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Comparative analysis of genome-wide-association studies signals for lipids, diabetes, and coronary heart disease: Cardiovascular Biomarker Genetics Collaboration

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Aims
To evaluate the associations of emergent genome-wide-association study-derived coronary heart disease (CHD)-associated single nucleotide polymorphisms (SNPs) with established and emerging risk factors, and the association of genome-wide-association study-derived lipid-associated SNPs with other risk factors and CHD events.

Methods and results
Using two case–control studies, three cross-sectional, and seven prospective studies with up to 25 000 individuals and 5794 CHD events we evaluated associations of 34 genome-wide-association study-identified SNPs with CHD.
risk and 16 CHD-associated risk factors or biomarkers. The Ch9p21 SNPs rs1333049 (OR 1.17; 95% confidence limits 1.11–1.24) and rs10757274 (OR 1.17; 1.09–1.26), MIA3 rs17465637 (OR 1.10; 1.04–1.15), Ch2q36 rs2943634 (OR 1.08; 1.03–1.14), APC rs383830 (OR 1.10; 1.02, 1.18), MTHFD1L rs6922269 (OR 1.10; 1.03, 1.16), CXCL12 rs501120 (OR 1.12; 1.04, 1.20), and SMAD3 rs17228212 (OR 1.11; 1.05, 1.17) were all associated with CHD risk, but not with the CHD biomarkers and risk factors measured. Among the 20 blood lipid-related SNPs, LPL rs17411031 was associated with a lower risk of CHD (OR 0.91; 0.84–0.97), an increase in Apolipoprotein AI and HDL-cholesterol, and reduced triglycerides. SORT1 rs599839 was associated with CHD risk (OR 1.20; 1.15–1.26) as well as total- and LDL-cholesterol, and apolipoprotein B. ANGPTL3 rs12042319 was associated with CHD risk (OR 1.11; 1.03, 1.19), total- and LDL-cholesterol, triglycerides, and interleukin-6.

Conclusion

Several SNPs predicting CHD events appear to involve pathways not currently indexed by the established or emerging risk factors; others involved changes in blood lipids including triglycerides or HDL-cholesterol as well as LDL-cholesterol. The overlapping association of SNPs with multiple risk factors and biomarkers supports the existence of shared points of regulation for these phenotypes.

Keywords

Coronary disease • Lipids • Genes • Risk factors

Introduction

Alterations in a number of intermediate phenotypes or biomarkers (such as blood lipid fractions, inflammation, or coagulation proteins) precede and are associated with a higher risk of coronary heart disease (CHD) events. With the exception of LDL-cholesterol (LDL-C) for which there is additional evidence from interventional trials, inability to exclude bias arising from confounding and reverse causation, precludes firm conclusions being drawn about their causal relevance using observational data alone.1 Genome wide association studies (GWAS) are less affected by confounding or by reverse association bias because genotype is determined by randomized allocation and fixed from conception.2–4 However single nucleotide polymorphisms (SNPs) associated with common diseases including CHD have typically been in non-coding DNA, distant from annotated genes, or in chromosomal regions where associated SNPs span several different equally plausible candidates.5–7 This can make it difficult to infer the mechanisms linking genome variation to clinical endpoints simply from genomic location.

Integration of information on genotype, intermediate phenotypes, and disease endpoints could provide insight into the mechanism by which disease-associated SNPs alter cardiovascular risk and help better define the causal relevance of cardiovascular biomarkers. However, the emphasis of most GWAS, thus far, has rightly been on the robust detection of genetic signals with a single phenotype (or narrow range of phenotypes, e.g. blood lipids) or one disease endpoint at a time.8

The large number of CHD cases needed to conduct adequately powered genetic association studies is most efficiently assembled using a case–control design,7 but preclinical risk factors and disease biomarkers have been best characterized in population-based cohort studies, which individually accrue fewer cases.9 Because genotype is determined at conception, and invariant, it becomes possible to undertake large-scale genetic meta-analyses that include information from both types of study to maximize available information on both biomarkers and disease endpoints.10,11

Using a collaboration of 12 studies, involving ~25 000 individuals, we typed the first SNPs to be identified from GWAS (from 2007 to 2008) that were associated either with myocardial infarction (MI) or with an intermediate phenotype previously associated with MI risk including LDL-C, HDL-cholesterol (HDL-C), triglycerides (TG), body mass index (BMI), or type-2 diabetes mellitus (T2DM).5,12–19 We studied the associations of these SNPs with a wider range of intermediate phenotypes, to elucidate shared points of regulation, and with CHD risk to assess if the biomarkers altered are likely to mediate or mark changes in the causal pathway to CHD.

