Apolipoprotein Isoform E4 Does Not Increase Coronary Heart Disease Risk in Carriers of Low-Density Lipoprotein Receptor Mutations

Summary: In humans, the E4 allele of the apolipoprotein E gene is associated with increased coronary heart disease risk next to its well-known association with Alzheimer disease risk. We investigated whether the presence of a low-density lipoprotein receptor mutation in patients clinically diagnosed with familial hypercholesterolemia was protective in E4/E4 carriers. In a cohort of 2400 patients clinically diagnosed with familial hypercholesterolemia, we found a low-density lipoprotein receptor gene mutation in 1383 patients, whereas in 1013 patients, such mutation was not present. In 92 patients homozygous for APOE4, the presence of a low-density lipoprotein receptor mutation conferred lower coronary heart disease risk (hazard ratio, 0.16; 95% confidence interval, 0.05–0.58; P = 0.005). From these results, we can conclude that the low-density lipoprotein receptor function is key to the detrimental effects of ApoE4 in humans. Further studies are now required to study the consequences of our observation for prevention of both coronary heart disease and Alzheimer disease.

Conclusion: Low-density lipoprotein receptor function is key to the detrimental effects of apolipoprotein E4 in humans. Kinetic studies in humans are now required to study the consequences of our observation for prevention of both coronary heart disease and Alzheimer disease.

Association of Genetic Variants and Incident Coronary Heart Disease in Multiethnic Cohorts: The PAGE Study

Summary: This study investigated the association of reported genome-wide, cross-sectional coronary heart disease (CHD) single-nucleotide polymorphisms (SNPs) with incident CHD. We replicated 9 CHD loci in individuals of European ancestry, including 9p21, 16q23.1, 6p24.1, 2q36.3, MTHFD2L, APOE, ZNF627, CXCL12, and LDL. The inclusion of coronary revascularization procedures among the incident CHD events introduced heterogeneity; for example, a 9p21 variant showed larger genetic effects in individuals age 55 years or younger and in women. The SNPs were not associated with CHD in black individuals and associations varied in other US minorities. Fine mapping of these regions may help to clarify these negative findings.

Conclusion: Prospective analyses of white participants replicated several reported cross-sectional CHD-SNP associations.

Lipoprotein(a) Genetic Variants Associated With Coronary and Peripheral Vascular Disease But Not With Stroke Risk in the Heart Protection Study

Summary: Recent genetic studies have demonstrated strong support for a causal role of plasma levels of lipoprotein(a) [Lp(a)] in coronary disease. The current findings from the Heart Protection Study, which are based on >12,000 prevalent disease cases and >3,000 incident events, increase our understanding of the relevance of Lp(a) for vascular disease risk. The Heart Protection Study demonstrates comparable strength of associations of an LPA genotype score, previously shown to explain more than half of the genetic variation in Lp(a) levels with coronary disease and peripheral vascular disease but not with stroke. These results indicate that Lp(a) may have effects on atherosclerotic and thrombotic diseases that are only relevant at specific sites. Furthermore, as indicated by the paradoxical results from prospective and randomized evidence for cholesterol and ischemic stroke risk, the lack of evidence of an association of LPA and stroke in the present study does not exclude the possibility that lowering Lp(a) could have beneficial effects on the risk of stroke or stroke subtypes. The results of large-scale randomized trials of agents that reduce plasma levels of Lp(a), such as niacin and cholesterylester transfer protein inhibitors, will help to assess the safety and efficacy of lowering Lp(a) levels on a broad range of vascular outcomes.

Conclusion: The comparable strength of associations of the LPA score with coronary disease and peripheral vascular disease but not with stroke suggest that Lp(a) may have effects on atherothrombotic vascular disease that are only relevant at specific sites.

