Reviewer’s report

Title: MTTP-297H Polymorphism Reduced Serum Cholesterol but Increased Risk of Non-Alcoholic Fatty Liver Disease

Version: 4  Date: 8 July 2015

Reviewer: M. Mahmood Hussain

Reviewer’s report:

This review was completed with the help of Ms. Meghan Walsh.

Summary:
This manuscript investigates associations between several MTTP polymorphisms and various metabolic parameters. They note that homozygosity for the H297 allele is associated with lower plasma LDL-C, lower plasma non-HDL-C, and higher risk of NAFLD.

Major Compulsory Revisions

1. There are many spelling, grammatical, and diction errors. It makes it very difficult to interpret and follow. Some of the statements contradict themselves. This document must be extensively edited before publication.

2. Results (paragraph 1) – The authors mention that there is linkage disequilibrium in the MTTP gene. In the discussion, they state (results paragraph 1), “there is strong linkage disequilibrium among these five polymorphisms (Figure 1)”. There was a high connection between haplotypes (G-493T and T128I) and (D98E and N166S).” Which alleles are linked? -493G with I128 or -493T with T128? D98 with N166 or D98 with 166S. Please be clear.

3. Throughout the manuscript, the missense mutations are reversed from the usual nomenclature. Usually, the major allele is listed first and the minor allele should be listed at the end. For example, T128I is I128T in most articles and databases. Only, N166S is listed correctly. Further the format is inconsistent throughout the manuscript. This particularly comes into play in Table 1. The missense mutation is listed as T128I. The genotypes are listed as TT:TC:CC. The CC substitution in DNA corresponds to the threonine, while the TT corresponds to isoleucine. This is confusing in the table. This also comes into play in paragraph 1 of the results. For example, T128I (T:C) implies that the T corresponds to the threonine and the C corresponds to isoleucine. This is not correct. The problem with the nomenclature extends further as it appears that your population has a completely opposite allele distribution than reported in most databases. Please confirm all SNPs and interpretation of results.

4. Results (second paragraph) – Authors state “subjects with CC genotype
The codons for glutamic acid are GAA and GAG. The codons for aspartic acid are GAT and GAC. There is a GAG (glutamic) => GAC (aspartic) transition resulting in a Glu to Asp transition. Thus, this statement is incorrect. Also, the conclusions need to modified to reflect these changes.

Minor Essential Revisions

1. Abstract (background) – Mutations in MTP is implicated in abetalipoproteinemia, not hypobetalipoproteinemia. One paper (Di Leo et al. 2005 Atherosclerosis) implicated MTP missense mutations in hypobetalipoproteinemia. However, this was not the paper that was cited.

2. Abstract (Background) – The author’s state “the precise mechanism linking dyslipidemia and NAFLD may be interactions with MTTP polymorphisms.” The term dyslipidemia is vague and misleading. This term can be used to describe elevated or reduced plasma lipids. Are they both linked to NAFLD? Which one is it? Also, the term “precise mechanisms” implies that MTP mutations are the reason the linkage. There are various other genetic and environmental factors that also affect plasma lipids and NAFLD.

3. Abstract (Conclusion): I would not use the term dyslipidemia. This implies that lipid values are out of range. All averages are within the normal range.

4. Background (paragraph 1) – MTP mutations are not widely implicated in Hypobetalipoproteinemia. Hypobeta can be removed from the manuscript.

5. Why did the authors use the AAI 25112.1 instead of the more commonly used NP_000244.2 accession number for the MTP template? Please correct.

6. Background (paragraph 2) – Author’s wrote “an imbalance of fatty acid homeostasis may contribute to the development of NAFLD, including dietary intake of chylomicrons.” The intestine makes chylomicrons to distribute dietary lipids from the intestine to peripheral tissues. Please revise.

7. Background (paragraph 3) – replace transfer catalytic activity with just transfer activity.

8. Throughout the paper, the authors are inconsistent with designations of the polymorphisms. For example, in statistical analysis paragraph one when describing a haplotype, they wrote “promoter -493 GG/D98/T128/S166/Q297H” replace with either Q297 or H297, whichever is correct. This is a consistent problem.

9. Several studies have investigated the correlation with plasma lipids and H297Q polymorphisms. None of them are cited. It should be noted they give conflicting results and discussed.

Bohme et al. 2008 Molecular Genetics and Metabolism

10. On the same note, the authors report, “Our study is the original report that carriers of the MTTP 297H were significantly associated with lower apoB-containing lipoproteins (LDL-C, non-HDL-C) and higher risk of NAFLD after adjustments for age, sex and insulin resistance.” See above papers.
11. Discussion (paragraph 3) please revise “proteoglycan binding site to apoB”.

12. Discussion (last paragraph) please revise the sentence “Absent of histologic diagnosis of NAFLD by liver biopsy would reduce the relevance of genetic effect”. It is unclear.

13. Please revise: “Because of the relatively large protein of MTP and apoB, clinical validation of the functional activities of these MTP polymorphisms, such as lipid binding or membrane transfer with apoB, are limited.”

14. Results (paragraph 4) – Authors state “(P= 0.0168, not shown in supplementary 2)”. Please show data both the ratio AST/ALT and the fatty liver formation since you are stating the results and giving a significant p-value.

- Discretionary Revisions

1. Results paragraph 5: Remove the sentence “aging has also been found to raise serum cholesterol and non-HDL-C a little.” It is out of place.

2. Discussion – Many papers report an association between -493 G/T and plasma lipids. It is the most characterized polymorphisms and shows the strongest correlations in the literature. Interestingly, the authors did not find a statistical correlation. It would be beneficial to point this out and discuss.

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

**Quality of written English:** Not suitable for publication unless extensively edited

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

**Declaration of competing interests:**

I declare I have no competing interests.