First, we hypothesized that comparative analysis of SNP associations with cardiovascular disease (CVD) risk factors/biomarkers and CVD endpoints would help delineate the mechanisms linking genetic variation to CVD events. Identifying which biomarker-associated SNPs also alter disease risk should help clarify which biomarkers lie in the causal pathway. Conversely, understanding which risk factors/biomarkers are altered by disease-associated SNPs should help elucidate the mechanisms underlying the disease association where these are uncertain. Second, we hypothesized that since non-genetic biomarkers are frequently correlated, SNPs identified for an index association with one CVD risk factor/biomarker would frequently be associated with a diverse array of other risk factors/biomarkers. Studying of the effect of a SNP on many phenotypes (a phenome scan) would define common points of regulation for diverse risk factors and help disentangle the causal from non-causal interconnections between correlated lipid and inflammation and coagulation markers. By helping to evaluate which SNP associations are exclusive to a single risk factor and which are more extensive, these analyses should also inform on the specificity of individual biomarkers for Mendelian randomization analyses of biomarkers and risk factors.

Methods

Data sets

Prospective studies

Seven UK prospective studies contributed to the collaboration: Northwick Park Heart Study II (NPHS II),20 British Regional Heart Study
(BRHS), English Longitudinal Study of Ageing (ELSA), Edinburgh Artery Study (EAS), the 1958 Birth Cohort (1958BC), the MRC 1946 Birth Cohort (MRC 1946), and the Whitehall II Study (WHII). Six studies (NPHS II, BRHS, ELSA, EAS, 1958BC, and MRC 1946) were population based, while Whitehall II was workplace based. The details of the sampling frame, inclusion criteria, duration of follow-up, and other details are listed in Supplementary material online, Methods 1 and Table S1.

Cross-sectional studies

Two cross-sectional studies contributed prevalent cases of CHD: the Southampton Atherosclerosis Study (SAS) and the Stockholm Heart Epidemiology Program (SHEEP). The SHEEP study included contemporaneous controls, while the representative, population-based cohort study NPHS II, in which participant recruitment overlapped geographically with that for SAS, acted as a control data set for SAS. Details of design, matching criteria, recruitment, main demographic details, definition of outcomes, and other measures are described in Supplementary material online, Methods 1 and Table S1. One cross-sectional study, the MRC-BHF British Genetics of Hypertension (BRIGHT) study included individuals without CHD but with high blood pressure (BP) (Table 1).

Measures

Demographic and other variables

Age, gender, BMI, and systolic and diastolic BP were available from all studies (Table 1).

Blood biomarkers

The availability of blood lipids and apolipoproteins (ApoBs), indices of glycaemic control, as well as inflammation, coagulation, and metabolic markers are listed in Table 1. All measurements were made using validated assays and protocols whose details have been reported previously (Supplementary material online, Methods 1) and all markers were assayed in subjects free from CHD at the time of sampling. For prospective studies, these samples were obtained either from the baseline survey or from a subsequent resurvey closest to the assessment at which the study DNA repository was established.

Clinical outcomes

We defined coronary heart disease as a composite endpoint of non-fatal MI, CHD death, or coronary revascularization procedure using prevalent and incident events. We used a similar approach to that adopted by the CARDioGRAM consortium for defining CHD endpoints. We defined incident events in cohort studies as occurring after establishment of the DNA repository and prevalent events as those non-fatal events preceding the establishment of a DNA repository. Subjects from the SAS study were sampled on the condition of having coronary stenosis ≥50% of the diameter in at least one major coronary artery (defined by coronary angiography rather than clinical CHD event).

