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Large Scale Association Analysis Identifies Three Susceptibility Loci for Coronary Artery Disease

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Abstract

Genome wide association studies (GWAS) and their replications that have associated DNA variants with myocardial infarction (MI) and/or coronary artery disease (CAD) are predominantly based on populations of European or Eastern Asian descent. Replication of the most significantly associated polymorphisms in multiple populations with distinctive genetic backgrounds and lifestyles is crucial to the understanding of the pathophysiology of a multifactorial disease like CAD. We have used our Lebanese cohort to perform a replication study of nine previously identified CAD/MI susceptibility loci (LTA, CELSR2-PSRC1-SORT1, CXCL12, MTHFD1L, WDR12, PCSK9, SH2B3, and SLCE2A3), and 88 genes in related phenotypes. The study was conducted on 2,002 patients with detailed demographic, clinical characteristics, and cardiac catheterization results. One marker, rs9622269, in MTHFD1L was significantly protective against MI (OR = 0.68, p = 0.0035), while the variant rs4977574 in CDKN2A-CDKN2B was significantly associated with MI (OR = 1.33, p = 0.0086). Associations were detected after adjustment for family history of CAD, gender, hypertension, hyperlipidemia, diabetes, and smoking. The parallel study of 88 previously published genes in related phenotypes encompassed 20,225 markers, three quarters of which with imputed genotypes. The study was based on our genome-wide genotype data set, with imputation across the whole genome to HapMap II release 22 using HapMap CEU population as a reference. Analysis was conducted on both the genotyped and imputed variants in the 88 regions covering selected genes. This approach replicated HNRNPA3P1-CXCL12 association with CAD and identified new significant associations of CDKL1, ST6GAL1, and PTPRD with CAD. Our study results provide evidence for the importance of the multifactorial aspect of CAD/MI and describes genes predisposing to their etiology.

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Competing Interests: Daniel E. Platt is employed at IBM. Ulla Grove Sidelmann is a researcher at Novo Nordisk, Denmark, and Frank Bonner is a researcher at Metabometrix Ltd, UK. The authors declare that this does not alter their adherence to all the PLoS ONE policies on sharing data and materials.

Introduction

Given increased life expectancy, prevention of diseases with severe manifestations and later life complications has become of tremendous importance. Researchers’ interest in identifying coronary artery disease (CAD) susceptibility genes reflects the very high mortality associated with both the disease [1] and one of its most serious complications: myocardial infarction (MI). While identifying the primary causes of CAD has been complicated by the multifactorial nature of the disease, genome-wide association studies (GWAS) have been moderately successful in revealing some of the polygenic structure of CAD. However, they only explain a modest fraction of the total heritability of CAD [2,3].

Linkage disequilibrium is the primary mechanism for generating direct (causal) and indirect (non-causal) genetic associations. Population stratification following population mixing [4,5] can lead to large numbers of correlated mutations [5,6,7], almost all of which are expected to be non-causal [8]. Population effects may also produce strong associations in some populations but not others by means of specific enrichment due to population genetic effects (e.g. drift and founder effect) [1,5,9,10]. Furthermore, in view of the multifactorial nature of CAD, the effects of genes
Materials and Methods
Study Subjects
A total of 2,002 subjects recruited between 2006 and 2009 for inclusion in the FGENTCARD database (www.well.ox.ac.uk/fgentcard) were selected. These subjects were referred to a catheterization care unit for clinical evaluation. The 4 main coronary arteries: the left main artery (LMCA), the left anterior descending artery (LAD), the left circumflex artery (LCx), and the right coronary artery (RCA) were visualized from different angles by angiography. The extent of stenosis in these vessels was assessed and recorded by percentage. Some of these patients were admitted to the hospital for having an MI as diagnosed by electrocardiogram and recorded by percentage. Others were admitted for reasons other than MI, such as unstable angina or cardiac diagnostic workup. Coronary event care unit for clinical evaluation. The 4 main coronary arteries: the left main artery (LMCA), the left anterior descending artery (LAD), the left circumflex artery (LCx), and the right coronary artery (RCA) were visualized from different angles by angiography. The extent of stenosis in these vessels was assessed and recorded by percentage. Some of these patients were admitted to the hospital for having an MI as diagnosed by electrocardiogram and recorded by percentage. Others were admitted for reasons other than MI, such as unstable angina or cardiac diagnostic workup.

