Phenotypic Differences Between Polygenic and Monogenic Hypobetalipoproteinemia

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GPR146 gene variants are associated with reduced plasma lipids and metabolic health: A novel role for GPR146 in hypolipidemia

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ABSTRACT

BACKGROUND
In mice, G-protein coupled receptor 146 (GPR146) deficiency causes hypolipidemia and protects against atherosclerosis. We here aim to evaluate whether a loss of *GPR146* in humans results in a similarly beneficial phenotype.

METHODS
We made use of common and rare genetic variants in the *GPR146* gene locus, as research instruments in population cohorts (UK-Biobank, >500 000 participants; Lifelines, ~165 000 participants and The Copenhagen-City Heart Study ~20 000) and in subjects with familial hypobetalipoproteinemia (FHBL).

RESULTS
Carriers of the common variant rs2362529 present with gene dose-dependent decreased low-density lipoprotein cholesterol (LDL-C) and apolipoprotein B. Further delineating the role of GPR146 in lipid metabolism, we show that the exchange of a highly conserved proline for a leucine residue at position (p.Pro62Leu; predicted to be damaging), present in 2615 participants of the UK-Biobank cohort, is associated with markedly lower LDL-C, HDL-C, but also triglycerides, compared to non-carriers. Remarkably, carriers of this variant also show reduced plasma levels of liver enzymes and C-reactive protein. In a family with FHBL, we finally show that the p.Pro62Leu variant segregates with low LDL-C.

CONCLUSIONS
This study shows that carriers of genetic variants in *GPR146* present with a beneficial lipidic and metabolic profile, low levels of LDL-C and reduced plasma liver enzymes which support the development of GPR146 antagonists against cardiometabolic diseases. The data furthermore support the hypothesis that loss of GPR146 is a novel cause of FHBL.

INTRODUCTION

Carriers of mutations in *APOB*, *MTTP*, *PCSK9* and *ANGPTL3* are all characterized by reduced plasma cholesterol levels and are protected from atherosclerosis[1–5]. When studying these genes in individuals with extremely low levels of low-density lipoprotein cholesterol (LDL-C), we previously showed that in 30% of the cases, we have no explanation for this phenotype[6,7], indicating that there is still room for new discoveries. The fact that genome-wide association studies (GWAS) (re)identified the above-mentioned key players in the metabolism of very low-density lipoprotein (VLDL), indicates that newly discovered genomic loci may play equally important roles.

One of the GWAS hits in 2013 was *GPR146*[8], encoding the G-protein coupled receptor 146. More specifically, a common DNA variation (rs1997243) in close proximity to the *GPR146* gene, present in 15% of individuals in the general population, was found to be associated with increased plasma levels of total cholesterol. Han *et* al. later showed that this increase in plasma cholesterol is caused by increased levels of *GPR146* mRNA[9], indicating that the encoded protein is directly involved in the regulation of cholesterol metabolism. Data in the public domain show that *GPR146* is mostly expressed in white adipose tissue where it has been reported to play a role in adipocyte differentiation[10] but this is unlikely to explain its effect on plasma cholesterol. In mice, we have provided evidence that loss of *Gpr146* in the liver reduces plasma cholesterol levels[11]. Murine *Gpr146* deficiency was furthermore shown to decrease the hepatic secretion of VLDL, the precursor of LDL, and to protect against diet-induced atherosclerosis in LDL receptor knock-out mice. Considering that GPRs are druggable targets[12], the latter finding nourished hope for the development of small molecules to inhibit GPR146 to improve the management of dyslipidemia and atherosclerosis, particularly in patients suffering from homozygous familial hypercholesterolemia (hoFH), lacking the LDL receptor[13,14]. The effects of GPR146 on plasma lipids in mice are associated with the fasting-refed state[11], but it is not known what the natural ligand for cellular GPR146 activation is. C-peptide, a cleavage product from insulin has been reported to play a role[15] but this was later disputed[16], and GPR146 remains an orphan receptor to date.

