Reviewer's report

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

**Version:** 1  **Date:** 28 March 2014

**Reviewer:** Sebastiano CALANDRA

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In a previous study the authors demonstrated that next generation sequencing (NGS) is suitable to detect candidate gene mutation in patients with Familial Hypercholesterolemic (FH) attending the lipid clinic.

In the present study they tried to assess the feasibility of NGS to identify mutations in hypercholesterolemic patients (suspected to have FH) which receive the healthcare outside the Hospitals or Lipid clinics.

Taking advantage of a repository of biological samples (GS:SFHS) in Scotland, they selected subjects for genetic analysis using criteria to enriched the test population for individuals likely to have FH. The introduce a clinical quality control to reduce the inclusion of subjects likely to have obesity and age related hyperlipidemia. After this filter they examined 193 individual with hypercholesterolemia (high cholesterol group), 232 with moderately elevated cholesterol some of whom under treatment with hypolipidemic drugs, and 192 age, sex matched controls.

They analysed LDLR gene, four exons of PCSK9 gene and the known hot spot in exon 26 of ApoB gene.

They found that in 2.1% of carriers of pathogenic mutations in high cholesterol group and subjects on cholesterol lowering therapy. In addition 1.4% of these subjects were carriers of missense variants of uncertain effect (VUCS). No pathogenic variants were detect in controls.

The study has been carefully conducted and the results are interesting and novel.

The study raises a major question which deserves some comment by the authors:

1) The percent of subjects with pathogenic mutations + VUCS is surprisingly low. This implies that 96% of hypercholesterolemic 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%?

Minor points.

1) intronic variants outside the spice sites were not considered. Was the frequency of some of these variants different in the three groups?
2) gain of function mutations of PCSK9 are rare causes of FH. Why the authors confined the analysis to only four exons of this gene?

3) 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.

Level of interest: An article of importance in its field

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 interests