Table 1. Description of the 10 SNPs used in the study.

| Chr / rs | Gene | Association with | Risk allele | Population site | Reference |
|----------|------|-----------------|-------------|----------------|-----------|
| 6p21 / rs1041981 | LTA | MI | A | Japan | Ozaki et al., 2002 |
| 9q21 / rs4977574 | CDKN2A-CDKN2B | MI | G | Italy | Kathiresan et al., 2009 |
| 1p13 / rs6646776 | CELSR2-PSRC1-SORT1 | MI | T | US | Kathiresan et al., 2009 |
| 1q41 / rs1746048 | CXL12 | MI | C | Spain | Kathiresan et al., 2009 |
| 6q25 / rs6922269 | MTHFD1L | MI | A | Finland | Kathiresan et al., 2009 |
| 2q33 / rs6725887 | WDR12 | MI | C | Sweden | Kathiresan et al., 2009 |
| 1p32 / rs11206510 | PCSK9 | MI | T | Iceland | Kathiresan et al., 2009 |
| 12q24 / rs3184504 | SH2B3 | MI | C | Asia | Gudbjartsson et al., 2009 |
| 12q24 / rs653178 | SH2B3 | MI | C | Asia | Gudbjartsson et al., 2009 |
| 6q26-27 / rs2048327 | SLC22A3 | CAD | C | Britain | Trégouët et al., 2009 |

For each SNP, genomic location, gene site, association with CAD and/or MI, risk allele, and populations in which association was found are displayed in left to right columns. For each association study, reference is indicated.

Abbreviations: Chr, chromosome; SNP, single nucleotide polymorphism; MI, myocardial infarction; CAD, coronary artery disease.

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9p21 Is Associated with Coronary Artery Disease
Table 2. Distribution of biological and biochemical characteristics of patients by stenosis category (n = 1,949).

|                         | Total (n = 1,949) | No stenosis (n = 425) | Stenosis (n = 1,524) | p-value |
|-------------------------|-------------------|-----------------------|----------------------|---------|
| **Males (%)**           | 72.7              | 57                    | 77.1                 | 2.4 × 10^{-16} |
| **Diabetes (%)**        | 29.7              | 16.2                  | 33.4                 | 9.5 × 10^{-13}  |
| **Hypertension (%)**    | 54.6              | 43                    | 58                   | 4.6 × 10^{-8}   |
| **Hyperlipidemia (%)**  | 47.9              | 36.2                  | 51.2                 | 3.8 × 10^{-8}   |
| **Smoking (%)**         | 64.4              | 55.6                  | 66.9                 | 2.8 × 10^{-5}   |
| **Family History of CAD (%)** | 61.8             | 65.4                  | 56.1                 | 0.0059 |
| **Age of onset (Median ±IQR)** | --               | --                    | 62 ± 16              | --    |
| **Glucose (mg/dl)**     | 118.8 ± 42.5      | 106.9 ± 33.7          | 122.0 ± 44.1         | 2.6 × 10^{-11} |
| **Total Cholesterol (mg/dl)** | 187.1 ± 48.4     | 187.7 ± 42.2          | 188.0 ± 49.9         | 0.925   |
| **HDL (mg/dl)**         | 41.3 ± 12.0       | 42.6 ± 13.0           | 41.0 ± 11.7          | 0.029   |
| **LDL (mg/dl)**         | 114.2 ± 41.1      | 114.1 ± 34.2          | 114.2 ± 42.7         | 0.95    |
| **Log triglycerides**   | 5.1 ± 0.5         | 5.0 ± 0.5             | 5.1 ± 0.5            | 0.15    |
| **BMI**                 | 29.0 ± 4.7        | 29.4 ± 5.2            | 28.9 ± 4.6           | 0.11    |

Left column lists the risk factors investigated upon recruitment of the study population. Averages values of biochemical characteristics are indicated for each of the total population, population without stenosis, and population with stenosis. The P-value is the chi-square.
Logistic regressions testing 600K SNP PCA k-means clusters (k = 4), capturing extremes of the leading Lebanese-specific principal components, did not predict MI or CAD, nor interact with risk factors or identified risk SNPs in the prediction of CAD or MI (data not shown). Population stratification identified in the cohort was shown not to have a significant effect in the association with a genomic control of \( \lambda = 1.033 \) (data not shown).