Protein Interaction–Based Genome-Wide Analysis of Incident Coronary Heart Disease

Summary: New approaches for the analysis of genome-wide association (GWA) data that allow for integration with complementary data are needed for phenotypes that do not support large-scale recruitment schemes or meta-analysis. Most GWA studies have focused on the identification of the strongest single-locus associations, but the identification of combined effects of many weakly associated variants is especially appealing for complex diseases, such as coronary heart disease (CHD), that are probably not caused by single variants or by a single biological pathway. We used a network-based analysis based on protein-protein interaction data and GWA results for CHD in 899 CHD cases and 1823 control subjects.
from the Nurses’ Health and the Health Professionals Follow-up Studies. Probability values for each gene were assigned according to the smallest probability values for single-nucleotide polymorphisms within 70 kb upstream and 20 kb downstream of the gene and corrected for the effective number of independent single-nucleotide polymorphisms for each gene. Networks of genes that represent direct protein-protein interactions were examined to identify protein complexes with evidence for association in aggregate. After correcting for the number of complexes tested, a significant association was observed between CHD and genes that encode proteins that interact with the β1-adrenergic receptor gene (ADRB1). This complex included 18 protein interaction partners that have not been identified as candidate loci for CHD. This comprehensive approach highlights that network-based analyses have the potential to reveal additional novel genes of interest to a phenotype beyond those discovered in GWA studies of common variants.

**Conclusion:** The integration of a GWA study with protein-protein interaction data successfully identifies a set of candidate susceptibility genes for incident CHD that would have been missed in single-marker GWA analysis.4

**Post-Genomic Update on a Classical Candidate Gene for Coronary Artery Disease: ESR1**

**Summary:** After age, male sex is the most important risk factor for cardiovascular disease; however, little research has been carried out to understand the underlying causes of sex-related risk, in comparison with work on modifiable risk factors. Although the physiological differences between the sexes are evident, the genetic differences are minimal because sex is determined by the presence (in males) of a single gene, SRY, on chromosome Y. This leads to the important conclusion that male sex itself is not a cardiovascular risk factor but a proxy variable that captures a large fraction of risk via other unknown, possibly modifiable, metabolic factors that differ between males and females. Sex hormone metabolism is a prime candidate system for explaining sex differences in risk. In this highly powered study, the authors perform an extensive survey of a broad range of genetic variation in the ESR1 gene, which encodes the principal candidate for explaining sex-related differences in coronary artery disease (CAD) risk, Estrogen receptor α (ERα). Despite ERα’s central role in sex hormone signaling, its widespread expression in vascular tissues, and the importance of sex for CAD risk, the authors find no evidence for the involvement of genetic variation in ESR1 in modifying CAD risk, either in the general population or separately in males and females. Against the context of a history of inconsistent results regarding this question, this study provides a reasonably conclusive answer and may stimulate a renewed effort to explore other elements of the sex hormone system to explain sex differences in CAD risk.

**Conclusion:** We suggest that future research on the genetic basis of sex-related differences in CAD risk should initially prioritize other genes in the reproductive steroid hormone biosynthesis system.5

**Heterogeneity of the Phenotypic Definition of Coronary Artery Disease and Its Impact on Genetic Association Studies**

**Summary:** The clinically heterogeneous nature of coronary artery disease (CAD) has caused well-recognized limitations in the phenotypic characterization of cases and control subjects for genetic studies of CAD. Experts in the field have recommended the use of standardized phenotypic definitions (eg, myocardial infarction and angiographic measures of disease burden) for all genetic analyses, although there currently is no empirical evidence to support the use of these “purer” phenotypes. In this project, we described the degree of heterogeneity of phenotypic definitions in individual genetic studies and investigated, with meta-analytic techniques, the impact of phenotypic definitions on the consistency and magnitude of genetic effects for CAD. We analyzed 965 individual studies for 32 genetic associations and found that the CAD phenotypes could be classified into 3 categories: acute coronary syndromes (44%); angiographically documented disease (34%); and broad, not otherwise specified CAD (22%). However, these clinical phenotypes were overlapping. Subgroup meta-analyses by phenotype showed discordant results, but phenotype classification generally explained small proportions of between-study heterogeneity. No CAD phenotype was consistently associated with larger or more homogeneous genetic effects in meta-analyses to support its preferential use in genetic studies or meta-analyses for CAD. Our findings reinforce the need of considering the totality of evidence for CAD phenotypes in future meta-analyses of genetic studies. Phenotypic-specific effects at the clinical phenotype level may exist, but these should be explored in secondary analyses after an association between a genetic marker and CAD has been established by an all-inclusive meta-analysis.

