerosis” (page 4).

   This typing error was changed accordingly (page 5, paragraph 3, line 5).

3. Page 6, first line from the bottom: “survey” is not understandable. This should be explained.

   To clarify, we changed the wording to “As part of the international WHO MONICA project, three independent cross-sectional population-based studies (surveys) covering the city of Augsburg (Germany) and two adjacent counties were conducted in 1984/85 (S1), 1989/90 (S2) and 1994/95 (S3) to estimate the prevalence and distribution of cardiovascular risk factors among individuals aged 25 to 64 (S1) or 25 to 74 years (S2, S3)” (page 7, paragraph 1, lines 3-8).

4. Table 1 could be improved with respect to presenting lifestyle. The last variable analysed – “survey” should be explained again in the legend of the table.

   We appreciate these remarks and have included a footnote explaining survey (page 24). Regarding lifestyle, we have presented the variables for smoking, alcohol consumption and frequency of exercise in the same way as they were analysed in the regression model.