

# A Breakthrough in Genetics and its Relevance to Prevention of Coronary Artery Disease in LMIC



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## ABSTRACT

More than 60 genetic risk variants predisposing to coronary artery disease (CAD) have been confirmed. The genetic risk for CAD is related to the number of genetic risk variants present and can be expressed as a genetic risk score (GRS), by summing the product of the number of high-risk variants inherited by each individual times the log of the odds ratio. Studies show risk stratification for CAD, based on the GRS, is more discriminatory than conventional risk factors and predicts the response to statin therapy. A prospective trial showed individuals with high GRS had 91% greater risk of cardiac events, and individuals with a healthy lifestyle had 46% fewer cardiac events than an unfavorable lifestyle. GRS remains the same throughout one's lifetime because your deoxyribonucleic acid does not change. GRS, determined as early as birth from saliva, is inexpensive and could transform the prevention of CAD in low- and middle-income countries.

The world is experiencing an era of globalization that has spread across all continents with an intensity never before experienced by mankind. Whereas globalization is affecting commerce, politics, and geopolitical issues, it is also influencing science and human health. The Western world in the 20th century was experiencing an epidemic of heart disease, whereas in the East, heart disease was less predominant. Today, heart disease is pandemic, being the number one killer in the world [1] with a significant decreasing trend in the West and an increasing trend in the East. This is based on the comprehensive assessment by the GBD (Global Burden of Disease) study launched by the World Bank and the World Health Organization [1]. GBD has assessed data from 187 countries involving 291 diseases associated with 1,160 sequelae. GBD claims these diseases are associated with 67 risk factors.

It has been recognized by epidemiologists for decades that genetic risk accounts for probably 40% to 60% of susceptibility for coronary artery disease (CAD). Nevertheless, modification of risk factors for common diseases has not included genetic risk, because the technology to discover these genetic variants was not available until about 2005 [2]. The appropriate current goal in preventing CAD is to treat the well-recognized traditional risk factors of cholesterol, hypertension, obesity, and smoking. Future prevention must be comprehensive and to do so must include modification of acquired and genetic risk factors and preferably be initiated prior to the development of the disease. CAD, particularly in low- and middle-income countries, is usually not detected until the disease is severe enough to manifest symptoms such as a myocardial infarction (MI). Early detection and prevention may require sophisticated techniques such as myocardial imaging or

cardiac catheterization, which can be expensive and in developing countries are not always freely available. The recent successful discovery of multiple genetic risk variants predisposing to CAD may be transforming, particularly in low- and middle-income countries, because it requires only a sample of saliva. Furthermore, genetic risk stratification for CAD can be determined as early as birth. This could transform primary and secondary prevention of CAD worldwide for individuals of all levels of income. To significantly attenuate the pandemic of heart disease may require physicians throughout the world to acquire a working knowledge of medical genetics.

## GENETIC PREDISPOSITION AND CAD

Extensive studies have documented that heritance plays a role in CAD. These results have been based on epidemiological and familial studies suggesting that 40% to 60% of susceptibility to CAD is due to genetic predisposition [3]. The Framingham study shows that a family history of CAD is associated with a 2.4-fold increased risk of CAD in men and a 2.2-fold increase in women [4]. The Danish Twin Registry [5] shows a higher frequency of CAD in monozygotic twins than in dizygotic twins, averaging 44% versus 14%. After correcting for other risk factors [6], the INTERHEART (Effect of Potentially Modifiable Risk Factors Associated With Myocardial Infarction in 52 Countries) study showed a family history of CAD increases the risk of CAD 1.5-fold. In the PROCAM (Prospective Cardiovascular Münster) study, a family history of MI documented an independent risk factor of CAD [7]. It is perhaps worthy of note that epidemiological studies suggest genetic predisposition exists for essentially all diseases.

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### THE GOLDEN AGE OF THE SINGLE-GENE ERA

The past 2 decades have been exciting for those of us interested in single-gene disorders. These disorders are rare and occur in much less than 1% of the population, most occurring with a frequency of less than one-tenth of 1%. For example, the most common single-gene disorder in cardiovascular disease is that of familial hypertrophic cardiomyopathy, which has an incidence of 1 in 500 [8]. In these disorders, the gene is associated with potent effects on the phenotype and the chromosomal location of the gene can be mapped once pedigrees of 2 or 3 generations affected with the disease have been genotyped. These disorders follow a Mendelian pattern of autosomal dominant or recessive inheritance. Using a few hundred deoxyribonucleic acid (DNA) markers to genotype the pedigree, one can analyze for genetic linkage to detect those DNA markers that occur more frequently in affected individuals. The linkage of those markers to the affected phenotype indicate that the DNA markers are in close proximity to the mutant gene responsible for the disorder. The DNA region is then sequenced and the mutant gene identified. It is estimated there are more than 7,000 single-gene disorders in humans, and the genes for more than one-half of them have already been identified [9].

### THE ERA OF GENOME-WIDE ASSOCIATION STUDIES FOR POLYGENIC DISORDERS

Genetic linkage analysis of families does not provide the resolution to discover genes associated with polygenic disorders, such as CAD. It has been expected for some time that these common disorders would be associated with polymorphisms in common genes and would be due to multiple genes having minimal effect rather than the single-gene potency observed in rare single-gene disorders. This would require a new approach referred to as case-control association studies, which would require thousands of cases and control subjects genotyped by hundreds of thousands of closely spaced DNA markers, with replication in a similar appropriate independent sample size [10]. The DNA markers became available in 2005 in the form of microarrays containing single-nucleotide polymorphisms (SNP). SNP are known to be distributed throughout the genome and account for more than 80% of human DNA variation, including susceptibility to disease [11]. The early microarrays contained 500,000 SNP with more recent versions of 1 million SNP [2]. In addition, HapMap [12] has annotated the location of more than 20 million SNP, which can be imputed and annotated to those SNP already genotyped by microarray analysis.

The microarray enabled one to genotype for SNP distributed throughout the genome and is referred to as a genome-wide association study (GWAS). This approach is unbiased and enables one to scan the whole genome without a preconception of which marker associates with the disease. The basis for the case-control association study is to determine the frequency of each SNP in cases and control subjects, and those SNP occurring

significantly more frequently in the cases compared with control subjects would indicate a DNA region associated with increased risk for the disease. The sample size must be extensive to account for false positives. If one is genotyping with 1 million SNP, by chance alone, there would be 50,000 associations, therefore a statistical correction is necessary to adjust for the multiple comparisons. It has become conventional to perform a Bonferroni correction, which requires the conventional p value of 0.05 to be divided by the number of comparisons, which in a GWAS is using 1,000,000 SNP (0.05/1,000,000) to achieve statistical significance. The resulting p value of 0.00000005 ( $5 \times 10^{-8}$ ) is conventionally referred to as “genome-wide significant” [13]. Those SNP in the discovery population identified to be significantly more frequent ( $p < 5 \times 10^{-8}$ ) in cases than in control subjects must be verified through replication in an appropriate and independent population of cases and control subjects. Previous case-control studies in the 1990s using the preconceived candidate-gene approach without replication were shown to be primarily flawed [14]. Currently, the preferred method is GWAS with appropriate independent replication. The results of the GWAS have been outstanding, identifying more than 2,800 genetic risk variants affecting more than 300 diseases [15].

