Author’s response to reviews

Title: Targeted genetic testing for familial hypercholesterolaemia using next generation sequencing: a population-based study

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Dear BMC Medical Genetics

Following recent submission of our manuscript by Norsworthy et al, “Targeted genetic testing for familial hypercholesterolaemia using next generation sequencing: a population based study”, MS:1832207559122547, we now have pleasure in submitting a revised manuscript.

We believe that our revised manuscript addresses all of the issues raised by referees regarding our original submission, as detailed in our point-by-point responses which are appended to this covering letter.

We thank you for consideration of our manuscript, which we hope will now be acceptable for publication in the journal.

Yours sincerely,

Professor Tim Aitman (for the authors)

Enc.
We provide below our point-by-point responses to the referees’ comments on our originally submitted manuscript, Norsworthy et al, MS:1832207559122547.

Reviewer 1: Zosia Miedzybrodzka

It was a well written paper addressing an important question. I think it should be published with revisions.

Key thing is that the paper assesses the rate of FH associated mutation in a people with higher than average "cholesterol" within a general practice selected cohort study. Those being tested are unselected for family history as the current clinical practice guidelines recommend. It is interesting that 2% of this population had mutations. It is not clear if these patients would have been selected for testing using the standard clinical diagnostic criteria (Simon Broome or MedPed). As family history information is available for the generation scotland cohort this should be added if possible. If not possible this limitation of the work should be discussed.

We thank the reviewer for her positive comments, and recommendation that the manuscript should be published with revisions. Regarding the use of standard clinical diagnostic criteria in this study, it was not possible to formally classify most of the high cholesterol group by Simon Broome or Dutch Lipid Clinic criteria, as the available clinical and diagnostic data in the GS:SFHS did not include phenotypes such as tendon xanthomata, LDL-cholesterol or age-of-onset of cardiovascular disease in relatives of index cases. However, it is noteworthy that a subset of 50 younger subjects in the high cholesterol group with total cholesterol 7-7.4mmol/l could be classified as not meeting Simon Broome criteria for FH, and that a molecular diagnosis of FH was made in one of these subjects. Family history of hypercholesterolaemia was also only available for study subjects whose relatives had been recruited to the study. None of the cholesterol-therapy group could be formally classified for FH by clinical criteria, as pre-treatment cholesterol measurements were not available from the GS:SFHS. Discussion of these points has now been added to the Discussion (Page 9, Lines 219-235) of the revised manuscript.

The paper gives its main purpose as looking for FH in a general practice population. I feel the paper would benefit from refocusing to indicate the general practice population in whom mutations were found were selected for "high cholesterol" not only being in general practice.

Although the title of the manuscript refers to 'Targeted Genetic Testing', we accept the need for more clarification of this point in the main text, and therefore we have included extra references to 'targeted' and 'selective' testing in a primary care population 'subset selected for hypercholesterolaemia' in the Abstract (Page 2, Lines 34-35), Background (Page 4, Lines 80-82) and Conclusions (Page 12, Lines 281 and 288) of the revised manuscript.

There are frequent references in the manuscript to patients with "high cholesterol". This should be more accurately defined throughout- in FH the abnormality is of high LDL cholesterol. The criteria for a definition of high cholesterol used in the study should be clearly set out in the methods. If only total cholesterol was available then this should be
explained, and the cut-off thresholds for each category should be defined. In discussion the relation of these to conventional diagnostic criteria should be discussed.

LDL-cholesterol data was not available from the GS:SFHS database and total cholesterol data was therefore used to select subjects for the study. This is now stated in the Methods section of the revised manuscript (Page 4, Lines 96-98). Cut-off thresholds are also defined in Figure 1. In addition, extra references to 'total cholesterol' have now been included within the Abstract (Page 2, Line 39) and Methods (Page 4, Line 101), the key word 'LDL-cholesterol' has now been changed to 'total cholesterol' (Page 3, Line 53), and it has now been clarified in the Methods that the term 'high cholesterol group' has been designated for high total cholesterol (Page 4, Line 100).

