Author's response to reviews

Title: The D9N, N291S and S447X variants in the lipoprotein lipase (LPL) gene are not associated with type III hyperlipidemia.

Authors:

David Evans (evans@uke.uni-hamburg.de)
Frank U Beil (beil@uke.uni-hamburg.de)

Version: Date: 16 May 2007

Author's response to reviews:

Dear Sir,

Enclosed is a copy of the revised manuscript together with our replies to the referees criticisms. Since the majority of the referees requested more information on the phenotypic characterisation of the patients we provide two new Tables giving these details. In addition we have grouped the patients from the clinic according to the form of hyperlipidemia and provided the frequencies of the LPL SNPs for each.

As R. Hegele points out this is an essentially negative study. The question of what additional genetic factors are required for the development of hyperlipidemia in patients with APOE2/2 genotype has been frequently posed but there is little data reported in the literature. The major problem being that due to the rarity of the condition it is difficult to obtain a large group of patients. Our collection of 100 patients is, to our knowledge, the largest employed to date in such a study. The fact that we could not confirm a widely cited (43 citations) report based on a smaller number of patients we believe provides the justification for publishing an "ultimately negative study."

The specific suggestions have been incorporated as detailed under the individual referees.

We thank the referees for their helpful criticisms

Yours faithfully

Dr David Evans

Robert Hegele
Major Points
1 and 2. We have included more data on the characterization of the patients in the new Tables 1 and 2. We have included the statement that Type III HLP is associated with an increased risk of atherosclerosis in the Introduction plus references to two review articles on Type III HLP.
3. See Table 1
4. Genotypes followed HWE with the exception of the N291S where in the patients with hypertriglyceridemia and mixed hypelipidemia there was a small overrepresentation of SS homozygotes, 3 rather than 0.65 expected and 3 rather than 1.1 expected respectively.
5 and 7 See Table 4
6. The point we wish to make in this report is our failure to reproduce in a study with four times the number of probands the observations reported in a widely cited (43 citations) paper.
8. The SNPs are functional. This is referred to in the revised text and references are given to the publications in which the functionality was demonstrated.
9. We consider it to be essentially a case of sample size.
10. No due to the small number of carriers of the rare alleles.

Minor Points

Have been corrected
Pierre Julien
The major criticism is the lack of phenotypic data in the original paper. We have provided such data in Tables 1 and 2. Lipid values are fasting values in the absence of lipid lowering medication. There is no discussion of LPL variants in the reference provided. We included the blood donor data to provide information on the frequencies of the LPL SNPs in a healthy population drawn from the same area as the patients. APOA5 variant frequencies have been incorporated into the text. All subjects included in the study were from the Hamburg area which is ethnically mixed, a statement to this effect has been included in the text. The possibility of multiple rare alleles in the LPL gene playing a role is discussed in the discussion. In contrast to the French Canadian population, the Hamburg population is ethnically diverse so that the possibility of a high incidence of a single, otherwise rare mutation due to founder effect such as the 207 found in French Canadians is low.

Kui Zhang
Major Points
1 Has been done (Table 3)
2 Has been done (Tables 1 and 2)
3 The point we wish to make in this report is our failure to reproduce in a study with four times the number of probands the observations reported in a widely cited (43 citations) paper.

Michael Hoffmann
Major Points
1 All patients are APOE2/2
2 Has been done
3 This a good point and we have incorporated it into the Discussion. In this communication our principle aim was to demonstrate that in a larger group of patients we could not confirm the role of the N291S SNP in Type III HLP. In addition we extended the study to include an additional two common SNPs. Thus the most common variants are not involved. The role of multiple rare alleles is a separate question which can be answered by sequencing the DNA of the LPL gene of all 100 patients.
Minor Points
1. We do not have a sufficiently complete data set.
2. Genotyping is not yet complete but at present we only have 5 N291S carriers who are also carriers of an APOA5 variant and a single D9N. With these numbers no conclusions can be drawn.

Jaroslav Hubacek
Major Point: Has been incorporated in the revised text, page 7.
Minor points
1 See Table 1 and 2
2 See Table 4
3 Has been done, page 2
4 See Materials and methods, page 4

Discretionary points
1. See Table 2
2. We wished to concentrate on the one specific question.
3. The material and methods section has been altered to make it clear that the patients were unrelated.
4. OK
5. Title has been altered.