**Conclusion:** Substantial phenotypic heterogeneity exists in CAD genetic associations, but differences in phenotype definition make a small contribution to between-study heterogeneity. We did not find a consistent effect in terms of the magnitude or homogeneity of summary effects for a specific phenotype to support its preferential use in genetic studies or meta-analyses for CAD.6

**Genomic Risk Variants at 1p13.3, 1q41, and 3q22.3 Are Associated With Subsequent Cardiovascular Outcomes in Healthy Controls and in Established Coronary Artery Disease**

**Summary:** The genomic risk loci at 1p13.3, 1q41, and 3q22.3 have been strongly associated with the risk of developing coronary heart disease in many large genome-wide association and cohort studies. This study investigated whether these important coronary artery disease risk variants may also contribute to disease progression and poorer outcomes in patients with established cardiovascular disease. Whether these risk loci also increase the risk of subsequently having a cardiovascular event in individuals with no known overt cardiovascular disease at the time of recruitment also was assessed. The findings from this study support previous associations between 1p13.3 and myocardial infarction and lipid levels. Extending these findings, we observed in patients with coronary heart disease an association between 1p13.3 and readmission for non–ST-segment elevation myocardial infarction and between 1q41 and the composite end point of death/cardiovascular disease readmission. The coronary artery disease risk locus at 3q22.3 was associated with subsequent survival/admission for cardiovascular disease event in individuals who were free of overt coronary artery disease at the time of study inclusion. These findings provide further evidence for an important role of 1p13.3, 1q41, and 3q22.3 in coronary artery disease. Furthermore, this study suggests that variants at 1p13.3 and 1q41 are independently associated with clinical outcomes in patients with established coronary heart disease and confirms 3q22.3 as a predictor of cardiovascular risk in individuals free of overt heart disease.

**Conclusion:** These data suggest that coronary artery disease genomic risk variants at 1p13.3 and 1q41 are associated with subsequent clinical outcome in heart patients and confirm rs9818870 at 3q22.3 as a predictor of cardiovascular risk in individuals free of overt heart disease.7

**Association of a Peripheral Blood Metabolic Profile With Coronary Artery Disease and Risk of Subsequent Cardiovascular Events**

**Summary:** Coronary artery disease (CAD) is the leading cause of death in industrialized countries, and many accepted risk factors for CAD are metabolic. However, we have an incomplete mechanistic...
understanding of CAD risk, and, equally important, there is a need to refine our ability to identify individuals at highest risk of cardiovascular events. New molecular profiling tools may help improve risk stratification and enhance our understanding of the disease process. Metabolomics is a novel technology used to study the small-molecule metabolites that are byproducts of cellular metabolism and that may be particularly useful for diagnosis of human disease. Therefore, in this study, we performed quantitative metabolomic profiling of 69 metabolites, including acylcarnitine species (byproducts of mitochondrial metabolism), amino acids, and conventional metabolites. Profiling was performed in peripheral blood samples from 2 independent CAD case-control datasets of participants enrolled in the Duke CATHGEN biorepository of patients undergoing cardiac catheterization. We observed that 2 metabolomic biosignatures were independently associated with CAD in both case and control datasets, 1 comprising metabolites from the branched-chain amino acid pathway and 1 from the urea cycle pathway. The metabolomic signatures also appeared to add discriminative capability to models with clinical risk factors alone. A third metabolomic biosignature in the blood was able to independently predict who would have future cardiovascular events (death or myocardial infarction). Hence, these metabolomic signatures may represent useful markers for the presence of CAD, potentially improve risk stratification, and identify novel mechanisms of CAD pathophysiology.

