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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.

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See page 290 for the editorial comment on this article (doi:10.1093/eurheartj/ehr225)

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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.7–9 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 mechanisms 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 SNPs 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),\textsuperscript{20} British Regional Heart Study (BRHS),\textsuperscript{21} English Longitudinal Study of Ageing (ELSA),\textsuperscript{22} Edinburgh Artery Study (EAS),\textsuperscript{23} the 1958 Birth Cohort (1958BC),\textsuperscript{24} the MRC 1946 Birth Cohort (MRC 1946),\textsuperscript{25} and the Whitehall II Study (WHII).\textsuperscript{26} 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),\textsuperscript{27} and the Stockholm Heart Epidemiology Program (SHEEP).\textsuperscript{28} 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. Two cross-sectional studies included individuals without CHD but with a diagnosis of T2DM (based on individuals with a fasting glucose level $\geq 11.1$ mmol/L, or self-reported use of anti-diabetic medication): the UCL Diabetes and Cardiovascular Disease Study (UDACS)\textsuperscript{29} and the Ealing Diabetes Study (EDS)\textsuperscript{30} (Supplementary material online, Methods 1 and Table S1). One cross-sectional study, the MRC-BHF British Genetics of Hypertension (BRIGHT) study\textsuperscript{31} 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.\textsuperscript{32} 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 $\geq 50\%$ 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 $< 10^{-7}$ 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 9p21 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 SNPplex platform; or the Centre for Cardiovascular Genetics UCL, using the ABI TaqMan platform (Table 1). The Whitehall II study provided genotypes from the IITMAT/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
Table 1  Studies contributing to the Cardiovascular Biomarker Genetics Collaboration (see Appendix 1 for further details)

| Study design<sup>a</sup> | NPHS-II | BRHS | ELSA | EAS | WH-II | 1958BC | MRC 1946 | SAS | SHEEP | UDACS | EDS | BRIGHT-cases | Total |
|--------------------------|---------|------|------|-----|-------|--------|----------|-----|-------|------|-----|-------------|-------|
| Study design<sup>a</sup> | Prospective cohort | Prospective cohort | Prospective cohort | Prospective cohort | Prospective cohort | Prospective birth cohort | Cases with angiographic coronary disease | Cases of CHD and controls | Swedish citizens | Diabetic patients | Diabetic patients | Cases from case-control study of hypertension |
| Sampling frame | General practices | Respondents of HSE | General practices | General practices | Workplace | Birth register | Birth register | CHD patients | Diabetic patients | Diabetic patients | General practices |
| N with DNA | 2775 | 3947 | 5274 | 940 | 1480 | 2700 | 1164 | 2698 | 575 | 311 | 1759 |
| Genotyping method | TaqMan | SNPlex | TaqMan | SNPlex | Affy 500k CVD chip | IBC 50k CVD chip | TaqMan | KASPAR | TaqMan | Affy 500k |
| % men | 100 | 100 | 48 | 50 | 77 | 50 | 76 | 69 | 59 | 60 | 40 |
| Years follow-up | 17 | 26 | 48 | 50 | 20 | 51 | 63 | 60 | 59 | 60 | 40 |
| Mean (SD) age of participants | 56.1 (3.4) | 64 | 64.3 (5.63) | 60.9 (6.0) | 45 | 53 at year of collection now 63 | 63.4 | 59.6 (7.15) | 66.7 (11.0) | 63.5 (13.8) | 54.6 (10.1) |
| Baseline year | 1989–94 | 1978–80 | 1998, 1999, 2001 | 1987 | 1985–88 | 1958 | 1946 | 2000–01 | 1992–94 | 2001 | 2001–02 | 1996 |
| Cases of CHD | 273<sup>b</sup> | 724<sup>b</sup> | 140<sup>c</sup> | 241<sup>c</sup> | NA | NA | 1164<sup>c</sup> | 1213<sup>c</sup> | NA | NA | NA | 3872 |
| Cases of T2D | 229<sup>b</sup> | 595<sup>b</sup> | 249<sup>c</sup> | 336<sup>c</sup> | NA | NA | NA | 600 | 331 | NA | 2340 |
| BMI | 2746 | 3863 | 5257 | 893 | 4789 | 1436 | 2455 | 310 | 1732 | 25 078 |
| Systolic BP | 2747 | 3860 | 5342 | 892 | 4803 | 1431 | 2439 | NA | 320 | 1759 | 25 204 |
| Diastolic BP | 2747 | 3860 | 5341 | 890 | 4803 | 1431 | 2439 | NA | 319 | 1759 | 25 195 |
| Pulse P | 2747 | 3860 | 5341 | 890 | 4803 | 1431 | 2439 | NA | 319 | 1759 | 24 145 |
| Total cholesterol | 2742 | 3845 | 5406 | 892 | 4799 | 1416 | 2321 | NA | 311 | 1502 | 25 286 |
| LDL cholesterol | 1735 | 3732 | 5264 | 887 | 4741 | 1338 | 2145 | NA | 269 | 1502 | 23 636 |
| HDL cholesterol | 1836 | 3735 | 5404 | 887 | 4799 | 1413 | 2155 | NA | 289 | 1503 | 24 064 |
| Triglycerides | 2742 | 2745 | 5406 | 892 | 4799 | 1415 | 2319 | NA | 289 | 1502 | 24 161 |
| ApoAI | 2344 | NA | NA | NA | 4637 | NA | NA | 1495 | NA | NA | 8476 |
| ApoB | 2344 | NA | NA | NA | 4637 | NA | NA | 1495 | NA | NA | 8476 |
| Homocysteine | 1361 | 3776 | NA | NA | 4637 | NA | NA | 1116 | NA | NA | 6253 |
| HbA1c | 3792 | 5405 | NA | 4759 | 1414 | 2333 | NA | 303 | 18 559 |
| Glucose | 3843 | 3224 | 891 | 4791 | NA | NA | NA | 315 | 14 983 |
| Insulin | 2717 | NA | NA | 4259 | NA | NA | NA | 303 | 8100 |
| Fibrinogen | 2733 | 3834 | 5436 | 874 | 4357 | 1416 | NA | 1403 | NA | NA | 20 053 |
| IL-6 | 2258 | NA | 628 | 4274 | NA | NA | 801 | 546 | NA | 8507 |
| C-reactive protein | 2279 | 3833 | 5404 | 605 | 4663 | 1418 | NA | 1115 | 545 | 82 | NA | 19 944 |