Genotyping

We typed 21 SNPs associated with LDL-C, HDL-C, TG, or BMI and a total of 16 SNPs previously shown to exhibit an association with CHD or T2DM from GWAS reported in 2007 and 2008. All SNPs that were significant with a \( P \)-value < 10^{-8} identified from GWAS were included for the analysis unless they were in linkage disequilibrium (LD). Where SNPs were in LD, the best proxy was chosen based on feasibility for genotyping to provide the maximum data available for the analysis. Two SNPs from the chromosome 9p21 region were selected as they had both been identified from GWAS. However, since rs10757274 had not been typed in HapMap at the time this analysis was initiated, and the degree of LD with other SNPs on chr9p21 was not known, both SNPs were included. Details are provided in Table 2. New genotyping was conducted using validated, high throughput genotyping platforms at the Genome Centre, Queen Mary University of London, using Kaspar technology or the ABI TaqMan platform; Medical Solutions, Nottingham, using the ABI SNPlex platform; or the Centre for Cardiovascular Genetics UCL, using the ABI TaqMan platform (Table 1). The Whitehall II study provided genotypes from the ITMAT/Broad Institute CARE consortium (IBC) Human CVD Beadchip (Illumina) while 1958BC and BRIGHT provided genotypes from the Affymetrix 500K whole genome array.

Analysis

We regarded a SNP association in CBGC with the same endpoint as that reported in the discovery GWAS as a replication analysis. Single nucleotide polymorphism associations in CBGC with outcomes distinct from the original discovery GWAS e.g., evaluation of associations of CHD-associated SNPs with risk factors and biomarkers were regarded as a discovery analysis.

Single nucleotide polymorphism associations with coronary heart disease

Seven studies including NPHS II, BRHS, ELSA, EAS, WHII, SAS, and SHEEP contributed to the meta-analysis of the association of genotype with CHD (Table 1). The meta-analysis utilized summary data from individual studies using a protocol agreed jointly by a central analysis subgroup in conjunction with principal investigators and statisticians from the participating studies. All analyses were restricted to individuals of Caucasian ethnicity and limited to subjects with complete data for gender, age, and genotype.

For the purposes of quality control and to allow evaluation of any genetic heterogeneity between studies, each study provided details of genotyping platform, call-rate, minor allele frequency (MAF), the exact \( P \)-value for a test of departure from Hardy–Weinberg equilibrium (HWE) in subjects without clinical evidence of CHD, and concordance rates for duplicate genotyping. We pre-specified a threshold call rate of 90%, but included SNPs with call rates >80% provided the MAF was concordant with other studies, genotype error rates were <1%, and the \( P \)-value for deviation for HWE exceeded 0.001. The pre-specified analysis plan is included as Supplementary material online, Methods 2. Genotypes were coded using a standardized designation for homozygous and heterozygous individuals. Individuals homozygous for the common allele served as the reference group for all the comparisons.

Each contributing study estimated an unadjusted and adjusted OR (and standard error) for CHD, for each additional rare allele carried (i.e. a trend analysis using an additive model on the logarithmic scale) as well as for subjects heterozygous or homozygous for the rare allele compared with those homozygous for the common allele. Variables used in the adjustment were age (in 5 year bands, e.g. 50–55, etc.), gender (male vs. female), and smoking (ever vs. never). A summary odds ratio (95% confidence interval) for the risk of CHD for each SNP was calculated by fixed effects meta-analysis using the Mantel–Hanzel method as well as by random effects meta-analysis using the DerSimonian and Laird method. Where available, we included estimates from the Wellcome Trust Case Control Consortium 1 (WTCCC1) study of CHD in the meta-analysis. A
Table I  Studies contributing to the Cardiovascular Biomarker Genetics Collaboration (see Appendix 1 for further details)

<table>
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<tr>
<th>Study design</th>
<th>NPHS-II</th>
<th>BRHS</th>
<th>ELSA</th>
<th>EAS</th>
<th>WH-II</th>
<th>1958BC</th>
<th>MRC 1946</th>
<th>SAS</th>
<th>SHEEP</th>
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<th>BRIGHT-cases</th>
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<td>Prospective</td>
<td>Prospective</td>
<td>Prospective</td>
<td>Prospective</td>
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<td>Birth cohort</td>
<td>Cases with angiographic coronary disease</td>
<td>CHD patients</td>
<td>Cases of CHD and controls</td>
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<td>Cases from case–control study of hypertension</td>
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<td>Respondents of HSE</td>
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<td>56.1 (3.4)</td>
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*a Incident cases only.
*b Both prevalent and incident cases.
*c Prevalent cases only; NA: not available.
false discovery rate (FDR)-adjusted P-value was calculated based on the number of hypotheses tested. Defining FDR as the proportion of falsely rejected hypotheses, i.e. for which the null was actually true, this new P-value, known as the q-value, is the minimum FDR when rejecting a null hypothesis from a list of tested null hypothesis, conditioned on at least one positive finding having occurred.