### IDENTIFICATION OF THE FIRST GENETIC RISK VARIANT (9P21) FOR CAD

In the pursuit of genes predisposing to CAD, the cases have included documented MI or coronary obstruction of >50% in  $\geq 1$  of the coronary arteries documented by coronary angiography. The 2 phenotypes are used interchangeably because MI almost always occurs because of superimposition of a thrombus on a ruptured atherosclerotic plaque. The control subjects are healthy asymptomatic individuals without documented CAD [2]. In 2007, independently and simultaneously, we [16] and deCODE genetics [17] identified the first genetic variant (9p21) associated with increased risk for CAD. The features characterizing 9p21 were virtually identical and confirmatory in the 2 studies. The 9p21 risk variant was shown to be extremely common with 1 or 2 copies occurring in 75% of the Caucasian population. Individuals heterozygous for the 9p21 risk variant were associated with 25% increased risk for CAD and homozygous individuals with 50% increased risk. In individuals with premature CAD, the 9p21 risk variant is associated with a 2-fold increased risk [18]. The major surprising and significant finding was the risk mediated by the 9p21 variant was independent of known risk factors such as cholesterol, hypertension, smoking, or diabetes. Within the next 2 years, 9p21 was confirmed to be a risk variant not only in Caucasians [16,17,19,20], but also in the Chinese [21], the Koreans [22], East Asians [23,24], and Japanese [25], but not in Africans or African Americans [26]. In these ethnic groups, the

TABLE 1. Genetic risk variants of genome-wide significance for CAD

| Lead SNP   | Chromosome | Nearest Genes             | Frequency of Allele Raising |                  |                         | Potential Mechanism of Action                     | Year Locus First Reported to Reach |                                      |
|------------|------------|---------------------------|-----------------------------|------------------|-------------------------|---|------------------------------------|--------------------------------------|
|            |            |                           | Risk                        | OR (95% CI)      | p Value                 |   | Genome-Wide Significance           | Consortium and/or Author [Reference] |
| rs11206510 | 1          | <i>PCSK9</i>              | 0.85                        | 1.08 (1.05–1.11) | $2.340 \times 10^{-8}$  | LDL levels  | 2009                               | MIGen [63]                           |
| rs7528419  | 1          | <i>SORT1</i>              | 0.79                        | 1.12 (1.10–1.15) | $1.970 \times 10^{-23}$ | LDL levels  | 2007                               | WTCCC and<br>Cardiogenics [19]       |
| rs515135   | 2          | <i>APOB</i>               | 0.79                        | 1.07 (1.04–1.10) | $3.090 \times 10^{-8}$  | LDL levels  | 2013                               | CARDIoGRAM +<br>C4D [35]             |
| rs6544713  | 2          | <i>ABCG5–ABCG8</i>        | 0.32                        | 1.05 (1.03–1.07) | $8.880 \times 10^{-7}$  | LDL levels  | 2013                               | CARDIoGRAM +<br>C4D [35]             |
| rs56289821 | 19         | <i>LDLR</i>               | 0.9                         | 1.14 (1.11–1.18) | $4.440 \times 10^{-15}$ | LDL levels  | 2009                               | MIGen [63]                           |
| rs4420638  | 19         | <i>APOE–APOC1</i>         | 0.17                        | 1.10 (1.07–1.13) | $7.070 \times 10^{-11}$ | LDL levels  | 2013                               | CARDIoGRAM +<br>C4D [35]             |
| rs3184504  | 12         | <i>SH2B3</i>              | 0.42                        | 1.07 (1.04–1.09) | $1.030 \times 10^{-9}$  | LDL levels, BP                                    | 2009                               | deCODE [64]                          |
| rs55730499 | 6          | <i>SLC22A3–LPAL2–LPA</i>  | 0.06                        | 1.37 (1.31–1.44) | $5.390 \times 10^{-39}$ | Lp(a) levels                                      | 2009                               | WTCCC and<br>Cardiogenics<br>[65]    |
| rs264      | 8          | <i>LPL</i>                | 0.85                        | 1.06 (1.03–1.09) | $1.060 \times 10^{-5}$  | TRIG levels                                       | 2013                               | CARDIoGRAM +<br>C4D [35]             |
| rs2954029  | 8          | <i>TRIB1</i>              | 0.55                        | 1.04 (1.03–1.06) | $2.610 \times 10^{-6}$  | TRIG levels                                       | 2013                               | CARDIoGRAM +<br>C4D [35]             |
| rs964184   | 11         | <i>ZNF259–APOA5–APOA1</i> | 0.18                        | 1.05 (1.03–1.08) | $5.600 \times 10^{-5}$  | TRIG levels                                       | 2011                               | CARDIoGRAM [29]                      |
| rs17609940 | 6          | <i>ANKS1A</i>             | 0.82                        | 1.03 (1.00–1.05) | $3.000 \times 10^{-2}$  | HDL levels, height                                | 2011                               | CARDIoGRAM [29]                      |
| rs3918226  | 7          | <i>NOS3</i>               | 0.06                        | 1.14 (1.09–1.19) | $1.690 \times 10^{-9}$  | BP  | 2015                               | 1GP CARDIoGRAM +<br>C4D [36]         |
| rs2681472  | 12         | <i>ATP2B1</i>             | 0.2                         | 1.08 (1.05–1.10) | $6.170 \times 10^{-11}$ | BP  | 2012                               | Lu et al. [66]                       |
| rs17514846 | 15         | <i>FURIN–FES</i>          | 0.44                        | 1.05 (1.03–1.07) | $3.100 \times 10^{-7}$  | BP  | 2013                               | CARDIoGRAM +<br>C4D [35]             |
| rs72689147 | 4          | <i>GUCY1A3</i>            | 0.82                        | 1.07 (1.05–1.10) | $6.070 \times 10^{-9}$  | BP, cell growth/<br>differentiation/<br>apoptosis | 2013                               | CARDIoGRAM +<br>C4D [35]             |
| rs11830157 | 12         | <i>KSR2</i>               | 0.36                        | 1.12 (1.08–1.16) | $2.120 \times 10^{-9}$  | BMI   | 2015                               | 1GP CARDIoGRAM +<br>C4D [36]         |
| rs663129   | 18         | <i>PMAIP1–MC4R</i>        | 0.26                        | 1.06 (1.04–1.08) | $3.200 \times 10^{-8}$  | BMI   | 2015                               | 1GP CARDIoGRAM +<br>C4D [36]         |
| rs4252185  | 6          | <i>PLG</i>                | 0.06                        | 1.34 (1.28–1.41) | $1.640 \times 10^{-32}$ | Coagulation                                       | 2013                               | CARDIoGRAM +<br>C4D [35]             |
| rs2519093  | 9          | <i>ABO</i>                | 0.19                        | 1.08 (1.06–1.11) | $1.190 \times 10^{-11}$ | Coagulation, LDL levels                           | 2011                               | CARDIoGRAM [29]                      |
| rs9349379  | 6          | <i>PHACTR1</i>            | 0.43                        | 1.14 (1.12–1.16) | $1.810 \times 10^{-42}$ | Arterial vessel wall<br>endothelial cell          | 2009                               | MIGen [63]                           |
| rs9319428  | 13         | <i>FLT1</i>               | 0.31                        | 1.04 (1.02–1.06) | $7.130 \times 10^{-5}$  | Arterial vessel wall<br>endothelial cell          | 2013                               | CARDIoGRAM +<br>C4D [35]             |

