Am J Transl Res 2009;1(3):221-234
www.ajtr.org/AJTR902004

Review Article

Genetics of coronary artery disease: focus on genome-wide association studies

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Received February 27, 2009; accepted March 5, 2009; available online March 5, 2009

Abstract: With the advent of the genome-wide association (GWA) study, a promising new avenue for identifying genetic markers for complex diseases like coronary artery disease (CAD) has been opened. This avenue, however, is not without challenges and limitations, including the need for carefully designed and executed studies and the risk of false positive associations. Nonetheless, new markers have been identified through such studies that could potentially revolutionize the ways that individuals with CAD are identified and managed.

Key words: atherosclerosis, coronary artery disease, genetics, genome wide association, genomics, myocardial infarction

Background

Coronary artery disease (CAD) and its associated complication, myocardial infarction (MI), is a leading cause of morbidity and mortality worldwide. CAD is a multifactorial disease that can be influenced by many different environmental and heritable risk factors. The progression of atherosclerotic disease involves a complex series of events, each event (e.g. foam cell formation, smooth muscle cell recruitment, etc.) involving multiple biological pathways and genes. Risk factors for CAD include hypertension, smoking status, male gender, age, body mass index, type 2 diabetes mellitus, and heredity. Analyte risk markers associated with CAD include lipid levels, cholesterol particle size and number, C-reactive protein, homocysteine, fibrinogen, and lipoprotein (a). However, many of the current traditional and novel risk markers are unable to fully predict who is at risk for CAD. For example, 35% of CAD has been shown to occur in people with total cholesterol levels <200 mg/dL [1]. Thus, other novel risk markers, including genetic risk markers, may be important for better refining individuals at risk for CAD and CV events.

Both twin and family studies have demonstrated that CAD has a strong heritable component. In one study examining 20,966 twins over a 36-year period, the heritability (h^2) for fatal CAD events was 57% for men and 38% for women [2]. Many studies have demonstrated that family history of CAD is an independent predictor of CAD [3-6]. Others have shown that distinct morphological features of CAD display high heritability [7, 8]. In addition, individuals with a family history of CAD generally have earlier onset, are male, have a history of smoking, and are hyperlipidemic [9]. The majority (72%) of early CAD cases (men < 55 years, women < 65 years) and 48% of all CAD cases, regardless of age, have a family history of CAD [10].

While multiple studies have concurred that CAD is highly heritable, the mechanisms underlying the heritable basis of CAD have been elusive. This is likely due to the complex nature of CAD. While a very small proportion of CAD is monogenic, or Mendelian in nature (e.g. familial hypercholesterolemia, familial defective apoB-100), the vast majority of CAD is genetically complex. Multiple genes are thought to contribute to CAD, each gene

contributing to only a small percent of the phenotype. In addition, many of the risk factors associated with CAD (e.g. blood pressure, diabetes) are themselves polygenic, further adding to the complexity, and hundreds of genes may be involved in CAD susceptibility [11]. Thus, while some genetic parameters, such as heritability, can be quantified for CAD, determining the number and scope of genes involved in CAD is challenging.

Many different family and population-based studies have identified candidate genes potentially associated with CAD. Unfortunately, the vast majority of results from linkage and association studies have not been reproducible or statistically significant, bringing to question the clinical utility of these markers [12]. However, recent technological and scientific advances have now made possible a new type of study: the genome-wide association (GWA) study. The advent of GWA studies has produced some novel and replicated associations in many disease states, including CAD.

Genome-wide association studies and CAD

GWA studies are essentially unbiased large-scale population-based studies evaluating the association of hundreds of thousands of markers (generally single nucleotide polymorphisms, or SNPs) across the genome with a particular phenotype. Part of the beauty of these types of studies is that they are not hypothesis-driven, allowing for the discovery of novel genetic markers. However, GWA studies are not without challenges and limitations and they must be carefully designed and executed. In the design of a GWA study, it is important to keep phenotypic heterogeneity of cases and misclassification of controls to a minimum [13]. Cases and controls should be well-matched to avoid confounders, such as population stratification. Furthermore, sample sizes should be large, generally in the thousands, and replication sample sizes in an independent population should be even larger. The risk for false positive or spurious associations is high in GWA studies, and strict quality control is essential.

One of the interesting aspects about GWA studies is that usually, loci are identified that have not been previously suspected as candidate genes/loci [14]. Several reasons could be behind this. First, the marker may be a spurious association. Second, the identified marker may be in linkage disequilibrium (LD) with the etiological marker. In this case, the effect of different populations with different LD structures must be taken into account [15]. Third, the identified marker may be an etiological marker, but has not been previously targeted as having a role in the disease. In the latter two cases, novel biological pathways involved in the disease state may be uncovered, leading to a better understanding of disease processes and, potentially, the development of novel therapies.

A search of the National Cancer Institute (NCI)-National Human Genome Research Institute (NHGRI)'s *Catalog of Published GWA Studies* (http://www.genome.gov/26525834) and the literature uncovered a total of 48 GWA studies examining CAD and/or associated traits or markers with $p \leq 10^{-5}$. Eight GWA studies with risk factor associations of $p \leq 10^{-5}$ have been published looking specifically at CAD or MI (**Table 1**). The remainder of the 40 identified GWA studies have been published examining traits or markers associated with CAD including coronary artery calcification, blood pressure, LDL cholesterol, HDL cholesterol, triglycerides, Lp(a), C-reactive protein, type 2 diabetes mellitus, and body mass index.

Chromosome 9, band p21.3

Multiple GWA studies have shown a highly significant association with various SNPs within a large LD block on chromosome 9 at band p21.3 (9p21.3) and CAD [16-21]. While results did not reach statistical significance to be included in the NHGRI *catalog of published GWA studies*, a GWA study involving the Framingham Heart Study also found an association with a 13 kb region on 9p21.3 with major CHD (p 2.5-3.5 x 10^{-4}) [22]. Additionally, they observed that 7 SNPs in a 76 kb region around 9p21 had $p<10^{-5}$ for major CHD and/or major cardiovascular disease (CVD).

Markers at the 9p21 locus have been shown to lead to a 15-20% risk for CAD in the 50% of Caucasian individuals heterozygous for the allele, and a 30-40% increased risk of CAD in the 25% of individuals homozygous for the allele [17]. Numerous follow-up case-control analyses have been performed and confirm

Table 1. Genome-Wide Association Studies of CAD and MI ($p<5 \times 10^{-5}$)