The current study provides a positive answer to the key question whether loss of *GPR146* in humans is associated with reduced plasma cholesterol as main factor for cardiovascular disease. We show that genetic impairment of GPR146 is a novel genetic cause of low LDL-C, similar to reports for mutations in *APOB*, *MTTP*, *ANGPTL3* and *PCSK9*, all targets for pharmaceutical intervention to lower plasma cholesterol[17]. Our findings support the idea that GPR146 is a relevant target to reduce plasma cholesterol with concomitant positive effects on liver function.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Association between genetic markers and *GPR146* gene expression

Expression quantitative trait loci (eQTL) analyses aim to connect genetic variants with the expression of genes, or specifically of nearby genes, in case of cis-eQTL. We used the eQTL dataset from the eQTLGen consortium[18], the largest eQTL repository available so far (**supplementary materials**). We tested the association of rs2362529 and rs1997243 with the expression of nearby genes including *GPR146* in blood. Additionaly, we studied the effects of rs2362529 and rs1997243 on *GPR146* expression in the human liver the eQTL dataset from GTEx (V8 release, https://gtexportal.org/)[19].

Genetic associations with plasma circulating biomarkers in the UK Biobank

The UK Biobank study is a population-based prospective cohort in the United Kingdom in which approximately 500,000 individuals aged between 40 and 69 years were recruited from 2006 through 2010. All participants have given informed consent for this study. The UK Biobank has ethical approval from North West - Haydock Research Ethics Committee (REC reference: 16/NW/0274). Details of the UK Biobank study have been described previously[20]. This research has been conducted using the UK Biobank resource under application number 15031. Clinical characteristics and biomarkers of individuals, variant genotyping, imputation and quality controls as well as genetic and statistical analyses are described in **supplementary materials, Tab.S1 and Fig.S2**.

Genetic association with coronary artery disease

Genetic association with coronary artery disease was assessed by using summary data from the meta-analysis published by Nikpay et al.[21] (http://www.cardiogramplusc4d.org/). In order to test the relevance of these effects, we compared it with well-established LDL-C associated variants (as presented by Ference *et* al. in the consensus statement from the European Atherosclerosis Society Consensus Panel[17]) using data from van der Harst et al.[22].

Selection of individuals with extreme LDL-C and targeted sequencing

Individuals with extreme dyslipidemia are likely to carry rare genetic variants (loss or gain of function variants) in genes with large effects on lipid metabolism, and we set out to find such mutations in *GPR146* in two large population-based prospective cohort studies: the Lifelines (n=167, 729 individuals from the Netherlands[6,23,24]) and the Copenhagen City Heart Study (CCHS n=20,000 individuals from Denmark[25,26]). We selected unrelated individuals with extremely low LDL-C (n=222 from Lifelines and n=195 from CCHS, LDL-C < 1st and 2nd percentiles respectively) and extremely high LDL-C (n=129 from Lifelines and n=194 from CCHS, LDL-C > 99th and 98th percentiles respectively) (**Tab.S2 and S3**). The next generation sequencing platform and analysis workflow that were used are detailed in the **supplementary materials**.

The proband of a familial case with hypocholesterolemia (LDL-C <5th percentile for age and sex) was recruited in the context of the HYPOCHOL trial (ClinicalTrials ID: NCT02354079). The genetic screening was performed using the DysliSEQ custom NGS panel as described previously[27].

Statistics and plots

Statistical analyses and tools used for plotting are described in the **supplementary materials**.

RESULTS

COMMON VARIANTS IN THE *GPR146* GENE LOCUS

Rs1997243, rs2362529 and GPR146 mRNA expression

GPR146 is ubiquitously expressed but its expression in the liver has been shown to dictate changes in plasma lipids in *Gpr146*$^{-/-}$ mice. With this in mind we first consulted GPR146 expression in the human liver using the eQTL dataset from GTEx.

We first studied rs1997243, the genetic variant that has led to the identification of *GPR146* as a candidate lipid gene[8,9,11,28] which is present in 14% of the general population. As can be appreciated from **Fig.S1A**, the alternative G-allele of rs1997243 tends to increase GPR146 expression as previously shown by Han *et* al.[9]. Following close examination of the *GPR146* gene locus, we noticed a second common *GPR146* variant, annotated as rs2362529. This variant has not been studied previously, and is present with a prevalence of 21% of the general population. Opposite to the findings of the alternative allele of rs1997243, the C-allele of rs2362529 tends instead to decrease *GPR146* expression **(Fig.S1B)**. In both cases, however, these changes did not reach statistical significance which is likely related to the small number of samples (n=208) in this dataset.