<sup>a</sup>Incident cases only.
<sup>b</sup>Both prevalent and incident cases.
<sup>c</sup>Prevalent cases only; NA: not available.
Table 2  Categories of single nucleotide polymorphisms typed by the Cardiovascular Biomarkers Genetics Collaboration

| SNP       | Gene    | Chr | Allele 1 | Allele 2 | Initial discovery study |
|-----------|---------|-----|----------|----------|-------------------------|
| CHD SNPs  |         |     |          |          |                         |
| rs599839  | SORT1   | 1   | A        | G        | Samani (2007)           |
| rs17465637| MIA3    | 1   | C        | A        | Samani (2007)           |
| rs17672135| FMN2    | 1   | T        | C        | WTCCC (2007)            |
| rs2943634 | intergenic | 2 | C        | A        | Samani (2007)           |
| rs383830  | APC     | 5   | T        | A        | WTCCC (2007)            |
| rs6922269 | MTHFD1L | 6   | G        | A        | Samani (2007)           |
| rs1333049 | CDKN2B  (d) | 9 | G        | C        | WTCCC (2007)            |
| rs10757274| CDKN2B (d) | 9 | A        | G        | McPherson (2007)        |
| rs501120  | CXCL12  (d) | 10 | C        | T        | Samani (2007)           |
| rs1994016 | ADAMTS7 | 15  | C        | T        | WTCCC (2007)            |
| rs17228212| SMAD3   | 15  | T        | C        | Samani (2007)           |
| rs8055236 | CDH13   (d) | 16 | T        | G        | WTCCC (2007)            |
| rs7250581 | VSTM2B  | 19  | A        | G        | WTCCC (2007)            |
| rs688034  | SEZ6L   | 22  | C        | T        | WTCCC (2007)            |
| 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)       |
| rs2611332 | 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   | 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 | LRAR1   (d) | 9 | T        | C        | Wallace (2008)          |
| rs6589566 | APOA5   | 11  | A        | G        | Wallace (2008)          |
| rs7229921 | CID15   (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); Wheaton (2007); McPherson (2007); Saxena (2007); Frayling (2007); Wallace (2008); Kathiresan (2008); Willer (2008). Comparing analysis of loci for lipids, diabetes, and CHD.