(continued)

TABLE 1. Continued

| Lead SNP   | Chromosome | Nearest Genes              | Frequency of Allele Raising |                  |                         | Potential Mechanism of Action   | Year Locus First Reported to Reach |  |
|------------|------------|----------------------------|-----------------------------|------------------|-------------------------|---|------------------------------------|--|
|            |            |                            | Risk                        | OR (95% CI)      | p Value                 |   | Genome-Wide Significance           | Consortium and/or Author [Reference]       |
| rs8042271  | 15         | <i>MFGE8-ABHD2</i>         | 0.9                         | 1.10 (1.06–1.14) | $3.680 \times 10^{-8}$  | Arterial vessel wall endothelial cell   | 2015                               | 1GP CARDIoGRAM + C4D [36]                  |
| rs7212798  | 17         | <i>BCAS3</i>               | 0.15                        | 1.08 (1.05–1.11) | $1.880 \times 10^{-8}$  | Arterial vessel wall endothelial cell   | 2015                               | 1GP CARDIoGRAM + C4D [36]                  |
| rs4593108  | 4          | <i>EDNRA</i>               | 0.8                         | 1.07 (1.05–1.10) | $8.820 \times 10^{-10}$ | Arterial vessel wall smooth muscle cell   | 2013                               | CARDIoGRAM + C4D [35]                      |
| rs17087335 | 4          | <i>REST-NOA1</i>           | 0.21                        | 1.06 (1.04–1.09) | $4.590 \times 10^{-8}$  | Arterial vessel wall smooth muscle cell   | 2015                               | 1GP CARDIoGRAM + C4D [36]                  |
| rs12202017 | 6          | <i>TCF21</i>               | 0.7                         | 1.07 (1.05–1.09) | $1.980 \times 10^{-11}$ | Arterial vessel wall smooth muscle cell   | 2011                               | CARDIoGRAM [29]                            |
| rs2107595  | 7          | <i>HDAC9</i>               | 0.2                         | 1.08 (1.05–1.10) | $8.050 \times 10^{-11}$ | Arterial vessel wall smooth muscle cell   | 2013                               | CARDIoGRAM + C4D [35]                      |
| rs2891168  | 9          | <i>CDKN2BAS</i><br>(9p21)  | 0.49                        | 1.21 (1.19–1.24) | $2.290 \times 10^{-98}$ | Arterial vessel wall smooth muscle cell   | 2007                               | McPherson et al., deCODE, WTCCC [16,17,20] |
| rs11191416 | 10         | <i>CYP17A1-CNNM2-NT5C2</i> | 0.87                        | 1.08 (1.05–1.11) | $4.650 \times 10^{-9}$  | Arterial vessel wall smooth muscle cell   | 2011                               | CARDIoGRAM [29]                            |
| rs4468572  | 15         | <i>ADAMTS7</i>             | 0.59                        | 1.08 (1.06–1.10) | $4.440 \times 10^{-16}$ | Arterial vessel wall smooth muscle cell   | 2011                               | C4D, Reilly, CARDIoGRAM [29-31]            |
| rs10840293 | 11         | <i>SWAP70</i>              | 0.55                        | 1.06 (1.04–1.08) | $1.280 \times 10^{-8}$  | Arterial vessel wall smooth muscle cell, inflammation/immune system/cell migration-adhesion | 2015                               | 1GP CARDIoGRAM + C4D [36]                  |
| rs17678683 | 2          | <i>ZEB2-ACO74093.1</i>     | 0.09                        | 1.10 (1.07–1.14) | $3.000 \times 10^{-9}$  | Cell growth/differentiation/apoptosis   | 2013                               | CARDIoGRAM + C4D [35]                      |
| rs2128739  | 11         | <i>PDGFD</i>               | 0.32                        | 1.07 (1.05–1.09) | $7.050 \times 10^{-11}$ | Cell growth/differentiation/apoptosis   | 2011                               | C4D [17]                                   |
| rs56062135 | 15         | <i>SMAD3</i>               | 0.79                        | 1.07 (1.05–1.10) | $4.520 \times 10^{-9}$  | Cell growth/differentiation/apoptosis   | 2015                               | 1GP CARDIoGRAM + C4D [36]                  |
| rs46522    | 17         | <i>UBE2Z</i>               | 0.51                        | 1.04 (1.02–1.06) | $1.840 \times 10^{-5}$  | Cell growth/differentiation/apoptosis   | 2011                               | CARDIoGRAM [29]                            |
| rs9970807  | 1          | <i>PPAP2B</i>              | 0.92                        | 1.13 (1.10–1.17) | $5.000 \times 10^{-14}$ | Inflammation/immune system/cell migration-adhesion  | 2011                               | CARDIoGRAM [29]                            |
| rs2487928  | 10         | <i>KIAA1462</i>            | 0.42                        | 1.06 (1.04–1.08) | $4.410 \times 10^{-11}$ | Inflammation/immune system/cell migration-adhesion  | 2011                               | C4D [17]                                   |