To further study GPR146 expression in carriers of both common variants, we made use the fact that GPR146 is also expressed in circulating blood cells and made use of the eQTL dataset from the eQTLGen consortium which comprises data of 31,684 human subjects. In line with findings in human liver, carriers of the rs1997243-G allele exhibit higher *GPR146* expression levels in circulating bood than non carriers ($p=3.3e^{-310}$). In contrast carriers of the rs2362529-C allele exhibit lower *GPR146* mRNA expression in blood than non carriers ($p=1.8e^{-21}$) (**Fig.1A**).

The two above described common *GPR146* variants are not in linkage disequilibrium (r^2= 0.0172 in the 1000 genomes population[29]). This indicates that rs1997243 and rs2362529 can be regarded as independent genetic research instruments with opposite effects on *GPR146* gene expression.

Rs1997243 and rs2362529, lipid traits, metabolic traits and CRP

To study the phenotypes of carriers of rs1997243 or rs2362529, we used the UK Biobank patients who passed our selection criteria (n=412,444 participants; **Tab.S1, Fig.S2**).

Plasma lipid traits

Fig.1B shows that carriers of the rs1997243-G allele (heterozygotes and homozygotes combined) present with increased total cholesterol, LDL-C, HDL-C, with concomitant increases in apolipoproteins compared to non-carriers, without changes in triglycerides and lipoprotein (a) (Lp(a)) (**Tab.S4)**. These effects were found to be gene-dose dependent (**Fig.S3A** and **Tab.S5**).

In carriers of the rs2362529-C allele, we observed the exact opposite, *i.e.* reduced levels of total cholesterol, LDL-C and HDL-C with concomitant reductions in apolipoproteins and no changes in plasma triglycerides and Lp(a) compared to non-carriers (**Fig.1B - Tab.S4**). Just like for the other variant the effects were gene dose-dependent (**Fig.S3B - Tab.S5**).

Metabolic traits and C-reactive protein

Although it has been reported that GPR146 plays a role in the differentiation of adipocytes[10], carriers of rs1997243-G or rs2362529-C do not show differences in body mass index (BMI) compared to

non-carriers (data not shown). Compared to non-carriers, carriers of the rs1997243-G allele are, to our surprise, characterized by increased levels of circulating gamma-glutamyltransferase, alkaline phosphatase, aspartate aminotransferase while the opposite is true for carriers of the rs2362529-C allele (**Fig.1C - Tab.S4,5**).

In addition, carriers of rs1997243-G or rs2362529-C allele(s) have increased or decreased levels of C-reactive protein (CRP) plasma levels, compared to non-carriers, respectively (**Fig.1D - Tab.S4,5**). Finallly, carriers of the rs2362529-C allele present with lower glycated haemoglobin (HbA_{1C}) levels than non-carriers (**Fig.1E - Tab.S4,S5**).

These results indicate that carriers of the rs1997243-G or rs2362529-C allele(s) have unfavorable or favourable lipid and metabolic profiles, respectively. Combined, these findings prompt the question whether these differences translate into an altered risk of coronary artery disease (CAD).

Rs1997243, rs2362529 and risk of CAD

In a meta-analysis from the CARDIoGRAMplusC4D Consortium, carriers of the rs2362529-C allele show a trend towards a decreased risk of CAD compared to non-carriers (**Fig.2A**). The reduction in CAD risk in carriers of *rs2362529* appears marginal but this was anticipated when considering the magnitude of the effect size of this variant on LDL-C levels: homozygotes for *rs2362529* show an average decrease of 2.24 mg/dl compared to carriers of the wild-type allele. To study this further, we investigated the effecs of both *GPR146* gene variants on LDL-C and CAD in the context of well characterized genetic variants in *APOB*, *PCSK9*, *HMGCR*, *ANGPTL3*[17]. As can be appreciated from **Fig.2B**, *GPR146 rs18997243* and *rs2362529* fit the regression line between the magnitude of LDL-C changes and the magnitude of effects on CAD data from van der Harst et al.[22]

RARE VARIANTS IN THE *GPR146* GENE

Identification of a missense variant (GPR146-p.Pro62Leu)

With the finding that common *GPR146* variants are associated with opposite effects on *GPR146* expression and plasma lipid traits, we set out to identify *GPR146* mutations with large effects on plasma levels of LDL-C. Taken that *Gpr146* knockout mice have reduced LDL-C and are protected against atherosclerosis[11], we hypothesized that loss or gain-of-function variants could be found in individuals with low and high LDL-C, respectively. In this effort, we sequenced genomic DNA of individuals with extreme LDL-C plasma levels in the Lifelines and CCHS cohorts (**Tab.S2 and S3**).