Phenotype	Chromosomal Region	Reported Gene(s)	SNP	Risk Allele Frequency	P-value	Odds ratio	95% CI	Reference
CAD	1p13.3	*CELSR2-PSRC1-SORT1*	rs5999839	0.23	4×10^{-19}	1.29	1.18-1.40	[18]
early-onset MI	1p13	*CELSR2-PSRC1-SORT1*	rs646776	0.81	1.5×10^{-8}	1.17	1.11-1.24	[21]
early-onset MI	1p32	*PCSK9*	rs11206510	0.81	9.6×10^{-9}	1.15	1.10-1.21	[21]
early-onset MI	1q41	*MIA3*	rs1746048	0.72	5.9×10^{-7}	1.13	1.08-1.18	[21]
CAD	1q41	*MIA3*	rs17465637	0.29	1×10^{-6}	1.2	1.12-1.30	[18]
CAD	1q43	NA	rs17672135	0.87	2×10^{-6}	1.43	1.23-1.64	[16]
early-onset MI	2q33	*WDR12*	rs6725887	0.14	1.3×10^{-8}	1.17	1.11-1.23	[21]
CAD	2q36.3	pseudogene	rs2943634	0.65	2×10^{-7}	1.21	1.13-1.30	[18]
CAD	3q22.3	*MRAS*	rs9818870	NA	7.4×10^{-13}	1.15	1.11-1.19	[92]
CAD	5q21	NA	rs383830	0.22	1×10^{-5}	1.60	1.16-2.21	[16]
early-onset MI	6p24	*PHACTR1*	rs12526453	0.65	1.3×10^{-9}	1.12	1.08-1.27	[21]
CAD	6q25	*MTHFD1L*	rs6922269	0.25	2×10^{-5}	1.17	1.04-1.32	[16]
CAD	6q25.1	*MTHFD1L*	rs6922269	0.25	3×10^{-8}	1.23	1.15-1.33	[18]
CAD	6q27	*SCL22A3-LPAL2-LPA*	rs2048327, rs3127599, rs7767084, rs10755578 (CTTG and CCTC haplotype)	NA	1.2×10^{-9} (CTTG), 4.2×10^{-15} (CCTC)	1.20 (CTTG), 1.82 (CCTC)	1.13-1.28 (CTTG), 1.57-2.12 (CCTC)	[86]*
early-onset MI	9p21	*CDKN2A, CDKN2B*	rs4977574	0.56	1.1×10^{-30}	1.28	1.23-1.33	[21]
MI	9p21.3	*CDKN2A, CDKN2B*	rs10757278	0.45	1×10^{-20}	1.28	1.22-1.35	[19]
CAD	9p21.3	intergenic	rs1333049	0.47	3×10^{-19}	1.36	1.27 – 1.46	[18]
CAD	9p21.3	*CDKN2A, CDKN2B*	rs1333049	0.47	1×10^{-13}	1.47	1.27–1.70	[16]
CAD	9p21.3	NA	rs10757274 and rs2383206	NA	NA	NA	NA	[17]
CAD	10q11.21	*CXCL12*	rs501120	0.13	9×10^{-8}	1.33	1.20-1.48	[18]
early-onset MI	10q11	*CXCL12*	rs1746048	0.84	3.4×10^{-5}	1.14	1.08-1.21	[21]
MI	12q24	*SH2B3*	rs3184504	0.38	8.6×10^{-8}	1.13	1.08-1.18	[82]
CAD	12q24.41	*HNF1A-C12orf43*	rs2259816	NA	5×10^{-7}	1.08	1.05-1.11	[92]
CAD	15q22.33	*SMAD3*	rs17228212	0.30	2×10^{-7}	1.21	1.13-1.30	[18]
CAD	16q23	NA	rs8055236	0.20	6×10^{-6}	1.91	1.33-2.74	[16]
early-onset MI	19p13	*LDLR*	rs1122608	0.75	1.9×10^{-9}	1.15	1.10-1.20	[21]
CAD	19q12	NA	rs7250581	0.22	3×10^{-5}	1.06	0.79-1.43	[16]
early-onset MI	21q22	*SLC5A3-MRPS6-KCNE2*	rs9982601	0.13	6.4×10^{-11}	1.20	1.14-1.27	[21]
CAD	22q12	NA	rs688034	0.31	4×10^{-6}	1.11	0.99-1.25	[16]

NA = not available
*Genome-wide haplotype analysis

the association of 9p21 and CAD [22-28]. A meta-analysis of case-control studies showed that the odds ratio per copy of the 9p21 risk allele was 1.29 (95% CI 1.22-1.37, p=0.0079) [29]. Abdullah et al demonstrated that four SNPs within the 9p21 region were significantly ($p=6.61 \times 10^{-7}$ to 1.87×10^{-8}) associated with premature and familial MI and CAD (average age of onset 40.3 +/- 5.1 years) [26]. Another group similarly made the connection between 9p21 and familial CAD in a high-risk population with familial hypercholesterolemia [25]. Although one study found an association between 9p21 and CAD, the association did not hold up with incident events or prevalent MI [27]. Additionally, the Rotterdam Study did not observe an association between 9p21 genotype and coronary heart disease or myocardial infarction in a large cohort of individuals aged 55 years and older [30]. Thus, while the vast majority of studies have identified a strong association with 9p21 and CAD, an association with this locus and MI may be population-dependent and garners further investigation.

The primary GWA studies were done in cohorts of individuals of Caucasian, Northern European, and Canadian descent. Follow-up studies in Korean, Japanese, and Italian populations also established the 9p21-CAD association in those populations [31-34]. Additionally, a multi-ethnic Atherosclerotic Disease, Vascular Function, and Genetic Epidemiology (ADVANCE) study confirmed the association in whites and extended it to U.S. Hispanics and U.S. East Asians, but not African Americans [35]. The lack of association in African Americans was consistent with what was observed by McPherson, et al. [17]. However, in both studies examining the association with African Americans and in

some of the other non-Caucasian-based studies, the sample sizes were not large, thus potentially limiting the statistical power of the studies. Nonetheless, the studies imply a consistent association of the 9p21 risk allele with CAD in individuals from a wide range of Asian and Caucasian backgrounds.

While the 9p21 association with CAD has been replicated on multiple occasions, associations between surrogate markers of atherosclerotic cardiovascular disease and 9p21 have remained less conclusive. Two studies did not identify an association between the 9p21 locus and carotid intima media thickness (IMT) [36, 37]. An additional study also did not demonstrate an association with 9p21 and abdominal aortic IMT [38]. Samani et al. postulated that CAD risk influenced by 9p21 occurs by a mechanism independent of carotid intimal thickening or endothelial dysfunction, and may instead affect coronary plaque stability [36]. Anderson et al. found that while 9p21 correlated with prevalence of angiographic coronary disease, it was unable to predict extent of disease [39]. The 9p21 locus was significantly correlated with angiographically characterized CAD in another study [24]. Horne et al., however, found that while 9p21 genotype was associated with the CAD phenotype, it was not associated with the severity or extent of CAD assessed angiographically [27]. They also did not find a strong association between history of MI and the 9p21 locus, as described above. They hypothesized that the 9p21 locus acted in the early stages, but not in the progression, of atherosclerosis. On the other hand, Ye et al. observed that the 9p21 genotype was associated with established carotid atherosclerosis and progression of atherosclerotic plaques as determined by carotid duplex scanning [37]. Thus, further investigations are necessary to help define where 9p21 fits into the picture of initiation or progression of atherosclerosis.

Other phenotypic correlations have been investigated with the 9p21 locus and CAD. In the Heart and Soul Study, 9p21 genotype was not associated with any echocardiographic parameter of cardiovascular structure and function (left ventricular hypertrophy, systolic dysfunction, diastolic disfunction, inducible ischemia, exercise capacity, mitral annular calcification, and aortic plaque) in individuals with known CAD [40]. Additionally, Chen et al. observed no significant association between 9p21 and quantitative indices of coronary atherosclerosis, such as minimal lumen diameter and number of coronary lesions or occlusions [41]. However, the 9p21 locus has been associated with coronary artery calcification, a reasonable marker of subclinical atherosclerosis [42].

In addition to CAD, the 9p21.3 locus has been associated with stroke, abdominal aortic aneurysms, and intracranial aneurysms [23, 28, 29, 43-46]. In part because of the relationship between the risk allele and stroke, and as discussed above, it has been suggested that the 9p21 risk allele may be related to plaque stability [36, 45]. The association of 9p21 with aneurysms also suggests an involvement in processes related to vessel wall integrity [23]. Bjorck et al. observed that the 9p21 locus was associated with abdominal aortic compliance and distensibility coefficients (measurements of arterial stiffness), further supporting a link between 9p21 and arterial wall diseases [38]. However, a recent GWA study of a Sardinian cohort did not report an association between 9p21 and arterial stiffness as determined by carotid-femoral pulse wave velocity [47].

Biology of 9p21

While the 9p21 association with CAD has been replicated on multiple occasions, the biological relevance of 9p21 is unclear at this time. The 9p21 region has been commonly implicated in the tumorigenesis of a variety of malignancies [48-51]. However, neither mouse models nor *in vitro* analyses have specifically implicated atherosclerotic processes with deletion or mutation of the 9p21 locus [52-55].

