Metabolic risk and cardiovascular disease: insights from large biobanks with genetic epidemiological approaches

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CHAPTER 3

The role of genetically-influenced phospholipid transfer protein activity in lipoprotein metabolism and coronary artery disease

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Abstract

Background: Phospholipid transfer protein (PLTP) transfers surface phospholipids between lipoproteins and as such plays a role in lipoprotein metabolism, but with unclear effects on coronary artery disease (CAD) risk. We aimed to investigate the associations of genetically-influenced PLTP activity with 1-H nuclear magnetic resonance (^{1}H-NMR) metabolomic measures and with CAD. Furthermore, using factorial Mendelian randomization (MR), we examined the potential additional effect of genetically-influenced PLTP activity on CAD risk on top of genetically-influenced low-density lipoprotein-cholesterol (LDL-C) lowering.

Methods: Using data from UK Biobank, genetic scores for PLTP activity and LDL-C were calculated and dichotomised based on the median, generating four groups with combinations of high/low PLTP activity and high/low LDL-C levels for the factorial MR. Linear and logistic regressions were performed on 168 metabolomic measures (N=58,514) and CAD (N=318,734, N-cases=37,552), respectively, with results expressed as β coefficients (in standard deviation units) or odds ratios (ORs) and 95% confidence interval (CI).

Results: Irrespective of the genetically-influenced LDL-C, genetically-influenced low PLTP activity was associated with a higher HDL particle concentration (β [95% CI]: 0.03 [0.01, 0.05]), smaller HDL size (-0.14 [-0.15, -0.12]) and higher triglyceride (TG) concentration (0.04 [0.02, 0.05]), but not with CAD (OR 0.99 [0.97, 1.02]). In factorial MR analyses, genetically-influenced low PLTP activity and genetically-influenced low LDL-C had independent associations with metabolomic measures, and genetically-influenced low PLTP activity did not show an additional effect on CAD risk.

Conclusions: Low PLTP activity associates with higher HDL particle concentration, smaller HDL particle size and higher TG concentration, but no association with CAD risk was observed.

Keywords: Phospholipid transfer protein (PLTP) activity; Lipoprotein metabolism; Genetic risk score; Coronary artery disease

1. Introduction

The phospholipid transfer protein (PLTP) facilitates the transfer of surface phospholipids between lipoprotein particles. For example, during the lipolytic conversion of triglyceride-rich lipoproteins (TRLs), PLTP transfers excess phospholipids to high-density lipoprotein (HDL), allowing TRL remnants to reduce in size (1-3). As such, PLTP plays an important role in the metabolism of both TRLs and HDL, and affects their particle size and composition. Evidence regarding the association of PLTP activity with coronary artery disease (CAD) is inconsistent. While several studies have suggested high PLTP activity is a risk factor for CAD (4-7), one study found this for men only (8), and another study suggested the opposite (9).

In a previous Mendelian randomization (MR) study, we observed that a specific lipoprotein risk profile (determined by principal component analysis) characterised by high levels of large HDL and low levels of small HDL was associated with increased risk of CAD (10). This association was found to be independent of apolipoprotein B (ApoB). Interestingly, the *PLTP* gene was one of the leading genetic variants responsible for this metabolomic risk profile. In addition, independent of our studies, a PLTP genetic score representing low PLTP activity was previously found to be associated with small HDL size, and lower cardiovascular risk (11).

Inspired by these observations, we hypothesized that PLTP activity could not only be involved in the HDL particle size distribution, but also in the association with CAD. An inverse association between HDL-cholesterol (HDL-C) and cardiovascular disease has long been established in robust prospective cohort analyses (12-14), but the causative role of HDL in CAD is complex and remains to be investigated further (15-18). Cumulative evidence suggests that potential atheroprotective functions of HDL, such as reverse cholesterol transport, cholesterol efflux, and inactivation of biohazards, may depend on specific HDL sub-particle characteristics which cannot be estimated via the simple measurement of HDL-C (19-21). Metabolomics analysis using 1-H nuclear magnetic resonance (^{1}H-NMR) spectroscopy provides detailed measures on the number, size and composition of the different lipoprotein (sub)classes that can serve as intermediate phenotypes between genetic variation and environmental influences that affect disease risk (22, 23). Thus, investigation of the association of PLTP activity with different metabolomic measures may provide additional insight into the biological mechanisms underlying the potential association of PLTP activity with CAD.

