Reviewer's report

Title: Multiple Splice Defects in ABCA1 cause Low HDL-C in a family with Hypoalphalipoproteinemia and Premature Coronary Disease

Version: 1 Date: 28 September 2008

Reviewer: Sebastiano CALANDRA

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The authors investigated the effect of two intronic mutations (IV7+6c>t and IVS31-1g>c respectively) of ABCA1 gene found in a subject with very low plasma HDL-C levels. Both mutations caused the formation of abnormal mRNAs as documented in peripheral blood cells.

The authors also looked at the transcripts of two minigenes harbouring the mutations in transfected COS cells. While the transcript of the mutant minigene harbouring the IVS31-1g>c was identical to that found in patient’s blood cells, the analysis of the transcript generated by the mutant minigene harbouring the IVS7-1g>c did not reveal mis-splicing. The authors suggest that this unexpected result may be related to the fact that part of the intronic regions were not included in this minigene construct.

The revised manuscript contains new experimental data and overall the study appears to be more convincing.

There are some points which deserve revision.

a) the sequence of the abnormally spliced cDNA should be given to support the schemes shown in figure 3, 4 and 5.

b) the size of the IVS7+ +6g>t minigene and that of the intronic regions deleted in the construction of the minigene must be provided, especially in view of the discrepancy between ex-vivo and in vitro results (discussed on page 12).

c) in figure 1 heterozygotes should be indicated by half shaded symbols and the compound heterozygotes (the proband and his sister) by a combination of the two symbols. In the inset of the figure the two mutations should be indicated as IVS7+6c>t and IVS31-1g>c instead of Exon 7 and Exon 31 skipping. The same applies to table 3.

d) figure 2 is in fact a table. I suggest to list the introns rather than the exons in the table. The headings “Acceptor Ri values” are mis-aligned.

e) the speculation concerning the failure to detect mis-splicing of IVS7+c>t minigene transcript (page 12) should be moved to the discussion.

f) the section “splice site analysis” (page 11) should follow the genomic sequencing results and precede the results of ex vivo cDNA analysis.

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:**

I declare that I have no competing interest.