### Table 2: Categories of single nucleotide polymorphisms typed by the Cardiovascular Biomarkers Genetics Collaboration

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<th>SNP</th>
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<th>Chr</th>
<th>Allele 1*</th>
<th>Allele 2</th>
<th>Initial discovery studyb</th>
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<td>rs688034</td>
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<td>22</td>
<td>C</td>
<td>T</td>
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T2D SNPs

| rs10811661 | CDKN2B (d) | 9 | C         | T        | Saxena (2007) |
| rs9939609  | FTO       | 16 | T         | A        | Frayling (2007) |

HDL SNPs

| rs17411031 | LPL       | 8  | C         | G        | Wallace (2008) |
| rs3890182  | ABCA1     | 9  | G         | A        | Kathiresan (2008) |
| rs261332   | LPC       | 15 | G         | A        | Wallace (2008) |
| rs9989419  | CETP      | 16 | G         | A        | Willer (2008) |
| rs16979595 | CLPTM1    | 19 | G         | A        | Wallace (2008) |

LDL SNPs

| rs599839   | SORT1     | 1  | A         | G        | Willer (2008) |
| rs562338   | APOB      | 2  | A         | G        | Willer (2008) |
| rs688      | LDLR      | 19 | C         | T        | Wallace (2008) |
| rs4420638  | APOC1/APOE | 19 | A         | G        | Willer (2008) |

Triglyceride SNPs

| rs12042319 | ANGPTL3   | 1  | G         | A        | Wallace (2008) |
| rs3917820  | SELP      | 1  | G         | A        | Wallace (2008) |
| rs12140698 | FAM5B (d) | 1  | C         | T        | Wallace (2008) |
| rs780094   | GCKR      | 2  | C         | T        | Willer (2008) |
| rs1471233  | intergenic | 4 | C         | T        | Wallace (2008) |
| rs2074755  | BAZ1B     | 7  | T         | C        | Wallace (2008) |
| rs17482753 | LPL       | 8  | G         | T        | Wallace (2008) |
| rs4740635  | intergenic | 9 | G         | C        | Wallace (2008) |
| rs7861175  | LPAR1 (d) | 9  | T         | C        | Wallace (2008) |
| rs6589566  | APOA5     | 11 | A         | G        | Wallace (2008) |
| rs7229921  | CIDEA (d) | 18 | A         | G        | Wallace (2008) |

(d) refers to the nearest downstream gene.

*Allele 1 is the reference allele for all comparisons.

Initial discovery studies are Samani (2007); WTCCC (2007); McPherson (2007); Saxena (2007); Frayling (2007); Wallace (2008); Kathiresan (2008); Willer (2008).

Single nucleotide polymorphism associations with continuous risk factors and biomarkers

Eleven studies including NPHS II, BRHS, ELSA, EAS, WHII, 1958BC, MRC 1946, SHEEP, UDACS, EDS, and BRIGHT contributed to the analysis of SNP associations with continuous risk factors and biomarkers. The distributions of TG, homocysteine, C-reactive
protein, and interleukin-6 (IL-6) were skewed in all studies and these variables were log transformed and analysed on the log-scale. For each SNP, we performed a linear regression analysis within study for each continuous biomarker assuming an additive effect of each variant allele. The per-allele regression coefficient, which is equal to the weighted per-allele mean difference, was then divided by the pooled standard deviation of the trait of interest, derived using information from each of the three genotypes categories; to calculate the standardized mean difference (and its corresponding standard error). Further details are provided in Supplementary material online, Methods 2 with information on the STATA do-files. Separate analyses were conducted unadjusted and adjusted for all of age (in 5 year bands, e.g. 50–55, etc.), gender (male vs. female), and smoking (ever vs. never). We used random and fixed effect meta-analysis to pool the within-study estimates for each trait to generate a summary per-allele standardized mean difference (and 95% confidence interval) to allow comparison of SNP effects across different traits on a common scale. For all traits, the mean of the standardized mean differences is 0 and the standard deviation 1. In the absence of consensus regarding statistical significance level for reporting SNP-associations with multiple traits or disease endpoints (pleiotropy), we reported all results as point estimates and 95% confidence limits (although P-values are available in the Supplementary material online). An FDR-adjusted P-value was also calculated based on the number of hypotheses tested.33 Supplementary material online, Methods 3 provides a detailed discussion of sample size and study power in the context of the range of confirmatory and exploratory analyses that we conducted.