Therapy aimed at lowering low-density lipoprotein cholesterol (LDL-C) is the mainstay of risk reduction in atherosclerotic cardiovascular disease prevention (24). Therefore, the

potential added value of PLTP activity as therapeutic target should be investigated on the background of LDL-C lowering therapy. A factorial MR design provides a method to investigate the interaction effects of two different treatments by using genetic variants as treatment proxies (25). This approach has been used previously to explore potential therapeutic targets on top of existing therapies (26-28).

In the present study, we set out to investigate the association of low PLTP activity, determined by genetic predisposition, with metabolomic measures and CAD, and examine its potential additional cardioprotective benefits on top of that of genetically-influenced low LDL-C levels.

2. Methods

2.1 Study population and design

The present study was embedded in the prospective UK Biobank (UKB) cohort, which recruited 502,628 participants aged 40-69 years across the entire United Kingdom during the baseline survey between 2006 and 2010. The UKB cohort study was approved by the North-West Multicentre Research Ethics Committee (MREC), and access for information to invite participants was approved by the Patient Information Advisory Group (PIAG) from England and Wales. All participants provided electronic written informed consent for the study. A detailed description of the UKB cohort study has been presented elsewhere (29). To minimize population stratification bias and influences from related participants, we restricted the study participants to 318,734 unrelated individuals with European ancestry, based on the estimated kinship coefficients for all pairs and the self-reported ancestral background (30).

A total of 58,514 unrelated European-ancestry UKB individuals with complete NMR metabolomics data and no history of CAD or cholesterol-lowering drug therapy were used to investigate the association with metabolomic measures, while 318,734 UKB individuals with 37,552 CAD cases and complete data were included to investigate the associations with CAD (Figure S1). A two-by-two factorial MR design was used to investigate the interaction effects of PLTP activity-lowering genetic variation and LDL-C-lowering genetic variation.

2.2 Coronary artery disease

According to the International Classification of Diseases edition 10 (ICD-10), CAD is defined as angina pectoris (I20), myocardial infarction (MI) (I21 and I22), and acute and chronic ischemic heart disease (IHD) (I24 and I25), all of which could be found online (https://biobank.ndph.ox.ac.uk/showcase/label.cgi?id=2409). These variables have been generated by the UKB data management team through a standard algorithm (https://biobank.ndph.ox.ac.uk/showcase/ukb/docs/first_occurrences_outcomes.pdf), combining self-reported health conditions from baseline and linked data from hospital admissions, primary care, and death registers. The linked data and its sources, mainly from National Health Service, were presented here (https://biobank.ndph.ox.ac.uk/showcase/exinfo.cgi?src=Data_providers_and_dates). The present study used the sum of prevalent and incident cases as main outcomes.

2.3 Metabolomic measures

The blood sample collection of UKB participants was undertaken at baseline between 2006 and 2010, and the blood sample handling and storage protocol has been previously described (31). The details of obtaining the NMR-based metabolomics data for UKB participants have been described elsewhere (https://biobank.ndph.ox.ac.uk/showcase/label.cgi?id=220) and in previous publications (32, 33). In brief, EDTA plasma samples collected at baseline recruitment were measured between June 2019 and April 2020 using a high throughput 1H-NMR-metabolomics platform (Nightingale Health, Helsinki, Finland).

This study included 168 direct metabolomic measures, with apolipoproteins (n = 2), lipoprotein particle sizes and concentration (n = 7), lipoprotein (sub)classes (n = 98), cholesterol (n = 15), triglycerides (n = 4), phospholipids and other lipids (n = 8), total lipids (n = 4), glycolysis related metabolites (n = 4), inflammation (n = 1), fluid balance (n = 2), fatty acids (n = 9), amino acids (n = 10), and ketone bodies (n = 4). Their characteristics in the study population are presented in Table S1. All metabolomic measures were processed using rank-based inverse normal transformation to approximate a normal distribution with standard deviation one and mean zero.