Risks associated with each allele expressed as odds ratios, odds ratios adjusted for gender, hypertension, hyperlipidemia, smoking, and diabetes, are displayed in Table S2 for CAD, and Table S3 for MI. Eight of the ten SNPs, rs4016981, rs1120651a, rs1740648, rs18264904, rs646776, rs635178, rs6725887, and rs2048327, showed no two-tailed significant associations with CAD or MI (Tables S2 and S3).

SNP rs4977574 was not significantly associated with CAD (Table S2), but was significantly associated with MI in a proportional odds logistic regression (OR = 1.86, 95%CI = 1.17–3.07, p = 0.012) for the GG genotype compared to the AA genotype (Table S3). In an adjusted analysis, this association with MI was unaltered (OR = 1.84, 95%CI = 1.14–3.09, p = 0.011) (Table S3). The heterozygous GJ genotype vs. AA showed odds ratios of 1.39 (unadjusted) and 1.42 (adjusted), but were insignificant (Table S3). Allele frequency results were also insignificant contrasting G vs. A alleles (OR = 1.38, 95%CI = 1.01–1.90, p = 0.039). All adjustment risk factors made highly significant contributions to the proportional odds logistic regression (Table 3).

The variant rs6922269, was seen to be robustly significant as protective against MI. The heterozygous odds ratio was highly significant (OR = 0.66, 95%CI = 0.48–0.9, p = 0.0087), and remained significant after adjustment (OR = 0.63, 95%CI = 0.45–0.85, p = 0.0037) (Table S3). The additive model risk showed an OR = 0.72, 95%CI = 0.56–0.91, p = 0.0083, and adjusted OR = 0.69, 95%CI = 0.53–0.88, p = 0.0035. It is interesting to note that the measured OR for the homozygous allele was 0.63, 95%CI = 0.31–1.15, p = 0.163 before adjustment, and OR = 0.60, 95%CI = 0.30–1.10, p = 0.121 after adjustment, though while as strong as the heterozygous risk, the error bars were much wider suggesting the population size of n = 11 for MI subjects carrying the homozygous allele was too small to resolve this risk, also suggestive of a protective power for the allele.

Analysis of our Illumina data allowed us to query much of the known SNP variations in the genome. Using an imputation method as a thorough survey of the variations from 88 genes, a total of 15,441 SNPs were imputed genotypes in addition to the method as a thorough survey of the variations from 88 genes, a total of 15,441 SNPs were imputed genotypes in addition to the.
the frequency of some genotypes and exclude any risk of bias consequent to low frequency representation. However such invasive measures of disease would be ethically impossible.

We have found that the variant rs4977574 located within the 9p21 locus confers an increased risk of developing MI in the GG state. Moreover, subjects homozygous for the G allele are at a much higher risk of MI. These results are in accordance with a study by Kathiresan where much higher risk of MI. These results are in accordance with a G state. Moreover, subjects homozygous for the phenotype, and more biological relevance. Furthermore, in the MAF, causative variants should have a stronger effect on the association. It is therefore important to go beyond the original chip used here provide a very good genome-wide coverage via tagSNPs of the losses of significance.

However, the tagSNPs provide statistical rather than functional numbers, especially in the reference category, may account for some development. For a number of these alleles, low power due to small since the differences in association across populations could indicate which best represent the underlying haplotypes. This should allow any of replication of association originally observed in Caucasian, it is possible that the reported SNP is not associated in our Lebanese cohort. Since our population is sufficiently closely related to the CEU, we could use HapMap catalogue of haplotypes to impute the genotypes of all the markers in regions of interest to test optimally for association between the gene and the phenotype.

This approach unraveled positive association of CAD with CXCL12, a gene that maps to 10q11. A CXCL12 intergenic SNP, rs1746048, had a C-risk allele associated with MI (OR = 1.17, p = 3 × 10^-6) [12]. The same rs1746048-C allele yielded positive association results with CAD in a recent study (OR = 1.09, p = 3 × 10^-6) [20]. Although rs1746048 was not associated with CAD in the Lebanese cohort, another SNP located in the same LD block, rs7900896, showed positive association results. The two SNPs are located 5' of CXCL12, 467 Kb away. They are not in the same HapMap, CEU-based, LD block. Our results imply that this genomic region contains several causative variants. Whether the associated SNPs are predisposing by themselves, or whether they are in LD with the real variant needs further investigations.