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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 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.

**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 effect meta-analysis to pool the within-study estimates for 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. An FDR-adjusted P-value (q-value) was also calculated based on the number of hypotheses tested. 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 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-analysis 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

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**Figure 1** Forest plot of associations of 34 single nucleotide polymorphisms identified by GWAS for association with coronary heart disease risk, type-2 diabetes or adiposity or blood lipids with coronary heart disease events. Odds ratios (95% CI) are presented for the Cardiovascular Biomarker Genetics Collaboration data with WTCCC1 data where available using a fixed effects model.
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.\(^5\,^6\,^38\,^39\,^40\)

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 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

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 SEZ6L, VSTM2B, CDH13, ADAMTS7, and FMN2 that were associated with CHD in initial GWAS,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 ≏11 000 cases of CHD or MI.41

Single nucleotide polymorphisms initially identified for an association with lipids. With the exception of three SNPs identified previously to be associated with TG (SELP rs3917820, Ch9p24 rs4740635, 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.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 CETP SNPs and treatment with a CETP-inhibitor.44 Genome wide association studies have also reported associations of SNPs in the HMGCR gene (which encodes the target for statins) with LDL-C and CHD risk.16,17 Single nucleotide polymorphisms in the gene PPARG that encodes the target for glitazone drugs have also been shown to influence the risk of T2DM.19,45 This suggests that the SNPs...
associated with blood lipids and other biomarkers could help to profile the likely effects of pharmacological modification of the same targets. The diverse effect profiles of SNPs associated with HDL-C indicate that not all therapeutic approaches for HDL-C elevation with the aim of coronary prevention are likely to be equally effective and that the choice of target may matter as much as the elevation of HDL-C per se.

The ANGPTL3 SNP rs12042319 was associated with increased CHD risk but with lower LDL-C, TG, and IL-6 levels, all of which have themselves been associated with increased risk of CHD.\textsuperscript{46–48} Although ANGPTL3 has been associated with TG and LDL-C levels,\textsuperscript{35} it has not been previously associated with CHD risk in GWAS analysis or in the recent pooled analysis.\textsuperscript{49} Therefore, the CHD association we observed should be considered hypothesis generating, and any relevant mechanism is deserving of further investigation. The LPL SNP rs17411031 was associated with a lower risk of CHD and with lower TG and higher HDL-C values. Other SNPs in this gene have previously been shown to affect TG, HDL-C, and CHD risk,\textsuperscript{14–17,19,50} and a recent analysis of a common variant in the APOA5 gene that is functionally linked with LPL, is also associated with TG, HDL-C, and CHD risk.\textsuperscript{51,52,47} These findings suggest further studies of the ApoAV/LPL pathway should be performed to evaluate it as a possible therapeutic target for coronary prevention.

