Published in final edited form as:

Trends Cardiovasc Med. 2011 May ; 21(4): 105–112. doi:10.1016/j.tcm.2012.03.007.

Circulating, imaging, and genetic biomarkers in cardiovascular risk prediction

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Abstract

In the primary prevention of cardiovascular disease, the study of biomarkers to identify at-risk individuals is an expanding field. Several developments have fueled this trend, including improved understanding of the pathophysiological processes underlying atherosclerosis, advances in imaging technology to enable the quantification of subclinical disease burden, and the identification of new genetic susceptibility variants for cardiovascular disease. Furthermore, the advent of high-throughput platforms for molecular profiling has increased the pace of biomarker discovery. The rising interest in biomarkers has been balanced by the recognition that standardized and rigorous statistical approaches are needed to evaluate the clinical utility of candidate risk markers. This article reviews the issues surrounding the evaluation of biomarkers, evidence from studies of existing biomarkers, and recent applications of biomarker discovery platforms.

INTRODUCTION

Cardiovascular disease is the leading cause of death in the United States and the world. The prolonged latent period separating risk factor exposures from the occurrence of acute events provides an important opportunity for primary prevention. A major challenge for prevention, however, is the accurate identification of “at risk” individuals. Traditional risk factors such as hyperlipidemia, hypertension, diabetes, and cigarette smoking are important risk-assessment tools that have led to the development of predictive models such as the Framingham 10-year risk score (Wilson et al. 1998). However, up to 60% of patients with coronary heart disease have been shown to have only one or none of these risk factors (Khot et al. 2003). The incentive to improve upon current models has led to a surge in interest in discovering, validating, and applying novel biomarkers. This article briefly reviews the issues surrounding the evaluation of biomarkers, evidence from studies of existing biomarkers, and recent advances in newer, unbiased biomarker discovery platforms.

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DISCLOSURES

Dr. Wang has participated on the scientific advisory board of Diasorin Inc., and has received assay support or research funding from Siemens, Brahms, Critical Diagnostics, Singulex, Diasorin, Pfizer, Roche, and LabCorp. Dr. Wang has received honoraria from Roche, Diasorin, and Quest Diagnostics. Dr. Wang is named as a co-inventor on patent applications relating to the use of metabolomic or neurohormonal biomarkers in risk prediction.

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BIOMARKERS: DEFINITION AND CHARACTERISTICS

A 2001 NIH committee provided the following definition for biomarkers: “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (Biomarkers Definitions Working Group 2001). This definition encompasses not only circulating biomarkers, but also genetic, tissue, or cellular markers, imaging results, and physical or electronic measurements. Furthermore, there are biomarkers for all phases in the evolution of disease, from risk factors to subclinical disease to overt disease. Reproducibility, safety, availability, and cost-effectiveness are among the core attributes that an ideal biomarker should possess (Morrow and de Lemos 2007).

In the particular case of cardiovascular risk prediction, the novel biomarkers should provide incremental information and influence clinical decision making (Manolio 2003). However, the determination of whether a novel biomarker adds incremental benefit to already existing clinical predictors is complex (Vasan 2006). Two characteristics, discrimination and reclassification, are frequently assessed. Discrimination is a measure of how well a model is able to distinguish between subjects who will have an event from those who will not. A test’s discriminatory ability is measured by its c-statistic, which is the area under the receiver operating characteristic curve (ROC) (Vasan 2006). The c-statistic can be interpreted as the probability that the model will correctly identify which member of a random case-control pair has the disease.

Nonetheless, two models may have similar c-statistics but different abilities to assign individuals to clinical risk categories. Reclassification examines whether the addition of a novel biomarker changes the predicted risk enough for subjects to be moved to a new risk category, thereby affecting clinical decision making. One way to measure the accuracy of reclassification is a metric known as the net reclassification improvement (NRI), which is the net difference between the proportions of rightfully and wrongfully reclassified individuals (Pencina et al. 2008) (Figure 1). In cardiovascular prevention, the impact of reclassification is perhaps most important for individuals at “intermediate” risk for disease, e.g. those in whom projected risk is high enough to warrant consideration of therapy, but not so high as to make this decision clear. An estimate of the NRI in a sample restricted to “intermediate-risk” individuals has been termed the “clinical NRI” (Ridker et al. 2008).