Following targeted sequencing, we selected rare variants which affect the protein sequence (or splicing regions) of *GPR146* (**Tab.S6**). Most of these rare variants were singletons, however, one missense variant, annotated as rs151124717, was found eight times in individuals with low LDL-C and only once in an individual with high LDL-C (**Fig.3A**). The nucleotide substitution (c.185C>T) results in the exchange of Proline for a Leucine residue at position 62 of the mature GPR146 protein (p.Pro62Leu) (**Fig.3B**). The missense variant is located in the first intracellular loop of GPR146 and affects an highly evolutionary conserved protein domain (**Fig.3C - Fig.S4A**). It is predicted to be damaging for the protein function by seven *in silico* algorithms (**Fig.S4B)**.

Carriers of the *GPR146*-p.Pro62Leu variant in the Lifelines and CCHS cohorts were all heterozygotes and did not carry mutations in genes that are known to cause familial forms of hypocholesterolemia (*APOB, MTTP, PCSK9, ANGPTL3*) or hypercholesterolemia (*LDLR, APOB,*

LDLRAP1, *APOE*, *PCSK9*). In a next step we screened the UK Biobank for carriers of the *GPR146*-p.Pro62Leu to examine their cadiometabolic phenotype.

Carriers of GPR146-p.Pro62Leu in the UK Biobank

Plasma lipid traits

In the UK Biobank, we identitifed 2,615 carriers of the *GPR146*-p.Pro62Leu variant (**Tab.S7)** which renders an allele frequency of 0.3% of the general population (in line with data from gnomAD, https://gnomad.broadinstitute.org/). In a direct comparison with carriers of the common rs2362529-C allele, it is clear that carriers of the *GPR146*-p.Pro62Leu present with larger reductions of total cholesterol, LDL-C and HDL-C, suggesting that this variant impairs the function of the protein as predicted (**Fig.3D**, **Tab.S4**, **Fig.S4B**).

Supporting, the relation between *GPR146* gene variants and atherosclerosis, the magnitude of the LDL-C lowering effect of *GPR146*-p.Pro62Leu (rs151124717) again correlates with the expected effect on CAD (**Fig.2B**). The effects of the *GPR146*-p.Pro62Leu variant on CAD did, however, not reach statistically significance, likely due to large standard errors.

Metabolic traits and CRP

Carriers of the *GPR146*-p.Pro62Leu variant presented with significant reductions in gamma-glutamyltransferase and alkaline phosphatase when compared to non-carriers (**Fig.3E - Tab.S4**). In addition, carriers of the *GPR146*-p.Pro62Leu also presented with significantly reduced CRP levels when compared to non-carriers (**Fig.3F and Tab.S4**).

Combined, these data illustrate that carriers of the *GPR146*-p.Pro62Leu variant are characterized by and overall beneficial cardiometabolic phenotype. The remarkable lipid profile of individuals carrying this variant, i.e. reductions in all major plasma lipids (**Fig.3D**), resembles those suffering from familial hypocholesterolemia[30], which prompted us to sequence the *GPR146* gene in familial cases with unexplained hypocholesterolemia.

Segregation of GPR146 p.Pro62Leu with hypocholesterolemia

In a familial case of hypobetalipoproteinemia from the HYPOCHOL clinical trial[31], in which mutations in canonical lipid genes were excluded, one proband was found to be heterozygous for the *GPR146*-p.Pro62Leu variant (**Fig.4**). This 24 year-old woman presented with very low levels of total cholesterol (124 mg/dL), LDL-C (59 mg/dL), apoB (60 mg/dL) and triglycerides (50 mg/dL), but normal HDL-C (55 mg/dL). We recruited six family members and tested the co-segregation of the *GPR146*-p.Pro62Leu variant with plasma LDL-C levels in this family (**Fig.4 - Tab.S8**). The results show the cosegregation of the genotype with the hypobetalipoproteinemia phenotype: all three individuals carrying the *GPR146*-Pro62Leu variant present with LDL-C <5th percentile for age and sex[32]. A broader screening of *GPR146* in FHBL cases is needed in order to further support *GPR146* as a monogenic origin of FHBL.