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

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**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 rs9939609) 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 C-reactive protein, 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, HbA1c, and C-reactive protein, 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, HbA1c, and C-reactive protein, as well as a lower HDL-C. Our findings are also in keeping with those reported previously from a meta-analysis of ~17 000 participants.\textsuperscript{53} Taken together, the findings suggest that targeting FTO itself or FTO-mediated effects may be effective
in reducing the risk of metabolic syndrome and, perhaps, its downstream consequences on disease risk as recently suggested.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 ~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 current analysis and Socio-economic Status and Health. Analyses of the UK Biobank, the English Longitudinal Study of Ageing (ELSIA) DNA Repository (EDNAR), received support under a grant (AG176440651) awarded by the National Institute on Ageing (NIA). ELSA was developed by a team of researchers based at the National Centre for Social Research, University College London and the Institute of Fiscal Studies. The data were collected by the National Centre for Social Research. The developers and funders of ELSA and the Archive do not bear any responsibility for the analyses or interpretations presented here. The Edinburgh Artery Study (EAS) was funded by the British Heart Foundation. The Whitehall II study has been supported by grants from the Medical Research Council; Economic and Social Research Council; British Heart Foundation; Health and Safety Executive; Department of Health; National Heart Lung and Blood Institute (HL36310), US; NIH: National Institute on Aging (AG13196), US; NIH: Agency for Health Care Policy Research (HS06516); and the John D and Catherine T MacArthur Foundation Research Networks on Successful Midlife Development and Socio-economic Status and Health. Analyses of the 1958 birth cohort data were funded by the Medical Research Council (G0601653) and undertaken at GOSH/UCL Institute of Child Health, which received a proportion of funding from the Department of Health’s NHHR Biomedical Research Centres funding scheme. DNA collection was funded by MRC grant G0000934 and genotyping was funded by Wellcome Trust grant 068545/Z/02. The 1946 British birth cohort is funded by the UK Medical Research Council. The Southampton Atherosclerosis Study (SAS) was funded by the British Heart Foundation PG/ 98183. This work was facilitated by the Barts and The London National Institute for Health Research Cardiovascular Biomedical Research Unit. The Stockholm Heart Epidemiology Program Study (SHEEP) was supported by grants from the Research Council of Sweden (0933), the Swedish Heart and Lung Foundation and Stockholm County Council (ALF). The BRIGHT study is supported by the Medical Research Council (G9521010D) and the British Heart Foundation (PG/02/128). The Barts and The London Charity funded the Barts and The London Genome Centre. This work forms part of the research themes contributing to the translational research portfolio of Barts and the London Cardiovascular Biomedical Research Unit which is supported and funded by the National Institute of Health Research. The BRIGHT study is also extremely grateful to all the patients who participated in the study and the BRIGHT nursing team. The Wellcome Trust Case Control Consortium was funded by the Wellcome Trust (grant number: 076113/B/04/Z). A.D.H. holds a British Heart Foundation Senior Fellowship (FS 05/125). L.S. holds a Wellcome Trust Senior Research Fellowship. R.S. is supported by a British Heart Foundation (Schillingford)

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 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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References

1. Brotman DJ, Walker E, Lauer MS, O'Brien RG. In search of fewer independent risk factors. Arch Intern Med 2005;165:138–145.

2. Katan M. Apolipoprotein E isoforms, serum cholesterol, and cancer. Lancet 1986;1:507–508.

3. Davey Smith G, Ebrahim S. ‘Mendelian randomization’: can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003;32:1–22.

4. Hingorani A, Humphries S. Nature’s randomised trials. Lancet 2005;366:1906–1908.

5. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–678.

6. Levy D, Eireth GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Uitterlinden AG, Heiss G, Fox CS, Witteman JCM, Boerwinkle E, O’Donnell CJ, Strachan D, Boettcher T, van Duijn CM. Genomewide association analysis study of blood pressure and hypertension. Nat Genet 2009;41:677–687.

7. Hardy J, Singleton A. Genomewide association studies and human disease. N Engl J Med 2006;359:1759–1768.

8. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. PNAS 2009;106:9362–9367.

9. Manolio TA. Cohort studies and the genetics of complex disease. Nat Genet 2009;41:5–6.

10. Heid IM, Boes E, Muller M, Kollieris B, Lamina C, Coassin S, Gieger C, Doring A, Klopp N, Frihke-Schmidt R, Tybring-Hansen A, Brandtstader A, Luchner A, Meitinger T, Wichmann HE, Kronberg F. Genome-wide association analysis of high-density lipoprotein cholesterol in the population-based KORA study sheds new light on intergenic regions. Circ Cardiovasc Genet 2008;1:10–20.

11. Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T, Voight BF, Bonnycastle LL, Jackson AU, Crawford G, Surti A, Guiducci C, Burt NP, Parish S, Clarke R, Ziegenhain D, Kubalaan KA, Marken MA, Scott LJ, Stringham HM, Galan P, Swift AJ, Kissuuto J, Bergmann RN, Junkins J, Laska M, Ferrucci L, Scheet P, Sanna S, Uda M, Yang Q, Lunetta KL, Dupuis J, de Bakker WP, O’Donnell CJ, Chambers JC, Kooper JS, Herr berg SD, Meneton P, Lakatta EG, Scutier A, Schlessinger D, Tuo mimelhoj J, Collins FS, Groop L, Alshuler D, Collins R, Lathrop GM, Melander O, Salomaa V, Peltonen L, Orho-Melander M, Ordovas JM, Boehnke M, Abecasis GR, McCarthy MI, Postmus D, Ingelsson E, Schunkert H, the WTCCC and the Cardiogenics Consortium. Genome-wide association analysis of coronary artery disease. N Engl J Med 2007;357:443–453.