The risk-allele SNPs within the 9p21.3 locus, which spans approximately 50-60kb, have been defined as being in a "desert zone of the genome" (**Figure 1**) [56]. The 3' end of *CDKN2B*, encoding the cyclin-dependent kinase inhibitor tumor suppressor $p15^{INK4B}$, is in a LD block with a cluster of tightly linked SNPS at the 9p21 locus. Weaker linkage disequilibrium extends through *CDKN2B* to *CDKN2A*, which encodes another tumor suppressor $p16^{INK4B}$. The cyclin dependent kinases are involved in cell cycle regulation and transforming growth factor-β (TGF-β) cell

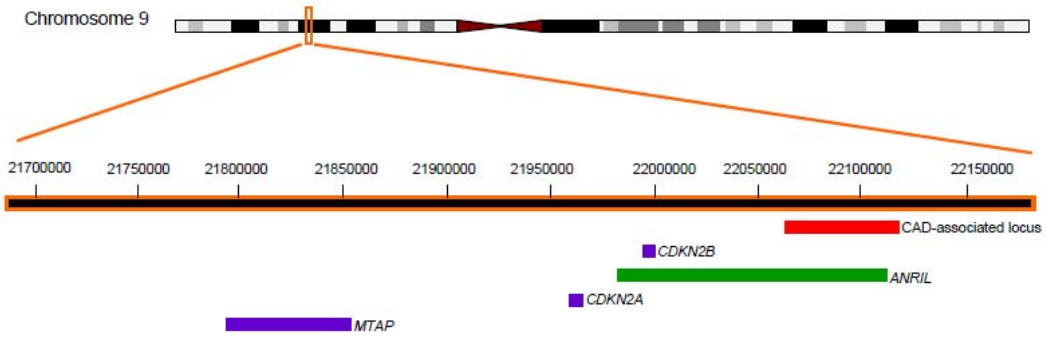

Figure 1. The chromosome 9, band p21 region. The location of the CAD-associated region is shown in red, annotated genes are shown in purple, and the *ANRIL* anti-sense non-coding RNA is shown in green.

cycle arrest [57]. In smooth muscle cells, TGF-β has been shown to have impaired signaling and reduced expression in atherosclerotic lesions and overexpression in abdominal aortic aneurysms [58-60]. Other studies have shown increased TGF-β levels in different stages of plaque development [61-63]. In fact, TGF-β1 has been proposed as a marker and potential therapeutic target for cardiovascular disease [60].

A gene encoding a large antisense non-coding RNA (*ANRIL*) spans almost the entire 9p21-CAD association region (**Figure 1**) [55]. *ANRIL* was discovered through deletion analysis of a family with hereditary melanoma-neural system tumors [64]. It was found that the expression of *ANRIL* coclusters with the expression of *ARF*, which is encoded for by an alternative exon 1 and exons 2 and 3 of *CDKN2A*. Atheromatous human vessels, abdominal aortic aneurysm walls, vascular endothelial cells, monocyte-derived macrophages, and coronary smooth muscle cells have all been shown to express *ANRIL* [43]. It has been speculated that transcription of the cyclin-dependent kinase genes may be regulated, at least in part, by *ANRIL* through RNA interference or some other mechanism [43, 44]. In fact, a $p15^{INK4B}$ antisense construct located in the 5' region of *ANRIL*, was shown to cause silencing of $p15^{INK4B}$ (encoded for by *CDKN2B*) in mouse embryonic stem cells through heterochromatin formation and DNA methylation [65]. Thus, a speculated mechanism for the 9p21 risk allele involves antisense regulation of *CDKN2B* (and/or *CDKN2A*), which could then affect signaling of TGF-β and/or additional cytokine(s) involved in cell cycle arrest/proliferation.

Another gene, *MTAP*, encoding methylthioadenosine phosphorylase, is part of a different LD block that is closer to the chromosome 9 telomere. *MTAP* encodes for methylthioadenosine phosphorylase (MTAP), an enzyme involved in polyamine metabolism that is deleted (along with $p16^{INK4B}$) in many cancers. Whether or not this enzyme is involved in CAD requires further investigation.

Clinical utility of genotyping 9p21.3

While the specific phenotypic associations of the 9p21 genotype are somewhat conflicting and not well defined at present, whether or not 9p21 has proven clinical utility in predicting cardiovascular risk is of significant importance. As described above, an approximate 15-40% increased risk for CAD has been observed (depending on number of 9p21 risk alleles carried by the individual). However the Women's Genome Health Study demonstrated that 9p21 genotype did not add significantly to prediction of cardiovascular risk compared to what can be assessed via established risk markers, high-sensitivity C-reactive protein (CRP), and family history of premature myocardial infarction [66]. Thus, while a 20% increased risk (for heterozygous 9p21 risk allele carriers) for CAD may be relevant in some populations, middle-aged women (who are generally at low risk for CAD) who carry the 9p21 allele will not have a high,

or even intermediate, risk for cardiovascular disease, despite the additional risk due to the presence of the 9p21 allele. Additionally, Talmud et al. demonstrated that 9p21 genotype did not add significantly to the CHD predictive utility by conventional risk factors in the Framingham risk score algorithm in a cohort of healthy middle-aged Caucasian men [67]. They did note, however, that 9p21 genotype did improve reclassification of CHD risk. Accordingly, based on studies performed in patients with early-onset angiographic CAD, Anderson et al. concluded that the clinical utility of 9p21 genetic assessment might be in refining CHD risk classification [39]. Thus, overall, it is suggested that 9p21 genotype may not be useful in stratifying risk in some low-risk populations but may provide discrimination in intermediate-risk individuals.

The launch of a clinical genotyping assay for 9p21, in October, 2007, was met with mixed enthusiasm (http://www.theheart.org/article/817629.do). Concerns regarding genetic testing for 9p21 include inherent problems with testing for the 9p21 locus in isolation, lack of biological knowledge of this locus and how it relates to CAD, uncertainty of the value and applicability of information obtained from genotyping, and potential for false negative reassurance in individuals who do not carry the risk allele. Thus, while 9p21 has been repeatedly associated with CAD, demonstrates a risk that is independent of traditional risk factors, and may be a useful marker in some populations (e.g. intermediate risk individuals), 9p21 genotyping as standard of care is debatable.

Chromosome 1p, band 13.3

A risk allele at 1p13 was found to be associated with CAD and early-onset MI, and this correlation was confirmed in a large scale association analysis [18, 20, 21]. This risk allele is in a 97-kb region of LD containing the *CELSR2*, *PSRC1*, and *SORT1* genes, and has been found to be strongly associated with low-density lipoprotein (LDL) and total cholesterol concentrations [68-74]. Based on expression studies correlated with LDL cholesterol levels, it was concluded that *SORT1* and *CELSR2* were the most likely candidate susceptibility genes at the 1p13.3 locus [75]. Sortilin is a pro-neurotrophin receptor encoded for by the *SORT1* gene, and is involved in adipocyte and muscle glucose metabolism. Sortilin expression is downregulated in obesity and has been implicated in insulin resistance [76]. Sortilin has also been shown to be involved in uptake and degradation of lipoprotein lipase, an important enzyme for lipid hydrolysis [77]. In relation to this, the gene encoding lipoprotein lipase (*LPL*) at 8p21 has also been implicated in multiple GWA studies as associated with high-density lipoprotein (HDL) cholesterol and triglyceride levels and other metabolic traits [69, 70, 73, 74, 78-80]. *CELSR2* encodes for cadherin EGF LAG seven-pass G-type receptor 2, but very little is known about its function. The third gene in the 1p13.3 gene complex is *PSRC1*, which encodes for proline/serine-rich coiled-coil 1, a microtubule-associated protein. The functional connection between *PSRC1* and CAD and/or lipid metabolism is not known. Thus, while there is a potential connection between the biology of *SORT1* and cholesterol levels and CAD, the picture involving *CELSR2* and *PSRC1* is less clear and further studies are needed to elucidate those mechanisms.