Conclusion: Metabolite profiles are associated with CAD and subsequent cardiovascular events.8

General Cardiovascular Risk Profile Identifies Advanced Coronary Artery Calcium and Is Improved by Family History: The Multiethnic Study of Atherosclerosis

Summary: Coronary artery calcium (CAC) provides prognostic information regarding the risk of cardiovascular events. As a result, the presence of advanced CAC has been proposed as a rationale for aggressive risk factor modification. Using the data from the Multiethnic Study of Atherosclerosis, we assessed the ability of the General Cardiovascular Risk Profile (GCRP) to identify individuals with advanced CAC and determined whether identification of advanced CAC is improved by adding 2 different definitions of a positive family history of coronary heart disease. We defined family history as a dichotomous variable (ie, having at least 1 first-degree relative with premature coronary heart disease) or by familial risk stratification (ie, as a weak, moderate, or strong family history, based on number of relatives with coronary heart disease, age at onset, and the presence of stroke or diabetes in the family). We observed that the GCRP worked well to identify individuals with positive CAC scores, and the addition of family history using either definition improved the discriminatory ability of this model. However, correct reclassification of individuals into lower or higher risk categories for a CAC score >300 was only improved with the addition of familial risk stratification to the GCRP variables. Our findings are consistent with the notion that the GCRP is a general model for assessing global cardiovascular disease risk, and we demonstrate that addition of family history to the GCRP (especially comprehensive familial risk assessment based on the number of relatives with coronary heart disease, age at onset, and the presence of stroke or diabetes in the family) further enhances the prediction of the presence of advanced CAC.

Conclusion: The GCRP identifies advanced CAC, an emerging indication for aggressive risk factor modification. Incorporation of family history, especially comprehensive familial risk stratification, provides incremental prognostic value.9

Design of the Coronary Artery Disease Genome-Wide Replication And Meta-Analysis (CARDIoGRAM) Study: A Genome-Wide Association Meta-Analysis Involving More Than 22 000 Cases and 60 000 Controls

Summary: Despite the recent progress in identification of coronary artery disease (CAD)/myocardial infarction genes, only a relatively limited fraction (<10%) of the overall genetic risk (heritability) of the disease is explained by the currently identified loci. One part of the explanation is likely to be the limited power of individual genome-wide association studies to detect such loci. The global Coronary Artery Disease Genome-Wide Replication And Meta-analysis (CARDIoGRAM) consortium will now analyze genome-wide information from >22 000 cases of CAD and >60 000 control subjects, and this will undoubtedly identify additional loci harboring common variants affecting CAD risk. Indeed, we anticipate a wealth of new information on heritable aspects of CAD and its risk factors, which probably will open multiple opportunities for scientific exploration. However, such a large experiment requires careful prospective planning of the methodology used. We describe how such a meta-analysis, including a replication study, could be conducted. In conclusion, CARDIoGRAM is a novel and powerful consortium poised to contribute to the understanding of common genetic variation affecting the risk for CAD and myocardial infarction. This information then can be used to derive mechanistic information on biological processes as well as used to identify potential targets for therapeutic intervention.

Conclusion: CARDIoGRAM is poised to contribute to our understanding of the role of common genetic variation on risk for CAD and myocardial infarction.10

Multiple Genetic Loci Influence Serum Urate Levels and Their Relationship With Gout and Cardiovascular Disease Risk Factors

Summary: Although the role of serum urate in the causal pathway for gout has been well characterized, substantial controversy exists regarding whether elevated serum urate also may be a cause of high blood pressure (BP), hyperglycemia, and chronic kidney disease; whether the association with serum urate observed in observational studies merely is a consequence of these conditions; or whether it is an artifact of uncontrolled confounding factors. Because the association between genes and disease generally is not subject to confounding by environmental factors or reverse causality, causal inferences between exposure and disease can be examined more specifically using mendelian randomization. In the present investigation, we tested the association of a genetic score constructed using 8 loci associated with serum urate with coronary heart disease (CHD) and its risk factors, including gout, glucose, systolic BP, diastolic BP, estimated glomerular filtration rate, and chronic kidney disease. Except for gout, none of the associations was statistically significant, and the lack of associations was replicated in another equally large independent sample. Although further confirmation is warranted, our study helps to elucidate the relationship between serum urate and CHD and its risk factors, which may contribute to a better understanding of the usefulness of controlling serum urate for preventing and designing treatments for CHD and its risk factors.

