R. Roberts, M.D.

GENETICS OF CORONARY ARTERY DISEASE: AN UPDATE

Robert Roberts, M.D.

University of Ottawa Heart Institute, Ottawa, Canada.

Abstract

In 2007, the first genetic risk variant, 9p21, was simultaneously discovered by two independent groups. 9p21 increases the risk of coronary artery disease in individuals with premature heart disease by twofold, and in the overall population the heterozygote is associated with a 25% increased risk and the homozygote with a 50% increased risk. It is of note that the risk mediated by 9p21 is independent of known risk factors. Since then, with the development of new technologies and the international consortium of CARDIoGRAM, there is now a total of 50 genetic risk variants confirmed and replicated for CAD. Of these 50, 35 mediate their risk by unknown mechanisms, indicating that the pathogenesis of atherosclerosis and myocardial infarction is due to additional factors as yet unknown. The role of genetic risk factors in the management of CAD is yet to be determined. Since many of them are independent of known risk factors, the genetic risk will in the future have to be incorporated into the guidelines, which recommend the target level of plasma LDL-C to be achieved based on the number of risk factors.

Introduction

Coronary artery disease (CAD), the number-one killer in the world, is largely preventable. Modification of conventional risk factors such as cholesterol has consistently shown a 30% to 40% reduction in mortality and morbidity.[1, 2] Genetic risk factors for CAD, well documented by epidemiological studies, have until recently been elusive.[3] The current explosion in the discovery of genetic risk variants for CAD will, in the future, provide more comprehensive primary prevention and the hope of further reduction in the incidence of CAD and its sequelae. It remains to be determined whether it will meet the challenge to eliminate or markedly attenuate CAD in the 21st century as claimed by several investigators.[4, 5]

21st Century: a Genetic Landfall for Coronary Artery Disease

The challenge to prevent CAD in the 21st century has had a great start. In 2007, we reported in this journal that the technology had arrived to pursue genes predisposing to polygenic disorders such as CAD.[3] The technology referred to is a chip containing 500,000 DNA markers selected to genotype the entire human genome, making possible the first genome-wide association studies (GWAS). These 500,000 DNA markers are single nucleotide polymorphisms (SNPs) occurring at a frequency greater than 1% that had been mapped to their chromosomal location in the human genome. For the human genome of 3 billion nucleotides, these 500,000 SNPs provided, on average, a marker every 6,000 nucleotides. Using the case-control association approach, one could genotype cases and controls and compare the frequencies of each DNA marker in cases to that of controls. Any DNA marker occurring statistically more frequently in cases than controls would reflect a DNA region that was associated with increased risk for that disease. The chip has since been updated to contain approximately one million SNPs.

The analysis of multiple SNPs requires a statistical correction, which by convention is a Bonferroni correction whereby a P value of 0.05 is divided by one million, giving a P value < 0.00000005 (i.e., P value < 5×10^{-8}); this is referred to as genome-wide significant.[6] Furthermore, the results have to be replicated in an appropriate independent population. Our discussion in this review focuses solely on the results of GWAS, in which the cases had documented CAD and the genetic risk variants discovered are genome-wide significant and have been replicated in an independent population.

To enrich for genetic predisposition, cases in the Ottawa Heart Genomics Study (OHGS) were required to be < 55 years for males and 65 years for females and to have obstruction ≥ 50% in one or more coronary vessels on a coronary angiogram or documented myocardial infarction (MI). Controls were required to be asymptomatic and ≥ 65 years for males and 70 years for females; in addition, those having had a coronary angiogram were required to have < 30% obstruction in either vessel. We phenotyped, genotyped, and performed a GWAS on individuals in the OHGS,[7] with replication in multiple independent populations from Texas (Houston and Dallas) and Denmark. The total sample size was greater than 23,000, enabling us to discover the first genetic risk variant for CAD, in 2007, located on the short arm (p) of chromosome 9, now commonly referred to as 9p21.[8] Simultaneously and independently, the deCODE group also discovered 9p21.[9] Within months, 9p21 was confirmed by multiple investigators around the world.[10-13] Subsequent technological advances markedly facilitated the pursuit of genetic risk for CAD, including the mapping of more than 16 million SNPs to their chromosomal location for use as DNA markers. Several GWAS were performed for CAD as well as other diseases, and by 2009, 12 genetic risk variants had been discovered.[14] It was realized from this data that multiple genetic risk variants contribute to CAD, each associated with only mild to moderate genetic influence. This