DISCUSSION

The key novel finding in this study is that carriers of new genetic variants affecting *GPR146* are characterized by low levels of plasma lipids. More specifically, we show that carriers of a common variant in the *GPR146* locus (rs2362529) have lower plasma levels of LDL-C, HDL-C, apoB, and apoAI with more pronounced effect in homozygotes compared to heterozygotes. The observed reduction in LDL-C in these carriers is shown to be concordant with the reduction in risk of CAD. These finding, combined with the notion that *Gpr146* deficient mice also have reduced LDL-C and HDL-C[9,11], have led us to hypothesize that mutations in *GPR146* may constitute a novel cause of hypolipidemia. Support for this concept comes from carriers of a rare coding variant, *GPR146*-p.Pro62Leu (predicted to be damaging for GPR146 functions), who are indeed characterized by reduced LDL-C, HDL-C but also triglycerides compared to non-carriers. Segregation of this rare variant with low LDL-C in a family with hypocholesterolemia provides further support for a novel role for GPR146 in human hypolipidemia.

Previous studies have already shown that GPR146 is associated with increased levels of plasma lipids[8,28,33]. Corroborating and extending these findings, we here show that carriers of rs1997243 or rs2362529, common variants in the *GPR146* locus, are characterized by opposite changes in plasma lipids. These findings bear similarity to opposite lipid profiles in carriers of rare variants in the *APOB* and *PCSK9* genes: loss-of-function mutations in *APOB* and *PCSK9* cause hypobetalipoproteinemia, while gain-of-function mutations cause hypercholesterolemia[34].

In a next step, we set out to identify *GPR146* mutations with larger effect sizes. In two white Caucasian general population samples, we found that *GPR146*-p.Pro62Leu was overrepresented in subjects with low LDL-C compared to individuals with high LDL-C. The proline residue at position 62 is highly evolutionary conserved and accordingly, substitution to a leucine residue is predicted to be deleterious on the function of GPR146. Notably, carriers of the rare *GPR146*-p.Pro62Leu variant are characterized by stronger reductions in plasma lipids compared to carriers of the common rs2362529 variant which suggests that *GPR146*-p.Pro62Leu may be a functional mutation. This is further supported by the cosegregation of the *GPR146*-p.Pro62Leu heterozygous variant with a low LDL-C phenotype in a family with hypocholesterolemia. However further functional investigations are needed to proof the functional impact of this variant in in-vitro/vivo models.

In contast to products of established genes in lipid metabolism such as *APOB*, *MTTP*, *APOC3*, *ANGPTL3*, and *LDLR*, the GPR146 protein is not directly involved in the machinery to synthesize lipoproteins or to clear them from the circulation. It is, in fact, the first protein known to date to control plasma lipid traits through cellular signaling pathways: GPR146 has previously been shown to increase *de novo* cholesterol synthesis in the liver through activation of the extracellular signal-regulated kinase signaling pathway[11]. The downstream effect on hepatic VLDL secretion was shown to contribute to the plasma lipid phenotype of *Gpr146* knock out mice. Whether these findings translate to humans needs to be establised.

Using two common *GPR146* variants and one rare (*GPR146*-p.Pro62Leu) variant as genetic instruments, the current study shows that *GPR146* is also associated with changes in circulating levels of hepatic enzymes and HbA_{1C}. When considering the previously suggested development of GPR146 antagonists[9,11,13,14], the current genetic data show that no adverse effects on the onset of type 2 diabetes, as described for genetic variants in *HMGCR* (the target of statins) and *PCSK9* (targets of evolocumab and alirocumab), are anticipated[35–37]. In addition, the association of GPR146 with plasma levels of CRP, a biomarker for systemic inflammation, is of particular interest[38,39] when considering the central role of inflammation in atherogenesis[40]. Finally, loss of GPR146 function is

not associated with elevated hepatic enzymes, commonly used as markers of hepatic steatosis or NAFLD, an adverse effect of Mipomersen (an *APOB* antisense oligonucleotide) and Lomitapide (a small molecule MTP inhibitor) used to treat hoFH[41]. In this regard we think it is of interest to note that, in mice, the protection against atherosclerosis is independent of the presence of the LDL receptor, which makes GPR146 an interesting therapeutic target for this group of patients[11].