Conclusion: The genetic urate score analysis suggested a causal relationship between serum urate and gout but did not provide evidence for one between serum urate and cardiovascular risk factors and CHD.11
Association of Single-Nucleotide Polymorphisms on Chromosome 9p21.3 With Platelet Reactivity: A Potential Mechanism for Increased Vascular Disease

Summary: Common genetic variants on chromosome 9p21.3 are associated with myocardial infarction, coronary artery disease, coronary artery calcification (CAC), and ischemic stroke. To gain insights into mechanisms underlying these associations, we examined variations in this region and platelet reactivity across multiple populations. One variant, rs10965219, showed consistent effects on platelet reactivity in 4321 subjects across 3 studies. The variant also was associated with CAC. Further, the association between the variant and platelet reactivity persisted after adjusting for variation in CAC. These results suggest that risk alleles at 9p21.3 may have pleiotropic effects on myocardial infarction, coronary artery disease, CAC, and stroke risk, possibly through their influence on platelet reactivity. Additional studies to further define the importance of platelet reactivity in those who carry the at-risk genotype may provide mechanistic insights toward personalized medicine through genotype-specific interventions that target platelet reactivity.

Conclusion: These results suggest that risk alleles at 9p21.3 locus may have pleiotropic effects on myocardial infarction/coronary artery disease and stroke risk, possibly through their influence on platelet reactivity.12

Improved Prediction of Cardiovascular Disease Based on a Panel of Single-Nucleotide Polymorphisms Identified Through Genome-Wide Association Studies

Summary: In the past 3 years, several genome-wide association studies have identified common genetic variants at multiple loci that are significantly associated with coronary artery disease (CAD) risk. The most robust of these is a risk allele at 9p21 that has been shown to modestly but inconsistently improve CAD risk prediction over and above conventional risk factors. However, the extent to which additional recently identified genetic variants of modest effect size cumulatively improve risk prediction remains controversial. In the present study, we demonstrate, in 2 large CAD case-control populations, that a collective of 12 previously replicated CAD-associated single-nucleotide polymorphisms confers significantly greater predictive capabilities than do traditional risk factors or traditional risk factors plus 9p21. We also show that logistic regression performs better than more complicated machine-learning approaches. Large meta-analyses of CAD case-control data sets including >100 000 individuals are underway. Relevant to the goal of personalized medicine, more informative risk prediction models can be expected as additional CAD-associated single nucleotide polymorphisms are identified and more refined phenotypic data become available.

Conclusion: Using the collective of 12 single-nucleotide polymorphisms confers significantly greater predictive capabilities for CAD than 9p21.3, whether or not traditional risks are considered. More accurate models probably will evolve as additional CAD-associated single-nucleotide polymorphisms are identified.13

Ancestry as a Determinant of Mean Population C-Reactive Protein Values: Implications for Cardiovascular Risk Prediction

Summary: Circulating C-reactive protein (CRP) concentration has been proposed as a biomarker for cardiovascular risk prediction and as a selection marker for initiating statin treatment. Most observational studies of CRP and cardiovascular disease have been conducted in Europeans, but average CRP concentrations are thought to differ in populations of non-European ancestry. We conducted a systematic review of published studies to precisely quantify circulating CRP levels in populations of diverse ancestry. Population average CRP values were higher in black and Hispanic individuals and lower in South and East Asian individuals in comparison with Europeans. The differences were not explained by study design or by the type of CRP assay and were preserved after adjustment for age and body mass index. We estimated that at age 60 years, 39% of East Asians but 65% of Hispanics have CRP values exceeding 2 mg/L, the cut-point used to define eligibility for rosuvastatin treatment for primary prevention of cardiovascular disease in men over 50 years and women over 60 years. Differences in blood CRP concentration in populations of diverse ancestry are sufficiently large to affect statin eligibility, based on a single CRP threshold of 2 mg/L, and may be only partially influenced by differences in variables related to cardiovascular risk. A single threshold value of CRP for cardiovascular risk prediction could potentially lead to inequalities in statin eligibility that may not accurately reflect the underlying levels of cardiovascular risk, a premise that warrants further study.