Other loci

Other loci that have been identified through multiple, independent GWA studies and confirmed in follow-up CAD association analyses include 1q41 and 10q11 (**Table 1**) [16, 18, 20, 21]. Interestingly, 10q11 was shown to have a significant relationship to CAD in women but not in men [20]. Another locus, 6q25, was also identified to be associated with CAD in multiple, independent GWA studies, but the correlation was not statistically significant in a large-scale association analysis [20]. Loci within the 12q23-24 region were found to be associated with CAD, MI, and coronary artery calcification in three separate GWA studies [42, 81, 82]. The reported genes in these studies are *DRIM* (12q23), *SH2B3* (12q24), and *HNF1A-C12orf43* (12q24.41). The *HNF1A* gene has also been implicated for its association with LDL cholesterol and C-reactive protein in other GWA studies [79, 83, 84].

Two genes, *LDLR* and *PCSK9*, in which mutations can lead to autosomal dominant hypercholesterolemia, were reported in a GWA study of early-onset myocardial infarction [21]. Mutations in *LDLR* (on 19p13), which encodes for the LDL receptor, leads to familial

hypercholesterolemia. Gain of function mutations in *PCSK9* (on 1p32), which encodes for proprotein convertase, subtilisin/kesin-type 9 and is involved in recycling of the LDL receptor, leads to autosomal dominant hypercholesterolemia 3. SNPs in these genes have also been identified in other GWA studies as associated with LDL cholesterol [69, 70, 74, 79].

Copy number variant and haplotype GWA analyses

Genome-wide association studies involving the univariate analysis of single nucleotide polymorphisms (SNPs) have been the standard to date. In contrast to polymorphisms at single base positions (SNPs), copy number variants (CNVs) are polymorphic deletions or duplications of large, (1 kilobase to several megabase) regions of the genome, and could likewise be associated with specific disease states. In that regard, The Myocardial Infarction Genetics Consortium explored the possibility of an association between copy number variants (CNVs) and early-onset myocardial infarction [21]. However, they did not detect a significant difference in CNVs in cases vs. controls, in genes vs. the genome, or at any individual locus.

In addition to univariate analysis of SNPs, there is the potential for SNPs in haplotype to be associated with certain phenotypes. Tregouet et al. recently performed a genome-wide haplotype association study in CAD, using a sliding-windows approach [85]. Using this type of analysis, a haplotype of four SNPs in the *SLC22A3-LPAL2-LPA* gene cluster was found to be associated with CAD. Of particular interest in this gene cluster is *LPA*, which encodes apolipoprotein (a), the protein component of lipoprotein (a) [Lp(a)]. Elevated levels of Lp(a) have been associated with an increased risk for CAD and MI [86-88].

Conclusion

GWA analyses are a powerful tool for evaluating genetic associations in complex disease. However, as described above, careful design and execution of GWA and follow-up association studies are imperative to providing robust, meaningful data. A major challenge with GWA analyses is separating the true markers from the spurious associations. Having stringent p-values is important, as is replicating results in independent cohorts. Additionally, comprehensive phenotyping of the cohort is also beneficial for understanding biological pathways. Extensively characterized cohorts will provide the foundation for ascertaining genetic and environmental risk factors in an integrated, interdependent manner.

For the vast majority of published GWA studies, the ethnic background of the primary study population has been European Caucasian. However, there is a need to extend association analyses to populations with other ethnic backgrounds. Linkage disequilibrium blocks and allele frequencies can vary widely between European Caucasians, African Americans, Asians, Hispanics, and other ethnic groups. Thus, associations found in one ethnic group may not translate to the same association in other ethnic groups. A case in point is 9p21.3, in which an association between this locus and CAD was observed in many different populations, but the association was not present in African Americans [17, 35].

In addition to confounding issues in association studies due to ethnicity, age also should be taken into account. In fact, a stronger impact of genetic factors on CAD is observed in younger individuals, possibly due to heterogeneity of effect associated with advancing age [2]. The 9p21 risk allele had a more pronounced effect in individuals with earlier onset MI, as observed by Helgadottir et al. [19]. Additionally, the Rotterdam Study was unable to make a correlation with 9p21 and CHD or MI in a prospective cohort study of nearly 8000 participants aged 55 years and older [30]. The authors cautioned, however, that the results of the study may be too underpowered to generalize them to an elderly population. Proper characterization of cohorts, as well as matching for age, gender, and ethnicity, is important to prevent misrepresentation of the findings.

While GWA studies have identified novel potential markers for CAD and other complex diseases, there are several limitations to the results of these studies [89]. These limitations include the risk of false positive results, lack of detection of rare variants due to the insensitive nature of the assay, biases from

incomplete phenotyping of cases and controls, and confounders due to population stratification. Furthermore, very few functional variants have been identified through GWA studies. Thus, further studies are necessary to ascertain the relationship of the identified variants to functional variants and biology of the disease.

Because of the nature of complex diseases, it is likely that multiple genetic risk alleles are needed to accurately assess risk. The risk alleles may be identified through association and linkage studies, but many of them may be identified by downstream studies investigating interactions between multiple risk loci. One study, using computer simulation, estimated that over 200 alleles were required to provide a reasonable assessment of CAD risk [90]. Thus, while GWA studies may be useful in identifying potential novel markers for complex disease, essential follow-up investigations involve determining the biology of the markers, interactions between loci, effect of risk markers in subclinical phenotypes, and association in other populations.

Acknowledgments

The author acknowledges helpful comments by Dr. Allan Jaffe.

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References

[1] Castelli WP. Lipids, risk factors and ischaemic heart disease. Atherosclerosis 1996; 124 Suppl: S1-9.

[2] Zdravkovic S, Wienke A, Pedersen NL, Marenberg ME, Yashin AI and De Faire U. Heritability of death from coronary heart disease: a 36-year follow-up of 20 966 Swedish twins. J Intern Med 2002; 252: 247-254.

[3] Lloyd-Jones DM, Nam BH, D'Agostino RB, Sr., Levy D, Murabito JM, Wang TJ, Wilson PW and O'Donnell CJ. Parental cardiovascular disease as a risk factor for cardiovascular disease in middle-aged adults: a prospective study of parents and offspring. JAMA 2004; 291: 2204-2211.

[4] Assmann G, Cullen P and Schulte H. Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Munster (PROCAM) study. Circulation 2002; 105: 310-315.

[5] Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J and Lisheng L. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004; 364: 937-952.

[6] Nasir K, Michos ED, Rumberger JA, Braunstein JB, Post WS, Budoff MJ and Blumenthal RS. Coronary artery calcification and family history of premature coronary heart disease: sibling history is more strongly associated than parental history. Circulation 2004; 110: 2150-2156.

[7] Fischer M, Broeckel U, Holmer S, Baessler A, Hengstenberg C, Mayer B, Erdmann J, Klein G, Riegger G, Jacob HJ and Schunkert H. Distinct heritable patterns of angiographic coronary artery disease in families with myocardial infarction. Circulation 2005; 111: 855-862.

[8] Fischer M, Mayer B, Baessler A, Riegger G, Erdmann J, Hengstenberg C and Schunkert H. Familial aggregation of left main coronary artery disease and future risk of coronary events in asymptomatic siblings of affected patients. Eur Heart J 2007; 28: 2432-2437.

[9] Harpaz D, Behar S, Rozenman Y, Boyko V and Gottlieb S. Family history of coronary artery disease and prognosis after first acute myocardial infarction in a national survey. Cardiology 2004; 102: 140-146.

[10] Williams RR, Hunt SC, Heiss G, Province MA, Bensen JT, Higgins M, Chamberlain RM, Ware J and Hopkins PN. Usefulness of cardiovascular family history data for population-based preventive medicine and medical research (the Health Family Tree Study and the NHLBI Family Heart Study). Am J Cardiol 2001; 87: 129-135.

[11] Lusis AJ, Mar R and Pajukanta P. Genetics of atherosclerosis. Annu Rev Genomics Hum Genet 2004; 5: 189-218.

[12] Morgan TM, Krumholz HM, Lifton RP and Spertus JA. Nonvalidation of reported genetic risk factors for acute coronary syndrome in a large-scale replication study. JAMA 2007; 297: 1551-1561.

[13] McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JP and Hirschhorn JN. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 2008; 9: 356-369.

