Genetic analysis of emerging risk factors in coronary artery disease

Short title: Genetic analysis of emerging CAD risk factors

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ABSTRACT

Background and aims: Type 2 diabetes (T2D), low-density lipoprotein-cholesterol (LDL-c), body mass index (BMI), blood pressure and smoking are established risk factors that play a causal role in coronary artery disease (CAD). Numerous common genetic variants associating with these and other risk factors have been identified, but their association with CAD has not been comprehensively examined in a single study. Our goal was to comprehensively evaluate the associations of established and emerging risk factors with CAD using genetic variants identified from Genome-wide Association Studies (GWAS).

Methods: We tested the effect of 60 traditional and putative risk factors with CAD, using summary statistics obtained in GWAS. We approximated the regression of a response variable onto an additive multi-SNP genetic risk score in the Coronary Artery DIsease Genomewide Replication And Meta-analysis (CARDIoGRAM) consortium dataset weighted by the effect of the SNP on the risk factors.

Results: The strongest association with risk of CAD was for LDL-c SNPs (*p=3.96E-34*). For non-established CAD risk factors, we found significant CAD associations for coronary artery calcification (CAC), Lp(a), LP-PLA2 activity, plaque, vWF and FVIII. In an attempt to identify independent associations between risk factors and CAD, only SNPs with an effect on the target trait were included. This identified CAD associations for Lp(a)(*p=1.77E-21*), LDL-c (*p=4.16E-06*), triglycerides (TG) (*p=1.94E-05*), Height (*p=2.06E-05*), CAC (*p=3.13E-23*) and Carotid plaque (*p=2.08E-05*).

Conclusions: We identified SNPs associated with the emerging risk factors Lp(a), TG, plaque, Height and CAC to be independently associated with risk of CAD. This provides further support for-ongoing clinical trials of Lp(a) and TG, and suggests that CAC and plaque could be used as surrogate markers for CAD in clinical trials.

KEYWORD: coronary disease; epidemiology; risk factors; genetics and cardiovascular diseases

INTRODUCTION

In recent years many genome wide association studies (GWAS) have been conducted for established and non-established risk factors for CAD (Supplementary Table 1)[1–33] with the aim to discovering genetic determinants of risk factors. For each of the 60 *a priori*-selected risk factors, Supplementary Table 1 lists the studies and their characteristics used in this analysis. Even if tested, in most cases the relative contribution of risk factors to CAD has not been comprehensively investigated, since each study typically tests for the association of only one or two traits with CAD at any one time.

Our goal was to comprehensively evaluate the associations of established and emerging risk factors with CAD using genetic variants identified from GWAS. The rationale is that this could identify risk factors for follow up studies, for example in much larger and more comprehensive Mendelian randomization (MR) studies, and/or provide support for ongoing clinical trials targeting selected biomarkers. Furthermore this may increase our understanding of the aetiological and genetic landscape of CAD.

METHODS

Trait selection

To identify GWAS of established and non-established risk factors for CAD we queried the NHGRI GWAS Catalogue (available at: http://www.ebi.ac.uk/gwas/) in May 2015. GWAS with summary level data on SNP, effect size, standard error of effect size, risk allele and risk allele frequency publicly available in the GWAS catalog or in the original paper were included in the analysis.

We considered T2D, LDL-c, BMI, blood pressure and smoking to be established risk factors for CAD. The non-established risk factors were selected from the GWAS Catalogue by reviewing the traits in the GWAS catalog, and select potential traits of interest. Each trait in the GWAS Catalogue which has been linked to CAD based on its pathophysiology in the literature by PubMed searches was included in the analysis; i.e. Height, Psoriasis, TG, HDL-c, LP-PLA2[5,6], adiponectin[9], MMP-1[4], homocysteine[23], white blood cell count(WBC)[34], glycated hemoglobin levels[35,36], hsCRP[10], coagulation disorders (vWF)[24], mean platelet volume(MPV)[33], platelet count[33], platelet aggregation[37], FVIII[24], Protein C[38], PAI-I[39], fibrinogen[8,10,31]); chronic inflammation (systemic lupus erythematosis [SLE][14–16], inflammatory bowel disease [IBD][19], rheumatoid arthritis[13,26]) and cardiac imaging (Coronary Artery Calcification (CAC)[22], cIMT[3]) or reported possible risk factors for CAD (vitamin D[1], glomerular filtration rate (GFR)[40], homoarginine levels[41],serum uric acid levels[42], serum dimethylarginine levels (symmetric)[43], serum dimethylarginine levels (assymetric/symmetric rate)[44], fat body mass(FBM)[45], and chronic kidney disease(CKD)[46]).