2.4 Genetic variants and genetic scores

The list of variants used for calculating the genetic scores for PLTP activity and LDL-C is given in Supplementary Table 2. For the genetic score of LDL-C, 58 independent genetic variants, which showed genome-wide significant associations ($P < 5 * 10^{-8}$) with LDL-C levels in Global Lipids Genetics Consortium (GLGC) (34), were selected based on the paper of Lotta et al., (26) which did not contain data from UKB. Those genetic variants were over 500 kb away from each other and had low linkage disequilibrium ($R^2 < 0.01$). More details on the selection of these LDL-C variants have been described by Lotta et al. (26) The genetic score of LDL-C was calculated by adding the number of 58 LDL-C-lowering alleles, weighted by their corresponding effects on LDL-C.

A previous study observed that the variants of rs6065904 (effect allele: A) and rs378144 (effect allele: C) were independently associated with lowering PLTP activity in both study samples (11), and another study also identified these two SNPs to be associated with PLTP activity in a general population cohort (35). Therefore, the unweighted genetic score of PLTP activity in the present study was calculated based on the total number of these two PLTP activity-lowering alleles, ranging from 0 to 4.

2.5 Factorial MR design

The LDL-C genetic score was dichotomized into low and high LDL-C levels based on the median value. Similarly, the PLTP activity genetic score was dichotomized into high and low PLTP activity levels.

According to the principles of factorial MR (25), participants were "naturally randomized" into 4 groups: (1) a reference group, with genetically-influenced high LDL-C and high PLTP activity levels, as a proxy for placebo (2) a group with genetically-influenced low PLTP activity levels, as proxy for lowering PLTP activity only and (3) a group with genetically-influenced low LDL-C levels, as proxy for LDL-C lowering therapy only (4), and a group with both genetically-influenced low PLTP activity and low LDL-C levels, as proxy for targeting PLTP activity-lowering therapy on top of LDL-C-lowering therapy. The LDL-C values in groups with genetically-influenced low/high LDL-C levels and in the four "naturally randomized" groups are presented in Table S3.

2.6 Statistical analysis

To estimate associations of the dichotomised PLTP activity genetic score with metabolomic measures and CAD, irrespective of the LDL genetic risk score, linear regression and logistic regression were performed, respectively, with results expressed as the β coefficients (in standard deviation (SD) units) or odds ratios (ORs) and 95% confidence interval (CI). To provide evidence regarding the effects of PLTP activity on CAD in additional settings and cohorts, we also investigated the associations of individual SNPs of PLTP activity (rs6065904 and rs378144) with CAD and extracted their corresponding estimated summary statistics from CARDIoGRAMplusC4D (N = 184,305 with cases = 60,801) and FinnGen consortia (N = 309,154 with cases = 33,628).

In factorial MR analyses, for each group relative to the reference group, the associations with plasma metabolomic measures were estimated by linear regression, and associations with CAD by logistic regression, with results expressed as the β coefficient and ORs, respectively. In addition, we performed interaction analyses for the continuous genetic scores of PLTP activity and LDL-C in order to test whether they had synergetic effects on the metabolomic measures and CAD, respectively.

To correct for possible population stratification, all regression models described above were adjusted for age, sex, and the first ten genomic principal components. Given the high intercorrelations between metabolomic measures, the 'effective number' designed to identify

independent metabolomic traits was used to correct for multiple testing according to Li et al (36). The statistically significant threshold of the *P* value was thus then defined as 1.79e-3 with an effective number of 28. We further examined the interaction effects of sex and continuous genetic score of PLTP activity on metabolomic measures and CAD, respectively, and then repeated the above main analyses for women and men separately to test the potential effect-measure modification by sex.

Moreover, the unweighted genetic score of PLTP activity constructed by only two genetic variants could potentially lead to missing information. Since the initial study we cited also found rs2294213 to be associated with PLTP activity in one of the two cohorts (11), we additionally included this SNP as a sensitivity analysis by calculating the genetic score of PLTP activity based on three SNPs, and examined its associations with the metabolomic measures and CAD. We also examined the associations between genetically-influenced PLTP activity and CAD in people with (N = 19,962, cases = 3,710) and without (N = 309,516, cases = 33,190) type 2 diabetes mellitus to assess whether there is effect-modification by type 2 diabetes mellitus.