Another distinction between the c-statistic and NRI is that improvement in the c-statistic depends partly upon the baseline model, a limitation that does not apply to the NRI. If the baseline model performs poorly with regard to discrimination (e.g. low c-statistic), the expected increment in the c-statistic from adding a new marker is higher, and vice versa (Pencina et al. 2012).

Ultimately, once a biomarker has established its predictive utility, rigorous assessment of the potential cost-effectiveness of strategies incorporating that biomarker is essential (Rapsomaniki et al. 2012). Examples of such analyses have recently appeared in the literature for high-sensitivity C-reactive protein (Lee et al. 2010).

CIRCULATING BIOMARKERS

The greatest amount of research and data exists for the category of circulating biomarkers (Greenland et al. 2010). Because a comprehensive review is outside the scope of this article, we will provide an overview of a few key candidates from different physiologic pathways.

Significant interest has been generated over sub-fractionating lipids beyond the traditional fasting profile (total cholesterol, HDL, LDL, and triglycerides). The ratio of Apolipoprotein

B (Apo B, which is contained in LDL and other atherogenic lipoproteins including VLDL, IDL, Lp(a)) to Apolipoprotein A-I (contained in HDL) has been shown to be a strong predictor of cardiovascular events (Yusuf et al. 2004). Whether ApoB or ApoB:Apo A-I provides a better risk estimation compared with a standard lipid panel has been examined in a number of studies, with some (Walldius et al. 2001, McQueen et al. 2008) but not all (Ridker et al. 2005, Ingelsson et al. 2007) showing incremental value. In the Framingham Offspring Study, measurement of ApoB or Apo A-I did not appear to improve discrimination or reclassification when compared with the total cholesterol:HDL-C ratio (Ingelsson et al. 2007).

Lp(a), another atherogenic lipoprotein, has been shown to be an independent predictor of coronary heart disease (Erqou et al. 2009). Studies using the “Mendelian randomization” approach support the hypothesis that Lp(a) plays a causal role in atherogenesis (Kamstrup et al. 2009). The principle of random gene assortment implies that, in a population, the distribution of risk alleles is largely independent of potential confounders. Thus, genetic variation in a pathway associated with high or low biomarker concentrations could create a “natural experiment” or “natural randomization” that might allow inferences about the effects of the biomarker (Smith and Ebrahim 2004). In the case of Lp(a), association of KIV-2 gene polymorphisms, one of the genetic determinants of Lp(a) levels, with risk of myocardial infarction may provide evidence of causality (Kamstrup et al. 2009).

High-sensitivity C-reactive protein (CRP), a marker of systemic inflammation, has been extensively studied for cardiovascular risk prediction. CRP is associated with the presence of conventional risk factors, as well as obesity and the metabolic syndrome (Miller et al. 2005). Furthermore, there are abundant data to suggest that elevated CRP predicts cardiovascular disease above and beyond conventional risk factors, as summarized in a recent U.S. Preventive Services Task Force meta-analysis (Buckley et al. 2009). Treatments associated with improved cardiovascular risk, such as exercise or statin therapy, have been shown to decrease CRP (Esposito et al. 2003, Ridker et al. 2008). On the other hand, the addition of CRP only provides minimal improvement in the c-statistic (Wilson et al. 2005, Cook et al. 2006). The ability of CRP to reclassify individuals also appears relatively modest. Investigators from the Physicians’ Health Study and the Framingham Heart Study reported significant NRIs of 5.3% and 5.6%, respectively, whereas investigators from the Malmö Diet and Cancer Study found a non significant NRI (Ridker et al. 2008, Wilson et al. 2008, Melander et al. 2009). Lastly, Mendelian Randomization studies have failed to detect an association between CRP genetic variants and cardiovascular risk, suggesting that CRP is a marker of risk but not a causal factor in atherogenesis (Elliott et al. 2009).

B-type natriuretic peptide (BNP), synthesized and released from the myocardium as a response to pressure and volume overload, plays a key role in volume homeostasis and ventricular remodeling (Levin et al. 1998). An important tool in the diagnosis and prognosis of heart failure, BNP has also been recently investigated for a role in ambulatory populations. Data from the Framingham Offspring Study showed that individuals with BNP values >80th percentile had a 62% increased risk for mortality and a 76% increased risk for major cardiovascular events (Wang et al. 2004). A subsequent large meta-analysis of 40 studies found that individuals in the top tertile of BNP levels had a relative risk of 2.82 for cardiovascular disease, compared with those in the bottom tertile (Di Angelantonio et al. 2009). On the other hand, evidence regarding the ability of BNP to raise the c-statistic or NRI has been mixed, with most studies suggesting performance comparable to or slightly better than CRP (Zethelius et al. 2008, Melander et al. 2009).