would require much larger sample sizes than initially expected to discover genetic risk variants for CAD. Many of the centers already pursuing GWAS for genetic predisposition for CAD agreed to collaborate and leverage their patients, expertise, and other resources. Together we formed an international consortium dedicated to the pursuit of discovering genes associated with CAD[15] that is the largest collaboration in the history of cardiology. The initial international consortium was referred to as the Coronary ARtery DIsease Genome-Wide Replication And Meta-Analysis (CARDIoGRAM) study, which involved 14 GWAS, each of which had been previously successful, and included investigators from the United Kingdom, Germany, United States, and Canada. This provided a sample size of 86,995 individuals (22,223 cases vs. 64,762 controls) of European ancestry for the discovery genotyping followed by replication of results in an independent population sample size of 56,682. The study led to the discovery of 13 new genetic risk variants for CAD and confirmation of 10 previously identified risk variants.[16] This was followed by the results from the Coronary Artery Disease C4D Genetics Consortium, which identified four additional genetic risk variants for CAD.[17] The IBC 50K CAD Consortium, using a 50K SNP array, identified three additional risk variants for CAD.[18] Subsequently, CARDIoGRAM joined with the C4D group to become CARDIoGRAMplusC4D with a total sample size of more than 190,000 individuals. Meta-analysis of this sample size led to the discovery and confirmation of 46 genetic risk variants associated with CAD.[19] There are currently a total of 50 genetic risk variants predisposing to CAD of genome-wide significance with confirmation in independent populations (Table 1).

Common Features in CAD Genetic Risk Variants

The widespread use of GWAS to discover genetic risk variants for common polygenic diseases has met with remarkable success. In just over 5 years, more than 2,800 genetic variants have been discovered as risk factors for more than 300 diseases.[20] The genetic risk variants for CAD have many features that are similar to genetic variants for other polygenic disorders:

1. The genetic risk variants for CAD are very common, occurring on average in 50% of the population with a frequency varying from 2% to 91% (Table 1).
2. The relative increased risk of each genetic variant is small, averaging 18% with an odds ratio varying from 2% to 90%.
3. For CAD as well as other common polygenic disorders, multiple genetic risk variants are inherited by everyone. Those at high genetic risk for CAD have a greater genetic risk burden due to inheritance of a greater number of common risk variants, as opposed to inheriting one or more genetic variant of high risk. In a CARDIoGRAM analysis of 23 genetic risk variants for CAD, the average number inherited per individual (case or control) was 17, varying from a minimum of 7 to a maximum of 37.
4. Most of the genetic risk variants for CAD are located in DNA sequences that do not code for protein. This means the risk variant mediates its increased risk for CAD directly or indirectly through regulation of DNA sequences that do code for protein.
5. All DNA genetic risk variants need only be assessed once, since one's DNA does not change over one's lifetime nor do genetic risk variants vary with time, meals, drugs, or gender.