All statistical analyses were performed using R (version 4.3.0) software, with 'circlize' package for circular plots (37).

3. Results

3.1 Participants characteristics

Characteristics of the study population for metabolomic measures (N = 58,514 with 43.1% men) and CAD (N = 318,734 with 45.8% men) are summarized in **Table 1**. The medians of the PLTP activity and LDL-C genetic scores, as well as the distribution of the "naturally randomized" genotype groups, were similar between the two study population. Both mean and median values of LDL-C were lower in the group with genetically-influenced low LDL-C level compared to the group with genetically-influenced high LDL-C level (Table S3).

Table 1. Characteristics of the study population included in the analyses for metabolomic measures and coronary artery disease

Characteristics	Study population for metabolomic measures	Study population for CAD
Participants, No.	58,514	318,734
Age (mean (SD))	55.69 (8.02)	56.75 (8.02)
Sex, men (No. (%))	25,236 (43.1)	146,031 (45.8)
GRS of LDL-C (median [IQR])	-3.31 [-3.50, -3.12]	-3.30 [-3.49, -3.11]
GRS of PLTP activity (median [IQR])	2.00 [1.00, 3.00]	2.00 [1.00, 3.00]
Genotype category (No. (%))		
high PLTP activity and high LDL-C levels	10,093 (17.2)	54,755 (17.2)
low PLTP activity and high LDL-C levels	19,164 (32.8)	104,612 (32.8)
high PLTP activity and low LDL-C levels	10,221 (17.5)	54,934 (17.2)
low PLTP activity and low LDL-C levels	19,036 (32.5)	104,433 (32.8)

CAD, coronary artery disease; GRS, genetic risk score; IQR, interquartile range; LDL-C, low-density lipoprotein-cholesterol; PLTP, phospholipid transfer protein; SD, standard deviation.

3.2 Associations of genetically-influenced PLTP activity with metabolomic measures and CAD irrespective of genetically-influenced LDL-C

The estimated associations between the dichotomised genetic score of PLTP activity and metabolomic measures are shown in **Figure 1**. Compared to participants with genetically-influenced high PLTP activity, the group with genetically-influenced low PLTP activity had a higher HDL particle concentration (β [95% CI]: 0.03 [0.01, 0.05]), lower levels of large and very large HDL particles and higher levels of small HDL particles resulting in a smaller average HDL particle size (β [95% CI]: -0.14 [-0.15, -0.12]). In addition, the group with genetically-influenced low PLTP activity had a higher level of triglycerides (TG) (β [95% CI]: 0.04 [0.02, 0.05]) and a larger very low-density lipoprotein (VLDL) particle size (β [95% CI]: 0.05 [0.03, 0.07]). The genetically-influenced PLTP activity was not associated with ApoB ($P = 0.38$). We observed that the associations between PLTP activity and metabolomic measures were directionally consistent for men and women, but the absolute effect sizes were larger in women for small and very large HDL particle concentration (*P*-values for interaction < 1.79e-3) (Figures S2 and S3).

We observed no evidence of an association between genetically-influenced PLTP activity and CAD (OR [95% CI]: 0.99 [0.97, 1.02]). The sensitivity analyses showed that, after including rs2294213, the effects of genetically-influenced PLTP activity, irrespective of LDL-C, on metabolomic measures were similar to those in the main analyses (Figure S6), and effects on CAD remained absent (OR [95% CI]: 0.99 [0.97, 1.02]). In addition, the effects of PLTP activity on CAD, irrespective of LDL-C, remained absent in people with and without type 2 diabetes mellitus, with ORs [95% CI] of 1.03 [0.96, 1.12] and 1.00 [0.97, 1.02], respectively. Additional association analyses of quantile groups of genetically-influenced PLTP activity with CAD showed that the groups with lower levels of genetically-influenced PLTP activity, compared to the group with the highest level of PLTP activity, were associated with ORs [95% CI] for CAD of 0.95 [0.91, 1.00], 0.95 [0.91, 1.00], and 0.96 [0.91, 1.00], respectively, and with *P*-values of 0.047, 0.042 and 0.060, respectively. When extracting the individual genetic PLTP variants from previously performed association studies in large consortia, we observed significant associations with CAD risk (Table S4).