|            |    |                            |      |                  |                         |   |      |                             |
|------------|----|----------------------------|------|------------------|-------------------------|---|------|-----------------------------|
| rs1870634  | 10 | <i>CXCL12</i>              | 0.64 | 1.08 (1.06–1.10) | $5.550 \times 10^{-15}$ | Inflammation/immune system/cell migration-adhesion  | 2007 | WTCCC and Cardiogenics [19] |
| rs1412444  | 10 | <i>LIPA</i>                | 0.37 | 1.07 (1.05–1.09) | $5.150 \times 10^{-12}$ | Inflammation/immune system/cell migration-adhesion  | 2011 | C4D [17]                    |
| rs6689306  | 1  | <i>IL6R</i>                | 0.45 | 1.06 (1.04–1.08) | $2.600 \times 10^{-9}$  | Inflammation/immune system/cell migration-adhesion, cell growth/differentiation/apoptosis | 2013 | CARDIoGRAM + C4D [35]       |
| rs67180937 | 1  | <i>MIA3</i>                | 0.66 | 1.08 (1.06–1.11) | $1.010 \times 10^{-12}$ | Extracellular matrix  | 2007 | WTCCC and Cardiogenics [19] |
| rs11838776 | 13 | <i>COL4A1/A2</i>           | 0.26 | 1.07 (1.05–1.09) | $1.830 \times 10^{-10}$ | Extracellular matrix  | 2011 | CARDIoGRAM [29]             |
| rs16986953 | 2  | <i>AKO97927</i>            | 0.1  | 1.09 (1.06–1.12) | $1.450 \times 10^{-8}$  | Other/unknown   | 2013 | CARDIoGRAM + C4D [35]       |
| rs7568458  | 2  | <i>VAMP5–VAMP8–GGCX</i>    | 0.45 | 1.06 (1.04–1.08) | $3.620 \times 10^{-10}$ | Other/unknown   | 2013 | CARDIoGRAM + C4D [35]       |
| rs6725887  | 2  | <i>WDR12</i>               | 0.11 | 1.14 (1.11–1.18) | $9.510 \times 10^{-18}$ | Other/unknown   | 2009 | MIGen [63]                  |
| rs9818870  | 3  | <i>MRAS</i>                | 0.14 | 1.07 (1.04–1.10) | $2.210 \times 10^{-6}$  | Other/unknown   | 2009 | Cardiogenics [67]           |
| rs273909   | 5  | <i>SLC22A4–SLC22A5</i>     | 0.12 | 1.06 (1.03–1.09) | $1.240 \times 10^{-4}$  | Other/unknown   | 2013 | CARDIoGRAM + C4D [35]       |
| rs6903956  | 6  | <i>ADTRP–C6orf105</i>      | 0.35 | 1.00 (0.98–1.02) | $9.600 \times 10^{-1}$  | Other/unknown   | 2011 | Wang et al. [33]            |
| rs56336142 | 6  | <i>KCNK5</i>               | 0.81 | 1.07 (1.04–1.09) | $1.850 \times 10^{-8}$  | Other/unknown   | 2013 | CARDIoGRAM + C4D [35]       |
| rs10953541 | 7  | 7q22                       | 0.78 | 1.05 (1.03–1.08) | $1.020 \times 10^{-5}$  | Other/unknown   | 2011 | C4D [17]                    |
| rs11556924 | 7  | <i>ZC3HC1</i>              | 0.69 | 1.08 (1.05–1.10) | $5.340 \times 10^{-11}$ | Other/unknown   | 2011 | CARDIoGRAM [29]             |
| rs10139550 | 14 | <i>HHIPL1</i>              | 0.42 | 1.06 (1.04–1.08) | $1.380 \times 10^{-8}$  | Other/unknown   | 2011 | CARDIoGRAM [29]             |
| rs216172   | 17 | <i>SMG6</i>                | 0.35 | 1.05 (1.03–1.07) | $5.070 \times 10^{-7}$  | Other/unknown   | 2011 | CARDIoGRAM [29]             |
| rs12936587 | 17 | <i>RAI1–PEMT–RASD1</i>     | 0.61 | 1.03 (1.01–1.05) | $8.240 \times 10^{-4}$  | Other/unknown   | 2011 | CARDIoGRAM [29]             |
| rs12976411 | 19 | <i>ZNF507–LOC400684</i>    | 0.91 | 1.49 (1.38–1.67) | $1.180 \times 10^{-14}$ | Other/unknown   | 2015 | 1GP CARDIoGRAM + C4D [36]   |
| rs28451064 | 21 | <i>KCNE2</i> (gene desert) | 0.12 | 1.14 (1.10–1.17) | $1.330 \times 10^{-15}$ | Other/unknown   | 2009 | MIGen [63]                  |
| rs180803   | 22 | <i>POM121L9P–ADORA2A</i>   | 0.97 | 1.20 (1.13–1.27) | $1.640 \times 10^{-10}$ | Other/unknown   | 2015 | 1GP CARDIoGRAM + C4D [36]   |
| rs1801251  | 2  | <i>KCNJ13–GIGYF2</i>       | 0.35 | 1.06 (1.04–1.08) | $1.46 \times 10^{-8}$   | Other/unknown   | 2017 | CARDIoGRAM Exom [68]        |
| rs3130683  | 6  | <i>C2</i>                  | 0.86 | 1.09 (1.06–1.13) | $7.87 \times 10^{-8}$   | Other/unknown   | 2017 | CARDIoGRAM Exom [68]        |
| rs11042937 | 11 | <i>MRV11–CTR9</i>          | 0.49 | 1.05 (1.03–1.07) | $3.21 \times 10^{-8}$   | Other/unknown   | 2017 | CARDIoGRAM Exom [68]        |

(continued)

TABLE 1. Continued

| Lead SNP   | Chromosome | Nearest Genes | Frequency of Allele Raising Risk | OR (95% CI)      | p Value                | Potential Mechanism of Action | Year Locus First Reported to Reach Genome-Wide Significance | Consortium and/or Author [Reference] |
|------------|------------|---------------|----------------------------------|------------------|------------------------|-------------------------------|---|--------------------------------------|
| rs11172113 | 12         | LRP1          | 0.41                             | 1.06 (1.04-1.08) | $1.78 \times 10^{-8}$  | Other/unknown                 | 2017  | CARDIOGRAM Exom [68]                 |
| rs11057830 | 12         | SCARB1        | 0.15                             | 1.09 (1.06-1.11) | $3.69 \times 10^{-10}$ | Vitamin E                     | 2017  | CARDIOGRAM Exom [68]                 |
| rs1800775  | 16         | CETP          | 0.51                             | 1.06 (1.04-1.08) | $2.21 \times 10^{-8}$  | HDL cholesterol               | 2017  | CARDIOGRAM Exom [68]                 |

BMI, body mass index; BP, blood pressure; CAD, Coronary Artery Disease Genetics Consortium; CAD, coronary artery disease; CARDIOGRAM, Coronary Artery Disease Genome-Wide Replication and Meta-Analysis; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MIGen, Myocardial Infarction Genetics Consortium; OR, odds ratio; TRIG, triglyceride(s); WTCCC, Wellcome Trust Case Control Consortium.

frequency was similar to that of the Caucasians, and the 9p21 risk was found to be independent of conventional risk factors for CAD. This confirmed the hypothesis that common genetic risk variants predispose to common diseases. It is estimated that more than 4 billion people in the world carry the 9p21 risk variant for CAD.