Results

Study populations and measures

The age range of the study populations was 44–67 years. Two studies were of men only (NPHS II and BRHS) while in the remainder, the proportion of men was between 48 and 72% of participants. Among the prospective studies with incident events, the length of follow-up was between 10 and 26 years. The two case–control studies (SHEEP and SAS) (2377 cases), and five of the seven prospective studies (1495 cases) contributed to the analysis of CHD outcomes (Table 1). The control subjects from SHEEP, participants with BP but no CHD from BRIGHT, and the participants from all cross-sectional and cohort studies contributed to the analyses of SNP effects on risk factors and biomarkers (Table 1). Information was available on BMI from 25 078 participants, and systolic and diastolic BP from 25 204 and 25 195 participants, respectively. The blood biomarkers measured spanned lipids and lipoproteins, indices of glycaemic control, inflammation, and coagulation including: total cholesterol (TC; \( n = 25\,286 \)), HDL-C (\( n = 24\,064 \)), LDL-C (\( n = 23\,636 \)), fasting, or non-fasting TGs (\( n = 24\,161 \)), ApoA1 (\( n = 8476 \)), ApoB (\( n = 8476 \)), C-reactive protein (\( n = 19\,944 \)), IL-6 (\( n = 8507 \)), fibrinogen (\( n = 20\,053 \)), fasting glucose (\( n = 14\,983 \)), fasting insulin (\( n = 8100 \)), glycated haemoglobin (HbA1c; \( n = 18\,559 \)), and homocysteine (\( n = 6253 \)).

Single nucleotide polymorphisms previously identified for an association with coronary heart disease

There was a strong concordance of MAFs across studies; see Supplementary material online, Tables S1–S3 for call rates, MAF and tests for departure from HWE. Of the 14 SNPs selected through an association with CHD, the following nine SNPs were associated with CHD events in a fixed effects meta-analysis: two at chromosome 9p21 (rs1333049, OR 1.17; 1.11–1.24 and rs10757274, OR 1.17; 1.09–1.26), and one each near SORT1 rs599839 (OR 1.20; 1.15–1.26), MIA3 rs17465637 (OR 1.10; 1.04–1.15), Ch2q36 rs2943634 (OR 1.08; 1.03–1.14), APC rs383830 (OR 1.10; 1.02–1.18), MTHFD1L rs6922269 (OR 1.10; 1.03–1.16), CXCL12 rs501120 (OR 1.12; 1.04–1.20), and SMAD3 rs17228212 (OR: 1.11; 1.05–1.17) (Figure 1 and Supplementary material online, Table S4). Supplementary material online, Figure S1 provides estimates from a random effects model. However, aside from rs599839, none of these CHD SNPs was associated with any of the wide range of risk factors and biomarkers analysed, despite available information from 20 000 or more participants for TC, LDL-C, HDL-C, TG, BMI, BP, fibrinogen, and C-reactive protein, over 15 000 for HbA1c, 10 000 for fasting glucose, and just under 10 000 for IL-6 and fasting insulin (Figures 2 and 3).

Single nucleotide polymorphisms previously identified for an association with blood lipids

Seventeen of 20 SNPs selected because of an initial association with a blood lipid component were associated with the same lipid fraction in the CBGC studies (Figure 3 and Supplementary material online, Table S4). The overlap with SNPs identified by the recent Global Lipids Genetics Consortium (GLGC) meta-analysis35 is shown in Supplementary material online, Table S7. However, in all cases the effect of this category of SNPs was found to extend beyond the initial reported lipid fraction. For example, ANGPTL3 rs12042319 was associated with BMI, TC, LDL-C, and IL-6 in addition to the reported association with TG (Supplementary material online, Figure S2). Another SNP, LIPC rs261332 whose initial reported association was with HDL-C, also showed additional associations with ApoAI, TC, fasting insulin, and TG (Supplementary material online, Figure S3).