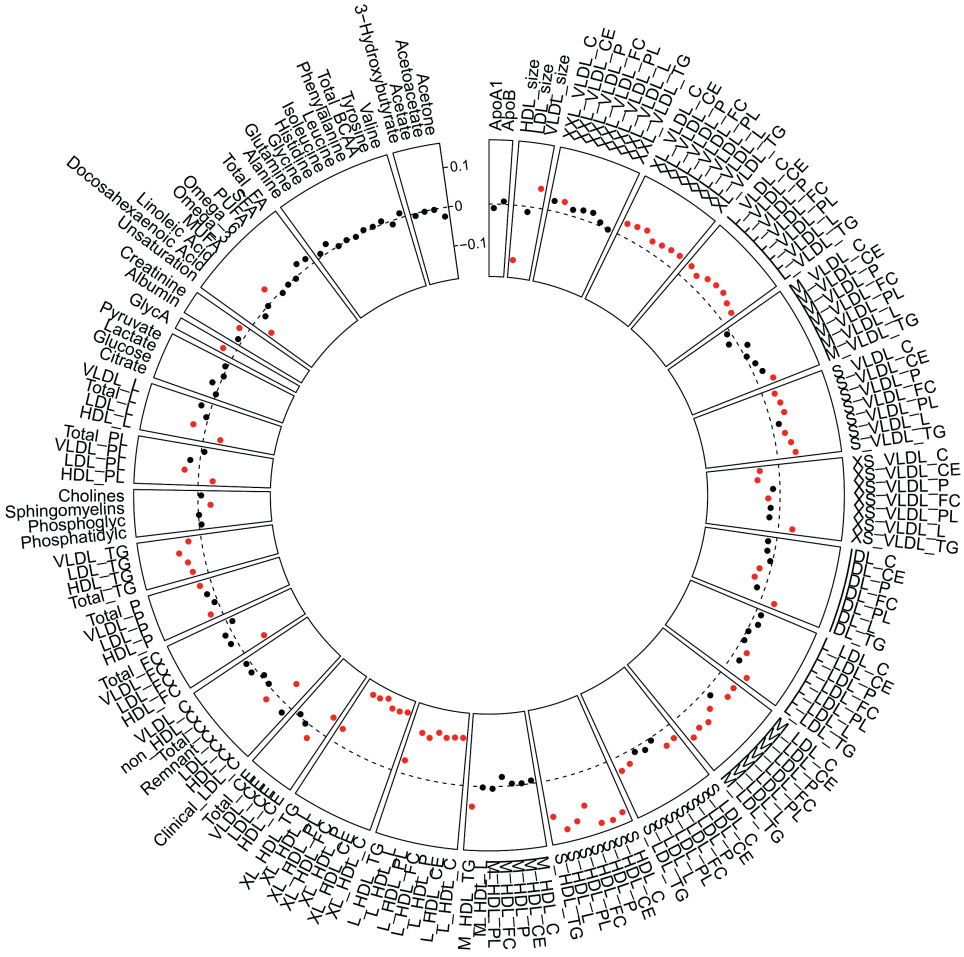

Figure 1. Estimated associations of genetically-influenced PLTP activity with 168 metabolomic measures. Participants with higher PLTP activity are in the reference group. Each dot represents the estimated beta coefficient from linear regression, which is expressed in standard deviation units. Red dots indicate statistically significant associations with $P < 1.79e\text{-}3$, and black dots indicate non-significant associations. Linear regression models are adjusted for age, sex, and the first ten genetic principal components. The definitions of the abbreviations in this figure are presented in Table S1.

