



## LETTER TO THE EDITOR

## Hypercholesterolemia risk-associated GPR146 is an orphan G-protein coupled receptor that regulates blood cholesterol levels in humans and mice

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Dear Editor,

Hypercholesterolemia is the leading risk factor for cardiovascular diseases. Current evidence suggests that the heritability for blood cholesterol levels is high, with 40%–60% in different cohorts.<sup>1,2</sup> Genome-wide association study (GWAS) is a powerful tool to ascertain the contribution of common genetic variants in population-wide disease variability. It has been performed extensively on blood lipids traits and hundreds of genome variants are associated with dyslipidemia. However, approximately 95% of these variants are located in genome noncoding regions and cluster in more than 300 loci in different populations.<sup>3</sup> We find that approximately 2/3 of these loci are located in noncoding regions and are not close to any gene that known plays a role in lipid metabolism. The disease-causing variants in these loci and the molecular mechanisms remain largely unknown, which prevents the interpretation of the GWAS results and their application in precision medicine. On the other hand, these noncoding regions may harbor novel genes or signaling pathways involved in lipid metabolism and be a valuable resource for further mechanistic studies.

We systematically analyzed these noncoding loci with unknown function. One such locus 7p22 is strongly associated with hypercholesterolemia in multiple cohorts<sup>4–6</sup> (Supplementary information, Figs. S1a, S2b). The lead SNP rs1997243 is a common noncoding variant with highest frequency in the European population and has a strong linkage disequilibrium (LD) non-synonymous variant rs11761941 (*GPR146* p. Gly11Glu) in some populations (Supplementary information, Figs. S1a, b, S2a). Both rs1997243 and rs11761941 are significantly associated with blood cholesterol levels (Supplementary information, Fig. S2b).<sup>5</sup> However, the *GPR146* p. Gly11 is not conserved and has been substituted with Asp, Asn or Ala in many other species except the *gray wolf* (Supplementary information, Fig. S2c). Bioinformatics analysis also predicts that *GPR146* p. Gly11Glu is benign and neutral (Supplementary information, Fig. S2d), rendering rs11761941 less likely to be the disease-causing variant.

We reasoned that any SNPs that have strong LD with the lead SNP could be the real disease-causing variant. For variants that are located in the noncoding region, the real disease-causing variant is most likely located in the regulatory region, such as regions marked by DNase I hypersensitivity and/or histone modification markers H3K27ac and H3K4me3.<sup>7–11</sup> A total of 125 linkage disequilibrium SNPs were identified for the lead SNP rs1997243, with 28 of them are located in genome active regions (Supplementary information, Fig. S3, Table S1). We then applied a luciferase reporter assay to compare the transcriptional activities between the minor allele and the major allele for each of them, with *APOA1* promoter served as a positive control. We found that only rs1997243 showed increased promoter activity compared with its reference allele (Fig. 1a, b; Supplementary information, Fig. S3). The rs1997243 does not change enhancer activity in the

enhancer luciferase reporter assay (Supplementary information, Fig. S3l), which is consistent with the enriched promoter-specific histone marker H3K4me3 at this position (Fig. 1a).<sup>7,10,11</sup>