Regarding the performance of the next-generation sequencing assay, the coverage of each base / fragment would not meet the recently accepted criteria for clinical grade sequencing. That does not mean these data are not valuable, but I think it would be reasonable to ask the authors to indicate that the assay would require more development before being used in routine clinical practice.

We accept this suggestion, and have amended the Methods section to draw attention to this point, with reference to standards now recommended by ASMG (Rehm et al, 2013 - reference 13 of resubmitted manuscript). We have also indicated that subsequent improvements to MiSeq protocols since this study was conducted would enable an improved assay design that would be likely to lead to current standards for clinical diagnostic sequencing to be met (Page 6, Lines 137-142).

Other specific recommendations:
Add numbers to the % in results.

We have revised the text so that the numbers of mutations can be more clearly seen (Page 7, Lines 166-167).

Reviewer 2: Sebastiano Calandra

The percent of subjects with pathogenic mutations + VUCS is surprisingly low. This implies that 96% of hypercholesterolaemic subjects in Scotland, despite a plasma cholesterol level in the range of classic molecular defined heterozygous FH, do not have mutations in the three major candidate genes. How these results fit with the mutation detection rate in FH patients in the lipid clinic that, ranges from 40% to 80%?

We thank the reviewer for his positive comments, in particular that the study has been carefully conducted, and is interesting and novel. With regard to the low mutation detection rate in our study, the population we have studied is very different from a lipid clinic population, where phenotypes and family history are highly characterised. The population we studied was selected with the aid of limited phenotypic and clinical data, which did not permit us to completely discriminate between individuals with monogenic and polygenic hypercholesterolaemia, or lifestyle related hypercholesterolaemia in the way that more detailed information such as presence or absence of tendon xanthomas and availability of LDL-cholesterol permit in Lipid Clinic populations. In addition, the Scottish population is known to have a relatively high prevalence of lifestyle-related hypercholesterolaemia (Roth et al, 2011 - reference 23 of resubmitted manuscript). Availability of more detailed clinical data and the study of populations with a lower background rate of cardiovascular disease would most likely allow for greater
enrichment for cases of FH than in the present study. These points have now been added to the Discussion of the revised manuscript (Page 10, Lines 241-251).

Intronic variants outside the splice sites were not considered. Was the frequency of some of these variants different in the three groups?

Because of the low frequency of known intronic mutations that cause FH and the difficulty of assigning pathogenicity to such sequence variants, intronic regions outside splice sites were not covered in this assay. We are therefore unable to comment on the frequency of intronic variants in the three study groups. This point is now made clear in the Methods section of the revised manuscript (Page 6, Lines 130-132 and Page 7, Lines 159-161).

Gain of function mutations of PCSK9 are rare causes of FH. Why the authors confined the analysis to only four exons of this gene?

The four PCSK9 exons included in the analysis represent the only exons in this gene to date that have been shown to contain gain-of-function FH-causing mutations, and as noted by the reviewer, these mutations account for a very small proportion of FH cases. We therefore considered that, given that the likelihood of detecting novel mutations in other PCSK9 exons was extremely low, and the difficulty of assigning pathogenicity to mutations in these regions, analysis of additional exons would not be informative for a routine clinical diagnostic test. Accordingly, PCSK9 exons not already shown to harbour FH-causing mutations were not included in the NGS assay design. This point has been addressed in the Methods section of the revised manuscript (Page 5, Line 124, and Pages 5-6, Lines 127-130).

Although the large majority APOB mutations causing FH are located in a restricted segment of exon 26, there may be mutations elsewhere in the gene which may reduce the capacity of apoB to bind to the LDLR.

APOB is a large gene, and although a very small number of FH mutations have been detected outside the main FH mutation locus in exon 26 (e.g. as in Thomas et al, 2013), considerations of practicality and informativeness for clinical assays, as discussed above, meant that we restricted analysis of this gene to exon 26. We have addressed this in the revised Methods section as for the previous point above (Pages 5-6, Lines 127-130).

References