### GWAS WAS SUCCESSFUL IN IDENTIFYING MULTIPLE GENES FOR CAD

The cost of genotyping with microarray technology decreased several fold and the studies tremendously increased in the pursuit of genes for CAD. In a very short interval, several groups mapped 11 other genetic risk variants for CAD [27]. Results clearly indicated these genetic risk variants occur commonly and impart minimal to moderate risk. It became apparent that the sample size to discover genetic risk variants with minimal effect, occurring in frequencies of 5% to 10% would have to be much larger than initially anticipated. This led to the formation of a consortium for meta-analysis involving several groups that had previously performed GWAS to identify genetic risk variants for CAD. This international consortium, CARDIOGRAM (Coronary Artery Disease Genome-Wide Replication and Meta-Analysis) [28], would be the largest collaboration in the field of cardiology and initially brought together investigators with a total sample size of 86,995 cases and control subjects and a replication sample size of 56,682, all of European ancestry. This initial effort led to the discovery of 13 new genetic risk variants for CAD and confirmation of the 10 that were previously identified [29]. This was followed by the discovery of 2 novel risk variants for CAD, ADAMTS7 and the ABO blood group locus [30]. The C4D (Coronary Artery Disease) Genetics Consortium mapped 4 novel genetic risk variants related to CAD [31]. Davies et al. [32] discovered a variant in the major histocompatibility locus to be associated with increased risk for CAD. Wang et al. [33] mapped a variant at 6p21 and the International BeadChip array 50K CAD Consortium [34] mapped another 4 novel risk variants for CAD. CARDIOGRAM enlarged its sample by adding the C4D Group's data, confirming previous genetic risk variants and identifying 15 additional genetic risk variants predisposing to CAD [35]. This was followed by other studies [36,37] that identified 14 new genetic risk factors for CAD. A total of 62 genetic risk variants predisposing to CAD of genome-wide significance have been replicated in appropriate independent populations, as shown in Table 1. These have been discussed extensively in a recent review [49].

### COMMON FEATURES OF GENETIC RISK VARIANTS FOR CAD

Genetic risk variants predisposing to CAD, like those for other polygenic disorders, have several features in common (Table 1):

1. The burden of risk relates to the number of genetic variants rather than any specific risk variant. In an

analysis of 23 genetic risk variants for CAD, the average number inherited per individual (case or control) was 17. Separation of cases from control subjects is most evident in those individuals with 20 or more genetic risk variants.

2. More than one-half of the genetic risk variants for CAD are very common, occurring in 50% of the population.
3. More than one-quarter of the genetic risk variants occur in more than 75% of the population.
4. The relative increased risk of each genetic variant for CAD averages only 18% with an odds ratio varying from 2% to 90%.
5. The majority of the genetic risk variants are located in DNA sequences that do not code for protein, implying the risk variant mediates the risk through regulation of downstream or upstream protein-coding sequences.
6. Genetic risk variants need only be assessed once as they do not change in an individual's lifetime, varying neither with time, nutrition, drugs, nor sex.

An analysis of [Table 1](#) indicates several genetic risk variants are associated with low-density lipoprotein cholesterol (LDL-C). This provides the opportunity to develop new therapies to lower plasma LDL-C levels that could be complementary to our current use of statins to inhibit the synthesis of cholesterol. In fact, the genetic variant in PCSK9 has already led to a new therapy that increases the removal of cholesterol and is shown to be very effective and safe in randomized placebo control trials. Another potential target for lowering cholesterol is the SORT1 genetic risk variant that involves a mechanism that enhances removal of LDL-C from the plasma. Similarly, the genetic variants associated with high-density lipoprotein cholesterol, triglycerides, and hypertension may materialize into new therapeutic approaches for the future.

Perhaps, the single most important observation to be learned from the genetic risk variants is the observation that only about one-third of the discovered genetic risk variants for CAD mediate their risk through known risk factors, such as cholesterol. This observation strongly suggests other mechanisms, as yet unknown, are involved in the pathogenesis of coronary atherosclerosis and MI. If one is to prevent CAD, the prevention must be comprehensive to reduce genetic as well as conventional risk.

### GENETIC RISK VARIANTS LEAD TO THE DEVELOPMENT OF NEW THERAPIES

Prevention and treatment of genetic risk may seem futuristic, but development of specific treatments will follow the same processes that we used previously to treat conventional risk factors, such as cholesterol. It was well known in the 1950s that cholesterol plays a role in the pathogenesis of CAD, and in the 1970s [50], a family with familial hypercholesterolemia was studied and shown to be due to a mutation in the LDL-C receptor. These individuals often develop MI within the second and third decade of life, which enhanced efforts to develop a drug to lower plasma levels of LDL-C. Dr. Endo

discovered the first cholesterol-lowering drug by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A. However, this drug, known as mevastatin, had significant side effects. This led to the discovery of lovastatin, which had the same mechanism of action and was approved by the U.S. Food and Drug Administration for marketing in 1987 [51]. Statins until recently were the only drug for primary and secondary prevention of hypercholesterolemia. It is now possible to use genetic risk variants and, through their dependent molecular pathways as targets, to develop drugs for prevention and treatment of CAD.

A new form of therapy has already evolved from the identification of mutations in the gene encoding for PCSK9. In 2003, Seidah et al. [52] discovered an enzyme, PCSK9, that increases the degradation of LDL receptors, delaying removal of plasma LDL-C and resulting in hypercholesterolemia with increased morbidity and mortality from CAD. Mutations were subsequently identified in the gene that encodes for PCSK9 and mutations inducing loss of function of PCSK9 were associated with a marked decrease in the risk for CAD and MI [53]. Monoclonal antibodies were developed to inhibit PCSK9 and phase I, II, and III clinical trials are ongoing. Preliminary results of these studies indicate inhibition of PCSK9 is safe and is associated with further decrease in plasma LDL-C over and above that achieved by statin therapy. African Americans that inherited hypocholesterolemia due to loss of function mutations in PCSK9 gene showed a mean reduction of 28% in plasma LDL-C levels and an 88% reduction in the risk of CAD. In a phase II trial of individuals with hypercholesterolemia receiving 80 mg of atorvastatin had a mean reduction of 17% in their plasma LDL-C levels versus 72% reduction in plasma LDL-C levels for those receiving 80 mg atorvastatin plus the PCSK9 antibody [54]. Based on genetic discovery, a new therapy has already been approved.

### GENETIC RISK VARIANTS FOR CAD PRIMARILY TARGET ATHEROSCLEROSIS

It is evident from [Table 1](#) that only 1 genetic risk variant has been discovered that relates directly to MI. In our present schema, a thrombus is precipitated by rupture of an atherosclerotic plaque leading to CAD and frequently MI. Only 1 genetic variant at the ABO locus was shown to be associated with MI, with all of the other variants apparently related to the pathogenesis of coronary atherosclerosis. Epidemiological studies have shown for decades a strong association between the ABO blood group at 9q34.2 and MI. This association was confirmed in CARDIoGRAM [30], showing the A and B risk variants increased the risk for MI by about 20%. The A and B genes encode for a protein (alpha-1-3N-acetylgalactosaminyltransferase) that transfers a carbohydrate on to von Willebrand factor (vWF)—this prolongs the half-life of vWF and predisposes the subject to coronary thrombosis and MI. The blood group O gene codes for the same transferase protein, but due to a

mutation, it lacks any biochemical activity and thus does not prolong the half-life of vWF or increase risk for CAD. In the recent Nurses' Health Study of more than 90,000 individuals, 4,070 developed heart disease. In this 20-year follow-up study, blood group A or B was associated with increased MI of about 10%; however, the combination of A and B blood groups increased the risk to 20% [55]. Plasma levels of vWF are about 25% higher in individuals with A, B, or AB blood groups as opposed to individuals with blood group O [56]. These results could have implications for antithrombotic therapy in the management of patients undergoing stent insertion, bypass surgery, or other artificial devices left in place on a chronic basis.