[14] Donnelly P. Progress and challenges in genome-wide association studies in humans. Nature 2008; 456: 728-731.

[15] Ioannidis JP. Prediction of cardiovascular disease outcomes and established cardiovascular risk factors by genome-wide association markers. Circulation Cardiovascular Genetics 2009; published

online Jan 23, 2009:
[16] Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447: 661-678.
[17] McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, Hinds DA, Pennacchio LA, Tybjaerg-Hansen A, Folsom AR, Boerwinkle E, Hobbs HH and Cohen JC. A common allele on chromosome 9 associated with coronary heart disease. Science 2007; 316: 1488-1491.
[18] Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, Dixon RJ, Meitinger T, Braund P, Wichmann HE, Barrett JH, Konig IR, Stevens SE, Szymczak S, Tregouet DA, Iles MM, Pahlke F, Pollard H, Lieb W, Cambien F, Fischer M, Ouwehand W, Blankenberg S, Balmforth AJ, Baessler A, Ball SG, Strom TM, Braenne I, Gieger C, Deloukas P, Tobin MD, Ziegler A, Thompson JR and Schunkert H. Genomewide association analysis of coronary artery disease. N Engl J Med 2007; 357: 443-453.
[19] Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, Jonasdottir A, Sigurdsson A, Baker A, Palsson A, Masson G, Gudbjartsson DF, Magnusson KP, Andersen K, Levey AI, Backman VM, Matthiasdottir S, Jonsdottir T, Palsson S, Einarsdottir H, Gunnarsdottir S, Gylfason A, Vaccarino V, Hooper WC, Reilly MP, Granger CB, Austin H, Rader DJ, Shah SH, Quyyumi AA, Gulcher JR, Thorgeirsson G, Thorsteinsdottir U, Kong A and Stefansson K. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science 2007; 316: 1491-1493.
[20] Consortium CAD. Large Scale Association Analysis of Novel Genetic Loci for Coronary Artery Disease. Arterioscler Thromb Vasc Biol 2009;
[21] Kathiresan S, Voight BF, Purcell S, Musunuru K, Ardissino D, Mannucci PM, Anand S, Engert JC, Samani NJ, Schunkert H, Erdmann J, Reilly MP, Rader DJ, Morgan T, Spertus JA, Stoll M, Girelli D, McKeown PP, Patterson CC, Siscovick DS, O'Donnell CJ, Elosua R, Peltonen L, Salomaa V, Schwartz SM, Melander O, Altshuler D, Ardissino D, Merlini PA, Berzuini C, Bernardinelli L, Peyvandi F, Tubaro M, Celli P, Ferrario M, Fetiveau R, Marziliano N, Casari G, Galli M, Ribichini F, Rossi M, Bernardi F, Zonzin P, Piazza A, Mannucci PM, Schwartz SM, Siscovick DS, Yee J, Friedlander Y, Elosua R, Marrugat J, Lucas G, Subirana I, Sala J, Ramos R, Kathiresan S, Meigs JB, Williams G, Nathan DM, Macrae CA, O'Donnell CJ, Salomaa V, Havulinna AS, Peltonen L, Melander O, Berglund G, Voight BF, Kathiresan S, Hirschhorn JN, Asselta R, Duga S, Spreafico M, Musunuru K, Daly MJ, Purcell S, Voight BF, Purcell S, Nemesh J, Korn JM, McCarroll SA, Schwartz SM, Yee J, Kathiresan S, Lucas G, Subirana I, Elosua R, Surti A, Guiducci C, Gianniny L, Mirel D, Parkin M, Burtt N, Gabriel SB, Samani NJ, Thompson JR, Braund PS, Wright BJ, Balmforth AJ, Ball SG, Hall AS, Schunkert H, Erdmann J, Linsel-Nitschke P, Lieb W, Ziegler A, Konig IR, Hengstenberg C, Fischer M, Stark K, Grosshennig A, Preuss M, Wichmann HE, Schreiber S, Schunkert H, Samani NJ, Erdmann J, Ouwehand W, Hengstenberg C, Deloukas P, Scholz M, Cambien F, Reilly MP, Li M, Chen Z, Wilensky R, Matthai W, Qasim A, Hakonarson HH, Devaney J, Burnett MS, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Epstein SE, Rader DJ, Scheffold T, Berger K, Stoll M, Huge A, Girelli D, Martinelli N, Olivieri O, Corrocher R, Morgan T, Spertus JA, McKeown PP, Patterson CC, Schunkert H, Erdmann J, Linsel-Nitschke P, Lieb W, Ziegler A, Konig IR, Hengstenberg C, Fischer M, Stark K, Grosshennig A, Preuss M, Wichmann HE, Schreiber S, Holm H, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Engert JC, Do R, Xie C, Anand S, Kathiresan S, Ardissino D, Mannucci PM, Siscovick D, O'Donnell CJ, Samani NJ, Melander O, Elosua R, Peltonen L, Salomaa V, Schwartz SM and Altshuler D. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet 2009; 41: 334-341.
[22] Larson MG, Atwood LD, Benjamin EJ, Cupples LA, D'Agostino RB, Sr., Fox CS, Govindaraju DR, Guo CY, Heard-Costa NL, Hwang SJ, Murabito JM, Newton-Cheh C, O'Donnell CJ, Seshadri S, Vasan RS, Wang TJ, Wolf PA and Levy D. Framingham Heart Study 100K project: genome-wide associations for cardiovascular disease outcomes. BMC Med Genet 2007; 8 Suppl 1: S5.
[23] Helgadottir A, Thorleifsson G, Magnusson KP, Gretarsdottir S, Steinthorsdottir V, Manolescu A, Jones GT, Rinkel GJ, Blankensteijn JD, Ronkainen A, Jaaskelainen JE, Kyo Y, Lenk GM, Sakalihasan N, Kostulas K, Gottsater A, Flex A, Stefansson H, Hansen T, Andersen G, Weinsheimer S, Borch-Johnsen K, Jorgensen T, Shah SH, Quyyumi AA, Granger CB, Reilly MP, Austin H, Levey AI, Vaccarino V, Palsdottir E, Walters GB, Jonsdottir T, Snorradottir S, Magnusdottir D, Gudmundsson G, Ferrell RE, Sveinbjornsdottir S, Hernesniemi J, Niemela M, Limet R, Andersen K, Sigurdsson G, Benediktsson R, Verhoeven EL, Teijink JA, Grobbee DE, Rader DJ, Collier DA, Pedersen O, Pola R, Hillert J, Lindblad B, Valdimarsson EM, Magnadottir HB, Wijmenga C, Tromp G, Baas AF, Ruigrok YM, van Rij AM, Kuivaniemi H, Powell JT, Matthiasson SE, Gulcher JR, Thorgeirsson G, Kong A, Thorsteinsdottir U and Stefansson K. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet 2008; 40: 217-224.
[24] Muendlein A, Saely CH, Rhomberh S,

Sonderegger G, Loacker S, Rein P, Beer S, Vonbank A, Winder T and Drexel H. Evaluation of the association of genetic variants on the chromosomal loci 9p21.3, 6q25.1, and 2q36.3 with angiographically characterized coronary artery disease. Atherosclerosis 2009; doi:10.1016/j.atherosclerosis.2008.10.035:

[25] van der Net JB, Oosterveer DM, Versmissen J, Defesche JC, Yazdanpanah M, Aouizerat BE, Steyerberg EW, Malloy MJ, Pullinger CR, Kastelein JJ, Kane JP and Sijbrands EJ. Replication study of 10 genetic polymorphisms associated with coronary heart disease in a specific high-risk population with familial hypercholesterolemia. Eur Heart J 2008; 29: 2195-2201.

[26] Abdullah KG, Li L, Shen GQ, Hu Y, Yang Y, MacKinlay KG, Topol EJ and Wang QK. Four SNPS on chromosome 9p21 confer risk to premature, familial CAD and MI in an American Caucasian population (GeneQuest). Ann Hum Genet 2008; 72: 654-657.