SNPs selection for association

For all analyses, we selected SNPs that reached a significance level of at least *p<5E-08* in the original GWAS of European individuals.

The goal was to test the association between the SNPs associated with risk factors and risk of CAD. For each trait we only selected independent SNPs (not in linkage disequilibrium (LD) with other SNPs for the same trait at r^2 cut-off<0.5). If two SNP were in LD this means that the alleles of both SNPs are inherited together more often than would be expected by chance. When we encountered SNPs in LD, the variant with the most significant p-value for the trait of interest was included in the analysis for that trait.

Traits with a significant association with CAD after the first analysis were selected for a secondary analysis to investigate independence of association by removing SNPs with pleiotropic effects. To do this, we categorized traits into two groups; “upstream” and “downstream” risk factors. This was in order to avoid removing SNPs that associated with several traits along a causal pathway from risk factor to disease. The upstream risk factors consist of the immediately modifiable risk factors BMI, lipids, lipoproteins, blood pressure, inflammation markers, coagulation traits and chronic inflammation traits. In contrast, downstream markers were much closer to the CAD phenotype and included CAC, cIMT and plaque. For upstream markers, we removed pleiotropic SNPs (by performing a pairwise LD-analysis using SNAP (https://www.broadinstitute.org/mpg/snap/) and removing those at r^2 >0.5) that were within the upstream group, but retained SNPs in LD with downstream markers (as this association could reflect a causal pathway). For downstream traits, we removed only those SNPs pleiotropic with other downstream traits.

Association of SNPs with CAD

To test the association between the selected risk factors and CAD we used the grs.summary function from R package Genetics ToolboX (http://cran.r-project.org/web/packages/gtx/index.html). This package implements a summary statistic method for approximating the regression of a response variable onto an additive multi-SNP genetic risk score in a given testing dataset weighting the association statistic by the effect of the SNP on the risk factors. This method uses single SNP association summary statistics: effect size, standard error of effect size and risk allele. Odds ratios (ORs) and confidence intervals (CIs) were transformed to effect sizes and standard error of effect sizes with an inverse natural logarithm, when necessary. We used the CARDIoGRAM GWAS publicly available data as our validation dataset[47]. In brief, CARDIoGRAM includes 22 233 cases of CAD and 64 762 controls[47]. An overly conservative Bonferroni corrected p-value significance of *8.33E-04* was set (0.05/60 traits), to account for the number of traits assessed. As a negative control, we tested SNPs associated with eye color for their association with risk of CAD.

We limited our study to GWAS performed in Caucasians since the CARDIoGRAM validation dataset is of the same ethnicity.

RESULTS

Trait selection

We included 69 studies in which a total of 60 risk factors were described. **Supplementary Table 1** provides an overview of the papers incorporated in the analysis, listing the unit of exposure per increased risk allele, the variance explained by the reported SNPs, the number of SNPs discovered and the sample size of the study.

SNPs selection for association.

The number of SNPs remaining after excluding duplicates and correcting for LD can be found in **Table 1.** Height was the trait with most SNPs (173) whereas some traits only had one SNP (e.g. smoking cessation). With these SNPs we performed a genetic association analysis with CAD using the CARDIoGRAM data.

Association of risk factors with CAD using all independent SNPs

In our analysis 15 out of 60 risk factors (**Table 1**) were significantly associated with CAD outcome in the CARDIoGRAM consortium. All established risk factors for CAD (LDL-c, SBP, DBP, BMI, T2D, smoking, HDL and TG) were identified to associate with CAD. The genetic risk score of LDL-c associated SNPs had the most significant p-value, with an OR_{CAD} 1.54, 95% CI: 1.44-1.65 *p=3.96E-34* per 1-SD increase in LDL-C. CAC had the most significant p-value for the non-traditional risk factors, OR_{CAD} 1.91,95% CI: 1.68-2.16 *p=3.13E-23*.