More recently, two new circulating biomarkers have been generating attention. The development of high-sensitivity assays for cardiac troponins (hsTnT or hsTnI) has enabled

the detection of this “myocardial injury” biomarker in the circulation of apparently healthy individuals. Recent studies have investigated whether high-sensitivity troponin can help predict events in such ambulatory populations. In the Dallas Heart Study, hsTnT was detectable in ~25% of middle-aged men and women (de Lemos et al. 2010). At a median follow-up of 6.4 years, all-cause mortality was 1.9% in participants without detectable hsTnT compared with 9.1% in those with detectable values. Cardiovascular mortality followed a similar trend, correlating in a stepwise manner with increasing levels of hsTnT. The addition of hsTnT also improved the c-statistic compared with a traditional risk factor model for predicting cardiovascular mortality (0.83 vs. 0.87). Similar findings have been reported from the Cardiovascular Health Study and the ARIC study (deFilippi et al. 2010, Saunders et al. 2011).

Growth differentiation factor 15 (GDF-15), a protein in the transforming growth factor superfamily produced by cardiomyocytes under stress, has recently been evaluated in two large community studies. In elderly individuals enrolled in the Rancho Bernardo Study, GDF-15 was shown to predict both cardiovascular and all-cause mortality (Daniels et al. 2011). Participants in the highest quartile of measured GDF-15 had a 2.4-fold increased risk of cardiovascular and non-cardiovascular mortality. Addition of GDF-15 to a traditional risk model had raised the c-statistic modestly (by 0.01) and was associated with an NRI of 5.6% for all cause-mortality. A recent report from the Dallas Heart Study found similar results (Rohatgi et al. 2011). Increased circulating GDF-15 levels were associated in a stepwise manner with both coronary artery calcification (CAC, a surrogate marker of atherosclerosis) and cardiovascular mortality. Participants in the highest pre-specified category of GDF-15 (> 1800 ng/L) had a 2.5-fold excess risk. The addition of GDF-15 resulted in a small increase in the c-statistic (about 0.02).

IMAGING BIOMARKERS

Overt coronary disease is preceded by a long period of asymptomatic, subclinical atherosclerosis (McGill and McMahan 1998). Thus, one approach to risk prediction is to identify individuals according to their subclinical burden of atherosclerosis using noninvasive imaging. Carotid intima-media thickness (CIMT) and coronary artery calcium (CAC) scores are the best-studied imaging modalities for this purpose.

CIMT, the combined thickness of the carotid intimal and medial layers, was initially described in the 1980s as an indirect measurement of the carotid atheroma burden (Pignoli et al. 1986). Measured using B-mode ultrasound, its association with myocardial infarction or stroke occurs in a stepwise fashion even after adjustment for traditional risk factors (O’Leary et al. 1999). A meta-analysis showed that each 0.1 mm increment at the common carotid IMT increased the risk of myocardial infarction by 10%–15% (Lorenz et al. 2007). A few studies have addressed the ability of CIMT to improve upon clinical risk prediction models. In the MESA cohort, addition of CIMT to traditional risk factors resulted in a minimal improvement in c-statistic (0.77 to 0.78) (Folsom et al. 2008). In the ARIC cohort, addition of CIMT had a small effect on the c-statistic (0.74 to 0.76), but improved reclassification with an NRI of 9.9%. Among intermediate risk individuals (10–20% 10-year risk), 24.8% were reclassified into a lower risk category whereas 13.5% were reclassified into the highest risk category (Nambi et al. 2010). More recently, data from the Framingham Offspring Study cohort have shown that the addition of internal carotid, but not common CIMT, improved reclassification, with an NRI of 7.6% (Polak et al. 2011). The majority of the reclassifications occurred in the intermediate risk group, with ~2/3 reclassified into a lower and ~1/3 reclassified into a higher risk group.