3.3 Factorial mendelian randomization analysis

Associations with metabolomic measures

In the factorial MR analyses, associations with metabolomic measures for other groups relative to the reference group are shown in **Figure 2**. Similar to the associations of the dichotomized PLTP activity genetic score with metabolomic measures described above, the group with genetically-influenced low PLTP activity and genetically-influenced high LDL-C level had higher levels of small HDL particles and TG, and lower levels of large and very large HDL particles, with smaller HDL particle size (β [95% CI]: -0.13 [-0.15, -0.11]) and larger VLDL particle size (β [95% CI]: 0.04 [0.02, 0.07]). The group with only genetically-influenced low level of LDL-C mainly showed lower levels of ApoB-containing lipoproteins and their lipids, such as LDL-C (β [95% CI]: -0.42 [-0.45, -0.39]). The group with both genetically-influenced low PLTP activity and low LDL-C levels had combined characteristics of the previous two groups. The results we similar for men and women (Figures S4 and S5). In addition, we observed no interaction between the PLTP activity genetic score and the LDL-C genetic score (P > 1.79e-3) for all metabolomic measures.

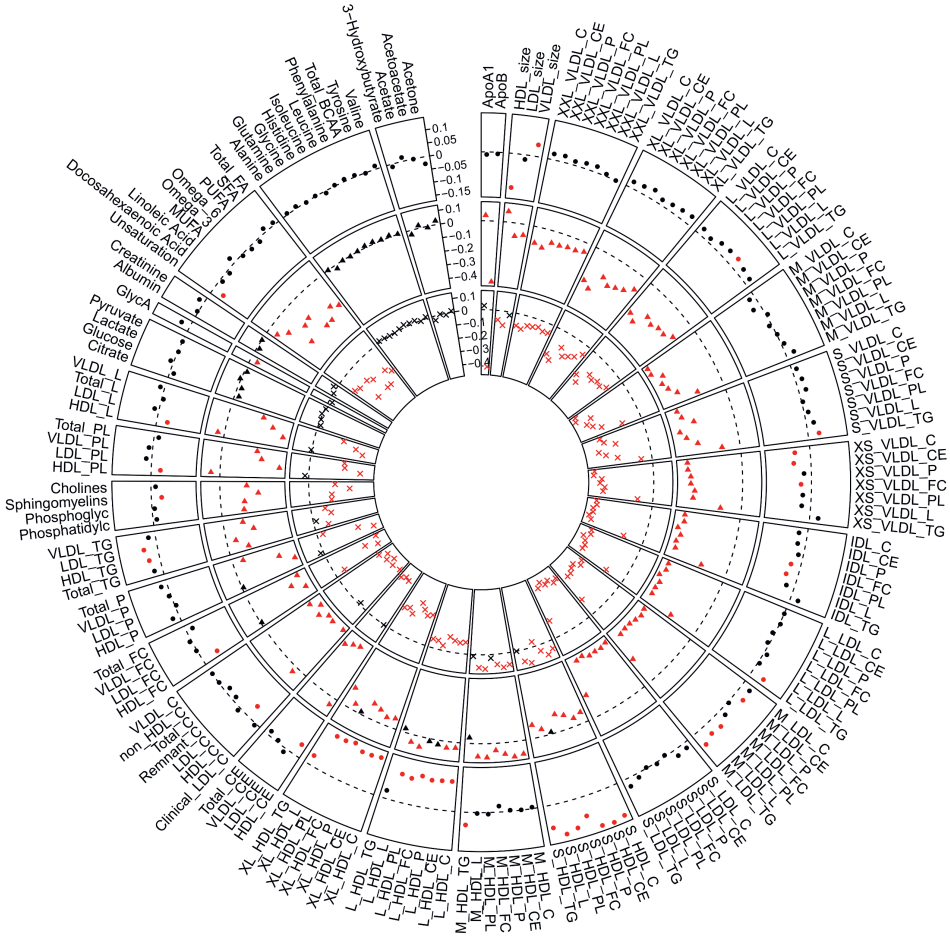

Figure 2. Estimated associations of three 'naturally randomized' groups relative to the reference group with 168 metabolomic measures. Dots, triangles and crosses represent the estimated β coefficients for the group with genetically-influenced low PLTP activity, the group with genetically-influenced low level of LDL-C, and the group with both genetically-influenced low PLTP activity and low LDL-C levels, respectively. Red colour indicates statistically significant associations with $P < 1.79e\text{-}3$, and black indicates non-significant associations. Linear regression models are adjusted for age, sex, and the first ten genetic principal components. The definitions of the abbreviations in this figure are presented in Table S1.

