The authors investigated the impact of common sequence variants located at 5’ end of the LDLR gene on the variations of plasma LDL-C level in patients with polygenic hypercholesterolemia (PH) and normolipidemic control subjects.

The study includes: i) the resequencing of a 3kb region of LDLR gene, containing part of the proximal promoter, exon 1 and intron 1, in 125 patients with PH; ii) the analysis of the frequency in PH patients and controls of some SNPs located in the promoter of LDLR gene found in silico to be potential binding sites for transcription factors; iii) the biological properties of a specific SNP (g.3131C>T) in terms binding nuclear proteins and intrinsic promoter activity in HepG2 cells.

The results indicate that the T-allele of the g.3131C>T SNP is associated with LDL-C levels, and explains part of the LDL-C variability. This allele appears to induce an increase in LDLR gene transcriptional activity which may explain the reduced plasma LDL-C levels found in carriers of CT + TT genotype.

Major compulsory revisions.

A) Resequencing the 3.1 kb region. Only data obtained in PH are presented in the Additional Table 2. This table contains some errors (124 patients instead of 125 as indicated in the text; the SNP rs60173709 corresponds to c.67 + 1698del T and is located in intron 1; the SNP rs6511720 corresponds to c.67+2015G>T as is located in intron 1).

B) Table 2 SNPs in the promoter region. The authors state that rs1742346, rs17242739, and rs171249120 presented a minor allele frequency >10%. That does not appear to be the case neither in PH patients nor in controls.

C) Table 3. The frequency of carriers of CT + TT genotype in controls is higher in the first quintile (Q1) of LDL-C level with respect to the fifth quintile (Q5). However this is also the case for Q2 and Q3 suggesting some kind threshold effect of the SNPs on plasma LDL-C. The authors should discuss this point. It would also be interesting to show the frequency distribution of CT + TT carriers in the quintiles of LDL-C in PH patients.

D) Fig. 1 A and B. Electrophoretic mobility assay. Fig. 1 A clearly shows the that the wild type oligonucleotide (carrying the wild type allele C) competed more easily with the binding of the T allele with respect to the C allele. Fig. 1 B shows that the slope corresponding to the T allele (0.0073) was higher than that corresponding to the C allele (0.0045). As specified in the methods (Riancho et al
2011) this would imply a lower oligonucleotide protein affinity in the case of the T allele (not a higher affinity as claimed by the authors).

E) Fig. 1C. Luciferase activity. The authors should show the complete data of this experiment (including pGL3 basic, and pRL-TK plasmid). If indeed the T allele has lower affinity for nuclear proteins, the data shown in fig. 1C would suggest that this allele binds less efficiently some repressors of LDLR gene transcription present in HepG2 cells. For this reason the transcription activity may be increased in carriers of g.3131T allele.

F) The authors should provide the evidence supporting the “T allele of g.3131C>T SNP as the “ancestor” allele.

Minor essential revisions

Abstract.

1) Primary hypercholesterolemia is indicated with the abbreviation PH that is also used in the text (and in the list of abbreviations) to indicate Polygenic Hypercholesterolemia.

2) 476 patients investigated instead of 477 as specified in the text

3) A 3103 bp from LDLR(c.2335 + c.67 +36) … is wrong. It should be replaced by ..-625 to + 2468 (as indicated in the methods)

4) four SNPs (…… ) the indication of the second SNP must be corrected “rs17241739” (add 1 to the number)

5) EMSA was carried out for the “rs27248720” … must be corrected rs 17248720 (the first number 2 must be replaced by 1)

6) g.3131 T>C… should be g.3131C>T

7) showed higher affinity for transcription factor (see comments on the data shown in Fig. 1)

8) g.3131 T>C… should be g.3131C>T

Table 1. Mean and standard deviation of each parameter should be indicated (as for apolipoprotein B).

Fig. 1B the term “alelo” should be replaced by the term “allele”

Additional table 1: “LDLDR” should be replaced by the “LDLR”

Discretionary revisions

Additional Table 2. It would be interesting to know the distribution of the SNPs also in control subjects.

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 interests.