The potential value of carotid ultrasound in assessing individuals at intermediate risk was noted in the most recent guidelines of the ACC/AHA (Class IIa recommendation) (Greenland et al. 2010). Because carotid ultrasound is non-invasive and does not involve ionizing radiation, there could be potential value of this test for tracking disease progression as well. However, an important limitation to CIMT is the potential for inter-operator or inter-reader variability. Although CIMT has frequently been used in clinical and epidemiologic studies, there is currently no clinical standard to train or certify ultrasonographers and readers (Stein et al. 2008). One potential way to address high inter-operator variability of CIMT is to focus on a simpler measure, carotid plaque (e.g. greater than or less than 25% plaque in the most involved carotid artery), which can be assessed relatively easily by ultrasound. Several studies have addressed risk prediction using carotid plaque measures, though there are less data concerning discrimination and reclassification (Cao et al. 2007, Nambi et al. 2010).

The relationship between CAC and coronary artery disease has been well-described in histopathologic studies (Rumberger et al. 1995). Using electron beam or multidetector row CT scans, the CAC score is derived based on the number, area, and radiodensity of calcific lesions (Agatston et al. 1990). Large-scale observational studies have demonstrated incremental cardiovascular risk associated with higher coronary calcium score, with a relative risk of 2.1 for individuals with calcium scores between 1 and 100, and a relative risk between 3 and 17 for those with scores > 100 (Pletcher et al. 2004). Furthermore, there is good evidence that CAC improves both discrimination and reclassification. In the MESA cohort, the addition of CAC to traditional risk factors improved the c-statistic from 0.77 to 0.81 (Folsom et al. 2008, Polonsky et al. 2010). The NRI was 25%, with reclassification largely driven by those in the intermediate-risk category. 292 participants (16%) from the intermediate risk group (n=1,847) were reclassified to the high risk category, whereas 712 participants (39%) were reclassified as low risk (Polonsky et al. 2010).

In the most recent ACC/AHA guidelines, measurement of CAC for individuals deemed at intermediate risk and low to intermediate risk received a Class IIa and IIb recommendation respectively (Greenland et al. 2010). There is also evidence that adding CAC may help in the management of cardiovascular risk factors, perhaps through encouraging better patient adherence. In the EISNER study, participants randomized to receiving CAC scan had modestly improved blood pressure, LDL, and Framingham risk score (FRS) at 4 years compared to a control group receiving standard care only (Rozanski et al. 2011).

Despite the encouraging data, there are still questions that remain to be addressed. Statins have not been shown to decrease CAC, although they may inhibit further progression (Achenbach et al. 2002). Thus, the role for serial CAC imaging is unclear, particularly in patients in whom a baseline CAC scan was the motivation for behavioral change. The issue of cost-effectiveness has only been recently investigated, with a model based on MESA data suggesting that CAC may be cost-effective in intermediate risk men, but not women (van Kempen et al. 2011). Lastly, the risk from increased exposure to ionizing radiation has not been fully quantified. Standardization of protocols is an important first step as the radiation dose can vary by 10 fold depending on the different protocols (Kim et al. 2009). In the future, randomized trials are needed to establish whether CAC imaging in primary prevention improves outcomes compared with standard risk assessment (Lauer 2010).

GENETIC BIOMARKERS

Heredity is known to play an important role in the development of cardiovascular disease. In the Framingham Offspring Study, premature parental cardiovascular disease was associated with a doubling of risk of developing coronary disease in the offspring (Lloyd-Jones et al.

2004). Twin studies have shown that in men, when one twin dies of coronary heart disease, the relative risk for the remaining one is as high as four-fold for dizygotes and eight-fold for monozygotes (Marenberg et al. 1994). Unlike traditional biomarkers, genetic information exists since birth, potentially allowing for risk prediction and therefore primary prevention to occur at a much earlier stage. In the ACC/AHA guidelines, obtaining family history is a Class I recommendation in the assessment of cardiovascular risk in asymptomatic individuals (Greenland et al. 2010).

The sequencing of the human genome in the last decade has enabled genome-wide association (GWA) studies for identifying common single nucleotide polymorphisms that are associated with cardiovascular disease (Table 1). The first validated locus, in the 9p21 region, was separately reported by 3 different groups (Helgadottir et al. 2007, McPherson et al. 2007, Samani et al. 2007). Individuals carrying two copies of the minor allele, comprising 20–25% of the analyzed population, had 30–60% increased risk. Since then, more than 20 genetic variants have been identified, many of which are not known to be related to traditional risk factors (Musunuru and Kathiresan 2010, Peden et al. 2011, Schunkert et al. 2011). Compared to 9p21, these loci have more modest associations with cardiovascular events, with per allele increased risk between 5% and 20%. The mechanism through which some of these variants confer an increased cardiovascular risk is still unclear. In the example of 9p21, the locus does not contain any known genes. Whether the region plays a regulatory function that affects the transcription of another part of the genome has been speculated (Musunuru and Kathiresan 2010). A recent study suggests that enhancer elements in the 9p21 region may bind key regulatory sequences that mediate inflammatory response (Harismendy et al. 2011).