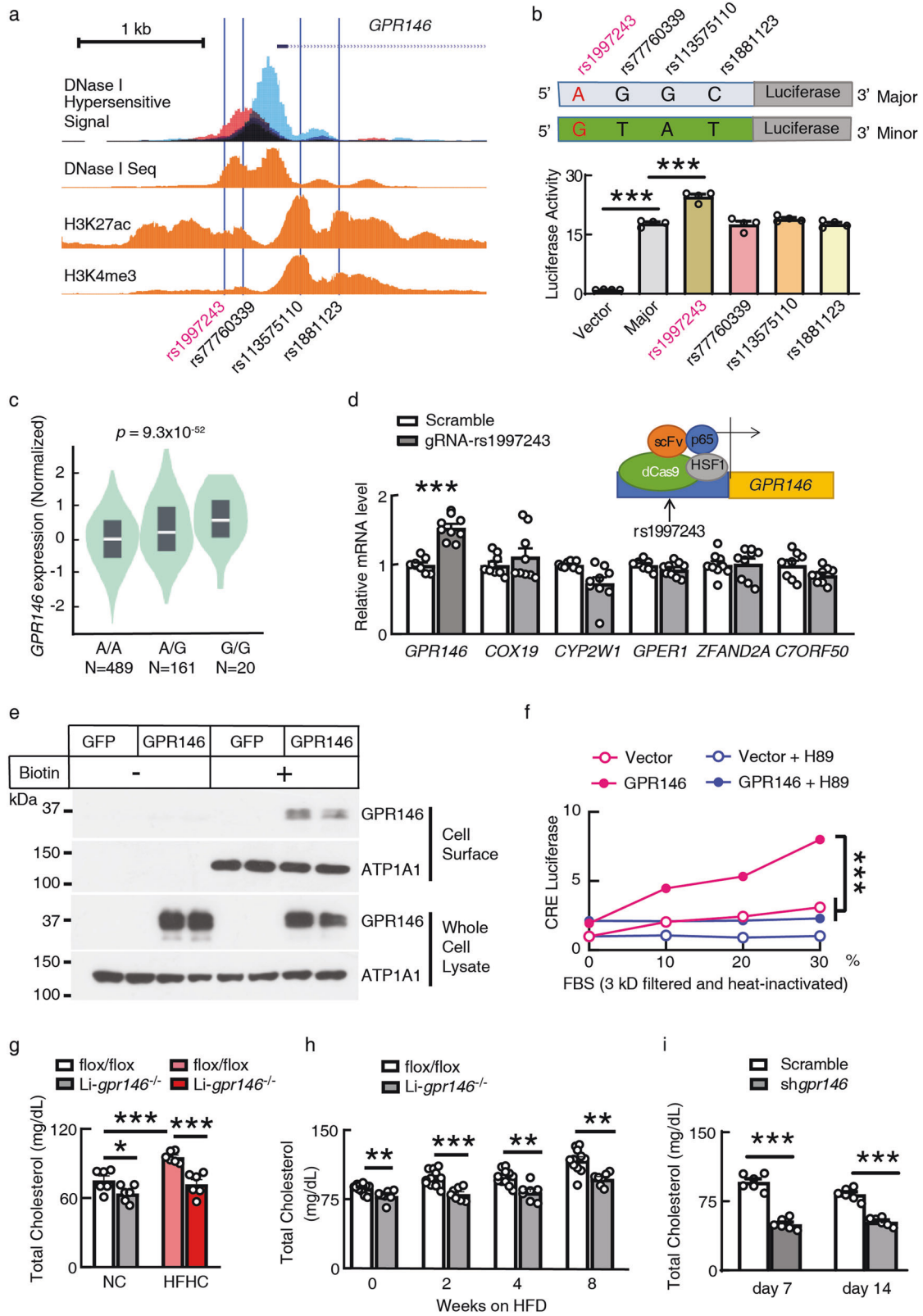
Expression quantitative trait loci (eQTL) analysis showed that the rs1997243 minor allele (G-allele) is strongly associated with increased expression of *GPR146* in humans (Fig. 1c). Targeted activation of the rs1997243 site with an enzymatic dead Cas9 (dCas9) system increased *GPR146* expression significantly, with no detectable change for other genes in this region (Fig. 1d; Supplementary information, Fig. S2e). These data suggest that rs1997243 is the disease-causing variant and may increase blood cholesterol levels by upregulating *GPR146* expression.