Pathological and Therapeutic Implications of Genetic Risk Variants for CAD

A brief analysis of Table 1 indicates that only 15 of the 50 genetic risk variants are associated with conventional risk factors for CAD: seven associated with low density lipoprotein-cholesterol (LDL-C); one with high density lipoprotein (HDL); two with triglycerides;

Chromosomal Location	SNP	Nearby Genes	Risk Allele Frequency (allele)	Odds Ratio	Delivery Route
Risk Variant Associated with LDL Cholesterol					
6q25.3	rs3798220	LPA	0.02 (C)	1.92 (1.48-2.49)	2009
2p24.1	rs515135	APOB	0.83 (G)	1.03	2012
1p13.3	rs599839	SORT1	0.78 (A)	1.29 (1.18-1.40)	2007
19p13.2	rs1122608	LDLR	0.77 (G)	1.14 (1.09-1.19)	2009
19q13.32	rs2075650	APOE	0.14 (G)	1.14 (1.09-1.19)	2011
2p21	rs6544713	ABCG5-ABCG8	0.29 (G)	1.07 (1.04-1.11)	2011
1p32.3	rs11206510	PCSK9	0.82 (T)	1.15 (1.10-1.21)	2009
Risk Variant Associated with HDL Cholesterol					
6p21.31	rs12205331	ANKS1A	0.81 (C)	1.04	2012
Risk Variant Associated with Triglycerides					
8q24.13	rs10808546	TRIB1	0.65 (A)	1.08 (1.04-1.12)	2011
11q23.3	rs964184	ZNF259, APOA5-A4-C3-A1	0.13 (G)	1.13 (1.10-1.16)	2011
Risk Variant Associated with Hypertension					
12q24.12	rs3184504	SH2B3	0.44 (T)	1.13 (1.08-1.18)	2009
10q24.32	rs12413409	CYP17A1, CNNM2, NT5C2	0.89 (G)	1.12 (1.08-1.16)	2011
4q31.1	rs7692387	GUCYA3	0.81 (G)	1.13	2012
15q26.1	rs17514846	FURIN-FES	0.44 (A)	1.04	2012

Chromosomal Location	SNP	Nearby Genes	Risk Allele Frequency (allele)	Odds Ratio	Delivery Route
Risk Variant Associated with Myocardial Infarction					
9q34.2‡	rs579459	ABO	0.21 (C)	1.10 (1.07-1.13)	2011
Risk Variant Mechanism of Risk Unknown					
9p21.3	rs4977574	CDKN2A,CDKN2B	0.46 (G)	1.25 (1.18-1.31) to 1.37 (1.26-1.48)	2007
1q41	rs17465637	MIA3	0.74 (C)	1.20 (1.12-1.30)	2007
10q11.21	rs1746048	CXCL12	0.87 (C)	1.33 (1.20-1.48)	2007
2q33.1	rs6725887	WDR12	0.15 (C)	1.16 (1.10-1.22)	2009
6p24.1	rs12526453	PHACTR1	0.67 (C)	1.13 (1.09-1.17)	2009
21q22.11	rs9982601	MRPS6	0.15 (T)	1.19 (1.13-1.27)	2009
3q22.3	rs2306374	MRAS	0.18 (C)	1.15 (1.11-1.19)	2009
10p11.23	rs2505083	KIAA1462	0.42 (C)	1.07 (1.04-1.09)	2010
1p32.2	rs17114036	PPAP2B	0.91 (A)	1.17 (1.13-1.22)	2011
5q31.1	rs2706399	IL5	0.48 (A)	1.02 (1.01-1.03)	2011
6q23.2	rs12190287	TCF21	0.62 (C)	1.08 (1.06-1.10)	2011
7q22.3	rs10953541	BCAP29	0.75 (C)	1.08 (1.05-1.11)	2011
7q32.2	rs11556924	ZC3HC1	0.62 (C)	1.09 (1.07-1.12)	2011
10q23.31	rs1412444	LIPA	0.34 (T)	1.09 (1.07-1.12)	2011
11q22.3	rs974819	PDGF	0.29 (T)	1.07 (1.04-1.09)	2011
13q34	rs4773144	COL4A1, COL4A2	0.44 (G)	1.07 (1.05-1.09)	2011
14q32.2	rs2895811	HHIPL1	0.43 (C)	1.07 (1.05-1.10)	2011
15q25.1	rs3825807	ADAMTS7	0.57 (A)	1.08 (1.06-1.10)	2011
17p13.3	rs216172	SMG6, SRR	0.37 (C)	1.07 (1.05-1.09)	2011
17p11.2	rs12936587	RASD1, SMCR3, PEMT	0.56 (G)	1.07 (1.05-1.09)	2011
17q21.32	rs46522	UBE2Z, GIP, ATP5G1, SNF8	0.53 (T)	1.06 (1.04-1.08)	2011
5p13.3*	rs11748327	IRX1, ADAMTS16	0.76 (C)	1.25 [1.18-1.33]	2011
6p22.1*	rs6929846	BTN2A1	0.06 (T)	1.51 (1.28-1.77)	2011
6p24.1**	rs6903956	C6orf105	0.07 (A)	1.65 (1.44-1.90)	2011
6p21.3	rs3869109	HCG27 and HLA-C	0.60 (C)	1.15	2012
1q21	rs4845625	IL6R	0.47 (T)	1.09	2012
Chr4	rs1878406	EDNRA	0.15 (T)	1.09	2012
7p21.1	rs2023938	HDAC9	0.10 (G)	1.13	2012
2p11.2	rs1561198	VAMP5-VAMP8	0.45 (A)	1.07	2012
Chr2	rs2252641	ZEB2-AC074093.1	0.45 (A)	1	2012
Chr5	rs273909	SLC22A4-SLC22A5	0.14 (C)	1.11	2012
6p21	rs10947789	KCNK5	0.76 (T)	1.01	2012
6q26	rs4252120	PLG	0.73 (T)	1.07	2012
8p22	rs264	LPL	0.86 (G)	1.06	2012
13q12	rs9319428	FLT1	0.32 (A)	1.1	2012

