A genetic basis for coronary artery disease

TRENDS IN CARDIOVASCULAR DISEASE

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Introduction

The most notable medical achievements of the 21ST Century will probably revolve around the application of genetics to prevention of disease, regenerative medicine and vaccines for global infections. In single gene disorders tremendous efforts were made from 1990 to 2010, which is referred to as the "Golden Age" for single gene disorders. Of the estimated 7,000 rare single gene disorders, a gene has been discovered for over 3,000***(1).*** The first cardiovascular disorder to be mapped was Hypertrophic Cardiomyopathy***(2),(3),*** then Dilated Cardiomyopathy***(4)*** followed by many others such as Wolff–Parkinson–White syndrome (WPW)***(5),*** Atrial Fibrillation***(6),*** Long QT Syndrome***(7)*** and Brugada Syndrome***(8).*** These are rare disorders occurring with a frequency of less than 1%, but more commonly, in about one-tenth of 1% of the population.

It is claimed that 80% of the deaths in the world are due to 20 common diseases. The combination of Coronary Artery Disease (CAD) and cancer account for over one-half of those deaths***(9).*** The prevention and treatment of CAD has been a major emphasis in the Western world and tremendous progress has been made with about 50% reduction in mortality from CAD in the last 50 years***(10).*** However, CAD is increasing in the developing world, making it the number one killer in the world***(11)***. It has been claimed by several investigators that CAD will be markedly attenuated, if not eliminated, in the 21st century***(12),(13)***. This optimism relates to the proven observation that CAD and its sequelae are, in large part, due to factors that can be prevented. Modification of conventional risk factors (e.g. cholesterol) for CAD in randomized, placebo, controlled clinical trials has been consistently associated with 30 to 40 percent reduction in mortality and morbidity from CAD***(14),(15).*** Epidemiological studies***(16)*** have long shown that genetic predisposition accounts for 40% to 60% of the risk or susceptibility for CAD. Coronary artery disease like most common diseases are polygenic, meaning several genes contribute to its predisposition, with each having only mild to moderate influence. Despite attempts to identify these genes, until recently, their discovery remained elusive.

Development of the technology to pursue Genome Wide Association Studies

Genes responsible for single gene disorders could be identified utilizing a few hundred DNA markers to genotype pedigrees of two or three generations in whom there were family members affected with the disease. This approach enabled one to differentially detect markers segregating individuals having the disease, versus those unaffected. Individuals co-inheriting both the disease and the marker means the marker is in close physical proximity to the gene responsible for the disease. The known chromosomal location of the marker makes it possible to clone and sequence the DNA region containing the gene and its mutation. This method is referred to as genetic linkage analysis. While highly sensitive and successful for single gene disorders, it does not have the sensitivity or resolution to identify genes responsible for polygenic disorders such as CAD.

To pursue genes associated with a polygenic disease such as CAD would require a case-control association study. In contrast to linkage analysis, the DNA genotyping is of unrelated cases and controls. In this approach, the frequency of DNA markers in cases would be compared to that of controls and if significantly more common in cases, it would indicate the marker is in a DNA region associated with increased predisposition to the disease(17). It was recognized in the 1990s that this approach would require hundreds of thousands of DNA markers to span the human genome. It would also require large sample sizes of several thousand cases and several thousand controls. In the absence of these DNA markers, the first approach to the case control analysis was to select Candidate genes based on their function and compare their frequency in cases versus controls. Studies reported over 100 candidates associated with genetic predisposition for CAD(18)***,(19),*** of which almost none would be confirmed in subsequent studies.