As a negative control, we tested eye color as a risk factor: no association between eye color and CAD risk was found OR_{CAD}=1.00, 95% CI: 0.99 – 1.02 *p=0.93.*

Association of risk factors with CAD using non-pleiotropic SNPs

Table 2 provides the number of SNPs that remained after pleiotropic SNPs were excluded. We analyzed 15 risk factors in our secondary analysis (**Table 2**) and found that six of the 15 traits had a significant association; the lowest p-value and highest effect size for CAD was for CAC OR_{CAD} 1.91,95% CI: 1.68-2.16 *p=3.13E-23*. In contrast to the primary analysis, no significant association was identified for LP-PLA2, T2D, HDL-c, SBP, DBP, MAP, vWF and FVIII when SNPs were thinned out by pleiotropy (**Figure 1**).

DISCUSSION

We conducted a comprehensive study to investigate the association of risk factors for their association with CAD using summary-level genetic data. Using all available SNPs, we found a significant association with CAD for the following traditional risk factors: LDL-C, HDL-C, SBP, DBP, BMI, T2D and TG. In addition, we found the following traits to associate with CAD: Height, CAC, Lp(a), LP-PLA2, plaque, factor VIII, von willebrand factor and mean arterial pressure. Of these emerging risk factors, when we removed potentially pleiotropic SNPs, associations with CAD persisted for Height, CAC, Lp(a), and plaque.

After removing potentially pleiotropic SNPs for FVIII and vWF, the association with CAD no longer persisted. Furthermore, the association between LP-PLA2 and CAD also diminished when limited to nominally non-pleiotropic SNPs, which is in keeping with a recent MR study[48]. This arises from an overlap in genetic variation between LP-PLA2 activity and lipid phenotypes in our data: 6 of 9 LP-PLA2 SNPs in our primary analysis where excluded in our secondary analysis because they were in LD with one of the lipid (LDL-C, HDL-C or TG) phenotypes. An explanation for this phenomena might be that in the bloodstream, two-thirds of LP-PLA2 circulates primarily bound to LDL; the remaining third is distributed between HDL and VLDL[49]. Measures of LP-PLA2 might thus partially reflect the concentration of proatherogenic lipoproteins. Recent clinical trials with the LP-PLA2 inhibitor darapladib in coronary heart disease patients yield similar results to our analysis[50,51]. In patients with stable CAD after optimal treatment for dyslipidemia, there was no added benefit of reducing LP-PLA2. Regarding vWF, other studies identified a weak association between vWF levels and CAD but it disappeared after adjusting for coexisting riskfactors[52,53]

For IMT and PAI-1, we do not find an association with CAD in our primary analysis. This is in contrast with other studies[3,17]. This may be due to low variance of the exposure explained by the SNPs for IMT (1.10%). Bis et al report two SNPs that are associated with IMT phenotype and CAD. Of the two SNPs, only rs17045031 near LRIG1 was significantly associated with IMT phenotype and therefore included in our analysis, the other SNP was not included in our analysis because of our threshold for significance, this might explain the discrepancy in results[3].

For PAI-1, out of the 10 SNPs identified by Huang et al, only two SNPs in ARNTL were nominally associated with CAD (rs6486122: OR 1.04; 95% CI: 1.01-1.07; rs3816360: OR 1.03; 95% CI: 1.01-1.06)[39]. In contrast, our chosen method incorporated all SNPs associated with PAI-1, which did not associate with CAD.

We did identify a clear association between Lp(a) and CAD (OR_{CAD} 1.25, 95% CI: 1.19-1.31 *p=3.92E-21),* and the association became stronger when we removed pleiotropic SNPs. This is in keeping with recent genetic studies including that by Clarke et al, which identified two common SNPs in the LPA gene that had an association with both the Lp(a) lipoprotein level and the risk of CAD[6].

The association between height and CAD (OR_{CAD} 0.87, 95% CI:0.80-0.93 p=2.06E-05) remained significant after removing pleiotropic SNPs. This is inline with recently published MR-studies by Nüesch et al. They conclude that taller individuals have a lower risk of developing CAD[54].

