Author's response to reviews

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

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Author's response to reviews: see over
Dear Dr. Edmunds:

We are submitting our revised manuscript and have made all of the requested suggestions by the reviewers. Listed below is a point-by-point response for each of the excellent suggestions made and now hope that the manuscript is suitable for publication in BMC:

Referee 1: Dr. Calandra

a) The sequence of the abnormally spliced cDNA should be given to support the schemes shown in figure 3, 4 and 5. As suggested, the sequences of the abnormally spliced cDNAs is now included at the bottom of new Figures 2 and 3.

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).

The total size of the insert was 3.5kb in size, which combined with the pTargeT vector (5.69kb), resulted in a total insert + vector size of 9.19kb. This information is now provided in the Materials and Methods section (page 9).

Because several intronic regions were too large to be incorporated in the vector (IVS6, 2.98kb; IVS7, 12.96kb; and IVS8, 4.96kb) truncated intronic regions of approximately 400bp were used (see Primers, Table 2). This information is now included in the text section of the Materials and Methods section (page 7).

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. Done.

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. Done

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. Done, see new Table 1.

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

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

**Referee 2: Dr. Marcil**

Minor Essential Revisions requested:

Page 12, Minigene transfection from the Results section. The discussion on IVS7 to explain the unexpected result, based on references 18, 19, 20, should appear rather in the Discussion section. **Done.**

Please correct some abbreviations: Calif should be corrected by CA; although authors have mentioned to have done this correction, 6 Calif abbreviations are still present in Material and Methods section; in page 8, ABC-A1 should be corrected by $ABCA1$ to use the standard abbreviation. **Done**

In Figure 1, the shading of the symbols used to represent differently exon 32 and exon 7 skipping mutation are quite confusing. Please use a clear pattern in these symbols. **Done.**

**Referee 3: Dr. Rogan:**

No changes requested.