Reviewer’s report

Title: Familial hypercholesterolemia in St.-Petersburg: the known and novel mutations found in the LDL receptor gene.

Version: 1 Date: 25 October 2004

Reviewer: Anne Soutar

Reviewer’s report:

General
This paper describes the use of SSCP analysis of amplified fragments to detect mutations in the LDL-receptor gene (LDLR) of patients with Familial Hypercholesterolemia (FH) who attend one of two clinics in St Petersburg, Russia. Although the study is not novel, the findings add to the number of different mutations known in the LDLR and shows that there is as much heterogeneity in FH in this region as in other parts of Europe. The data are sound and, for the most part, support the conclusions drawn.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. In view of the lack of space restraints, it would be very useful to provide some information about how many base pairs flanking each exon are included in the PCR products analysed.

2. I am not convinced that SSCP only misses a small minority of point mutations — data from other labs suggests this is not the case unless many different conditions are used. In this study the authors have detected a preponderance of insertion/deletion mutations, which suggests that they may be missing some single base substitutions; this part of the discussion should be modified. Indeed, their stated clinical criteria for FH are very stringent, and it is surprising that they have found mutations in only 25/45 patients.

3. They also state that large deletions are a “rare cause of FH in this population” and that “such deletions were excluded in most of these patients” – the experimental basis for these two statements should be given.

4. Currently, one of the most interesting questions about FH is the number of patients who truly do not have a mutation in the LDLR (or other known gene), and the authors should pay more attention to this aspect of their study. For example, have they sequenced all the PCR products in any of the patients in whom they failed to find a mutation by SSCP? Ideally this should have been done for any patients with a clinical diagnosis of “definite FH”. Or have they sequenced PCSK9 or at least screened for the published mutations in PCSK9 that have been found to co-segregate with hypercholesterolaemia in some families with FH? Even if this has not been done, some comment about how many patients they are convinced do not have known mutations should be included in the discussion.

5. The authors have listed also listed a silent mutation as P548P, but codon 548 is not proline – do they mean P518 or P542, which are the only Pro residues in exon 11?. And whichever it is, the authors should consider whether the base change might introduce a new splice site.
6. The authors have found a number of single base substitutions that are listed here as mutations that affect function because they have appeared in other databases, even though there is no experimental evidence that they affect function. The authors should comment in the discussion about how or why these affect LDL receptor function. For example, although the variant that is predicted to substitute Val806 with Ile was listed as FH NY-5 by Hobbs and co-workers, there is no experimental evidence that this affects LDL receptor function. Since it occurs in the Val residue in the NPVY sequence (often listed as NPxY) in which it is known that the Val residue can be substituted with Ala without loss of function, it cannot be assumed that such a conservative substitution would severely affect LDL receptor activity. The second such example is the mutation listed as Val776Met; the single base substitution that is predicted to cause this occurs in the codon at the junction between exons 16 and 17, and since it substitutes the final invariant G at the splice junction with A, it is more likely to affect splicing than to result in a simple amino acid substitution.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. At the top of p4, the authors describe a mutation as making the LDL receptor “invalid” – this does not seem to be quite correct usage, maybe “defective” would be better.

Discretionary Revisions (which the author can choose to ignore)

1. The T705I variant has been found in tight linkage disequilibrium with a variation in intron 7, upstream of exon 8 (nt1061-8T, Heath et al, J Med Genet 2000, 37:723 and Mozaz et al, Hum Mutat 2000, 15: 483). Was this variant detected in association with T705I in this study? Also, I am not sure it is correct to list T705I as a “silent” variant, because it is predicted to change an amino acid; perhaps it would be better to designate it as a variant that is not associated with hypercholesterolaemia.

2. It would be interesting to know why FH patients were diagnosed in the Institute for Human Brain.

What next?: Accept after minor essential revisions

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

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

None