Author's response to reviews

Title: Microsomal triglyceride transfer protein -164 T>C gene polymorphism and risk of cardiovascular disease: results from the EPIC-Potsdam case-cohort study.

Authors:

Romina di Giuseppe (romina.digiuseppe@dife.de)
Sonali Pechlivanis (Sonali.Pechlivanis@uk-essen.de)
Eva Fisher (FisherE@rki.de)
Maria Arregui (Maria.Arregui@dife.de)
Beate Weikert (beaweikert@gmx.de)
Sven Knüppel (sven.knueppel@dife.de)
Brian Buijsse (Brian.Buijsse@dife.de)
Andreas Fritsche (Andreas.Fritsche@med.uni-tuebingen.de)
Stefan N Willich (stefan.willich@charite.de)
Hans-Georg Joost (joost@dife.de)
Heiner Boeing (boeing@dife.de)
Susanne Moebus (Susanne.Moebus@uk-essen.de)
Cornelia Weikert (weikert@dife.de)

Version: 2 Date: 23 January 2013

Author's response to reviews: see over
Nuthetal, 23 January 2013

To the Editor of BMC Medical Genetics

Dear Editor,

Thank you very much for your consideration of our manuscript “Microsomal triglyceride transfer protein -164 T>C gene polymorphism and risk of cardiovascular disease: results from the EPIC-Potsdam case-cohort study” (track number 7357587137840582) by di Giuseppe R. et al., and request for a revised version.

According to the suggestions of reviewers we have made the changes to our manuscript that in our view has improved and benefited. A point by point description of every single change we made has been reported in the rebuttal letter, submitted as a new manuscript file.

The current version of the manuscript has been read and approved by all qualified authors who have examined both the text and illustrations in detail.

The manuscript is original work that has not been published previously and is not under consideration elsewhere. There are not actual or potential conflicts of interest in relation to this manuscript.

We hope that in this new form our manuscript will be considered for publication in BMC Medical Genetics

Thank you in advance for your attention.

Yours sincerely,

Romina di Giuseppe, on behalf of all co-authors

Corresponding author: Romina di Giuseppe, PhD.
Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbrücke, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany.
+49 (0)33200 88 2714. Fax: +49 (0)33200 88 2721. E-mail: romina.digiuseppe@dife.de
We thank the Referees for their efforts in reviewing our manuscript. According to their suggestions we have made the changes to our manuscript MS: 7357587137840582.

1) Reviewer's report

**Title:** Microsomal triglyceride transfer protein -164 T>C gene polymorphism and risk of cardiovascular disease: results from the EPIC-Potsdam case-cohort study.

**Version:** 1  **Date:** 28 November 2012

**Reviewer:** Catherine Phillips

**Reviewer's report:**

di Giuseppe et al., investigated whether the -164T>C polymorphism in MTTP alters the risk of CVD, depending on the cholesterol levels, in a case-cohort study. While this SNP was not associated with CVD risk, they report interaction between genotype and cholesterol whereby increased CVD risk was observed among individuals with low cholesterol levels, which seemed to be abolished among risk allele carriers with high cholesterol levels. An independent cohort was used to verify findings in the original cohort. Despite OR in the same direction, no significant associations were replicated in the independent cohort, probably due to limited number of CVD cases. The methods are appropriate, data is sound and study limitations have been adequately described.

**Minor Essential Revisions**

1. Before the “statistical analysis” section there is a sentence “(I wrote to Susanne Moebus, and I’m waiting for a reply), which should be removed.

   *We apologize for this inaccuracy. The sentence has now been removed.*

2. Genetic analyses – please clarify whether this was the only SNP determined or whether additional genotyping was performed.

   *This SNP was not the only SNP genotyped. This SNP is part of GWAS platforms and was genotyped using Illumina Hap300, Illumina Hap550, Illumina Human660W-Quad and Illumina HumanOmni1-Quad.*
3. Please correct spelling of Qiagen

*Page 8, Line 184: Qiagen has now been corrected.*

4. and provide genotyping platforms used in replication cohort (4 platforms are mentioned)

*As already mentioned above this SNP, as part of GWAS platforms, was genotyped using 4 different platforms. Page 9, Lines: 191-194: “The MTTP SNP –I128T (rs3816873) was genotyped by Illumina Hap300, Illumina Hap550, Illumina Human660W-Quad and Illumina HumanOmni1-Quad”.*

5. also please provide genotyping metrics for the replication cohort (call rate, accuracy etc)

*Page 9: Lines 193-194: “The call rate for this SNP was 99.9%”.