The first major breakthrough came in 2005 with the development of a micro-array of 500,000 DNA markers(20) followed by arrays containing 1 million DNA markers(21). These markers were single nucleotide polymorphisms (SNPs), which had been characterized and annotated to their chromosomal location by the HapMap Project(22). Given the human genome has 3.2 billion nucleotides, this means a marker exists on average for every six thousand nucleotides. This approach, referred to as a Genome Wide Association Study (GWAS), represents an unbiased approach whereby markers were distributed throughout the human genome, with no preference in terms of their selection. However, with a p-value of 0.05 utilizing 1 million markers the chances of finding false positives would be about 25,000. To minimize or eliminate these false positives, it would be necessary to do some form of statistical correction which was recommended(23) to be the Bonferroni correction, whereby a p-value of 0.05 is divided by 1 million, giving a corrected p-value of 0.00000005 ($p<5x10^{-8)}$s referred to as genome wide significant(24). Secondly, a marker showing an association with CAD of genome wide significance in the discovery population had to be confirmed in an independent population, referred to as the replication sample. This review will summarize only those genetic risk factors discovered to be of genome wide significance and also replicated in an independent population.

Discovery of 9p21, the first genetic risk variant associated with CAD

In our first attempt to perform a GWAS in pursuit of genes for CAD, we recruited the Ottawa Heart Genomic Study (OHGS)(25). The criterion for CAD cases was individuals with documented coronary artery disease, diagnosed in males before 55 years of age and in females before 65 years. The diagnosis of CAD required 50% obstruction in one or more coronary vessels on a coronary angiogram or documented myocardial infarction (MI). Controls were required to be asymptomatic and over 65 years for males and over 70 years for females. A GWAS(25) was performed with the OHGS as the discovery population, with replication in multiple independent populations from Texas (Houston and Dallas) and Denmark. The total sample size exceeded 23,000. A genetic risk variant associated with CAD was located on the short arm of chromosome 9, now commonly referred to as 9p21(26). Simultaneously and independently, the deCode Group also discovered 9p21(27). Within months, investigators from around the world confirmed that 9p21 was a significant risk factor for CAD(28). A carrier of 9p21 with one copy (heterozygote) was associated with increased risk of 25% and with two copies (homozygote), a 50% increased risk. In individuals with premature coronary artery disease, 9p21 increases the risk for CAD by about two-fold(27)**.** The unexpected and most surprising finding was that the risk of 9p21 for CAD is independent of all known risk factors for CAD. Secondly, it was indeed very common, with 9p21 occurring in over 75% of the population. These observations were confirmed around the world in all ethnic groups except Africans and African Americans*(28)*. In the latter group 9p21 appears to carry no increased risk due to the 9p21 region having been broken up into several smaller regions, each associated with minimal, if any, increased risk. Subsequent studies show 9p21 risk variant contributes to increased risk for intracranial and abdominal aortic aneurysms(29)*,* and Alzheimer's disease(30). The molecular mechanism whereby 9p21 mediates its risk for CAD remains unknown. The various experimental and clinical studies exploring the mechanism of action of 9p21 were discussed and summarized in a recent review(31)**.** While the molecular mechanism of 9p21 is unknown, its site of action has been confirmed. All studies have consistently shown the 9p21 risk variant relates to coronary atherosclerosis and only secondarily to myocardial infarction***(32),(33),(34),(35)***. Some studies have also suggested 9p21 to be associated with the rate of progression of coronary atherosclerosis(32)***,(35).*** However, there are other studies(33)***,(36),*** which have not confirmed this correlation. All of these studies have been cross-sectional and related to incidental events, such as MI or cardiac death. Determining if the 9p21 risk variant is related to progression of coronary atherosclerosis may require a longitudinal study assessing progression of coronary atherosclerosis on coronary angiography, rather than the sequelae such as myocardial infarction.

Success of GWAS in Discovery of Genetic Variants for CAD

Following the discovery of 9p21, several GWAS were performed for CAD as well as for other diseases and by 2009, twelve other genetic risk variants associated with CAD were discovered(28)**.** An analysis of the data indicated there were multiple risk variants associated with CAD, each having only minimal genetic effect. This emphasized to the investigators that it would require even larger sample sizes than expected to identify genetic risk variants for CAD. Several centres performing GWAS for CAD agreed to collaborate and pool their samples, expertise and other resources to perform a meta-analysis. The International consortium,