A summary profile of associations for all the SNPs analysed with all the biomarkers measured is shown in Figure 3. The range and direction of associations was distinctive for each SNP. For example, of the five SNPs associated with HDL-C (CLPTM1 rs16979595, LPL rs17411031, LIPC rs261332, ABCA1 rs3890182, and CETP rs9989419), all had a different pattern of association with other risk factors and biomarkers. For some SNPs, the associations extended across a wider range of CHD biomarkers e.g. for GCKR rs780094, associations encompassed lipids and ApoB (TC and TG) as well as the inflammation markers C-reactive protein and fibrinogen (Supplementary material online, Figure S4).
Of the SNPs in this category, only three were associated with CHD (Figure 1): LPL rs17411031 whose initial association was with HDL-C (OR 0.91; 0.84–0.97) (Figure 4), SORT1 rs599839 whose initial association was with LDL-C (OR 1.20; 1.15–1.26), and ANGPTL3 rs12042319 whose initial association was with TG (OR 1.11; 1.03–1.19) (Supplementary material online, Figure S2).

Single nucleotide polymorphisms previously identified for an association with type-2 diabetes or adiposity

Neither of the two SNPs typed whose initial association was with T2DM (Ch9p21 rs10811661 and FTO rs9939609) or adiposity (FTO rs9939609) was associated with CHD in the current analysis.
However, we observed associations of the FTO SNP with variables incorporated in one or more definitions of the metabolic syndrome including systolic BP, TG and HDL-C (but not LDL-C), and C-reactive protein, in addition to fasting insulin and glucose, HbA1c, and BMI (Figures 3 and 5).

**Discussion**

Genome wide association studies have had resounding success in identifying genetic variants contributing to individual differences in the levels of established and emerging risk factors and CHD events.\(^5\,6\,11\,19\,35\,36\) Genome wide association studies thus far have typically been designed to assess associations of many hundreds of thousands of SNPs usually with a single risk factor or disease endpoint at a time. However, many cardiovascular risk factors and biomarkers are correlated, and scores of alleles are thought to contribute to any common disease or traits,\(^37\) so it has been hypothesized that overlapping genetic associations with multiple phenotypes are likely to be frequent. For example, SNPs in the SH2B3 gene has been associated with celiac disease, rheumatoid arthritis, eosinophil count, high BP, and MI.\(^5\,6\,38\,39\)

In the current analysis, we systematically tested the extent to which SNPs identified through an initial association with a lipid fraction, diabetes, or CHD risk are related to a wider range of intermediate phenotypes. The aim was to identify common points of regulation among correlated risk factors and biomarkers and to evaluate which intermediate phenotypes are likely to lie in or mark changes in the causal pathways involved in CHD.

**Single nucleotide polymorphisms initially identified for an association with coronary heart disease events**

The well-studied Ch9p21 SNPs (rs1333049 and rs10757274) were significantly associated with the endpoint of CHD events, defined as prevalent MI (in SHEEP), incident/prevalent CHD events (in the prospective observational studies) or angiographic CAD (in SAS). However, neither of these SNPs nor the lead SNPs in SMAD3, MIA3, CXCL12, MTHFD1L, or Ch2q36 were associated with T2DM or any of the CHD biomarkers studied including BMI, BP, blood lipids, and ApoBs, the inflammation markers IL-6, fibrinogen, and C-reactive protein, or a number of markers of glycaemic status including fasting glucose and insulin as well as HbA1c. Given that

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**Figure 2** Association of rs1333049 in the CDKN2B region on chromosome 9 with coronary heart disease-related risk factors and biomarkers.
the combined data set included over 20,000 observations for BMI, BP, TC, HDL-C and LDL-C, TG, C-reactive protein, and fibrinogen and over 10,000 for fasting glucose and HbA1c and the fact that these traits are continuous rather than dichotomous measures, the effect estimates we obtained are likely to be precise and based on adequately powered analysis. The FDR for many of the identified trait associations have a q-value < 10\(^{-5}\),

suggesting that the majority of these are likely to be true. However, we could not exclude very minor effects on these or other traits. Prior studies of these CHD-associated SNPs (including the initial GWAS) have reported a lack of association with blood lipids and BP or with clinically defined hypertension or hyperlipidaemia, but the breadth and detail of intermediate phenotypes studied previously has not been as great as in the current study.\(^5,12,13,41,42\)