Associations with coronary artery disease

Compared to the reference group with both genetically-influenced high PLTP activity and high LDL-C levels, the OR [95% CI] of the CAD risk was 0.99 [0.96, 1.02] for the group with genetically-influenced low PLTP activity. For the group with genetically-influenced low level of LDL-C, and the group with both genetically-influenced low PLTP activity and low LDL-C levels, the ORs [95% CI] were 0.86 [0.83, 0.89] and 0.85 [0.83, 0.88], respectively (**Figure 3**). There was no evidence of an interaction between the PLTP activity genetic score and the LDL-C genetic score for CAD (*P*-value for interaction = 0.84). Besides, stratification analyses showed a greater reduction of CAD risk in men than in women for each genotype group (**Figure 3**).

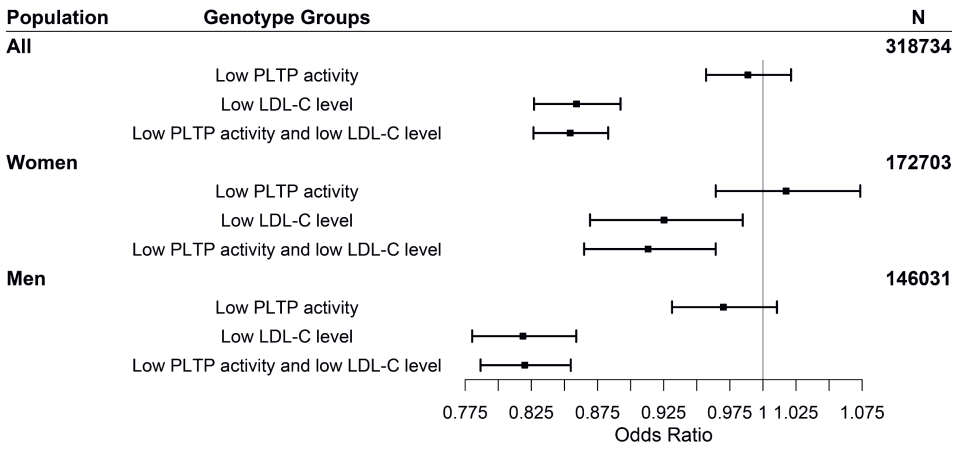

Figure 3. Estimated associations of three 'naturally randomized' genotype groups relative to the reference group with coronary artery disease. LDL-C, low-density lipoprotein-cholesterol; PLTP, phospholipid transfer protein. Logistic regression models are adjusted for age, sex, and the first ten genetic principal components.

4. Discussion

We investigated the association of genetically-influenced PLTP activity with metabolomic measures and CAD, and the potential effect of genetically-influenced PLTP activity on top of that of genetically-influenced LDL-C lowering by factorial MR design. Genetically-influenced lower PLTP activity was associated with lower numbers of large HDL particles, higher numbers of small HDL particles, as well as higher level of TG. We did not observe an association between genetically-influenced PLTP activity and the risk of CAD, irrespective of genetically-influenced LDL-C. In factorial MR analyses, the present study found that the effects of genetically-influenced PLTP activity and genetically-influenced LDL-C level on metabolomic measures were independent. However, genetically-influenced low PLTP activity did not show an additional effect on CAD risk on top of that of genetically-influenced low LDL-C.

The absence of an effect of genetically-influenced low PLTP activity on CAD risk irrespective of genetically-influenced LDL-C was unexpected. Consistent with a previous publication (11), we found that genetically-influenced low PLTP activity was associated with higher levels of small HDL particles. However, different from the previous study, no association of genetically-influenced PLTP activity with CAD risk was found in the present study. This apparent discrepancy may be due to the characteristics of the cohorts used for the analyses, but this remains to be further investigated.

