Author's response to reviews

Title: A possible role for miRNA silencing in disease phenotype variation in Swedish transthyretin V30M carriers

Authors:

Malin Olsson (malin.olsson@medbio.umu.se)
Nina Norgren (nina.norgren@medicin.umu.se)
Konen Obayashi (konen@fc.kuh.kumamoto-u.ac.jp)
Violaine Plante-Bordeneuve (vplante@free.fr)
Ole B Suhr (ole.suhr@medicin.umu.se)
Kristina Cederquist (Kristina.Cederquist@vll.se)
Jenni Jonasson (Jenni.Jonasson@vll.se)

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Author's response to reviews: see over
Dear Sir

Thank you for the constructive comments from the referees. Please bring forward our appreciation on the work they have laid down on our manuscript.

Please find below a point-to-point answer to the questions raised by the referees.

**Referee: 1**

**Reviewer:** Wenqiang Yu

Major compulsory revisions:
In this manuscript, Malin Olsson et al have identified those 3 SNPs on the TTR gene may contribute to the FAP phenotype. According to the rs62093482 SNP on 3’-UTR of TTR gene and prediction of binding sites changes for miRNA, the author proposed the hypothesis that miRNA may take some roles on the low penetrance and high age onset of the disease in Swedish patient population. The result sounds interesting and may help on understanding the mechanism of FAP. The author discover that 4 miRNA especially Hsa-miR-643 may be affected and contributed to the phenotype of FAP. In order to make this points clear the author need to do following experiments:

**Q1.** Make the construction placing 3 UTR of TTR WT gene and polymorphism downstream of report gene, transfecting this plasmid and Hsa-miR-643 to the cell line and find out whether the miRNA can make any difference on the report gene.

**A1.** We have started setting up a series of experiments to clarify the effects of the newly discovered SNP in the 3’UTR region of TTR. One of these experiments will be a luciferase construct containing the polymorphic 3’UTR or the wt 3’UTR of TTR, that will be co-transfected into a cell line together with the predicted miRNAs. This will only be a small part of the project, that later will come together in a separate study. miRNA expression levels will also be included in this study (see below, A2).

**Q2.** If possible, check the expression level of miRNA hsa-miR-643 and TTR gene in the FAP patient and controls which can make the points clear.

**A2.** Since miRNA has different expression levels in different tissues the appropriate thing to do is to investigate the expression levels in liver tissues of FAP V30M patients and controls since TTR is predominantly expressed in the liver. We have started to collect liver biopsies from FAP V30M patients that are undergoing liver transplantation and at the same time collect liver biopsies from the liver donor as control material, but patient material is limited (project therefore beyond three months extension offered).

**Referee: 2**
**Reviewer:** Simin D. Maleknia

**Q1.** The manuscript will greatly improve by incorporating two key areas (1) epigentics, the role of environmental effects in etiology of amyloid formation, and (2) the role of genetic interactions among the multiple loci rather than single-locus.

**A1.** See below:

**Q2.** Page 4, last paragraph: The authors note that “environmental factors have an impact on phenotype . . “ but did not provide any references to this important area of previous research. For example, *Free Radical Biology & Medicine, 2009, 46, 1241-1249,* describes the role of oxidative stress in AD. With respect to TTR and V30M mutation, preliminary results on the role of oxidative stress in amyloid fibril formation have been reported (*FEBS J, 2006, 73, 5400-5406*).

**A2.** Two references for the environmental effects have been added to the article (*See corrections in background*). A section on the epigenetic involvement in the disease phenotype has been added to the discussion section (*See corrections in discussion*).

**Q3.** The manuscript does not address the important role of interactions among multiple loci (Sores, et al, *Human Molecular Genetics, 2005, 14 (4), 543-553*). It is important to introduce the readers to this area of research, and to expand the discussion section.

**A3.** A section on interactions among multiple loci has been added to the discussion (*See corrections in discussion*).

**Referee: 3**

**Reviewer:** Joel Buxbaum

**Q1.** With respect to the analysis the authors do not appear to have corrected the significance values for multiple comparisons.

**A1.** We have corrected all the p-values for multiple comparison (*See corrections in Method/statistics, Results and in table 1*).

**Q2.** While the speculation regarding microRNA binding sites is interesting they provide no evidence that it is the case. If they showed some correlation with serum TTR levels of individuals with each allele then the argument concerning the role of microRNA suppression of expression would have been more valid.

In addition most TTR mutations in most populations are associated with lower TTR serum levels (although levels of abnormal protein have not been determined in all) yet the Swedes have reduced penetrance.

**A2.** It is possible to measure the relative amounts of wt versus mutated TTR in plasma, but the levels vary between individuals, due to e.g age. Therefore a better approach would be to use allele specific expression instead. This will be incorporated in the follow-up study (see referee 1, Wenqiang Yu). A paragraph on other populations has been added to the discussion (*See corrections in discussion*).

**Q3.** The SNP analysis was clearly worthwhile but they have not done appropriate follow up experiments to justify the hypotheses generated by the computational analyses.

**A3.** Follow up experiments are ongoing. Please see comments for referee 1, Wenqiang Yu.
We believe that our manuscript has improved with the suggested changes and clarifications, and hope that it acceptable for publication in BMC Medical Genetics-journal

Sincerely

Malin Olsson PhD, Corresponding author

Medical and Clinical Genetics
Building 6M, 3rd floor
Umeå University Hospital
SE 901 85 Umeå
Sweden
E-mail: malin.olsson@medbio.umu.se
Telephone +46 90 7851767
FAX +46 90 130760