### 9P21 AND ITS MECHANISM OF ACTION

The 9p21 risk variant for CAD has been the most studied risk variant and is present in more than 4 billion individuals. Unfortunately, 9p21 risk variant is in a region where there is a long noncoding ribonucleic acid referred to as Anril. Because the 9p21 risk variant is not present in the mouse genome, it makes for great difficulty in determining its function. The 9p21 risk variant also contributes to increased risk for intracranial and abdominal aortic aneurysms [44]. The 9p21 risk variant, despite its implications for an inflammatory role, does not associate with C-reactive protein [45,46]. We have shown the 9p21 risk variant associates with coronary atherosclerosis and not with MI—a finding that has been consistently confirmed by others [30,57-59]. Several studies have also indicated that the 9p21 risk variant increases the progression of coronary atherosclerosis as indicated by the correlation between the number of vessels involved and the number of copies of the 9p21 risk variant inherited [57,59]. This was confirmed in a recent large meta-analysis study [60]. Other previous studies do not confirm a co-relation between the 9p21 risk variant and progression of coronary atherosclerosis [22,25,58]. Thus, the molecular mechanism mediating the risk for 9p21 remains unknown even though its site of action is clearly at the vessel wall and not related to plaque rupture or thrombosis. Unfortunately, the mechanisms remain unknown for most genetic risk variants. Results of studies using computerized molecular pathways suggest some of these genetic risk variants are involved in inflammatory pathways that contribute to the pathogenesis of atherosclerosis [61].

### GENETIC RISK SCORE IMPROVES RISK STRATIFICATION FOR CAD

A major hope that stimulated the pursuit of genetic risk factors predisposing to CAD was for improved risk stratification to enable more appropriate and early preventive therapy. Risk stratification of CAD based on genetic risk variants is largely independent of known risk factors and could be more discriminatory than conventional risk factors. The use of a single genetic risk variant such as 9p21 offers no advantage over conventional risk factors [43]. This is expected knowing there are multiple risk variants and the increase risk of any genetic variant is minimal.

This was exemplified by an analysis of 9p21 [62], showing no improvement over conventional risk factors such as the Framingham Risk Score or that of the American College of Cardiology/American Heart Association guidelines. The burden of genetic risk for CAD is due to the accumulative risk imparted by the total number of genetic risk variants inherited by that individual. A single value for a genetic risk score (GRS) can be obtained by summing the product of the number of high-risk variants inherited by each individual for each susceptibility variant and the log of the odds ratio as determined by previous studies [39,43].

Mega et al. [48] demonstrated that genetic risk is independent of conventional risk factors by using a GRS based on 27 genetic risk variants previously shown to predispose to CAD. The study consisted of a sample size of 48,421 individuals and 3,477 events. The investigators genotyped the DNA of individuals enrolled in 2 primary prevention trials (JUPITER [Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin] and ASCOT [Anglo-Scandinavian Cardiac Outcomes Trial]) and 2 secondary prevention trials (CARE [Cholesterol and Recurrent Events] and PROVE IT-TIMI 22 [Pravastatin or Atorvastatin Evaluation and Infection Therapy—Thrombolysis In Myocardial Infarction 22]). Risk stratification based on the GRS divided the group into low, intermediate, and high risk. GRS also predicted the response to statin therapy. In the primary prevention trial JUPITER, GRS required treatment of 66 individuals to prevent 1 cardiac event in 10 years in individuals at low genetic risk, intermediate risk required 42, and high genetic risk required 25; in the ASCOT trial, the numbers were 57, 47, and 20, respectively. Another study [40] evaluated 50 SNP that included the 27 SNP used by Mega et al. [48]. Results showed that the 23 additional SNP improved discrimination ( $p < 0.0001$ ) and reclassification ( $p < 0.0001$ ). Individuals with high GRS had a 2.4-fold greater risk than those with low GRS [58]. A study using GRS based on 49,310 SNP [69] confirmed the increased predictive value of genetic risk variants over that of conventional risk factors in 5 prospective population cohorts with a total sample size of 12,676 subjects. Recent studies by Ganna et al. [38] and Ripatti et al. [41] also confirmed GRS for CAD is independent of conventional risk factors and for risk stratification is superior to conventional risk factors. The addition of genetic risk variants to conventional risk factors further enhances the power of predicting cardiac events.

### PROSPECTIVE RISK STRATIFICATION WITH GRS SHOWS A HEALTHY LIFESTYLE REDUCES CARDIAC EVENTS BY 46% RELEVANT TO UNHEALTHY LIFESTYLE

A recent clinical trial conducted by Khara et al. [47] used a GRS to stratify for cardiac events. GRS was derived from previously proven genetic risk variants for CAD. Four prospective cohorts were genotyped composing a sample size of 55,685 participants that were identified from the



ARIC (Atherosclerosis Risk in Communities), the WHS (Women's Genome Health Study), the MDCS (Malmo Diet and Cancer Study), and the cross-sectional BiImage Study. The objective was to determine whether a healthy lifestyle decreases cardiac events. A healthy lifestyle was defined on the basis of 4 factors (no current smoking, no obesity, regular physical activity, and a healthy diet). The investigators then categorized the participants into a favorable lifestyle (3 of 4 healthy lifestyle factors) or an unfavorable lifestyle (1 or no healthy lifestyle factors). Participants stratified as having a high GRS for CAD had a 91% higher risk of cardiac events than those participants with low GRS. A favorable lifestyle was associated with a 46% lower risk of cardiac events than an unfavorable lifestyle. The standardized 10-year incidence of coronary events was 5.1% for a favorable lifestyle versus 10.7% for an unfavorable lifestyle. This study confirms the clinical applicability of risk stratification for CAD based on a score using genetic risk variants and its superiority over conventional risk factors. Furthermore, it refutes the myth that genetic risk cannot be treated. It is worth noting that genetic risk is treated in the same manner as treatment for environmental or acquired risk.