**Figure 3** (A) Matrix of associations between the 34 single nucleotide polymorphisms analysed, continuous risk factors and biomarkers, and coronary heart disease events. Each cell is colour coded according to the P-value for the relevant association. (B) Matrix of associations between the 34 single nucleotide polymorphisms analysed, continuous risk factors and biomarkers, and coronary heart disease events. Each cell is colour coded according to the beta-coefficient from the pooled regression analysis (i.e. effect size) for the relevant association.
The association of these SNPs with clinical events despite the absence of association with a wide range of established and emerging cardiovascular risk factors suggests their effects are mediated through a previously unsuspected disease mechanism. Additional fine mapping analyses will be required to demarcate the likely causal variants and functional studies to help identify the disease mechanisms.

Lead SNPs in genomic regions including \textit{SEZ6L}, \textit{VSTM2B}, \textit{CDH13}, \textit{ADAMTS7}, and \textit{FMN2} that were associated with CHD in initial GWAS,\textsuperscript{5,12} were not associated with CHD events in the current data set, broadly consistent with a recent analysis from the Coronary Artery Disease Consortium which included \textasciitilde 11 000 cases of CHD or MI.\textsuperscript{41}

Single nucleotide polymorphisms initially identified for an association with lipids

With the exception of three SNPs identified previously to be associated with TG (\textit{SELP} rs3917820, Ch9p24 rs4740635, \textit{ODEA} rs7229921), we replicated associations of 17 other SNPs with LDL-C, HDL-C, or TG. Each of these 17 variants had additional effects on other lipids or ApoBs, at least equal in size to the index association, and some also had effects on other phenotypes such as inflammation markers or glycaemic indices.

Although many of these phenotypes are inter-correlated, the profile of associations was distinctive for each SNP (Figure 3), arguing that SNPs associated with several phenotypes have a true biological basis. For example, of the five SNPs associated in the same direction with HDL-C, only two were associated with TG; however, the direction of the effect was different. A similar situation was observed for SNPs originally associated with TG. These marked differences in the patterns of SNP associations contrast with the observed almost invariable association between HDL-C and TG levels.\textsuperscript{43} Genetic studies in populations have been likened to natural randomized trials and we have previously reported on concordant effects on blood lipids and lipoproteins of \textit{CETP} SNPs and treatment with a CETP-inhibitor.\textsuperscript{44} Genome wide association studies have also reported associations of SNPs in the \textit{HMGCR} gene (which encodes the target for statins) with LDL-C and CHD risk.\textsuperscript{16,17} Single nucleotide polymorphisms in the gene \textit{PPARG} that encodes the target for glitazone drugs have also been shown to influence the risk of T2DM.\textsuperscript{19,45} This suggests that the SNPs

![Figure 4](https://example.com/figure4.png)

**Figure 4** Association of rs17411031 in \textit{LPL} with triglycerides, HDL-cholesterol, and Apolipoprotein AI.
Lipid Traits | Subjects | SMD (95% CI)
--- | --- | ---
ApoA1 | 8273 | -0.01 (-0.04, 0.03)
ApoB | 8273 | 0.01 (-0.02, 0.04)
TC | 24098 | 0.01 (-0.01, 0.03)
HDL-C | 22912 | -0.02 (-0.04, 0.00)
LDL-C | 22509 | 0.01 (-0.01, 0.03)
Triglycerides | 22979 | 0.02 (0.00, 0.04)

Adiposity and Glycaemic Control | Subjects | SMD (95% CI)
--- | --- | ---
BMI | 23957 | 0.07 (0.05, 0.09)
Glu | 14295 | 0.03 (0.01, 0.06)
HbA1c | 17586 | 0.03 (0.01, 0.06)
Insulin | 8001 | 0.05 (0.01, 0.10)

Blood Pressure | Subjects | SMD (95% CI)
--- | --- | ---
DBP | 24055 | 0.02 (-0.01, 0.04)
PulseP | 23049 | 0.02 (0.00, 0.04)
SBP | 24058 | 0.03 (0.01, 0.05)

Emerging Biomarkers | Subjects | SMD (95% CI)
--- | --- | ---
Fibr | 18891 | 0.01 (-0.01, 0.03)
CRP | 18817 | 0.02 (-0.00, 0.04)
Hcy | 6095 | -0.01 (-0.04, 0.03)
IL-6 | 8416 | 0.01 (-0.02, 0.04)

Figure 5 Association of rs9936909 in FTO with components of the metabolic syndrome.