Conclusion: Differences in CRP concentration in populations of diverse ancestry are sufficiently large to affect statin eligibility, based on a single CRP threshold of 2 mg/L, and only partially influenced by differences in variables related to cardiovascular risk. A single threshold value of CRP for cardiovascular risk prediction could lead to inequalities in statin eligibility that may not accurately reflect underlying levels of cardiovascular risk.14

Altered Hepatic Gene Expression Profiles Associated With Myocardial Ischemia

Summary: Acute coronary syndrome (ACS) is accompanied by systemic changes in inflammation, coagulation, and metabolism, which may affect the outcome and prognosis of ACS. These systemic reactions are not explained by cardiac events alone. Several lines of evidence suggest that patients with fatty liver disease have a high risk of developing cardiovascular diseases, and it is possible to speculate that the liver is involved in a systemic reaction that modifies the pathogenesis of ACS. However, the relation between liver and myocardial ischemia in the acute ischemic phase has not been elucidated so far. In this investigation, we simultaneously analyzed the gene expression profiles of the liver and heart during acute myocardial ischemia in mice and observed the presence of humoral factors that intervened between the heart and liver. These humoral factors were released from the heart and influenced the liver to secrete important tissue remodeling factors. One of these humoral factors, osteopontin, a widely expressed glycoprotein, was increased in the ischemic heart and altered the gene expression of hepatocytes to produce important tissue remodeling factors (such as vascular endothelial growth factor-A). Our observations suggest that hepatic gene expression is potentially regulated by humoral factors of cardiac origin provoked by myocardial ischemia, and we provide direct evidence that the liver is involved in a systemic reaction that accompanies ACS. Our findings provide potential new insights into the pathophysiology of ACS.

Conclusion: Hepatic gene expression is potentially regulated by cardiac humoral factors under myocardial ischemia. These results provide new insights into the pathophysiology of acute coronary syndrome.15

Genetic and Clinical Correlates of Early-Outgrowth Colony-Forming Units

Summary: Cells termed “endothelial progenitor cells” (EPCs) have attracted interest as potential biomarkers and even cellular therapies for cardiovascular disease. This enthusiasm stems from studies suggesting that lower EPC number is associated with increased risk of cardiovascular disease and events and the hypothesis that EPCs, broadly defined, contribute to endothelial repair. However, this literature encompasses several different phenotypic definitions of EPCs and small to medium-sized patient samples. In the present
Peroxisome Proliferator-Activated Receptor Pathway Gene Polymorphism Associated With Extent of Coronary Artery Disease in Patients With Type 2 Diabetes in the Bypass Angioplasty Revascularization Investigation 2 Diabetes Trial

Summary: In patients with diabetes mellitus, atherosclerosis is more extensive and rapidly progressive. The reasons for this are not known but may relate in part to genetic variation that influences vascular disease severity. Using microarray technology to interrogate >3000 tagged single-nucleotide polymorphism markers of peroxisome proliferator-activated receptor pathway genes, we have discovered a polymorphism in the TLLI gene that is significantly associated with angiographic extent of coronary artery disease among patients with type 2 diabetes mellitus. This genetic polymorphism explains more variance of the phenotype than previously determined clinical factors. This association was initially observed in white patients with type 2 diabetes mellitus in the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial and then validated specifically in the diabetic subgroups of 2 other independent white patient cohorts with quantitatively coronary imaging. TLLI encodes a metalloproteinase that regulates procollagen processing and the metabolism of inhibitors of bone morphogenetic proteins involved with atherosclerotic calcification. These observations identify a novel putative genetic contributor to the biology of diabetic coronary atherosclerosis and suggest that targeting the TLLI/bone morphogenetic proteins pathway may afford specific therapeutic intervention for diabetic vascular disease.

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Coronary Artery Disease Etiology, Genetic Epidemiology, and Pharmacogenomics

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