*GPR146* is an orphan G-protein coupled receptor (GPCR) that is highly expressed in the livers and adipose tissues of humans and mice. In the liver, it is specifically expressed in hepatocytes (Supplementary information, Fig. S4a–c). It contains seven typical transmembrane domains by prediction and is located on plasma membrane of cultured cells (Fig. 1e; Supplementary information, Fig. S4d–f). GPCRs typically signal through  $G\alpha_s$ ,  $G\alpha_i/o$ ,  $G\alpha_q/11$ ,  $G\alpha_{12/13}$  or  $G\beta/\gamma$  and regulate cAMP level,  $Ca^{2+}$  mobilizations, ERK/MAPK activity or small G protein RhoA activity.<sup>12</sup> We found that *GPR146* responds to serum that has been filtered by 3 kD cut-off and activates the transcriptional activity of the cAMP response element (CRE) (Supplementary information, Fig. S4g). Moreover, this response is preserved when the serum is further heat-inactivated by boiling and can be fully blocked by the PKA inhibitor H89 (Fig. 1f). Our data indicates that *GPR146* is a cell signaling receptor that responds to serum stimulation and activates the CRE activity.

To further study its function in vivo, we generated *gpr146* knockout mice with the *Cre-LoxP* system. Totally we got 6 lines of F1 mice and they were genotyped by genome sequencing (data not shown), southern blot and PCR genotyping (Supplementary information, Fig. S5a–c). Line 92 was used for all experiments unless otherwise indicated. We generated whole-body, liver-specific and adipose tissue-specific knockout mice by crossing with Cre recombinase driven by *CMV*, *albumin* and *adiponectin* promoters respectively. Whole-body and liver-specific *gpr146*<sup>−/−</sup> mice (Li-*gpr146*<sup>−/−</sup>), but not adipose tissue-specific *gpr146*<sup>−/−</sup> mice, had significantly decreased blood cholesterol levels and are protected from high-fat high-cholesterol (HFHC) diet induced hypercholesterolemia, which suggest that *gpr146* regulates blood cholesterol levels mainly through the liver (Fig. 1g; Supplementary information, Figs. S5d–g, S6a–d). Consistent with this, both ApoB-100 and ApoB-48 protein levels were significantly decreased in plasma of Li-*gpr146*<sup>−/−</sup> mice, especially under HFHC diet feeding. ApoA1 was also slightly decreased in Li-*gpr146*<sup>−/−</sup> mice fed with HFHC diet (Supplementary information, Fig. S6e, f). Moreover, Li-*gpr146*<sup>−/−</sup> mice were protected from high-fat diet induced hypercholesterolemia (Fig. 1h; Supplementary information, Fig. S7a). Acutely suppressing *gpr146* expression in adult mice also dramatically

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decreased blood cholesterol levels, which indicates that blocking *gpr146* function will be an effective strategy to decrease blood cholesterol in adults (Fig. 1i; Supplementary information, Fig. S7b). These results were confirmed in Li-*gpr146*<sup>-/-</sup> mice derived from an independent F1 line (Supplementary information, Fig. S7c, d). Taken

together, our results clearly demonstrate that GPR146 positively regulates blood cholesterol levels, which is consistent with increased cholesterol levels in humans with the rs1997243 risk allele.

In summary, we found a noncoding disease-causing variant rs1997243 that upregulates the expression level of an orphan

**Fig. 1** rs1997243 is a non-coding variant that regulates blood cholesterol levels through upregulating *GPR146* expression. **a** rs1997243 and three other SNPs that have strong LD with rs1997243 are located in the genome active region. **b** Minor allele of rs1997243 increases its promoter activity. **c** Minor allele of rs1997243 is significantly associated with increased *GPR146* expression in humans. **d** Targeted activation of rs1997243 site increases *GPR146* expression in HepG2 cells. **e** *GPR146* is located on plasma membrane of 293 T cells. **f** *GPR146* responds to heat-inactivated serum and activates CRE activity in HepG2 cells. Part of the cells was treated together with PKA inhibitor H89 (10  $\mu$ M). **g** Li-*gpr146*<sup>-/-</sup> mice have decreased blood cholesterol levels with normal chow (NC) and high-fat high-cholesterol (HFHC) diets feeding. Samples were collected after overnight fasting ( $n = 6$ , female, 10–13 weeks). **h** Li-*gpr146*<sup>-/-</sup> mice are protected from high-fat diet (HFD)-induced hypercholesterolemia. Samples were collected after overnight fasting ( $n = 7$ –11, male, 10–12 weeks). **i** Knocking down *gpr146* in adult mice decreases blood cholesterol levels ( $n = 6$ , male, 8 weeks). Samples were collected after 4 h fasting in the morning. All data are expressed as means  $\pm$  SEM and  $P$  values were calculated with Student's test (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