The clear association between TG and CAD remained significant after removing pleiotropic SNPs: OR_{CAD}=1.45, 95% CI: 1.22– 1.72 *p=1.94E-05*. This is in line with the findings by Do et al[55], They used 185 common SNPs to examine the role of TG on risk for CAD. They show that loci with only a strong magnitude of association with TG are associated with CAD. Furthermore, Holmes et al. performed a Mendelian randomization analysis based on individual participant level data from 62000 individuals with >12.000 CHD events. They found a causal role for TG in the analysis restricted to SNPs only associated with TG and no association with any other lipid trait[56]. More recently, White et al used 140 SNPs and identified a consistent causal association of TG with risk of CAD using different MR approaches[57].

Although HDL-C associated with CAD on initial analysis, when we limited the genetic instrument to only non-pleiotropic SNPs, this association diminished. This is in keeping with prior reports[56–59] To take one example, in the paper by Voight et al., a Mendelian randomization analysis was conducted using a genetic risk score consisting of 14 common SNPs that associated predominantly with HDL cholesterol and tested this score in up to 12,482 cases of myocardial infarction and 41,331 controls. They found that a 1 SD increase in

HDL cholesterol due to genetic score was not associated with risk of myocardial infarction (OR 0.93, 95% CI 0.68-1.26, *p=0.63*)[58].

Interestingly, in our analysis restricted to non-pleiotropic SNPs, the established causal risk factors SBP and DBP were no longer associated with CAD. This arose because restricting to non-pleiotropic SNPs resulted in the removal of the majority of SNPs with only 3 SNPs remaining for DBP and 1 SNP for SBP. SNPs were removed because they were also associated with MAP, PP, HTN. While the causal role of blood pressure in CAD is well-established, this suggests that our approach overly-penalized some traits that have a highly pleiotropic genetic architecture.

The association of CAC and plaque with CAD retained after removing pleiotropic SNPs and is worthy of further comment. Over the past few years there has been a move to use surrogate markers of CHD in clinical trials as a marker of "hard" clinical outcomes, but at a lower cost than conducting a full outcome-based clinical trial. Given that these downstream traits are proximal to the CAD phenotype, they are less likely to be confounded compared to a trait that is more distant (or upstream trait, such as a blood lipid profile). Our data therefore suggest that, given the relationship of these traits with risk of CAD, that they may represent a proxy for CAD, however further investigations are needed, for example relating SNPs such as LDL-C and SBP on plaque and CAC[60] to see whether they associate with these traits.

Our study has several strengths and weaknesses that merit discussion. To the best of our knowledge we are the first to report on the association between SNPs associated with multiple non-established risk factors and risk of CAD. We are, however restricted to published studies, which might have resulted in selection bias. Furthermore, the data was derived from the publications, and no source data quality assessment could be performed, we were dependent on the quality of the published papers. As an example, traits where the genetic landscape is not fully captured by current GWAS might bias our results. Therefore, at the moment we cannot rule out any association between these risk factors and CAD. Because summary level data were used for analyses, we were not able to perform age and gender corrections (however these would have been conducted in the original GWAS); we are therefore dependent on the corrections performed in the published papers. Finally, this study represents a rather broad but crude genetic analysis; more sophisticated analyses such as multivariate MR[61] and/or MR-Egger[62] would represent next steps to more comprehensively assessing and

accounting for pleiotropy of genetic instruments and testing for independence of causal estimates across multiple risk factors.

In conclusion, our multiple trait genetic analysis of established and emerging risk factors for CAD provides further evidence that TG and Lp(a) should be prioritized as potential therapeutic targets for CAD prevention, and suggests that CAC and plaque could be potential surrogate markers for CAD.

Conflict of interest: None to declare.

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Table 1: Association Results of the primary analysis including all SNPs associated with exposures. OR_{CAD} arises from regressing SNP-exposure and SNP-CAD associations and represent the odds ratios per SD increase in continuous traits, and comparing presence vs absence of a binary trait.