Another SNP, rs9295489 in CDKAL1, showed positive association with CAD in our cohort. As a part of the Cardiovascular Health Study (CHS) GWAS that aims to identify genetic variants associated with cardiovascular risk factors (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id = phs000226.v2.p1), rs2206734 in CDKAL1 was associated with MI occurrence (p = 5.05 × 10^-8). The two SNPs are intragenic, 345 Kb away, defining separate LD blocks. While our study confirms that variants near CDKAL1 are associated with risk for cardiovascular anomalies, no published data has associated CDKAL1 with CAD phenotype. Interestingly, since 2007, numerous studies have associated CDKAL1 SNPs with a cardiovascular disease (CVD) risk factor: Type 2 Diabetes (T2D) [19,40,41,42,43]. Thus, rs9295489 in CDKAL1 may influence T2D levels and consequently affect the risk for CVD.

Two genes, ST6GAL1 and PTPRD (See Table S1 for positive SNPs), were shown to be associated with CAD for the first time in our study. Both genes are known to carry common variants predisposing to Diabetes Mellitus [18,19,44,45]. Rs16861329 in ST6GAL1 locus was associated with T2D and pancreatic beta-cell function [18]. The fact that in our study ST6GAL1 has a protective effect on CAD occurrence suggests an inverted linkage between the active alleles or different alleles arising in various impacts on gene function, and thus, on health. PTPRD, previously shown to be a susceptibility locus for T2D [19,44,45], carries variants predisposing to CAD in the Lebanese cohort, showing again a genetic link between T2D and CAD.

A Bonferroni correction per interrogated marker (20,224) would be very over conservative since imputation identified many markers in perfect LD to be tested. A correction for the number of regions tested (88) would be more appropriate, and would retain the significance of these results even though these regions can encompass independent variants. We therefore consider these candidate genes to be of strong interest, but that they require further validation.

This association study investigates genetic factors underlying CAD/MI in individuals of Lebanese ancestry, a population with increased susceptibility to CAD. We identified common genetic variants at two loci previously identified as CAD/MI genetic risk factors (9p21 and CXCL12), and two loci newly associated with CAD (CDKAL1 and PTTPRD). We identified MTHFDL and ST6GAL1 as having a protective effect against CAD/MI. Cardiovascular diseases coexist with metabolic risk factors including central obesity and diabetes. Our study provides new insights into the
genetic link between metabolic and cardiovascular traits. Some of the identified loci have implicated metabolic genes without a previously known connection with CAD. Future studies will build on these successes by identifying additional variants and by determining the functional impact of the underlying genes. Further, our findings show the potential for new discovery from genetic association studies in populations of non-European ancestry.

Supporting Information

Table S1 Variants’ imputed genotype probabilities. Imputed genotype probabilities for variants in 88 candidate genes with +/- 50Kb on each side. The test was considered significant when P<0.01, relative information >0.4 and minor allele frequency >5%.

Table S2 Proportional Odds Logistic regression predicting CAD in graded categories from 10 SNPs. Proportional Odds Logistic regression predicting CAD in graded categories from 10 SNPs, both with additive and independent homozygous/heterozygous odds, without and with adjustment by family history of CAD, history of smoking, diagnoses of diabetes, hyperlipidemia, hypertension, and gender are represented. Odds ratio tests of disease vs. haplotype frequency are also indicated.

Table S3 Logistic regression predicting MI from 10 SNPs. Analysis was performed both with additive and independent homozygous/heterozygous odds, without and with adjustment by family history of CAD, history of smoking, diagnoses of diabetes, hyperlipidemia, hypertension, and gender. Odds ratio tests of disease vs. haplotype frequency are also indicated.

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Author Contributions

Conceived and designed the experiments: JH MF DG DEP PAZ. Performed the experiments: SS SY. Analyzed the data: SS DAB SY DEP PAZ. Contributed reagents/materials/analysis tools: JSY MH RO NS SK HEB EC AS BD. Wrote the paper: SS DAB SY DEP PAZ.
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