Data concerning the discrimination and reclassification value of these variants are limited. An earlier study showed that variants in the 9p21 region helped more accurately reclassify 13.5% of the studied male population (Talmud et al. 2008). A subsequent study from the Women's Genome Health Study, did not replicate this finding, as neither the c-statistic nor the NRI was significantly changed when compared to the Reynolds risk score, a model which incorporates family history (Paynter et al. 2009). Polymorphisms associated with cholesterol have also been examined for their ability to improve risk stratification. A nine loci genotype score was shown to modestly improve reclassification (NRI=6%), although it had no impact upon the c-statistic (Kathiresan et al. 2008). Recently, a genetic risk score derived from 13 loci associated with coronary heart disease, including 9p21, was not shown to improve c-statistic or reclassification over traditional risk factors and family history (Ripatti et al. 2010). Notably however, in that study, the association of the genetic risk and coronary heart disease was not attenuated by adjustment for family history; thus, if a useful panel of genetic variants can be identified, it would be expected to add on top of reported family history (Wray et al. 2010).

NEWER TECHNOLOGIES: METABOLOMICS

Measurements of metabolic changes have been made possible through newer technologies such as nuclear magnetic resonance (NMR) or mass spectroscopy (MS). Metabolites, small biochemicals and end products of complex regulatory pathways (transcriptional, translational, and post-translational), stand at the crossroads between genetics and environment (Figure 2). In the search for novel cardiac biomarkers, metabolomics presents unique opportunities and challenges. Because metabolites can reflect the end responses of physiologic processes, they may be more directly linked with phenotypes that have environmental determinants, such as cardiovascular disease, when compared with genetic variants or other “upstream” factors (Lewis et al. 2008). At the same time, however, metabolite concentrations may be affected by other biological (diet, medication, stress) and

experimental (sample storage, extraction) factors unrelated to the condition of interest (Mayr 2008). For example, an early study using NMR technology reported >90% sensitivity and specificity for identifying advanced three-vessel disease (Brindle et al. 2002). However, the case and control samples differed substantially with respect to age, gender, and other risk factors. A subsequent study enrolling only men and stratifying by statin treatment was unable to replicate the results (Kirschenlohr et al. 2006).

Recently, a matched case-control study, enrolling participants from a cardiac catheterization laboratory, identified components from branched-chain amino acid and urea cycle metabolites as independent cardiovascular risk factors. The addition of these two groups of measures to a clinical model modestly improved the discriminative ability (c-statistic 0.76 vs. 0.78) (Shah et al. 2010). Another research group, focusing on lipids, has identified metabolites associated with cardiovascular disease. Three of them, phosphatidylcholine-choline, trimethylamine *N*-oxide, and betaine, were subsequently shown to promote atherosclerosis when supplemented in a mouse model (Wang et al. 2011).

Certain metabolite profiles have also been associated with the development of cardiovascular risk factors. In the Framingham Offspring Study, five branched chain or aromatic amino acids (isoleucine, leucine, valine, tyrosine, and phenylalanine) were predictive of future diabetes (Wang et al. 2011). For each amino acid, a standard deviation increment in concentration was associated with a 57% to 102% increased odds of developing diabetes. Individuals in the top quartile of an amino acid score had a 6-fold increased risk for diabetes compared with those in the lowest quartile.

The studies above utilized targeted metabolomics profiling, which focused on tens or hundreds of metabolite biomarkers. Other studies using more “unbiased” approaches are likely to add further information by discovering different biomarkers in a “hypothesis-free” manner (Herder et al. 2011).

CONCLUSION

Cardiovascular primary prevention, identifying and treating at-risk individuals, remains a major public health priority. Although many biomarkers have been strongly associated with increased cardiovascular risk, few have been rigorously shown to improve upon current risk stratification algorithms by more than a modest margin. Advances in genomics, transcriptomics, proteomics, and metabolomics, however, have opened vast new areas of investigation and led to the discovery of numerous potential biomarkers. New concepts in biostatistics, such as reclassification, are providing tools to evaluate these novel markers. Ultimately, further studies will also be needed to translate advances in disease prediction into improved clinical outcomes. The gold-standard by which any screening test is proven to be beneficial remains the randomized controlled trial, which has thus far only been performed in limited settings for cardiovascular screening (Lauer 2010).