We previously identified a specific lipoprotein profile (denoted as PC4) characterised by higher levels of large HDL particles and lower levels of small HDL particles, which showed a reduced CAD risk in MR analysis (10). Moreover, in factorial MR analyses, genetically-influenced lowering PC4 showed an additional beneficial effect on CAD on top of genetically lowered LDL-C (data not shown). Although the *PLTP* gene was a major factor contributing to the genetic score of PC4, the data in this study show that low genetically-influenced PLTP activity *per se* does not explain the reduced risk of CAD of PC4. A distinctive difference in the lipoprotein profile associated with genetically-influenced PC4 versus PLTP is that, in the latter, total TG is increased. We and others have previously shown that genetically-influenced TG is a causal contributor to CAD (28, 38). Thus, on the premise that increasing the number of small HDL particles has atheroprotective effects (10, 39, 40), which may be explained by smaller HDL particles being more efficient in inducing cellular cholesterol efflux (19-21), the absence of an effect of genetically-influenced PLTP on CAD may be explained by the proatherogenic effect of the higher TG resulting in a net null effect.

Although the composite genetic score of PLTP activity was not associated with CAD, the individual SNPs in this genetic score were associated with CAD in the summary statistics from several large consortia studies (Table S4). This indicates that some other unknown aspect associated with the PLTP locus, beyond activity as represented by the genetic risk score, may be involved in CAD risk. It should also be noted that the associated effect sizes were small, with ORs > 0.96 and 95% confidence intervals bordering 1. In addition, not all cohorts included in the previously performed meta-analysis consistently showed the association of genetic-influenced PLTP activity with cardiovascular disease, such as the Rotterdam study and the Stockholm Heart Epidemiology Programme study (11). One study showed that the atheroprotective effects of lowering PLTP activity was confined to men (8), another study found an association between high PLTP activity and increased CAD risk in statin-treated individuals only (7). Our analyses of the associations of quantile groups of genetically-influenced PLTP activity with CAD, showed that compared to the group with highest genetically-influenced PLTP-activity, the groups with lower PLTP activity had ORs ≥ 0.95 and upper limits of confidence intervals bordering 1.00, indicating small marginal association. Considering the evidence thus far, the effect of plasma PLTP activity on CAD in the general population seems modest if present at all. It cannot be excluded that under specific circumstances PLTP activity may actually affect CAD risk. However, this remains to be explored further.

A previous study in a transgenic mouse model has suggested that PLTP plays a role in the production of VLDL (41), but the generalizability of this finding to humans needs to be interpreted with caution. Our study did not find an association between genetically-influenced low PLTP activity and the VLDL particle concentration nor ApoB level, but rather did show a negative association with TG and VLDL size. This suggests that PLTP activity is rather involved in the catabolism of TG in VLDL, consistent with a function of PLTP in the transfer of excess surface phospholipids that result from the hydrolysis of TG in VLDL to HDL resulting in the conversion of small HDL into larger HDL (42-44). Consistent with these studies, we confirmed that PLTP has an effect on HDL size distribution, with the main finding being a positive association between genetically-influenced PLTP activity and HDL size.

To the best of our knowledge, the present study is the first to simultaneously investigate the associations of genetically-influenced PLTP activity with extensive NMR-based metabolomic measures and CAD, as well as its potential role on top of that of lowering LDL-C using genetic instruments. Embedded in the UK Biobank encompassing a large number of participants, the statistical analyses have ample power. However, several limitations in this study should also be taken into account when interpreting the results. Since PLTP activity could also be influenced by non-genetic factors, especially since glucose has been shown to regulate PLTP expression (35, 45, 46), the two SNPs selected according to reference (11)

may not fully reflect the actual PLTP activity of the participants in our study, given the different population characteristics. In addition, due to the lack of continuous medication data for all included participants from baseline to the end of follow-up, participants taking cholesterol-lowering medication were not excluded for the associations of genetically-influenced PLTP activity with CAD, which could have led to underestimated CAD risk.

In conclusion, our data reinforce the notion that plasma PLTP activity plays an important role in lipoprotein metabolism, showing that low PLTP activity associates with higher HDL particle concentration, smaller HDL particle size, higher TG concentration. Our analyses further provide genetic evidence that lowering plasma PLTP activity does not significantly reduce CAD risk, which may be due to counterbalancing pro- and anti-atherogenic effects of PLTP activity.

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