Table 1. Chronological list of 50 genetic variants (genome-wide significant) associated with coronary artery disease or myocardial infarction.

*Variant identified only in Japanese; **Variant identified only in Han Chinese; ‡ The risk variant at 9q34.2 is associated with myocardial infarction but not with coronary atherosclerosis. A: adenine; C: cytosine; CI: confidence interval; G: guanine; OR: odds ratio; SNP: single nucleotide polymorphism; T: thymine.

four with hypertension; and one with coronary thrombosis. The remaining 35 risk variants operate through mechanisms yet to be determined. While the hope is to ultimately use these genetic risk variants for more effective primary and secondary prevention, the immediate surprise is that many other factors contribute to the pathogenesis of atherosclerosis and CAD that are yet unknown. Research can now be directed towards these new genetic risk factors with the hope of identifying new pathways that lead to CAD. This implies a great opportunity to develop new biomarkers for detecting early CAD as well as unique targets for novel therapy. Just as 10 of these genetic risk variants mediate their risk through lipids, it is expected that the 35 genetic risk variants of unknown function will mediate their risk through only a few pathways. Identification of these molecular pathways will provide for early detection and more effective prevention of this disease. It is self-evident that until we identify these pathways, we are unlikely to be comprehensive in our prevention of CAD. The identification of PCSK9 has already led to the development of new therapies for CAD as described below.

Genetics Leads to New Therapy for CAD: PCSK9 Inhibition

Evidence that cholesterol plays a major role in the pathogenesis of atherosclerosis has been known for more than five decades. However, one of the major observations confirming the link between cholesterol and heart disease was from human genetics. In the 1970s, members of a family with hypercholesterolemia, due to a mutation in the LDL-receptor, experienced heart attacks in their 2nd and 3rd decade of life.[21] This observation catalyzed efforts to find a drug to lower plasma levels of LDL-C. Two decades later, a drug that inhibits cholesterol synthesis was introduced; all drugs with this mechanism are referred to as statins. Statins are essentially the only drug for primary and secondary prevention of hypercholesterolemia. The worldwide budget for statins alone is more than $70 billion.