12. Meitinger T, Pertzemilis A, Kavaslar N, Stewart A, Roberts R, Cox DR, Hinds DA, Pennacchio LA, Tybring-Hansen A, Folsum AR, Boerwinkle E, Hobbis HH, Cohen JC. A common allele on chromosome 9 associated with coronary heart disease. Science 2007;316:1488–1491.

13. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, Heath SC, Timpson NJ, Najjar SS, Stringham HM, Strait J, Duren WL, Maschio A, Busonero F, Pulas A, Alba G, Swift AJ, Morten MA, Naranu S, Bennett D, Parish S, Shen H, Galan P, Menelon F, Hertzberg S, Ziegenhain D, Chen WM, Li Y, Scott LJ, Scheet PA, Sundvall J, Watanabe RM, Nagaraia R, Ebrahim S, Lawlor DA, Ben-Shlomo Y, v.egg-Smhi G, Shuldiner AR, Collins R, Bergman RN, Uda M, Tuomilehto J, Cao A, Collins FS, Lakatta E, Lathrop GM, Boehnke M, Schlessinger D, Mohlke KL, Aebi M, Thompson J, Morley D, Coward H, Stenson DP, Zeggini E, Freathy RM, Lindgren CM, Perry RB, Elliott KS, Lango H, Rayner NW, Shields B, Harnies LW, Barrett JC, Ellis S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJF, Barroso I, Wareham NH, Karpe F, Owen KN, Cardon LR, Walker M, Hitman GA, Palmer CNA, Doney ASF, Morris AD, Smith GD, The Wellcome Trust Case Control Consortium, Hattersley AT, McCarthy M, Willer C, Nagorsen D, Ordovas JM, Berglund G, Vattanai E, Josullivan E, Hedblad B, Taskinen MR, Newton-Cheh C, Salomaa V, Peltonen L, Groop L, Alshuler DM, Orho-Melander M. Six new loci associated with low blood density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet 2008;40:189–197.

14. Aulchenko YS, Voight BF, Lyssenko V, Burtt NP, de Bakker PIW, Chen H, Roix JJ, Visscher MP, Liu C, Voight BF, Florez JC, Groop L, Ardlie K, Bengtsson Bostrom K, Isomaa B, Meitinger T, Braund P, Wichmann HE, Barrett JH, Konig IR, Stevens SE, Dominiczak AF, Lathrop GM, Webster J, Farrall M, Spector TD, Samani NJ, Caulfield MJ, Munroe PB. Genome-wide association study identifies genes for body mass index and predisposes to childhood and adult obesity. Science 2007;316:889–894.

15. Saxena R, Voight BF, Lyssenko V, Burt NP, de Bakker PW, Chen H, Roix JJ, Kathiresan S, Hirschorn JN, Daly MJ, Hughes TE, Group A, Alshuler D, Almgren P, Florez JC, Meyer J, Andlue K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speleos JK, Taskinen MR, Tuomi T, Guiducci C, Benjamin A, Carlson D, Gudnason V, Hackett R, Hill L, Hollopasted J, Laurie CA, Sigmund M, Stermin S, Aulchenko Y, Madsen SV, Mennsens VW, Tewey H, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Saff A, Gibbons J, Handshaler H, Healy C, Nguyen K, Gates C, Soucze G, Gage D, Nizzara M, Gabriel S, Orus N, Witt J, Gudnason V, Hackett R, Hill L, Hofvendahl J, Lauerman D, Pastorek J, Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, Davet Institutes of BioMedical Research. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 2007;316:1331–1336.

16. Miller GJ, Bauer KA, Barzegar S, Foley AJ, Mitchell JP, Cooper JA, Rosenfeld RD. The effects of quality and timing of venepuncture on markers of blood coagulation in healthy middle-aged men. Thromb Haemost 1995;73:82–87.

17. British Regional Heart Study. http://www.ucl.ac.uk/jspcr/research/vbhr/index.htm. 2009. Ref Type: Internet Communication.