The genotype distribution of the –I128T (rs3816873) SNP followed the HWE (P=0.71) (Page 11, Lines: 242-243) and the minor allele frequency (C allele) was 0.26 (Page 11, Line: 246).*

6. Although the 2 SNPs which have been examined are in LD, why was the same SNP not determined in the discovery and validation cohorts?

*In the replication cohort the MTTP –I128T SNP (rs3816873) was already measured on a random selected sample of 1 513 HNR participants out of 4 814. In the revised manuscript we have now better explained this concept: Page 7; Lines 134-138: “The genotyping of the MTTP SNP –I128T (rs3816873) was already available on a random selected sample of n=1 513 Heinz Nixdorf Recall participants out of 4 814. After exclusion of participants with a history of CVD and/or diabetes at baseline and/or treated with anti-hyperlipidemic drugs, the final replication cohorts consisted of n=1 218 individuals (30 CVD and 1 188 non-cases).*

Given the relationship between MTTP and triglycerides, was their interaction with respect to CVD analysed?
Yes, we already analyzed the interaction between MTTP and triglycerides in relation to CVD and found non-significant results. In the revised manuscript we have now reported also this finding. Page 13, Lines 290-293: “We performed additional analyses to test both the multiplicative and additive interactions for triglycerides, HDL- and LDL-cholesterol concentrations. The interactions between MTTP/triglycerides and MTTP/HDL-cholesterol in relation to CVD were not significant (P=0.18 and P=0.11, respectively)...

7. Similarly given the link between MTTP and SREBPs, and the relationship between SREBPs and HDL and LDL cholesterol, it would be worthwhile to examine HDL and LDL modulation of genetic risk to ascertain which particular cholesterol component is driving the observations?

This is an interesting question that unfortunately we cannot fully answer as we don’t have direct measurements of LDL-cholesterol concentrations, and therefore we had to use the Friedewald equation to estimate them. Nevertheless, based on your suggestions we reported these additional results in the revised manuscript.

Page 13, Lines 290-303: “We performed additional analyses to test both the multiplicative and additive interactions for triglycerides, HDL- and LDL-cholesterol concentrations. The interactions between MTTP/triglycerides and MTTP/HDL-cholesterol in relation to CVD were not significant (P=0.18 and P=0.11, respectively), whereas they were significant and in the same direction as those found for total cholesterol when LDL-cholesterol was analyzed (multiplicative interaction: P=0.023; SI_\text{additive} interaction = 0.33 95% CI (0.15-0.73) and RERI_\text{additive} interaction = -1.17 95% CI (-2.01—0.33). Stratified analysis according to the 2 LDL-cholesterol categories (<130 and \geq 130 mg/dL) showed an increased CVD (HR_\text{additive}: 1.24; 95% CI: 0.98 to 1.56; HR_{dominant}: 1.51; 95% CI: 1.09 to 2.08) and IS risk (HR_\text{additive}: 1.30; 95% CI: 0.96 to 1.75; HR_{dominant}: 1.66; 95% CI: 1.07 to 2.57) in the low LDL group when MTTP was considered in a dominant fashion. A decreased CVD (HR_\text{additive}: 0.80; 95% CI: 1.58 to 1.09; HR_{dominant}: 0.69; 95% CI: 0.47 to 1.00) and MI (HR_\text{additive}: 0.74; 95% CI: 0.51 to 1.07; HR_{dominant}: 0.62; 95% CI: 0.39 to 0.96) risk was observed, instead, in the high LDL group, always in a dominant fashion (data not shown)."

We furthermore added the following discussions: Page 14; Lines 317-321: “Similar relationships were observed considering LDL-cholesterol with levels lower and higher than 130 mg/dl suggesting that LDL is the driving cholesterol component. However, the value of
LDL levels seems to be limited as they had to be estimated based on the Friedewald formula [46]. In fact further studies are needed to replicate these findings.”

For your knowledge only we reported here the table showing the results stratified by LDL-cholesterol.