In 2003, Seidah et al. discovered PCSK9, an enzyme that increases the degradation of LDL receptors.[22] Since LDL receptors are a major mechanism for the removal of LDL-C, PCSK9 is associated with hypercholesterolemia and increased mortality from heart disease. Subsequently, other mutations in the gene encoding for PCSK9 have been identified. Those associated with increased function are associated with higher cholesterol levels and increased cardiac morbidity and mortality. This is in contrast to mutations inducing loss of function of PCSK9, which are associated with hypocholesterolemia and a decreased incidence of MI and death. It was well recognized and recently confirmed in a U.K. study that only 28% of individuals receiving a statin reached the recommended target for plasma LDL-C.[23] There are several reasons for not obtaining this target, but one is intolerance associated with high doses of statins. Inhibition of PCSK9 provides a complementary therapy to statins since it can lower the plasma levels of LDL-C without affecting the synthesis of cholesterol. African Americans that inherited hypocholesterolemia due to loss of function mutations in PCSK9 showed a mean reduction of 28% in plasma LDL-C levels and a mean reduction of 88% in the risk of CAD. Despite these families being exposed to hypocholesterolemia throughout their lives, there were no adverse side effects.[24] Several therapies have been developed to inhibit PCSK9 and are now undergoing clinical trials.[25-28] The one appearing most promising is a monthly injection of a monoclonal antibody.[27, 28] Results of phase I trials showed no significant side effects and LDL-C reductions of 41% to 58%.[29] Phase II trials were in individuals with hypercholesterolemia receiving atorvastatin treatment. Those receiving 80 mg of atorvastatin alone had a mean reduction of 17% in their LDL-C versus a 72% reduction in LDL-C for those receiving 80 mg atorvastatin plus the PCSK9 antibody.[29] Phase III clinical trials are currently ongoing. In just a few years, since this genetic discovery, a new and potent therapy is emerging for the treatment of hypercholesterolemia. Thus, genetic observations have again provided us new insight and novel therapy for CAD.

Blood Groups A and B are Risk Variants for CAD with Therapeutic Implications

In a CARDIoGRAM study, a GWAS was performed in 4,372 patients with documented CAD by angiography and confirmed MI and in 2,739 patients with documented CAD without MI.[30] There was a strong association between the ABO blood group locus at 9q34.2 and MI but no association with CAD. This was replicated in an independent population. Epidemiologists have claimed for decades that blood group O offers protection from MI. Blood groups A, B, and O are different forms of the same gene at 9q34.2. The A and B genes encode for a protein (alpha 1, 3N-acetylgalactosaminyltransferase) that transfers a carbohydrate moiety onto von Willebrand Factor (vWF). This prolongs the life of vWF and predisposes to coronary thrombosis and MI. The blood group O gene codes for a protein that has been mutated and lacks any biochemical activity and thus does not transfer the carbohydrate moiety onto vWF. As a result, individuals with blood group O show no increased risk for MI.

The frequency of the gene that encodes for A or B blood group occurs in about 57% of Caucasians. The average relative increased risk for MI is about 20% depending on the genotype. In the recent Nurses' Health Study and Health Professionals Follow-up Study of more than 90,000 individuals, 4,070 developed heart disease. In this 20-year follow-up study, having blood group A or B alone was associated with an increased risk of MI of about 10%; however, the combination of A and B blood groups increased the risk to 20%.[31] It also has been shown that plasma levels of vWF complex are approximately 25% higher in individuals with A, B, or AB blood groups as opposed to blood group O.[32]

These results have important implications for people undergoing angioplasty, bypass surgery, and other such procedures. For example, should individuals of blood group A or B receive some form of antiplatelet therapy such as aspirin?