18. Gardener EA, Huppert FA, Guralnik JI, Melzer D. Middle-aged and mobility-limited prevalence of disability and symptom attributions in a national survey. J Gen Intern Med 2006;21:1091–1096.

19. Fowkes FG, Housley E, Cawood EH, MacIntyre CCA, Ruckley CV, Prescott RJ. Edinburgh Artery Study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. Int J Epidemiol 1991;20:384–392.

20. Power C, Elliott J. Cohort profile: 1958 British birth cohort (National Child Development Study). Int J Epidemiol 2006;35:44–41.

21. Wadsworth M, Kirk D, Richards M, Hardy R. Cohort profile: the 1946 National Birth Cohort (MRC National Survey of Health and Development). Int J Epidemiol 2006;35:59–54.
26. Marmot MG, Stanfeld S, Patel C, North F, Head J, White I, Brunner E, Feeney A, Marmot MG, Smith GD. Health inequalities among British civil servants: the Whitehall II study. Lancet 1997;337:1387–1393.

27. Ye S, Dunleavy L, Bannister W, Day L, Tapper W, Collins AR, Day IN, Simpson I. Independent effects of the -219 G>T and epsilon3/epsilon3 polymorphisms in the apolipoprotein E gene on coronary artery disease: the Southampton Atherosclerosis Study. Eur J Hum Genet 2003;11:437–443.

28. Theorell T, Tsutsumi A, Hallquist J, Reuterwall C, Hogstedt C, Fredlund P, Emlund N, Johnson JV. Decision latitude, job strain, and myocardial infarction: a study of working men in Stockholm. The SLEEP Study Group. Stockholm Heart epidemiology program. Am J Public Health 1998;88:382–388.

29. Stephens J, Hurel S, Acharya J, Humphries S. An interaction between the interleukin-6 -174 G>C gene variant and urinary protein excretion influences plasma oxidative stress in subjects with type 2 diabetes. Cardiovas Diabetol 2004;3:2.

30. Ireland H, Kontoulas CJ, Cooper JA, Hawe E, Humphries SE, Mather H, Goodall AH, Hogwood J, Jhanu-Vague J, Yudkin JS, Di Minno G, Margaglione M, Hamsten A, Miller GJ, Bauer KA, Kim YT, Stearns-Kurosawa DJ, Kurosawa S. EPCR Ser219Gly: Elevated sEPCR, prothrombin F1+2, risk for coronary heart disease, and increased sEPCR shedding in trophoblast. Atherosclerosis 2005;183:282–292.

31. Caulfield MJ, Munroe PB, Pembroke J, Samani NJ, Dominiczak A, Brown M, Simpson I. Independent effects of the -219 G>T polymorphism in the apolipoprotein E gene on coronary artery disease, and increased sEPCR shedding in vitro EPCR Ser219Gly: Elevated sEPCR, prothrombin F1+2, risk for coronary heart disease, and increased sEPCR shedding in trophoblast. Atherosclerosis 2005;183:282–292.

32. Preuss M, König IR, Thompson JR, Erdmann J, Absher D, Assimes TL, Dahmus E, Lebovitz H, Quyyumi AA, Levey AI, Vaccarino V, Reilly MP, Rader DJ, Williams MJ, van Rij AJ, James G, Tzabetti E, Malerba G, Pignati P, Boretti A, Pezzoli G, McManus P, Wijmenga C. Van Heel DA. Newly identified genetic risk variants for celiac disease related to the immune response. Nat Genet 2008;40:395–402.

33. Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, Li Y, Kurekeman FAS, Zemelnikova A, Hinks A, Guiducci C, Chen R, Alfredsson L, Amos CI, Ardle KG, Barton A, Bowes J, Brouwer E, Butt NP, Catessen J, Coblyn J, Coenen MJ, Costenbader KH, Croswell LA, Crusius B, Jui C, de Bakker P, De Jager PL, Ding B, Emery P, Flynn E, Harrison P, Hindling K, Huidzeng JW, Kastner DL, Ke X, Lee AT, Liu X, Martin P, Morgan AW, Padyukov L, Posthumus MD, Radstake TRDJ, Reid DM, Seielstad M, Seldin MF, Shadick NA, Steer S, Tak P, Thomson AW, Tseng J, Toes REM, de Vries N, Begovich AB, Worthington J, Siminovitch KA, Gervenkeszen PK, Klein Rettig AL, Mezey E, Smit JH, Sinisalo J, Whitfield JB, Waterworth DM, Wareham NJ, Waeber G, Vollenweider P, Wijmenga C, Wilson A, Wolfskehl D, Wurtele A. A genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Genet 2010;42:508–514.