dedicated to discovering genes associated with CAD(37)***,*** would be the largest international collaboration in the history of cardiology. The consortium was referred to as the Coronary Artery Disease Genome Wide Replication And Meta-Analysis (CARDIoGRAM) study, which involved investigators from the UK, Germany, USA, Iceland and Canada. The initial sample size was 86,995 of which all were of European ancestry. This consortium led to the discovery initially of 14 new genetic risk variants for CAD and the confirmation of 10 previously identified risk variants(38)***.*** This was followed by the results of another GWAS, the Coronary Artery Disease C4D Genetics Consortium(39)***,*** resulting in 4 additional genetic risk variants for CAD and The IBC 50K CAD Consortium identified 3 additional risk variants for CAD(40)***.*** CARDIoGRAM and C4D subsequently joined together to form CARDIoGRAM plusC4D with a sample size of over 240,000. Utilizing these resources a meta-analysis was performed, which led to the discovery and confirmation of 46 genetic risk variants associated with CAD***(41).*** There are currently a total of 50 genetic risk variants predisposing to CAD, all of genome wide significance with confirmation in independent populations *(see Table 1)*. In addition to the progress for CAD, GWAS has experienced remarkable success in the past eight years, having discovered more than 3,000 genetic risk variants as risk factors for more than 300 diseases(42).

Genetic risk variants confirm the long suspected genetic predisposition for CAD

The results of GWAS confirm the epidemiological studies indicating that CAD has a genetic predisposition as expected for most diseases. The 50 genetic variants are far less than expected to account for the 40% to 60% of genetic predisposition. It would appear there are many more genetic variants for CAD to be identified. All of the genetic variants are very common, which proves the theory that common diseases relate to common genetic variants. The plethora of genetic risk variants for CAD have strong implications for the prevention and treatment of this disease. The goal of this century is to intensify the prevention of CAD, which will require comprehensive prevention programs.

Th**e source and evolution of human DNA variation**

The DNA sequence of the human genome is 99.5% identical(43). Thus, the variation in human features, such as the color of one's hair, eyes or predisposition to disease is due to the 0.5% difference in DNA sequences. Over 80% of human variation is due to SNPs***(43),*** of which each human genome has about 3 million. The human genus Homo separated from its higher primate ancestry about 6 million years ago***(44),(45)***. Over this interval, several species evolved with Homo sapiens appearing about 300,000 years ago(46)***,(47),*** which is now regarded as the modern human. The origin of human DNA variation is primarily due to DNA copying errors during replication of the two strands of DNA ***(48),(49)***. DNA turns over every few days with less than one error per billion bases created, nevertheless, over thousands of generations it is enough to induce a significant number of mutations which, when they occur in the egg or sperm, can be transmitted to the next generation. About 94% percent of the copying errors are substitutions of a single nucleotide***(48),(49).*** The recently estimated mutation rate of 1.4×10-8***(50)*** leads to 40 new mutations per generation. The world's population of 7 billion has 400 billion new mutations in the current generation and over 90% of these new mutations are SNPs which are contained in the 3 million SNPs in each genome. These SNPs in the initial offspring

are extremely rare and their fate will be determined by Darwinian principles based on whether they are favorable, detrimental or benign to survival. If the SNP has a favorable effect on human survival, it will increase in frequency with each succeeding generation, while those SNPs that are harmful, will remain rare with the tendency for them to be eliminated from the genome.

The genetic variants associated with risk for CAD appear to be "paradoxical"

The 50 genetic variants associated with increased risk for CAD have many features in common with each other as well as with genetic risk variants associated with other diseases. All of the genetic variants occur frequently in the population and are distributed throughout the genome. Since the genetic risk variants for CAD are very common, it would indicate that these SNPs increased in frequency with each succeeding generation because they are associated with improving human survival. Their deleterious effect of contributing to the pathogenesis of CAD is a secondary incidental function that is probably age-dependent and represents only an unintended residual. Several of the genetic risk variants for CAD are involved with innate or adaptive immunity which, through their inflammatory response to infections and other forms of injury, have prolonged the lifespan of mammals such as humans. However, repeated bouts of inflammation, while a necessary prerequisite to fight off infections, can over the years contribute to some of the components known to induce atheroma, the hallmark of CAD. Inflammation, long suspected to contribute to the pathogenesis of atherosclerosis, may be further understood through elucidating the mechanisms whereby genetic variants mediate their risk.