9p21 Predisposes to Coronary Atherosclerosis and not Myocardial Infarction

The 9p21 risk variant for CAD is perhaps the most robust genetic variant and the most studied of those risk variants with unknown function. This risk variant is contained in a long non-protein coding RNA (LncRNA) of 126,000 bps referred to as Anril, which remains of unknown function. The 9p21 risk variant was not introduced into the genome until the arrival of higher primates and is highly conserved in the human genome. The 9p21 risk allele occurs in 75% of humans except for Africans (50% heterozygous, 25% homozygous). Each risk variant is associated with an increased relative risk for CAD of about 25%. The risk of 9p21 is consistently observed by investigators throughout the world to be independent of conventional risk factors such as cholesterol, diabetes, or hypertension. In individuals with premature CAD, 9p21 homozygosity is associated with a 2-fold increased risk for CAD. The 9p21 risk variant also contributes to increased risk for intracranial and abdominal aortic aneurysms[33] and Alzheimer's disease[34] and has recently been associated with

periodontitis[35] and gout,[36] diseases with a marked inflammatory component. It is of note that the 9p21 risk variant does not associate with C-reactive protein.[37, 38]

Determining the function of 9p21 is further complicated by the observation that the risk variant is not present in the mouse genome, the favorite animal model for assessing gene function. Harismendy et al. had suggested that interferon-gamma may mediate the risk of 9p21 for CAD.[39] However, we have recently shown that interferon-gamma acts independently of the 9p21 risk variant.[40]

All studies have consistently shown that the 9p21 risk variant is associated with atherosclerosis and not with MI.[30, 41-43] Several studies have also indicated that the 9p21 risk variant is associated with progression of coronary atherosclerosis as suggested by the correlation between the number of vessels involved and the number of copies of the 9p21 risk variant.[41, 43] However, there are other studies that have not confirmed the correlation between 9p21 and progression of CAD.[42, 44, 45]

Genetic Risk Variants and Management of CAD

Where do these genetic risk variants fit in the management of CAD? Currently, the answer would be that they do not. One might argue that until there is some therapy to alter their risk, why would one screen for these genetic risk variants? If one has to await the development of drug therapy, it could certainly be 10 years away other than what has already been identified for PCSK9 or antiplatelet therapy for blood groups A and B. One approach to incorporating independent genetic risk variants such as 9p21 into the management of CAD is on the basis of increased burden of risk as outlined by the Adult Treatment Panel III (ATP III). Currently, the ATP III recommends that LDL-C ≥ 190 mg/dL be reduced in individuals with one other risk factor and that LDL-C ≤ 160 mg/dL be reduced in those with two other risk factors. One of these other risk factors could be an independent genetic risk factor such as 9p21, since there is universal agreement that 9p21, like the 34 other genetic risk factors, is independent of conventional risk factors. The ATP panel could then assess whether individuals positive for one or more of these genetic risk variants should have LDL-C treated since it provides for an independent risk factor. It is important to note that in individuals with premature CAD, 9p21 is associated with a 2-fold increased risk—greater than the risk from smoking or that associated with a moderate increase in blood pressure or plasma LDL-C.

The Hope for the Future

The challenge for the next decade will be to identify the molecular mechanisms mediating the risk of those genetic risk variants that do not act through known conventional risk factors. There is good evidence that several of these genetic risk variants predispose to CAD through inflammatory pathways.[19, 46] This would appear to be a major pathway in keeping with previous epidemiological suggested evidence. Genetic observations have already contributed to the mainstay therapy of prevention, namely, statin drugs to prevent CAD, and PCSK9 inhibition that will likely enhance and complement statin therapy based on its effects in ongoing clinical trials. It is reasonable to assume that genetic risk variants will lead to markers for earlier detection of CAD as well as drug therapies to interrupt or attenuate the risk. This is occurring along with the overall trend of personalized medicine, in which the disease and the individual will be treated with more specific therapies to match their genome susceptibilities.

Conflict of Interest Disclosure: Dr. Roberts is a consultant to Cumberland Pharmaceuticals and Celera.