[27] Horne BD, Carlquist JF, Muhlestein JB, Bair TL and Anderson JL. Association of Variation in the Chromosome 9p21 Locus With Myocardial Infarction Versus Chronic Coronary Artery Disease. Circulation Cardiovascular Genetics 2008; 1: 85-92.

[28] Karvanen J, Silander K, Kee F, Tiret L, Salomaa V, Kuulasmaa K, Wiklund PG, Virtamo J, Saarela O, Perret C, Perola M, Peltonen L, Cambien F, Erdmann J, Samani NJ, Schunkert H and Evans A. The impact of newly identified loci on coronary heart disease, stroke and total mortality in the MORGAM prospective cohorts. Genet Epidemiol 2008;

[29] Schunkert H, Gotz A, Braund P, McGinnis R, Tregouet DA, Mangino M, Linsel-Nitschke P, Cambien F, Hengstenberg C, Stark K, Blankenberg S, Tiret L, Ducimetiere P, Keniry A, Ghori MJ, Schreiber S, El Mokhtari NE, Hall AS, Dixon RJ, Goodall AH, Liptau H, Pollard H, Schwarz DF, Hothorn LA, Wichmann HE, Konig IR, Fischer M, Meisinger C, Ouwehand W, Deloukas P, Thompson JR, Erdmann J, Ziegler A and Samani NJ. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. Circulation 2008; 117: 1675-1684.

[30] Dehghan A, van Hoek M, Sijbrands EJ, Oostra BA, Hofman A, van Duijn CM and Witteman JC. Lack of association of two common polymorphisms on 9p21 with risk of coronary heart disease and myocardial infarction; results from a prospective cohort study. BMC Med 2008; 6: 30.

[31] Shen GQ, Li L, Rao S, Abdullah KG, Ban JM, Lee BS, Park JE and Wang QK. Four SNPs on chromosome 9p21 in a South Korean population implicate a genetic locus that confers high cross-race risk for development of coronary artery disease. Arterioscler Thromb Vasc Biol 2008; 28: 360-365.

[32] Shen GQ, Rao S, Martinelli N, Li L, Olivieri O, Corrocher R, Abdullah KG, Hazen SL, Smith J, Barnard J, Plow EF, Girelli D and Wang QK. Association between four SNPs on chromosome 9p21 and myocardial infarction is replicated in an Italian population. J Hum Genet 2008; 53: 144-150.

[33] Hiura Y, Fukushima Y, Yuno M, Sawamura H, Kokubo Y, Okamura T, Tomoike H, Goto Y, Nonogi H, Takahashi R and Iwai N. Validation of the association of genetic variants on chromosome 9p21 and 1q41 with myocardial infarction in a Japanese population. Circ J 2008; 72: 1213-1217.

[34] Hinohara K, Nakajima T, Takahashi M, Hohda S, Sasaoka T, Nakahara K, Chida K, Sawabe M, Arimura T, Sato A, Lee BS, Ban JM, Yasunami M, Park JE, Izumi T and Kimura A. Replication of the association between a chromosome 9p21 polymorphism and coronary artery disease in Japanese and Korean populations. J Hum Genet 2008; 53: 357-359.

[35] Assimes TL, Knowles JW, Basu A, Iribarren C, Southwick A, Tang H, Absher D, Li J, Fair JM, Rubin GD, Sidney S, Fortmann SP, Go AS, Hlatky MA, Myers RM, Risch N and Quertermous T. Susceptibility locus for clinical and subclinical coronary artery disease at chromosome 9p21 in the multi-ethnic ADVANCE study. Hum Mol Genet 2008; 17: 2320-2328.

[36] Samani NJ, Raitakari OT, Sipila K, Tobin MD, Schunkert H, Juonala M, Braund PS, Erdmann J, Viikari J, Moilanen L, Taittonen L, Jula A, Jokinen E, Laitinen T, Hutri-Kahonen N, Nieminen MS, Kesaniemi YA, Hall AS, Hulkkonen J, Kahonen M and Lehtimaki T. Coronary artery disease-associated locus on chromosome 9p21 and early markers of atherosclerosis. Arterioscler Thromb Vasc Biol 2008; 28: 1679-1683.

[37] Ye S, Willeit J, Kronenberg F, Xu Q and Kiechl S. Association of genetic variation on chromosome 9p21 with susceptibility and progression of atherosclerosis: a population-based, prospective study. J Am Coll Cardiol 2008; 52: 378-384.

[38] Bjorck HM, Lanne T, Alehagen U, Persson K, Rundkvist L, Hamsten A, Dahlstrom U and Eriksson P. Association of genetic variation on chromosome 9p21.3 and arterial stiffness. J Intern Med 2009; 265: 373-381.

[39] Anderson JL, Horne BD, Kolek MJ, Muhlestein JB, Mower CP, Park JJ, May HT, Camp NJ and Carlquist JF. Genetic variation at the 9p21 locus predicts angiographic coronary artery disease prevalence but not extent and has clinical utility. Am Heart J 2008; 156: 1155-1162 e1152.

[40] Farzaneh-Far R, Na B, Schiller NB and Whooley MA. Lack of association of chromosome 9p21.3 genotype with cardiovascular structure

and function in persons with stable coronary artery disease: The Heart and Soul Study. Atherosclerosis 2009; doi:10.1016/j.atherosclerosis.2008/12.026:

[41] Chen SN, Ballantyne CM, Gotto AM, Jr. and Marian AJ. The 9p21 susceptibility locus for coronary artery disease and the severity of coronary atherosclerosis. BMC Cardiovasc Disord 2009; 9: 3.

[42] O'Donnell CJ, Cupples LA, D'Agostino RB, Fox CS, Hoffmann U, Hwang SJ, Ingellson E, Liu C, Murabito JM, Polak JF, Wolf PA and Demissie S. Genome-wide association study for subclinical atherosclerosis in major arterial territories in the NHLBI's Framingham Heart Study. BMC Med Genet 2007; 8 Suppl 1: S4.

[43] Broadbent HM, Peden JF, Lorkowski S, Goel A, Ongen H, Green F, Clarke R, Collins R, Franzosi MG, Tognoni G, Seedorf U, Rust S, Eriksson P, Hamsten A, Farrall M and Watkins H. Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. Hum Mol Genet 2008; 17: 806-814.

[44] Bown MJ, Braund PS, Thompson J, London NJM, Samani NJ and Sayers RD. Association between the coronary artery disease risk locus on chromosome 9p21.3 and abdominal aortic aneurysm. Circ Cardiovasc Genet 2008; 1: 39-42.

[45] Smith JG, Melander O, Lovkist H, Heblad B, Engstrom G, Nilsson P, Carlson J, Berglund G, Norrving B and Lindgren A. Common genetic variants on chromosome 9p21 confers risk of ischemic stroke: a large-scale genetic association study. Circ Cardiovasc Genet 2009; 10.1161/CIRCGENETICS.108.835173:

[46] Thompson AR, Golledge J, Cooper JA, Hafez H, Norman PE and Humphries SE. Sequence variant on 9p21 is associated with the presence of abdominal aortic aneurysm disease but does not have an impact on aneurysmal expansion. Eur J Hum Genet 2008;

[47] Tarasov KV, Sanna S, Scuteri A, Strait JB, Orru M, Parsa A, Lin P-I, Maschio A, Lai S, Piras MG, Masala M, Tanaka T, Post WS, O'Connell JR, Schlessinger D, Cao A, Nagaraja R, Mitchell BD, Abecasis GR, Shuldiner AR, Uda M, Lakatta E and Najjar SS. COL4A1 is associated with arterial stiffness by genome wide association scan. Circ Cardiovasc Genet 2009; DOI: 10.1161/CIRCGENETICS.108.823245:

[48] Pollock PM, Pearson JV and Hayward NK. Compilation of somatic mutations of the CDKN2 gene in human cancers: non-random distribution of base substitutions. Genes Chromosomes Cancer 1996; 15: 77-88.