The current study presents *GPR146* as a novel candidate gene for human hypolipidemia. The findings support the development of strategies to antagonize GPR146 to reduce plasma levels of cholesterol and atherosclerosis. This could be achieved through hepatic mRNA silencing approaches such as employed for PCSK9, ANGPTL3, APOC3 and LPA[42] (https://clinicaltrials.gov/) or through targeting the genomic DNA in hepatocytes with CRISPR[43]. As a G-coupled protein receptor, GPR146 also offers the option to find small molecule inhibitors but at this point deorphanization of GPR146 is warranted to unravel the molecular mechanisms that underlie the observations in this study.

Limitations

Despite great efforts by our groups, we have not been able to provide evidence that *GPR146*-p.Pro62Leu is a functional mutation. Our *in vitro* as well as *in vivo* systems have been inadequate to address the functionality of GPR146 variants. This is clearly complicated by the notion that the ligand for GPR146 as well as specific readouts of GPR146 activity remains to be identified. However, the circumpstancial evidence that we provide in this study combined with the clearcut evidence that *Gpr146*$^{-/-}$ mice present with lower plasma lipids, makes, in our opinion, a strong case of the actual involvement of attenuated GPR146 function in low levels of plasma lipids and a favourable metabolic phenotype in humans. Hopefully the consequences of human genetic *GPR146* deficiency will soon give the required unequivocal evidence. It is in this regard good to note that whole body ablation of *Gpr146* in mice is compatible with life.

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The authors have nothing to disclose

LIST OF SUPPLEMENTAL MATERIALS

Online Figures S1-S4
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HIGHLIGHTS

- lower *GPR146* gene expression, is associated with beneficial effects on plasma: -lipids, -liver enzymes and hsCRP, with a concordant reduction in CAD risk.
- The *GPR146*-p.Pro62Leu coding rare variant induces larger reductions of plasma lipids, liver enzymes and CRP compared to the common variant.
- GPR146 deficiency may be a new genetic cause of familial hypobetalipoproteinemia and a potential target for intervention to reduce plasma cholesterol levels and atherosclerosis with a particularly good metabolic safety profile.

FIGURES LEGENDS

FIGURE 1

Panel A: Effects of rs2362529 and rs1997243 on the expression of *GPR146* in whole blood (Source: https://www.eqtlgen.org). The upper part of the figure shows the effects of rs2362529 (green arrows) while the bottom part shows the effects of rs1997243 (red arrows) on nearby genes. + and – signs show the direction of the effects with corresponding p values.

Panel B: Lipids, lipoproteins, and apolipoproteins in plasma as a function of the C-allele of rs2362529 (green symbols) and the G-allele of rs1997243 (red symbols) in individuals from UK Biobank cohort. The effects are shown as beta-coefficients (per z-score unit) per C-allele or G-allele compared with non-carriers respectively. Horizontal bars depict standard errors. N; number of individuals included in the analysis, LDL; low density lipoprotein, ApoB; apolipoprotein B, ApoAI; apolipoprotein AI, Lp(a); lipoprotein a.

Panel C: Plasma liver enzymes as a function of the C-allele of rs2362529 (green symbols) and the G-allele of rs1997243 (red symbols) in individuals from UK Biobank cohort. The effects are shown as beta-coefficients (per z-score unit) per C-allele or G-allele compared with non-carriers respectively. Horizontal bars depict standard errors. N; number of individuals included in the analysis, ALT; alanine transaminase, AST; aspartate transaminase, GGT; ammaglutamyl-transferases, ALP, alkaline phosphatase.