Funding/Support: Dr. Roberts receives grant support from CIHR #MOP82810 (RR)/Canada and CFI #11966 (RR)/Canada.

Keywords: single nucleotide polymorphisms; genome; GWAS; genome-wide association studies; genetic risk variants; coronary artery disease

References

1. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, Macfarlane PW, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med. 1995 Nov 16;333(20):1301-8.
2. Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, et al., for the AFCAPS/TexCAPS Research Group. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels. JAMA. 1998 May 27;279(20):1615-22.
3. Roberts R. Personalized medicine: an idea whose time is approaching. Methodist Debakey Cardiovasc J. 2007;3(1):3-7.
4. Wald NJ, Law MR. A strategy to reduce cardiovascular disease by more than 80%. BMJ. 2003;326:1419-23.
5. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al.; INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004 Sep 11-17;364(9438):937-52.
6. Petretto E, Liu ET, Aitman T. A gene harvest revealing the archeology and complexity of human disease. Nat Genet. 2007 Nov;39(11):1299-301.
7. 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 Aug;23 Suppl A:7A-15A.
8. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al. A common allele on chromosome 9 associated with coronary heart disease. Science. 2007 Jun 8;316(5830):1488-91.
9. Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science. 2007 Jun 8;316(5830):1491-3.
10. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007 Jun 7;447(7145):661-78.
11. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University and Novartis Institutes for BioMedical Research, Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PIW, Chen H, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007 Jun 1;316(5829):1331-6.
12. Broadbent HM, Peden JF, Lorkowski S, Goel A, Ongen H, Green F, et al. 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 Mar 15;17(6):806-14.
13. Palomaki GE, Melillo S, Bradley LA. Association between 9p21 genomic markers and heart disease: a meta-analysis. JAMA. 2010 Feb 17;303(7):648-56.
14. Dandona S, Stewart AF, Roberts R. Genomics in coronary artery disease: past, present and future. Can J Cardiol. 2010 March;26 Suppl A:56A-9A.
15. Preuss M, Konig IR, Thompson JR, Erdmann J, Absher D, Assimes TL, et al. 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 Oct 1;3(5):475-83.