[49] Smith-Sorensen B and Hovig E. CDKN2A (p16INK4A) somatic and germline mutations. Hum Mutat 1996; 7: 294-303.

[50] Foulkes WD, Flanders TY, Pollock PM and Hayward NK. The CDKN2A (p16) gene and human cancer. Mol Med 1997; 3: 5-20.

[51] Liggett WH, Jr. and Sidransky D. Role of the p16 tumor suppressor gene in cancer. J Clin Oncol 1998; 16: 1197-1206.

[52] Krimpenfort P, Quon KC, Mooi WJ, Loonstra A and Berns A. Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. Nature 2001; 413: 83-86.

[53] Sharpless E and Chin L. The INK4a/ARF locus and melanoma. Oncogene 2003; 22: 3092-3098.

[54] Chin L, Pomerantz J and DePinho RA. The INK4a/ARF tumor suppressor: one gene--two products--two pathways. Trends Biochem Sci 1998; 23: 291-296.

[55] Serrano M, Lee H, Chin L, Cordon-Cardo C, Beach D and DePinho RA. Role of the INK4a locus in tumor suppression and cell mortality. Cell 1996; 85: 27-37.

[56] Topol EJ, Murray SS and Frazer KA. The genomics gold rush. JAMA 2007; 298: 218-221.

[57] Hannon GJ and Beach D. p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. Nature 1994; 371: 257-261.

[58] Kalinina N, Agrotis A, Antropova Y, Ilyinskaya O, Smirnov V, Tararak E and Bobik A. Smad expression in human atherosclerotic lesions: evidence for impaired TGF-beta/Smad signaling in smooth muscle cells of fibrofatty lesions. Arterioscler Thromb Vasc Biol 2004; 24: 1391-1396.

[59] Fukui D, Miyagawa S, Soeda J, Tanaka K, Urayama H and Kawasaki S. Overexpression of transforming growth factor beta1 in smooth muscle cells of human abdominal aortic aneurysm. Eur J Vasc Endovasc Surg 2003; 25: 540-545.

[60] Redondo S, Santos-Gallego CG and Tejerina T. TGF-beta1: a novel target for cardiovascular pharmacology. Cytokine Growth Factor Rev 2007; 18: 279-286.

[61] Bobik A, Agrotis A, Kanellakis P, Dilley R, Krushinsky A, Smirnov V, Tararak E, Condron M and Kostolias G. Distinct patterns of transforming growth factor-beta isoform and receptor expression in human atherosclerotic lesions. Colocalization implicates TGF-beta in fibrofatty lesion development. Circulation 1999; 99: 2883-2891.

[62] Piao M and Tokunaga O. Significant expression of endoglin (CD105), TGFbeta-1 and TGFbeta R-2 in the atherosclerotic aorta: an immunohistological study. J Atheroscler Thromb 2006; 13: 82-89.

[63] Panutsopulos D, Papalambros E, Sigala F, Zafiropoulos A, Arvanitis DL and Spandidos DA. Protein and mRNA expression levels of VEGF-A and TGF-beta1 in different types of human coronary atherosclerotic lesions. Int J Mol Med 2005; 15: 603-610.

[64] Pasmant E, Laurendeau I, Heron D, Vidaud M, Vidaud D and Bieche I. Characterization of a germ-line deletion, including the entire

INK4/ARF locus, in a melanoma-neural system tumor family: identification of ANRIL, an antisense noncoding RNA whose expression coclusters with ARF. Cancer Res 2007; 67: 3963-3969.
[65] Yu W, Gius D, Onyango P, Muldoon-Jacobs K, Karp J, Feinberg AP and Cui H. Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA. Nature 2008; 451: 202-206.
[66] Paynter NP, Chasman DI, Buring JE, Shiffman D, Cook NR and Ridker PM. Cardiovascular disease risk prediction with and without knowledge of genetic variation at chromosome 9p21.3. Ann Intern Med 2009; 150: 65-72.
[67] Talmud PJ, Cooper JA, Palmen J, Lovering R, Drenos F, Hingorani AD and Humphries SE. Chromosome 9p21.3 coronary heart disease locus genotype and prospective risk of CHD in healthy middle-aged men. Clin Chem 2008; 54: 467-474.
[68] Wallace C, Newhouse SJ, Braund P, Zhang F, Tobin M, Falchi M, Ahmadi K, Dobson RJ, Marcano AC, Hajat C, Burton P, Deloukas P, Brown M, Connell JM, Dominiczak A, Lathrop GM, Webster J, Farrall M, Spector T, Samani NJ, Caulfield MJ and Munroe PB. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia. Am J Hum Genet 2008; 82: 139-149.
[69] 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, Mulas A, Albai G, Swift AJ, Morken MA, Narisu N, Bennett D, Parish S, Shen H, Galan P, Meneton P, Hercberg S, Zelenika D, Chen WM, Li Y, Scott LJ, Scheet PA, Sundvall J, Watanabe RM, Nagaraja R, Ebrahim S, Lawlor DA, Ben-Shlomo Y, Davey-Smith 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 and Abecasis GR. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet 2008; 40: 161-169.
[70] Kathiresan S, Melander O, Guiducci C, Surti A, Burtt NP, Rieder MJ, Cooper GM, Roos C, Voight BF, Havulinna AS, Wahlstrand B, Hedner T, Corella D, Tai ES, Ordovas JM, Berglund G, Vartiainen E, Jousilahti P, Hedblad B, Taskinen MR, Newton-Cheh C, Salomaa V, Peltonen L, Groop L, Altshuler DM and Orho-Melander M. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet 2008; 40: 189-197.
[71] Sandhu MS, Waterworth DM, Debenham SL, Wheeler E, Papadakis K, Zhao JH, Song K, Yuan X, Johnson T, Ashford S, Inouye M, Luben R, Sims M, Hadley D, McArdle W, Barter P, Kesaniemi YA, Mahley RW, McPherson R, Grundy SM, Bingham SA, Khaw KT, Loos RJ, Waeber G, Barroso I, Strachan DP, Deloukas P, Vollenweider P, Wareham NJ and Mooser V. LDL-cholesterol concentrations: a genome-wide association study. Lancet 2008; 371: 483-491.
[72] Samani NJ, Braund PS, Erdmann J, Gotz A, Tomaszewski M, Linsel-Nitschke P, Hajat C, Mangino M, Hengstenberg C, Stark K, Ziegler A, Caulfield M, Burton PR, Schunkert H and Tobin MD. The novel genetic variant predisposing to coronary artery disease in the region of the PSRC1 and CELSR2 genes on chromosome 1 associates with serum cholesterol. J Mol Med 2008; 86: 1233-1241.
[73] Aulchenko YS, Ripatti S, Lindqvist I, Boomsma D, Heid IM, Pramstaller PP, Penninx BW, Janssens AC, Wilson JF, Spector T, Martin NG, Pedersen NL, Kyvik KO, Kaprio J, Hofman A, Freimer NB, Jarvelin MR, Gyllensten U, Campbell H, Rudan I, Johansson A, Marroni F, Hayward C, Vitart V, Jonasson I, Pattaro C, Wright A, Hastie N, Pichler I, Hicks AA, Falchi M, Willemsen G, Hottenga JJ, de Geus EJ, Montgomery GW, Whitfield J, Magnusson P, Saharinen J, Perola M, Silander K, Isaacs A, Sijbrands EJ, Uitterlinden AG, Witteman JC, Oostra BA, Elliott P, Ruokonen A, Sabatti C, Gieger C, Meitinger T, Kronenberg F, Doring A, Wichmann HE, Smit JH, McCarthy MI, van Duijn CM and Peltonen L. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. Nat Genet 2009; 41: 47-55.
[74] Sabatti C, Service SK, Hartikainen AL, Pouta A, Ripatti S, Brodsky J, Jones CG, Zaitlen NA, Varilo T, Kaakinen M, Sovio U, Ruokonen A, Laitinen J, Jakkula E, Coin L, Hoggart C, Collins A, Turunen H, Gabriel S, Elliot P, McCarthy MI, Daly MJ, Jarvelin MR, Freimer NB and Peltonen L. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nat Genet 2009; 41: 35-46.
[75] Schadt EE, Molony C, Chudin E, Hao K, Yang X, Lum PY, Kasarskis A, Zhang B, Wang S, Suver C, Zhu J, Millstein J, Sieberts S, Lamb J, GuhaThakurta D, Derry J, Storey JD, Avila-Campillo I, Kruger MJ, Johnson JM, Rohl CA, van Nas A, Mehrabian M, Drake TA, Lusis AJ, Smith RC, Guengerich FP, Strom SC, Schuetz E, Rushmore TH and Ulrich R. Mapping the genetic architecture of gene expression in human liver. PLoS Biol 2008; 6: e107.
[76] Kaddai V, Jager J, Gonzalez T, Najem-Lendom R, Bonnafous S, Tran A, Le Marchand-Brustel Y, Gual P, Tanti JF and Cormont M. Involvement of TNF-alpha in abnormal adipocyte and muscle sortilin expression in obese mice and humans. Diabetologia 2009;
[77] Nielsen MS, Jacobsen C, Olivecrona G, Gliemann J and Petersen CM. Sortilin/neurotensin receptor-3 binds and mediates degradation of lipoprotein lipase. J