<table>
<thead>
<tr>
<th>LDL-cholesterol</th>
<th>CVD</th>
<th>MI</th>
<th>IS</th>
<th>CVD</th>
<th>MI</th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-cholesterol &lt; 130 mg/dL</td>
<td>CVD</td>
<td>MI</td>
<td>IS</td>
<td>CVD</td>
<td>MI</td>
<td>IS</td>
</tr>
<tr>
<td>C allele</td>
<td>1.24</td>
<td>1.18</td>
<td>1.30</td>
<td>0.80</td>
<td>0.74</td>
<td>0.93</td>
</tr>
<tr>
<td>P_additive</td>
<td>0.069</td>
<td>0.299</td>
<td>0.087</td>
<td>0.153</td>
<td>0.112</td>
<td>0.760</td>
</tr>
<tr>
<td>Dominant</td>
<td>1.51</td>
<td>1.38</td>
<td>1.66</td>
<td>0.69</td>
<td>0.62</td>
<td>0.85</td>
</tr>
<tr>
<td>P_dominant</td>
<td>0.013</td>
<td>0.154</td>
<td>0.023</td>
<td>0.051</td>
<td>0.033</td>
<td>0.587</td>
</tr>
<tr>
<td>Recessive</td>
<td>0.67</td>
<td>0.75</td>
<td>0.57</td>
<td>1.03</td>
<td>0.97</td>
<td>1.16</td>
</tr>
<tr>
<td>P_recessive</td>
<td>0.301</td>
<td>0.573</td>
<td>0.319</td>
<td>0.933</td>
<td>0.932</td>
<td>0.794</td>
</tr>
</tbody>
</table>

8. Results page 9, sentence “According to genotype no significant differences in common prevalent diseases and socio-demographic characteristics were observed in subjects with or without CVD (Table 1).” Would read better replacing “common prevalent diseases” with central obesity, obesity and hypertension.

Following the Referee’s suggestion the sentence has now been replaced: “According to genotype no significant differences in central obesity, obesity and hypertension and socio-demographic characteristics were observed in subjects with or without CVD (Table 1)” (page 11; Lines: 253-255).

9. Table 1 - Please clarify abdominal obesity in Table 1 – what criteria and cut-offs were used?

To define abdominal obesity we used the ATP III criteria (Reference [41]) based on the following waist circumference cut-off points: men ≥ 102 cm and women ≥ 88 cm. These cut-offs have now been specified in Table 1.

Page 26, Lines 621-623:
Abdominal obesity was defined according to the ATP III criteria [41] based on the following waist circumference cut-off points: men ≥ 102 cm and women ≥ 88 cm.

Obesity was defined as BMI ≥ 30 kg/m².

10. Also no methods for LDL or TG are provided in the methods section, please include.

On page 8 Lines 174-177 we reported that “…triglycerides were measured with an automatic analyzer (ADVIA 1650, Siemens Medical Solutions, Erlangen, Germany). LDL-cholesterol was calculated using Friedewald’s formula [40].”

11. Although already referenced the discussion in relation to MTP SNPs and circulating lipids would benefit from inclusion of the findings from Phillips et al., (ref 12). While this was a small study there is detailed fasting and postprandial lipoprotein composition data for high CVD risk subjects (T2DM) according to -493 G/T.

We have now included in the discussion the findings of this study, which indeed emphasize the concept we already expressed regarding the hypothesized differential lipid regulation of MTTP in the presence or absence of disease. Page 14, Lines 332-337: “Furthermore, Phillips et al. in a small study including 82 patients with type 2 diabetes mellitus (T2DM) of a Caucasian population found that the subjects heterozygous for the -493 G/T had lower LDL-cholesterol and, in the postprandial phase, higher apoB48 levels in the VLDL fraction. The authors suggested that the -493 G/T polymorphism seemed to confer protection against atherosclerosis in T2DM patients [12].”

12. Genotype frequencies were not different between CVD and CVD free subjects. This finding should be included in the abstract.

This finding has now been included in the abstract. Page 2, Line 42: “Genotype frequencies were not different between CVD and CVD free subjects (P=0.79).”

13. Following on from this the conclusions in the abstract and discussion that “the -164T>C polymorphism might have implications for the development of cardiovascular disease” need to be amended, as there is no genetic association but
rather an interaction between genotype and total cholesterol levels which predisposes risk allele carriers with low cholesterol levels to increased CVD risk, which seems to be abolished among risk allele carriers with high cholesterol levels.

We thank the referee for pointing this out. We followed her suggestion and changed our conclusions in the abstract and in the manuscript accordingly:

Abstract

Conclusions:

Pages 2-3, Lines 52-55: “Our study suggests an interaction between MTTP -164T>C functional polymorphism with total cholesterol levels. Thereby risk allele carriers with low cholesterol levels may be predisposed to an increased risk of developing CVD, which seems to be abolished among risk allele carriers with high cholesterol levels.”

Manuscript conclusions

Pages 16-17, Lines: 389-393: “The findings of this study suggest that in the subjects investigated an interaction between MTTP -164T>C functional polymorphism with total cholesterol levels predisposes risk allele carriers with low cholesterol levels to an increased risk of developing CVD, which seems to be abolished among risk allele carriers with high cholesterol levels. However, further studies are warranted in order to shed more light on these complex mechanisms.”