Single nucleotide polymorphisms initially identified for an association with body mass index or diabetes

We studied two SNPs identified with an index association with T2DM, one of which (FTO rs9936909) is thought to act through a primary effect on adiposity and BMI. Neither of these SNPs was associated with CHD in this study, however, the FTO SNP was associated with higher BMI, systolic BP, fasting insulin and glucose, HbA1c, TG, and CRP, as well as a lower HDL-C. The International Diabetes Federation (http://www.idf.org/metabolic_syndrome) defines metabolic syndrome as the presence of central obesity (indexed by waist circumference) together with the presence of two of the following: raised BP, raised TGs, raised fasting glucose, and reduced HDL-C. Our findings are also in keeping with those reported previously from a meta-analysis of ~17,000 participants.57 Taken together, the findings suggest that targeting FTO itself or FTO-mediated effects may be effective.

LPL pathway should be performed to evaluate it as a possible therapeutic target for coronary prevention.
in reducing the risk of metabolic syndrome and, perhaps, its downstream consequences on disease risk as recently suggested.\textsuperscript{54}

**Limitations**

Our analysis was limited to SNPs identified by the first wave of GWAS, and although the list of variants influencing CHD, blood lipids, diabetes, and BMI has since increased substantially, the first SNPs to be identified are likely to represent the largest effect sizes. Our analysis represents one of the first attempts to study the effect of SNPs from GWAS relevant to cardiovascular disease on a wide range of cardiovascular phenotypes as well as CHD. The study is well powered to achieve this, and for example, a sample size of \( \sim 7000 \) subjects would be required to be able to discover SNPs that explain as little as 0.5\% of the variance with 80\% power, and for the majority of traits we far exceed this number. The approach we have taken could now be extended to incorporate both a wider range of SNPs from subsequent GWAS as well as a wider range of phenotypes, to build a more comprehensive picture of the repertoire of SNPs affecting each of the cardiovascular risk factors and biomarkers as well as the repertoire of traits affected by any given SNP, and to integrate this information with the risk of clinical disease endpoints.

The GLGC analysis was a recent hugely important and successful effort to discover and replicate additional loci for four lipid traits: total-, LDL-, and HDL-C, as well as TGs. For the SNPs that were present in both our study and the GLGC, the directions of effect were concordant as shown in Supplementary material online, Tables S5–S7. However, the GLGC did not have the opportunity to study the wide range of inflammation, coagulation, and glycaemic markers that we have been able to report on in the present work. Although the GLGC analysis investigated the association of lipid-associated loci with CHD risk in 24 607 CAD cases compared with 5794 cases in the current analysis (see Supplementary material online, Table S7), many of the SNPs we studied were already identified for their association with CHD risk. Nevertheless, our findings should be interpreted within the context of the available power of the study and the potential for false positive association. There may also have been heterogeneity of effect estimates among the different sample collections evidenced by the high \( I^2 \) values for many of the pooled analyses, and the reasons for this potential heterogeneity are worthy of further investigation, though some could be attributed to the differences in study design. Ongoing collaborative GWAS in CHD should help to clarify the role of these loci and also unveil additional smaller effect loci underlying susceptibility to CHD.

**Conclusions**

The unique properties of genotype, which are distinct from other natural differences between individuals, provide new opportunities for evaluating causal links between associated intermediate phenotypes and between phenotypes and disease. Our findings demonstrate that there are likely to be important unsuspected disease mechanisms and therapeutic targets for CHD; and that there may be points of regulation for diverse cardiovascular risk factors and biomarkers. In future, enhancing the level of detail at both genetic and phenotypic level, incorporating transcriptomics, metabolomics, as well as structural imaging should provide a more comprehensive understanding of the mechanisms linking genome variation with disease. In turn, this should help the development of additional effective therapies for cardiovascular disease prevention.

**Supplementary material**

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: A.D.H. is on the editorial board of Drug and Therapeutics Bulletin, a BMJ Group Publication; has provided non-renumerated advice to GlaxoSmithKline, and London Genetics; and has received honoraria for speaking at educational meetings relating to risk factor management and primary prevention. J.W. is 90% employed at GlaxoSmithKline while retaining a 10% appointment at London School of Hygiene and Tropical Medicine.

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