Trait	#SNPS	OR_{CAD}	95% CI	P-value
LDL-c	54	1.542	1.438-1.653	3.96E-34
CAC	2	1.906	1.678-2.164	3.13E-23
TG	48	1.399	1.305-1.499	3.01E-21
Lp(a)	5	1.249	1.193-1.308	3.92E-21
Diastolic Blood pressure	27	1.486	1.355-1.631	5.19E-17
Systolic Blood pressure	25	1.492	1.359-1.639	5.47E-17
LP-PLA2 (activity)	9	1.377	1.257-1.510	8.09E-12
HDL-c	74	0.789	0.737-0.845	1.34E-11
T2D	92	1.221	1.132-1.317	2.19E-07
Plaque	2	1.348	1.175-1.547	2.08E-05
Height	173	0.866	0.800-0.932	2.06E-05
BMI	69	1.082	1.042-1.123	3.85E-05
Factor VIII	5	2.249	1.504-3.363	7.83E-05
von Willebrand factor	8	0.786	0.696-0.888	1.09E-04
Mean arterial pressure	22	1.342	1.152-1.563	1.54E-04
hsCRP	20	0.893	0.833-0.958	1.63E-03
Hypertension	11	1.300	1.101-1.535	2.00E-03
GFR	3	1.031	1.010-1.053	3.86E-03
cIMT	3	1.521	1.226-1.816	5.33E-03
Homoarginine levels	1	0.856	0.757-0.967	1.26E-02
Glycated hemoglobin levels	12	1.124	1.024-1.235	1.43E-02
ADMA/SDMA	1	0.879	0.780-0.991	3.49E-02
SDMA	1	1.119	1.008-1.242	3.49E-02
Ulcerative colitis	19	1.071	1.004-1.143	3.89E-02
White blood cell count	9	0.675	0.456-1.000	5.01E-02
Psoriasis	15	1.041	0.997-1.084	7.35E-02
Inflammatory Bowel Disease	96	1.037	0.996-1.081	7.62E-02
Smoking Cessation	1	0.491	0.203-1.185	1.14E-01
Body Fat Mass	1	1.311	0.921-1.867	1.33E-01
Stroke	6	1.097	0.968-1.243	1.48E-01
Platelet count	55	1.040	0.984-1.099	1.60E-01
Protein C	4	1.025	0.990-1.061	1.62E-01

Mean Platelet Volume	27	0.977	0.943-1.012	1.92E-01
Serum Uric Acid	3	1.073	0.947-1.217	2.70E-01
Fibrinogen	28	1.015	0.987-1.044	2.95E-01
Vitamin D	10	1.006	0.993-1.019	3.76E-01
SLE	23	0.983	0.945-1.022	3.86E-01
Platelet Aggregation: ADP aggregation (GS)	3	0.940	0.806-1.097	4.34E-01
Chronic kidney disease	3	1.018	0.974-1.064	4.38E-01
Smoking Initiation	2	1.135	0.818-1.574	4.50E-01
Platelet Aggregation: epinephrine aggregation(FHS)	3	0.956	0.847-1.079	4.68E-01
Platelet Aggregation: epinephrine aggregation(GS)	3	1.062	0.886-1.273	5.14E-01
Pulse Pressure	11	0.949	0.788-1.143	5.83E-01
Platelet Aggregation: ADP aggregation (FHS)	3	0.967	0.848-1.101	6.11E-01
Adiponectin	13	1.003	0.991-1.014	6.57E-01
Alcohol consumption	2	1.001	0.997-1.005	6.64E-01
COPD	9	1.001	0.998-1.003	6.73E-01
Crohn's disease	25	0.992	0.940-1.047	7.83E-01
Rheumatoid Arthritis	40	1.006	0.960-1.055	7.98E-01
Matrix metalloproteinase-1	2	1.007	0.952-1.065	8.17E-01
NT-proBNP	3	1.010	0.900-1.134	8.69E-01
Smoking	3	1.025	0.754-1.393	8.74E-01
Homocysteine	20	1.004	0.943-1.070	8.92E-01
Caffeine	2	0.969	0.548-1.711	9.13E-01
PAI-1	8	1.007	0.870-1.166	9.24E-01
Eye color	7	1.001	0.986-1.016	9.31E-01
Platelet Aggregation: collagen lag time (FHS)	1	0.996	0.892-1.114	9.49E-01
Platelet Aggregation: collagen lag time (GS)	1	0.987	0.667-1.462	9.49E-01