Biol Chem 1999; 274: 8832-8836.
[78] Chambers JC, Elliott P, Zabaneh D, Zhang W, Li Y, Froguel P, Balding D, Scott J and Kooner JS. Common genetic variation near MC4R is associated with waist circumference and insulin resistance. Nat Genet 2008; 40: 716-718.
[79] 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, Burtt NP, Parish S, Clarke R, Zelenika D, Kubalanza KA, Morken MA, Scott LJ, Stringham HM, Galan P, Swift AJ, Kuusisto J, Bergman RN, Sundvall J, Laakso M, Ferrucci L, Scheet P, Sanna S, Uda M, Yang Q, Lunetta KL, Dupuis J, de Bakker PI, O'Donnell CJ, Chambers JC, Kooner JS, Hercberg S, Meneton P, Lakatta EG, Scuteri A, Schlessinger D, Tuomilehto J, Collins FS, Groop L, Altshuler D, Collins R, Lathrop GM, Melander O, Salomaa V, Peltonen L, Orho-Melander M, Ordovas JM, Boehnke M, Abecasis GR, Mohlke KL and Cupples LA. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet 2009; 41: 56-65.
[80] Kooner JS, Chambers JC, Aguilar-Salinas CA, Hinds DA, Hyde CL, Warnes GR, Gomez Perez FJ, Frazer KA, Elliott P, Scott J, Milos PM, Cox DR and Thompson JF. Genome-wide scan identifies variation in MLXIPL associated with plasma triglycerides. Nat Genet 2008; 40: 149-151.
[81] Erdmann J, Grosshennig A, Braund PS, Konig IR, Hengstenberg C, Hall AS, Linsel-Nitschke P, Kathiresan S, Wright B, Tregouet DA, Cambien F, Bruse P, Aherrahrou Z, Wagner AK, Stark K, Schwartz SM, Salomaa V, Elosua R, Melander O, Voight BF, O'Donnell CJ, Peltonen L, Siscovick DS, Altshuler D, Merlini PA, Peyvandi F, Bernardinelli L, Ardissino D, Schillert A, Blankenberg S, Zeller T, Wild P, Schwarz DF, Tiret L, Perret C, Schreiber S, Mokhtari NE, Schafer A, Marz W, Renner W, Bugert P, Kluter H, Schrezenmeir J, Rubin D, Ball SG, Balmforth AJ, Wichmann HE, Meitinger T, Fischer M, Meisinger C, Baumert J, Peters A, Ouwehand WH, Deloukas P, Thompson JR, Ziegler A, Samani NJ and Schunkert H. New susceptibility locus for coronary artery disease on chromosome 3q22.3. Nat Genet 2009; 41: 280-282.
[82] Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, Thorleifsson G, Helgadottir H, Steinthorsdottir V, Stefansson H, Williams C, Hui J, Beilby J, Warrington NM, James A, Palmer LJ, Koppelman GH, Heinzmann A, Krueger M, Boezen HM, Wheatley A, Altmuller J, Shin HD, Uh ST, Cheong HS, Jonsdottir B, Gislason D, Park CS, Rasmussen LM, Porsbjerg C, Hansen JW, Backer V, Werge T, Janson C, Jonsson UB, Ng MC, Chan J, So WY, Ma R, Shah SH, Granger CB, Quyyumi AA, Levey AI, Vaccarino V, Reilly MP, Rader DJ, Williams MJ, van Rij AM, Jones GT, Trabetti E, Malerba G, Pignatti PF, Boner A, Pescollderungg L, Girelli D, Olivieri O, Martinelli N, Ludviksson BR, Ludviksdottir D, Eyjolfsson GI, Arnar D, Thorgeirsson G, Deichmann K, Thompson PJ, Wjst M, Hall IP, Postma DS, Gislason T, Gulcher J, Kong A, Jonsdottir I, Thorsteinsdottir U and Stefansson K. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet 2009; 41: 342-347.
[83] Reiner AP, Barber MJ, Guan Y, Ridker PM, Lange LA, Chasman DI, Walston JD, Cooper GM, Jenny NS, Rieder MJ, Durda JP, Smith JD, Novembre J, Tracy RP, Rotter JI, Stephens M, Nickerson DA and Krauss RM. Polymorphisms of the HNF1A gene encoding hepatocyte nuclear factor-1 alpha are associated with C-reactive protein. Am J Hum Genet 2008; 82: 1193-1201.
[84] Ridker PM, Pare G, Parker A, Zee RY, Danik JS, Buring JE, Kwiatkowski D, Cook NR, Miletich JP and Chasman DI. Loci related to metabolic-syndrome pathways including LEPR,HNF1A, IL6R, and GCKR associate with plasma C-reactive protein: the Women's Genome Health Study. Am J Hum Genet 2008; 82: 1185-1192.
[85] Tregouet DA, Konig IR, Erdmann J, Munteanu A, Braund PS, Hall AS, Grosshennig A, Linsel-Nitschke P, Perret C, Desuremain M, Meitinger T, Wright BJ, Preuss M, Balmforth AJ, Ball SG, Meisinger C, Germain C, Evans A, Arveiler D, Luc G, Ruidavets JB, Morrison C, van der Harst P, Schreiber S, Neureuther K, Schafer A, Bugert P, El Mokhtari NE, Schrezenmeir J, Stark K, Rubin D, Wichmann HE, Hengstenberg C, Ouwehand W, Ziegler A, Tiret L, Thompson JR, Cambien F, Schunkert H and Samani NJ. Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease. Nat Genet 2009; 41: 283-285.
[86] Rhoads GG, Dahlen G, Berg K, Morton NE and Dannenberg AL. Lp(a) lipoprotein as a risk factor for myocardial infarction. JAMA 1986; 256: 2540-2544.
[87] Bostom AG, Cupples LA, Jenner JL, Ordovas JM, Seman LJ, Wilson PW, Schaefer EJ and Castelli WP. Elevated plasma lipoprotein(a) and coronary heart disease in men aged 55 years and younger. A prospective study. JAMA 1996; 276: 544-548.
[88] Armstrong VW, Cremer P, Eberle E, Manke A, Schulze F, Wieland H, Kreuzer H and Seidel D. The association between serum Lp(a) concentrations and angiographically assessed coronary atherosclerosis. Dependence on serum LDL levels. Atherosclerosis 1986; 62: 249-257.
[89] Pearson TA and Manolio TA. How to interpret a genome-wide association study. JAMA 2008; 299: 1335-1344.

[90] Ortlepp JR, Lauscher J, Janssens U, Minkenberg R, Hanrath P and Hoffmann R. Analysis of several hundred genetic polymorphisms may improve assessment of the individual genetic burden for coronary artery disease. Eur J Intern Med 2002; 13: 485-492.