34. Cardiovascular Disease Consortium. Large scale association analysis of novel genetic loci for coronary artery disease. Arterioscler Thromb Vasc Biol 2009;29:774–780.

35. Karvonen J, Silander K, Kee F, Tietz L, Salomaa V, Kuusela K, Wiklund PG, Virtamo J, Saarahe O, Perret C, Perola M, Peltonen L, Cambi F, Erdmann J, Samani NJ, Schunkert H, Evans A. The impact of recently identified loci on coronary heart disease, stroke and total mortality in the MORGAM prospective cohorts. Genet Epidemiol 2009:33:237–246.

36. Solberg S, Hjemseth D, Hofvander Y, Bagger C, Hamsten A, Nordeng H, Hjelte L, Bøg, Madsen M, Mikkelsen S, Christiansen K, Jørgensen J, Lindblad P, Skak SJ, Skak S, Schrøder C, Schröder D, Gudmundsdottir L, Gille D, Olivier M, Martinelli N, Vrijenhoek B, Vrijenhoek D, Eijffloff GJ, Atiam, D, Theute G, Deichmann C, Thompson P, Wijmenga C. Robertson K, Wijk M, Hall IP, Postma DS, Gislason T, Schulte J, Jonk A, Jonkonda I, Thunström Dahl SS, Gislason I, Halldorsson B, Stefansson K. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet 2009;41:342–347.

37. Gudbjartsson DF, Bjornsdottir US, Halipai E, Helogdrat A, Sulem P, Jonsson GM, Thorleifsson G, Helgadottir H, Stefansson F, Williams C, Hui J, Beilby J, Warington NM, James A, Palmer LJ, Koppelman GH, Heinmä R, Kruemer M, Boezen HM, Wheatley A, Altmüller J, Shin SD, Uh ST, Cheong JS, Bagnust J, Gislason B, Gislason D, Park CS, Lumsden RM, Porsberg C, Hansen JW, bakker V, Weter J, Tarsa J, Jones S, So NY, Mcalpine S, Blankenberg S, de Paepe A, Moyer MS, Boezen H, Mckinlay S, Quyyumi AA, Levey AI, Vaccarino V, Reilly MP, Rader DJ, Williams MJ, van Rij AJ, Jones G, Tzabetti E, Malerba G, Pignati P, Boretti A, Pezzoli G, McManus P, Wijmenga C. Van Heel DA. Newly identified genetic risk variants for celiac disease related to the immune response. Nat Genet 2008;40:395–402.

38. Erdman J, Grozhennig A, Blong PS, Konig IR, Hengstenberg C, Hall AS, Linsel-Nitschke P, Katheres S, Wright B, Tregouet DA, Camben F, Bruse P, Ahrrahzou R, Wagner AK, Stottm K, Schwartz SM, Salomaa V, Eloraus R, Melander O, Voigt BF, D'Onnello CJ, Petilon L, Sissick DV, Altschuler D, Merini P, Peviani F, Bernardi L, Arndiso D, Shillert A, Blankenberg S, Zeller T, Wild P, Schwartz DF, Tietl R, Perret C, Schreiber S, Morag MEB, Schafer A, Marz W, Renner W, Buger P, Kluter H, Schrezenji J, Rubin D, Ball SG, Balfomh AJ, Wichmann HE, Metinger T, Fischer M, Meisinger C, Baumert JP, Peters A, Wuwehand VW, Deloukas P, Thompson JR, Ziegler A, Samani NJ, Schunkert H. New susceptibility locus for coronary artery disease on chromosome 3q2.3. Nat Genet 2009;41:280–282.

39. Vischer PM, Montgomery GW. Genome-wide association studies and disease: from trickle to flood. JAMA 2009;302:2018–2029.
the mechanism-based and off-target actions of cholesteryl ester transfer protein inhibitors with CETP gene polymorphisms. Circulation 2010;121:52–62.