### RISK STRATIFICATION BASED ON GRS—A PARADIGM SHIFT IN PRIMARY PREVENTION OF CAD FOR ALL LEVELS OF INCOME

Currently, a 40-year-old woman having a plasma LDL-C of 180 mg/dl and no other risk factors according to the Clinical Practice Guidelines [42] would not receive any therapy other than being checked periodically. If that same person underwent a GRS and was in the intermediate- or high-risk group, she could be advised of lifestyle changes and if necessary receive statin therapy to reduce plasma LDL-C to levels of 70 to 80 mg/dl. GRS has the advantage over conventional risk factors because it is not dependent on age. GRS can be determined at birth or any time period in one's life because the DNA does not change in one's lifetime. The potential to identify early asymptomatic individuals at high risk for CAD could indeed transform primary prevention, particularly in women prior to menopause. Analysis of a single saliva sample can determine those individuals at high risk for CAD and make available appropriate early prevention to individuals of all levels of incomes. The use of GRS to stratify risk for CAD has been shown in several studies and modification of the genetic risk has successfully reduced cardiac events [47]. Although these genetic risk variants can be genotyped and assessed for prevention, routine guidelines and recommendations have yet to decide their role in routine clinical management. According to the guidelines, the presence of additional risk factors mandate more intensive therapy of known risk factors such as plasma LDL-C. The practice guidelines will have to assess whether individuals determined to have high genetic risk should receive more aggressive treatment of plasma LDL-C. Despite the lack of

an approved algorithm for risk stratification of CAD using these risk variants, the future appears very promising. In addition, the genetic risk variants confirm that other as yet unknown factors contribute to the pathogenesis of CAD. Research on the mechanisms and the targets of these newly discovered genetic risk variants will not only transform prevention but also the diagnosis, prevention, and treatment of CAD in the future.

### SUMMARY

Genome-wide association studies have discovered and replicated more than 60 genetic risk variants for CAD. Only about one-third of the variants mediate their risk through the known risk factors (e.g., cholesterol). Second, they occur at high frequency in the human genome and each is associated with minimal risk. The total genetic risk for CAD is proportional to the number of risk variants and when combined into a GRS are more effective in risk stratification than conventional risk factors. The added advantage of the GRS is the risk can be determined at any age and is likely to enable a paradigm shift in primary prevention of CAD. A new therapy of PCSK9 inhibitors for prevention of CAD has evolved, and other novel drugs targeted to genetic risk variants are likely to emerge.

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### REFERENCES

1. Murray CJ, Lopez AD. Measuring the global burden of disease. *N Engl J Med* 2013;369:448–57.
2. Roberts R, Stewart AF, Wells GA, Williams KA, Kavaslar N, McPherson R. Identifying genes for coronary artery disease: an idea whose time has come. *Can J Cardiol* 2007;23(Suppl A):7A–15A.
3. Chan L, Boerwinkle E. Gene-environment interactions and gene therapy in atherosclerosis. *Cardiol Rev* 1994;2:130–7.
4. Schildkraut JM, Myers RM, Cupples LA, Kiely DK, Kannel WB. Coronary risk associated with age and sex of parental heart disease in the Framingham study. *Am J Cardiol* 1989;64:555–9.
5. Allen G, Harvald B, Shields JP. Measures of twin concordance. *Acta Genet Stat Med* 1967;17:475–81.
6. Yusuf S, Hawken S, Ounpuu S, et al., for the INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): Case-control study. *Lancet* 2004;364:937–52.
7. Cooper JA, Miller GJ, Humphries SE. A comparison of the PROCAM and Framingham point-scoring systems for estimation of individual risk of coronary heart disease in the Second Northwick Park Heart Study. *Atherosclerosis* 2005;181:93–100.
8. Hejtmančík JF, Brink PA, Towbin J, et al. Localization of gene for familial hypertrophic cardiomyopathy to chromosome 14q1 in a diverse US population. *Circulation* 1991;83:1592–7.
9. Kaiser J. Human genetics: affordable “exomes” fill gaps in a catalog of rare diseases. *Science* 2010;330:903.
10. Hirschhorn JN, Daly MJ. Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet* 2005;6:95–108.
11. Stranger BE, Forrest MS, Dunning M, et al. Relative impact of nucleotide and copy number variation on gene expression phenotypes. *Science* 2007;315:848–53.