G-protein coupled receptor *GPR146*. *GPR146* positively regulates blood cholesterol levels mainly through the liver. We believe that the increased expression level of *GPR146* can at least partially explain the disease-causing effect of rs1997243 in humans.

In contrast to the causal variants in Mendelian disease, which typically confer large effects, the common variants from GWAS usually have modest effects. This is especially true for GWAS SNPs that are located in noncoding regions. However, variants that explain a small proportion of the traits may provide substantial biological or therapeutic insights. The rs1997243 confers a modest effect on the total blood cholesterol level with an effect size of 0.033.<sup>4</sup> However, by combining bioinformatics analysis and functional studies, we found that the downstream target gene *GPR146* has a large impact on blood cholesterol levels. Our study also reveals that *GPR146* responds to an endogenous ligand in the serum and activates the CRE activity, which suggest that *GPR146* is a functional GPCR and has therapeutic potential. Thus our study provides an example that the common noncoding variant with modest effect may provide important biological or therapeutic insights. The strategy we developed here can be applied to other noncoding loci with unknown mechanisms as well.

Our study should be interpreted within the context of its limitations. First, we systematically analyzed all SNPs in a 200 kb window across the locus and found that rs1997243 is the only one that changes its promoter activity and increases the expression level of *GPR146*. We cannot exclude the possibility that other variants extremely far away from the lead SNP may mediate the disease-causing effect together with rs1997243. However, our study provides compelling evidence that rs1997243 is a disease-causing variant and increases *GPR146* expression, which contributes to the increased blood cholesterol in humans. Second, our animal models strongly suggest that *Gpr146* regulates blood cholesterol levels mainly through the liver. However, eQTL analysis in humans showed that the strongest association with *GPR146* expression is in whole blood cells. Thus, we cannot exclude the possibility that *GPR146* may regulate blood cholesterol levels through other tissues together with the liver in humans. Third, we found that *gpr146* knockout mice have decreased blood cholesterol levels, however the underlying mechanism needs further investigation.

During preparation of this manuscript, Dr. Cowan's group reported the phenotypic characterization of *gpr146* knockout mice.<sup>13</sup> They reported that *gpr146* knockout mice have decreased blood cholesterol levels, which is consistent with our results. However, our results provide genetic evidence that *GPR146* regulates blood cholesterol levels not only in mice but also in humans. First, although the 7p22 locus is known strongly associated with hypercholesterolemia, we are the first to show that rs1997243 is the disease-causing variant in this locus. Second, we provide multiple lines of evidence that the rs1997243 risk allele specifically upregulates the expression level of *GPR146*. Third, by generating *gpr146* knockout mice, we provide strong evidence that *Gpr146* positively regulates blood cholesterol levels mainly through the liver. Altogether, our results indicate that *GPR146* is an important regulator of blood cholesterol levels in both humans

and mice. We believe *GPR146* will be an attractive drug target for hypercholesterolemia and atherosclerotic cardiovascular diseases.

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## AUTHOR CONTRIBUTIONS

F.H., X.L., C.C., Y.L., M.D., and Y.W. designed the study, performed experiments and wrote the paper. Y.Z., Y.L., B.-L.S., and H.H.H. contributed to data analysis and interpretation. Y.W. supervised the work and obtained the funding.

## ADDITIONAL INFORMATION

**Supplementary information** accompanies this paper at <https://doi.org/10.1038/s41422-020-0303-z>.

**Competing interests:** The authors declare no competing interests.

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