45. Scott LJ, Mohlke KL, Bonnycastel L, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narsisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely NM, Riessow NL, Sprau AG, Tang M, White PP, Herick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Sarames J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doherty KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M. A Genome-Wide Association Study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007;316:1341–1345.

46. Cholesterol Treatment Trialists’ (CTT) Collaboration. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet 2010;376:1670–1681.

47. Triglyceride Correlation of Statin Lipid Studies. The Emerging Risk Factors Collaboration. Triglyceride-mediated pathways and coronary disease: collaborative analysis of 101 studies. Lancet 2010;375:1634–1639.

48. Danesh J, Kaptoge S, Mann AG, Sarwar N, Wood A, Angleman SB, Wensley F, Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Cholesterol Treatment Trialists’ (CTT) Collaboration. Triglyceride-mediated pathways and coronary disease: collaborative analysis of 101 studies. Lancet 2010;375:1634–1639.

49. Nahrstaedt J, Nelson CP, Nothen MM, Olivieri O, Patel RS, Patterson CC, Peters A, Peyvandi F, Qu L, Quyyumi AA, Rader DJ, Valli, Rice C, Rosendaal FR, Rubin D, Salomaa V, Sampaio ML, Sandhu MS, Schadt E, Schaefer A, Schillert A, Schreiber S, Schrezenmeier H, Schwartz SM, Siscovick DS, Sivanathan M, Sivapalaratnam S, Smith A, Smith TB, Sorensen JR, Sokoloski T, Sparkes AJ, Stark K, Stirrups K, Stoll M, Tang WH, Tannstedt S, Thorpe J, Thorpe J, Thorpe J, Tomazewski M, Utterlinden AG, van Rij AM, Voght BF, Wareham NJ, Wells G, Wichmann HE, Wild PS, Willenborg C, Wittman J, Wright BJ, Ye S, Zeller T, Ziegler A, Cambien F, Goodall AH, Cupples LA, Quertermous T, Marz W, Hengstenberg C, Blankenberg S, Ouwenda WH, Hall AS, Deloukas P, Thompson JR, Stefansson K, Roberts R, Thorsteinsdottir U, O’Donnell CJ, McPherson R, Erdmann J. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 2011 March 6; advance online publication.

50. Sagoo GS, Tatt I, Salani G, Butterworth AS, Sarwar N, van Maarel M, Jukema JW, Wiman B, Kastelein JJP, Bennet AM, de Faire U, Danesh J, Higgins JT. Seven lipoprotein lipase gene polymorphisms, lipid fractions, and coronary disease: a HuGE association review and meta-analysis. Am J Epidemiol 2008;168:1233–1246.

51. Talmud P, Cooper J, Hattori H, Miller I, Miller G, Humphries S. The apolipoprotein A-V genotype and plasma apolipoprotein A-V and triglyceride levels: prospective risk of type 2 diabetes. Results from the Northwick Park Heart Study II. Diabetologia 2006;49:2337–2340.

52. Vaessen SFC, Schap FF, Kuivenhoven JA, Groen AK, Huttin BA, Boekholt SM, Hattori H, Sandhu MS, Bingham SA, Luben R, Palmen JA, Wareham NJ, Humphries SE, Kastelein JJP, Talmud P, Khaw KT. Apolipoprotein A-V: triglycerides and risk of coronary artery disease: the prospective Epic-Norfolk Population Study. J Lipid Res 2006;47:2064–2070.

53. Freathy RM, Timpson NJ, Lawlor DA, Pouta A, Ben-Shlomo Y, Ruokonen A, Ebrahim S, Shields B, Zeggini E, Weedon MN, Lindgren CM, Lango H, Melzer D, Ferrucci L, Paolizzo G, Neill MJ, Karpe F, Palmer CNC, Morris AD, Elliott P, Faber J, Smith G, McCarthy MI, Hattersley AT, Frayling TM. Common variation in the FTO gene alters diabetes-related metabolic traits to the extent expected given its effect on BMI. Diabetes 2008;57:1419–1426.

54. Zimmermann E, Kring S, Berentsen TL, Holst C, Pers TH, Hansen T, Pedersen O, Sorensen TIA, Jørgensen T. Fatness-associated FTO gene variant increases mortality independent of fatness—in cohorts of Danish men. PloS ONE 2009;4:e4428.