Panel D: Plasma C-reactive protein as a function of the C-allele of rs2362529 (green symbols) and the G-allele of rs1997243 (red symbols) in individuals from UK Biobank cohort. The effects are shown as beta-coefficients per z-score unit) per C-allele or G-allele compared with non-carriers respectively. Horizontal bars depict standard errors, N; number of individuals included in the analysis, CRP; C-reactive protein.

Panel E: Plasma glucose or HbA_{1C} levels as a function of the C-allele of rs2362529 (green symbols) and the G-allele of rs1997243 (red symbols) in individuals from UK Biobank cohort. The effects are shown as beta-coefficients (per z-score unit) per C-allele or G-allele compared with non-carriers. Horizontal bars depict standard errors. N; number of individuals included in the analysis, HbA_{1C}; glycated haemoglobin.

FIGURE 2

Panel A: Odds ratio for coronary artery disease as a function of the C-allele of rs2362529 and the G-allele of rs1997243 compared with non-carriers. The effects are shown as odds-ratio per C-allele or

G-allele respectively compared with non-carriers. Horizontal bars depict standard errors. N; number of individuals included in the analysis, CAD; coronary artery disease.

Panel B: Proportional risk of CAD associated with different genetic variants that affect LDL-c levels. All values are presented as magnitude of change (positive values). The regression line shows the proportional risk of CAD as a function of genetically determined change in LDL cholesterol (in mmol/L). The vertical and horizontal error bars are standard errors for the risk of CAD and LDL-c levels respectively.

FIGURE 3

Panel A: Selection of individuals with extreme LDL-C plasma levels (<1st or 2nd percentile for age and sex from Lifelines cohort and CCHS, respectively and >99th or 98th percentile for age and sex from Lifelines cohort and CCHS respectively). Green crosses depict carriers of the *GPR146*-p.Pro62Leu.

Panel B: Schematic view of the *GPR146* gene located on the 7p22.3 locus and the variant *GPR146*-p.Pro62Leu (chr7:1057700 (GRCh38.p13); NM_138445.4:c.185C>T; NP_612454.1:p.Pro62Leu).

Panel C: Schematic view of the predicted amino-acids sequence and protein structure of GPR146. The red arrow indicates the Proline residue at position 62 of the protein located in the first intracellular loop. The total protein consists in 333 amino-acids.

Panel D: Lipids, lipoproteins, and apolipoproteins in plasma as a function of the T-allele of rs151124717 (green symbols) and the C-allele of rs2362529 (gray symbols) in individuals from the UK Biobank cohort. The effects are shown as beta-coefficients (per z-score unit) per T-allele or C-allele compared with non-carriers respectively. Horizontal bars depict standard errors. N; number of individuals included in the analysis, LDL; low density lipoprotein, ApoB; apolipoprotein B, ApoAI; apolipoprotein AI, Lp(a); apolipoprotein a.

Panel E: Plasma liver enzymes as a function of the minor T-allele of rs151124717 (green symbols) and the minor C-allele of rs2362529 (gray symbols) in individuals from the UK Biobank cohort. The effects are shown as beta-coefficients (per z-score unit) per T-allele or C-allele compared with non-carriers respectively. Horizontal bars depict standard errors. N; number of individuals included in the analysis, ALT; alanine transaminase, AST; aspartate transaminase, GGT; gammaglutamyl-transferases, ALP, alkaline phosphatase.

Panel F: Plasma C-reactive protein levels as a function of the minor T-allele of rs151124717 (green symbols) and the minor C-allele of rs2362529 (gray symbols) in individuals from the UK Biobank cohort. The effects are shown as beta-coefficients (per z-score unit) per T-allele or C-allele compared with non-carriers. Horizontal bars depict standard errors. N; number of individuals included in the analysis, CRP; C-reactive protein.

FIGURE 4

Pedigree of a family with familial hypobetalipoproteinemia. Carriers of the GPR146-p.Pro62Leu variant are depicted with green symbols (II:6, III:3, and III:5/proband) and all have LDL-C values below the 5^{th} percentile. Abbreviations: TC (total cholesterol), LDL-C (low-density lipoprotein cholesterol), HDL-C (high-density lipoprotein cholesterol) and triglycerides plasma levels are presented in mg/dL. Circles = women and squares = men; *, Values between brackets show percentile for corresponding values adjusted for age and sex.