16. Schunkert H, Konig IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet. 2011 Apr;43(4):333-8.
17. The Coronary Artery Disease (C4D) Genetics Consortium. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. Nat Genet. 2011 Apr;43(4):339-44.
18. IBC 50K CAD Consortium. Large-scale gene-centric analysis identifies novel variants for coronary artery disease. PLoS Genet. 2011 Sep;7(9):e1002260.
19. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet. 2013 Jan;45(1):25-33.
20. Wise AL, Gyi L, Manolio TA. eXclusion: toward integrating the X chromosome in genome-wide association analyses. Am J Hum Genet. 2013 May 2;92(5):643-7.
21. Brown MS, Goldstein JL. Familial hypercholesterolemia: a genetic defect in the low-density lipoprotein receptor. N Engl J Med. 1976 Jun 17;294(25):1386-90.
22. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, 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 Feb 4;100(3):928-33.
23. Hovingh GK, Davidson MH, Kastelein JJ, O'Connor AM. Diagnosis and treatment of familial hypercholesterolaemia. Eur Heart J. 2013 Apr:34(13):962-71.
24. 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 Mar 23;354(12):1264-72.
25. Graham MJ, Lemonidis KM, Whipple CP, Subramaniam A, Monia BP, Crooke ST, et al. Antisense inhibition of proprotein convertase subtilisin/kexin type 9 reduces serum LDL in hyperlipidemic mice. J Lipid Res. 2007 Apr;48(4):763-7.
26. Wierzbicki AS, Hardman TC, Viljoen A. Inhibition of pro-protein convertase subtilisin kexin 9 [corrected] (PCSK-9) as a treatment for hyperlipidaemia. Expert Opin Investig Drugs. 2012 May;21(5):667-76.
27. Sjouke B, Kusters DM, Kastelein JJ, Hovingh GK. Familial hypercholesterolemia: present and future management. Curr Cardiol Rep. 2011 Dec;13(6):527-36.
28. Ni YG, Di MS, Condra JH, Peterson LB, Wang W, Wang F, et al. A PCSK9-binding antibody that structurally mimics the EGF(A) domain of LDL-receptor reduces LDL cholesterol in vivo. J Lipid Res. 2011 Jan;52(1):78-86.
29. Stein EA, Mellis S, Yancopoulos GD, Stahl N, Logan D, Smith WB, et al. Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. N Engl J Med. 2012 Mar 22;366(12):1108-18.
30. Reilly MP, Li M, He J, Ferguson JF, Stylianou IM, Mehta NN, 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 Jan 29;377(9763):383-92.
31. He M, Wolpin B, Rexrode K, Manson JE, Rimm E, Hu FB, et al. ABO blood group and risk of coronary heart disease in two prospective cohort studies. Arterioscler Thromb Vasc Biol. 2012 Sep;32(9):2314-20.
32. 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 Jun;69(6):1691-5.
33. Helgadottir A, Thorleifsson G, Magnusson KP, Gretarsdottir S, Steinthorsdottir V, Manolescu A, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet. 2008 Feb;40(2):217-24.
34. Emanuele E, Lista S, Ghidoni R, Binetti G, Cereda C, Benussi L, et al. Chromosome 9p21.3 genotype is associated with vascular dementia and Alzheimer's disease. Neurobiol Aging. 2011 Jul;32(7):1231-5.
35. Schaefer AS, Richter GM, Groessner-Schreiber B, Noack B, Nothnagel M, El Mokhtari NE, et al. Identification of a shared genetic susceptibility locus for coronary heart disease and periodontitis. PLoS Genet. 2009 Feb;5(2):e1000378.
36. Wang B, Meng D, Wang J, Liu S, Zhou S, Miao Z, et al. Genetic association of polymorphism rs1333049 with gout. Rheumatology (Oxford). 2011 Sep;50(9):1559-61.
37. 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 Jul;13(3):306-19.
38. Wang W, Peng WH, Lu L, Zhang RY, Zhang Q, Wang LJ, 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 Jan;124(1):66-71.
39. Harismendy O, Notani D, Song X, Rahim NG, Tanasa B, Heintzman N, et al. 9p21 DNA variants associated with coronary artery disease impair interferon-gamma signalling response. Nature. 2011 Feb 10;470(7333):264-8.
40. Almontashiri NA, Fan M, Cheng BL, Chen HH, Roberts R, Stewart AF. Interferon-gamma activates expression of p15 and p16 regardless of 9p21.3 coronary artery disease risk genotype. J Am Coll Cardiol. 2013 Jan 15;61(2):143-7.
41. Dandona S, Stewart AF, Chen L, Williams K, So D, O'Brien E, et al. Gene dosage of the common variant 9p21 predicts severity of coronary artery disease. J Am Coll Cardiol. 2010 Aug 3;56(6):479-86.
42. 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 Dec;1(2):85-92.
43. Ardissino D, Berzuini C, Merlini PA, Mannuccio Mannucci P, Surti A, Burtt N, et al. Influence of 9p21.3 genetic variants on clinical and angiographic outcomes in early-onset myocardial infarction. J Am Coll Cardiol. 2011 Jul 19;58(4):435-7.
44. Hinohara K, Nakajima T, Takahashi M, Hohda S, Sasaoka T, Nakahara K, 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(4):357-9.
45. Shen GQ, Li L, Rao S, Abdullah KG, Ban JM, Lee BS, 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 Vascul Biol. 2008 Feb;28(2):360-5.
46. Huan T, Zhang B, Wang Z, Joehanes R, Zhu J, Johnson AD, et al. A systems biology framework identifies molecular underpinnings of coronary heart disease. Arterioscler Thromb Vasc Biol. 2013 Jun;33(6):1427-34.