Legend:

*OR; Odds Ratio(per 1-SD increment) (for binary traits this represents per-log-odds increase),CAD: Coronary Artery Disease 95\% CI; 95\% Confidence Interval, LDL-c; Low-density-lipoprotein-cholesterol,CAC; Coronary Artery Calcification, TG; Triglycerides, Lp(a); Lipoprotein(a), LpPLA2; Lipoprotein-associated phospholipase A2,HDL-c; High-density Lipoprotein-cholesterol, T2D; Type 2 diabetes,BMI;Body mass index,hsCRP;High sensitive C-reactive protein,GFR;Glomerular filtration rate ,cIMT; Carotid Intermedia thickness, ADMA; asymmetric dimethylarginine, SDMA,SLE; Systemic Lupus Erythematosus,FHS; Framingham Heart Study, GS; Genetic Study of Atherosclerosis,COPD;Chronic Obstructive Pulmonary Disease,NT-proBNP;N-terminal pro b-type natriuretic peptide,PAI-1; Plasminogen Activator Inhibitor-1

Text:

The association of 60 risk factors with CAD was tested. Of these 15 traits reached after multiple testing corrected significance. Fifteen risk factors were put forward to secondary analysis. The column N SNPs provides the number of independent (R^2<0.5) SNPs tested per risk factor.

Table 2: Association Results of secondary analysis using only SNPs that are specific for the exposure of interest. OR_{CAD} arises from regressing SNP-exposure and SNP-CAD associations and represent the odds ratios per SD increase in continuous traits, and comparing presence vs absence of a binary trait.

Trait	#SNPS	OR_{CAD}	95%CI	P-value
CAC	2	1.906	1.678-2.164	3.13E-23
Lp(a)	3	1.293	1.226-1.363	1.77E-21
LDL-c	31	1.293	1.159-1.443	4.16E-06
TG	27	1.448	1.222-1.716	1.94E-05
Plaque	2	1.348	1.175-1.547	2.08E-05
Height	172	0.867	0.801-0.934	2.54E-05
BMI	63	1.080	1.029-1.134	1.75E-03
Diastolic Blood pressure	3	1.456	1.038-2.044	2.96E-02
T2D	47	1.198	1.012-1.418	3.55E-02
HDL-c	47	0.890	0.799-0.992	3.56E-02
Systolic Blood pressure	1	1.708	0.935-3.120	8.18E-02
LP-PLA2 (activity)	3	1.135	0.976-1.320	1.01E-01
Mean Arterial Pressure	7	1.210	0.913-1.604	1.85E-01
Factor VIII	2	1.548	0.249-9.618	6.39E-01
Von Willebrand factor	5	0.997	0.648-1.534	9.89E-01

Legend:

*OR; Odds Ratio (per 1-SD increment) (for binary traits this represents per-log-odds increase), CI; Confidence Interval, CAC; Coronary Artery Calcification, Lp(a); Lipoprotein (a), LDL-c; Low-density Lipoprotein-cholesterol, BMI; Body Mass Index, TG; Triglycerides, HDL-c; High-density Lipoprotein-cholesterol, LP-PLA2; Lipoprotein-associated phospholipase A2.

Text:

In secondary analysis we excluded SNPs that overlapped between traits or were in linkage disequilibrium across traits from analysis. As can be noted, there was a large overlap between the genetic variation for Systolic and Diastolic blood pressure. Six traits were significantly associated with CAD

Figure 1:Association results of the 15 significant risk factors in the primary analysis and their associations in the pruned analysis. OR_{CAD} arises from regressing SNP-exposure and SNP-CAD associations and represent the odds ratios per SD increase in continuous traits, and comparing presence vs absence of a binary trait.

Text:

In secondary analysis we excluded SNPs that overlapped between traits or were in linkage disequilibrium across traits from analysis. Five traits are also associated with CAD in our secondary analysis. Odds ratios (grey bars) and 95% confidence intervals (black lines) are shown for both methods. Traits with an asterisk are associated with CAD in both the primary and secondary analysis.