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Figure 1.

Model 1 (a–d) represents a traditional risk stratification model whereas Model 2 (e–h) represents one incorporating a useful biomarker (reclassification). Correctly reclassified cases are represented in green whereas incorrectly reclassified cases are in red. For individuals who did not experience an event (blue), 120 were correctly reclassified (high risk to low risk) and 20 were incorrectly reclassified (low risk to high risk), yielding $(120 - 20)/900 = 11\%$. For individuals who did experience an event (red), 60 were correctly reclassified (low risk to high risk) and 60 were incorrectly reclassified (high risk to low risk), yielding $(60 - 60)/500 = 0\%$. The NRI is the sum of these two percentages, which in this case is equal to 11%. Reproduced with permission from (Drawz and Sedor 2011).

Figure 2. Metabolites, end products of complex regulatory pathways. Reproduced with permission from (Lewis et al. 2008)

Table 1

Association of common variants polymorphism with coronary artery disease

Chr	SNP	Risk allele frequency (%)	Odds ratio (95% CI) per risk allele	Gene(s) of interest within or near associated interval
6q26–6q27	rs3798220	2	1.47 (1.35–1.60)	<i>LPA</i>
	rs10455872	7	1.68 (1.43–1.98)	<i>LPA</i>
9p21	rs4977574	56	1.29 (1.25–1.34)	<i>CDKN2A-CDKN2B-ANRIL</i>
21q22	rs9982601	13	1.20 (1.14–1.27)	<i>SLC5A3-MRPS6-KCNE2</i>
1p13	rs646776	81	1.19 (1.13–1.26)	<i>CELSR2-PSRC1-SORT1</i>
1p32	rs17114036	91	1.17 (1.13–1.22)	<i>PPAP2B</i>
10q11	rs1746048	84	1.17 (1.11–1.24)	<i>CXCL12</i>
2q33	rs6725887	14	1.17 (1.11–1.23)	<i>WDR12</i>
3q22	rs9818870	15	1.15 (1.11–1.19)	<i>MRAS</i>
1p32	rs11206510	81	1.15 (1.10–1.21)	<i>PCSK9</i>
19p13	rs1122608	75	1.15 (1.10–1.20)	<i>LDLR</i>
1q41	rs17465637	72	1.14 (1.10–1.19)	<i>MIA3</i>
11q23	rs964184	13	1.13 (1.10–1.16)	<i>ZNF259, APOA5-A4-C3-A1</i>
12q24	rs3184504	40	1.13 (1.08–1.18)	<i>SH2B3</i>
6p24	rs12526453	65	1.12 (1.08–1.17)	<i>PHACTR1</i>
10q24	rs12413409	89	1.12 (1.08–1.16)	<i>CYP17A1, CNNM2, NT5C2</i>
9q34	rs579459	21	1.10 (1.07–1.13)	<i>ABO</i>
7q32	rs11556924	62	1.09 (1.07–1.12)	<i>ZC3HC1</i>
6q23	rs12190287	62	1.08 (1.06–1.10)	<i>TCF21</i>
15q25	rs3825807	57	1.08 (1.06–1.10)	<i>ADAMTS7</i>
12q24	rs2259816	37	1.08 (1.05–1.11)	<i>HNF1A</i>
6p21	rs17609940	75	1.07 (1.05–1.10)	<i>ANKK1A</i>
14q32	rs2895811	43	1.07 (1.05–1.10)	<i>HHIPL1</i>
13q34	rs4773144	44	1.07 (1.05–1.09)	<i>COL4A1, COL4A2</i>
17p13	rs216172	37	1.07 (1.05–1.09)	<i>SMG6, SRR</i>
17p11	rs12936587	56	1.07 (1.05–1.09)	<i>RASD1, SMCR3, PEMT</i>
17q21	rs46522	53	1.06 (1.04–1.08)	<i>UBE2Z, GIP, ATP5G1, SNF8</i>

Table adapted with permission from Musunuru and Kathiresan (Musunuru and Kathiresan 2010), with additional polymorphisms from (Schunkert et al. 2011).