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: No competing interests to declare.
2) Reviewer's report

Title: Microsomal triglyceride transfer protein -164 T>C gene polymorphism and risk of cardiovascular disease: results from the EPIC-Potsdam case-cohort study.

Version: 1 Date: 30 November 2012

Reviewer: Kenji Okumura

Reviewer's report:

General Comments

The authors studied the association of microsomal triglyceride transfer protein (MTTP) -164 T>C gene polymorphism and risk of cardiovascular disease using the subjects of the EPIC-Postdam Study. This study is a case-cohort and prospective study. When the subjects were divided into two groups according to cholesterol levels, the rare allele, that is C allele, had a dominant effect on the occurrence of cardiovascular disease, in particular, ischemic stroke in the subjects with cholesterol levels of < 200mg/dL. They concluded that MTTP -164 T>C is a functional polymorphism regarding the development of cardiovascular disease.

Specific Comments

1. P 12, lines 1-2 “The association seemed to be stronger for IS than for MI, although the difference was not significant.”

According to Table 2, only ischemic stroke, not MI, is associated with MTTP -164 T/C polymorphism. What do you mean “the difference was not significant”.

We thank the referee for this question as we realized that what we wrote is perhaps not so clear. At this point we performed a competing risk analysis to compute the Wald test for assessing the difference in association between MTTP -164T>C and myocardial infarction (MI) versus MTTP -164T>C and ischemic stroke (IS). The competing risk analysis yielded a P value of 0.28 and 0.20, respectively, for the additive and the dominant model. This means that the positive relation between MTTP -164T>C and incident CVD, in individuals with cholesterol levels <200 mg/dL, seemed stronger for IS than for MI although the Wald test (i.e. the formal test used to check whether the association between MI and IS differed) did not reach the typical statistical significance level of 0.05 (as reported above Wald test P value=0.28 for the additive model).

In the revised manuscript we have now better explained this concept: Page 14, Lines 314-315: “The association seemed to be stronger for IS than for MI, but differences in the associations were not supported by competing risk analysis.”
2. The authors explained that the reason of the different effect of MTTP genotype is that MTTP gene expression and activation is linked to cholesterol levels, resulting in the accumulation of lipids in tissues in the C allele subjects with lower cholesterol. However, the reverse effect in the subjects with cholesterol levels of > 200 mg/dL could not be explained.

We fully agree with the Referee regarding this point consequently we continue to stress the importance of further investigations. Indeed, this represents a limitation of observational researches.

3. How did you determine the cutoff value of cholesterol at 200 mg/dL?

We used the ATP III criteria to determine the cut-off value of cholesterol (<200 mg/dL: desirable; 200-239 mg/dL: borderline high; ≥240 mg/dL: high) (Reference [41]). We, however, combined the borderline high/high categories (Page 9, Line: 206-209) in order to test the biological interaction —as we needed four disjoint categories (low cholesterol and non-gene, low cholesterol and gene, high cholesterol and non-gene, high-cholesterol and gene) (Reference 42: Andersson T, Alfredsson L, Källberg H, Zdravkovic S, Ahlbom A: Calculating measures of biological interaction. European Journal of Epidemiology 2005, 20:575-79). Furthermore, by combining the two above mentioned categories we also had enough subjects to perform stratified analysis.

4. Do the results from this study suggest that cholesterol levels should be increased in the subjects with the MTTP C allele?

No, absolutely not. Results from this observational study suggest an interaction between MTTP -164T>C functional polymorphism with total cholesterol levels. Thus risk allele carriers with low cholesterol levels may be predisposed to an increased risk of developing CVD, which seems to be abolished among risk allele carriers with high cholesterol level (albeit this reverse effect could not be explained, as highlighted above). Furthermore, these findings should also be “carefully” interpreted as those mimicking MTTP inhibitors, in particular in the light of the long term side effects MTTP inhibition may generate (as already
discussed on Page 16, Lines 374-377. We, however, continue to stress the importance of further studies in order to shed more light on these complex mechanisms.

**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 declare that I have no competing interests' below.

After statistical review, in the revised version of the manuscript we now also have reported that the concentrations of total, HDL-, LDL-cholesterol and triglycerides were multiplied by 1.1 to account for citrate’s dilution factor (as we didn’t consider it before). This has only slightly changed the numerical values of Hazard Ratios without changing or altering the meaning of the main findings.

**EDITORIAL REQUIREMENT**

Also, please make the following formatting changes during revision of your manuscript.

1) Please include all author details on the title pages, and rename the disclosure section as "Competing interests".

Done.