12. International HapMap Consortium. A haplotype map of the human genome. *Nature* 2005;437:1299–320.
13. Petretto E, Liu ET, Aitman TJ. A gene harvest revealing the archeology and complexity of human disease. *Nat Genet* 2007;39:1299–301.
14. Pare G, Serre D, Brisson D, et al. Genetic analysis of 103 candidate genes for coronary artery disease and associated phenotypes in a founder population reveals a new association between endothelin-1 and high-density lipoprotein cholesterol. *Am J Hum Genet* 2007;80:673–82.
15. Wise AL, Gyi L, Manolio TA. eXclusion: toward integrating the X chromosome in genome-wide association analyses. *Am J Hum Genet* 2013;92:643–7.
16. McPherson R, Pertsemlidis A, Kavaslar N, et al. A common allele on chromosome 9 associated with coronary heart disease. *Science* 2007;316:1488–91.
17. Helgadottir A, Thorleifsson G, Manolescu A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science* 2007;316:1491–3.
18. McPherson R. Chromosome 9p21 and coronary artery disease. *N Engl J Med* 2010;362:1736–7.
19. Samani NJ, Erdmann J, Hall AS, et al., for the WTCCC and the Cardiogenics Consortium. Genomewide association analysis of coronary artery disease. *N Engl J Med* 2007;357:443–53.
20. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–78.
21. Ding H, Xu Y, Wang X, et al. 9p21 is a shared susceptibility locus strongly for coronary artery disease and weakly for ischemic stroke in Chinese Han population. *Circ Cardiovasc Genet* 2009;2:338–46.
22. Shen GQ, Li L, Rao S, et al. 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;28:360–5.
23. Kumar J, Yumnam S, Basu T, et al. Association of polymorphisms in 9p21 region with CAD in North Indian population: replication of SNPs identified through GWAS. *Clin Genet* 2011;79:588–93.
24. Saleheen D, Alexander M, Rasheed A, et al. Association of the 9p21.3 locus with risk of first-ever myocardial infarction in Pakistanis: case-control study in South Asia and updated meta-analysis of Europeans. *Arterioscler Thromb Vasc Biol* 2010;30:1467–73.
25. Hinohara K, Nakajima T, Takahashi M, et al. Replication of the association between a chromosome 9p21 polymorphism and coronary artery disease in Japanese and Korean populations. *J Hum Genet* 2008;53:357–9.
26. Kral BG, Mathias RA, Sukhtipat B, et al. A common variant in the CDKN2B gene on chromosome 9p21 protects against coronary artery disease in Americans of African ancestry. *J Hum Genet* 2011;56:224–9.
27. Dandona S, Stewart AF, Roberts R. Genomics in coronary artery disease: past, present and future. *Can J Cardiol* 2010;26(Suppl A):56A–9A.
28. Preuss M, König IR, Thompson JR, et al., for the CARDIoGRAM Consortium. 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. *Circ Cardiovasc Genet* 2010;3:475–83.
29. Schunkert H, König IR, Kathiresan S, et al., for the CARDIoGRAM Consortium. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat Genet* 2011;43:333–8.
30. Reilly MP, Li M, He J, et al. Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. *Lancet* 2011;377:383–92.
31. Coronary Artery Disease (CAD) Genetics Consortium. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. *Nat Genet* 2011;43:339–44.
32. Davies RW, Wells GA, Stewart AF, et al. A genome-wide association study for coronary artery disease identifies a novel susceptibility locus in the major histocompatibility complex. *Circ Cardiovasc Genet* 2012;5:217–25.
33. Wang F, Xu CQ, He Q, et al. Genome-wide association identifies a susceptibility locus for coronary artery disease in the Chinese Han population. *Nat Genet* 2011;43:345–9.
34. IBC 50K CAD Consortium. Large-scale gene-centric analysis identifies novel variants for coronary artery disease. *PLoS Genet* 2011;7:e1002260.
35. Deloukas P, Kanoni S, Willenborg C, et al., for the CARDIoGRAM Consortium. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet* 2013;45:25–33.
36. Nikpay M, Goel A, Won HH, et al., for the CARDIoGRAM Consortium. A comprehensive 1,000 genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet* 2015;47:1121–30.
37. Webb TR, Erdmann J, Stirrups KE, et al., for the Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia Investigators. Systematic evaluation of pleiotropy identifies 6 further loci associated with coronary artery disease. *J Am Coll Cardiol* 2017;69:823–36.
38. Ganna A, Magnusson PK, Pedersen NL, et al. Multilocus genetic risk scores for coronary heart disease prediction. *Arterioscler Thromb Vasc Biol* 2013;33:2267–72.
39. Thanassoulis G, Vasan RS. Genetic cardiovascular risk prediction: will we get there? *Circulation* 2010;122:2323–34.
40. Tada H, Melander O, Louie JZ, et al. Risk prediction by genetic risk scores for coronary heart disease is independent of self-reported family history. *Eur Heart J* 2016;37:561–7.
41. Ripatti S, Tikkanen E, Orho-Melander M, et al. A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. *Lancet* 2010;376:1393–400.
42. Goff DC Jr, Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2014;129(Suppl 2):S49–73.
43. Goldstein BA, Knowles JW, Salfati E, Ioannidis JP, Assimes TL. Simple, standardized incorporation of genetic risk into non-genetic risk prediction tools for complex traits: coronary heart disease as an example. *Front Genet* 2014;5:254.
44. Helgadottir A, Thorleifsson G, Magnusson KP, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. *Nat Genet* 2008;40:217–24.
45. Beckie TM, Beckstead JW, Groer MW. The association between variants on chromosome 9p21 and inflammatory biomarkers in ethnically diverse women with coronary heart disease: a pilot study. *Biol Res Nurs* 2011;13:306–19.
46. Wang W, Peng WH, Lu L, et al. Polymorphism on chromosome 9p21.3 contributes to early-onset and severity of coronary artery disease in non-diabetic and type 2 diabetic patients. *Chin Med J (Engl)* 2011;124:66–71.
47. Khera AV, Emdin CA, Drake I, et al. Genetic risk, adherence to a healthy lifestyle, and coronary disease. *N Engl J Med* 2016;375:2349–58.
48. Mega JL, Stitzel NO, Smith JG, et al. Genetic risk, coronary heart disease events, and the clinical benefit of statin therapy: an analysis of primary and secondary prevention trials. *Lancet* 2015;385:2264–71.
49. Assimes TL, Roberts R. Genetics: implications for prevention and management of coronary artery disease. *J Am Coll Cardiol* 2016;68:2797–818.
50. Brown MS, Goldstein JL. Familial hypercholesterolemia: a genetic defect in the low-density lipoprotein receptor. *N Engl J Med* 1976;294:1386–90.
51. Simons J. The \$10 billion pill. *Fortune* 2003;147: 58–62, 66, 68.
52. Seidah NG, Benjannet S, Wickham L, et al. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci U S A* 2003;100:928–33.
53. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med* 2006;354:1264–72.
54. Stein EA, Mellis S, Yancopoulos GD, et al. Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. *N Engl J Med* 2012;366:1108–18.

55. He M, Wolpin B, Rexrode K, et al. ABO blood group and risk of coronary heart disease in two prospective cohort studies. *Arterioscler Thromb Vasc Biol* 2012;32:2314–20.
56. Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ Jr, Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood* 1987;69:1691–5.
57. Dandona S, Stewart AF, Chen L, et al. Gene dosage of the common variant 9p21 predicts severity of coronary artery disease. *J Am Coll Cardiol* 2010;56:479–86.
58. Horne BD, Carlquist JF, Muhlestein JB, Bair TL, Anderson JL. Association of variation in the chromosome 9p21 locus with myocardial infarction versus chronic coronary artery disease. *Circ Cardiovasc Genet* 2008;1:85–92.
59. Ardissino D, Berzuini C, Merlini PA, et al., for the Italian Atherosclerosis, Thrombosis, and Vascular Biology Investigators. Influence of 9p21.3 genetic variants on clinical and angiographic outcomes in early-onset myocardial infarction. *J Am Coll Cardiol* 2011;58:426–34.
60. Chan K, Patel RS, Newcombe P, et al. Association between the chromosome 9p21 locus and angiographic coronary artery disease burden: a collaborative meta-analysis. *J Am Coll Cardiol* 2013;61:957–70.
61. Huan T, Zhang B, Wang Z, et al., for the CARDIoGRAM Consortium, ICBP Investigators. A systems biology framework identifies molecular underpinnings of coronary heart disease. *Arterioscler Thromb Vasc Biol* 2013;33:1427–34.
62. Palomaki GE, Melillo S, Bradley LA. Association between 9p21 genomic markers and heart disease: a meta-analysis. *JAMA* 2010;303:648–56.
63. Myocardial Infarction Genetics Consortium. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants [Erratum in *Nat Genet*. 41:762]. *Nat Genet* 2009;41:334–41.
64. Gudbjartsson DF, Bjornsdottir US, Halapi E, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009;41:342–7.
65. Tregouet DA, Konig IR, Erdmann J, et al. Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease. *Nat Genet* 2009;41:283–5.
66. Lu X, Wang L, Chen S, et al. Genome-wide association study in Han Chinese identifies four new susceptibility loci for coronary artery disease. *Nat Genet* 2012;44:890–4.
67. Erdmann J, Grosshennig A, Braund PS, et al. New susceptibility locus for coronary artery disease on chromosome 3q22.3. *Nat Genet* 2009;41:280–2.
68. Emdin CA, Kherr AV, Nataraja P, et al. Phenotypic characterization of genetically lowered human lipoprotein(a) levels. *J Am Coll Cardiol* 2016;68:2761–72.
69. Abraham G, Havulinna DSc, Bhalala OG, et al. Genomic prediction of coronary artery disease. *Eur Heart J* 2016;37:3267–78.