Common features of Genetic risk variants for CAD

The wide-spread use of GWAS to discover genetic risk variants for common polygenic diseases has met with remarkable success. In just over five years, over 2800 genetic variants have been discovered as risk factors for more than 300 diseases***(42).*** The genetic risk variants for CAD have many features in common with each other, and 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 the frequency varying from 2% to 91% ***(see Table 1)***.
2. The relative increased risk of each genetic variant is small, averaging 18% with an Odds Ratio (OR) varying from 2% to 90%.
3. The genetic risk for CAD is related to the number of risk variants inherited, as opposed to the potency of a genetic variant. In a CARDIoGRAM analysis of 23 genetic risk variants for CAD, the average number of genetic risk variants 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, as for other common diseases, are located in DNA sequences that do not code for protein. This means the risk variant mediates its risk for CAD directly or indirectly through regulation of up-stream or down-stream DNA sequences that code for protein.
5. All DNA genetic risk variants need only be assessed once per lifetime, since one's DNA does not change over one's lifetime, and neither do they vary with time, meals, drugs or gender.

Genetic risk variants for CAD indicate factors in addition to cholesterol contribute to the pathogenesis of atherosclerosis and myocardial infarction

It is worthy of note 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; four with hypertension; and one with coronary thrombosis]. The remaining 35 risk variants operate through mechanisms yet to be determined. The immediate surprise is that many other factors other than cholesterol contribute to the pathogenesis of atherosclerosis. This has important implications for both primary and secondary prevention of CAD, as well as treatment for the disease. It has long been assumed that cholesterol is the main culprit and for that reason, our primary and secondary prevention are all aimed at decreasing plasma LDL–C by utilizing drugs that decrease the synthesis of cholesterol or by modifying the diet to decrease cholesterol intake. To prevent CAD will require more comprehensive prevention of both genetic and other environmental risk factors. Research can now be directed towards these new genetic risk factors in 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 of the 35 genetic risk variants of unknown function, their risk will be mediated through only a few pathways. It is self-evident that until we identify these pathways, we are unlikely to be successful in prevention of CAD. The identification of mutations in PCSK9 has already led to the development of new therapies for CAD as described below.

Genetics catalyzed the first drug to lower plasma cholesterol and recent mutations in PCSK9 have again led to the development of a novel therapy

One of the major observations confirming the link between cholesterol and heart disease was from human genetics. In the 1970s, a family with hypercholesterolemia, due to a mutation in the LDL-receptor, experienced heart attacks in their 2nd and 3rd decade of life***(51)***. This observation catalyzed efforts to find a drug to lower plasma levels of LDL-C. Two decades later, a drug which inhibits cholesterol synthesis was introduced. All drugs acting through this mechanism are referred to as statins. Statins provide the main and essentially the only drug, for primary and secondary prevention of hypercholesterolemia with a world-wide budget for statins alone of over $70 billion.

One of the genetic risk variants shown in Table 1 is that of PCSK9, which increases the degradation of LDL–receptors and is associated with hypercholesterolemia***(52)***, and increased mortality from heart disease. Subsequently, mutations in the gene encoding for PCSK9 were identified, and those associated with increased function were associated with higher cholesterol levels and increased cardiac morbidity and mortality. In contrast, mutations inducing loss of function of PCSK9, were associated with hypocholesterolemia and a decreased incidence of MI and death. 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 a lifelong duration of hypocholesterolemia, there were no adverse side effects***(53)***. Several therapies***(54),(55),(56),(57)*** have been developed to inhibit PCSK9 and are now undergoing

clinical trials. The one appearing most promising is a monthly injection of a monoclonal antibody***(56),(57)***. Results of Phase I trials showed no significant side effects and LDL-C reductions of 41% to 58%***(58)***. Phase II trials were in individuals with hypercholesterolemia receiving atorvastatin treatment. Those receiving 80mg atorvastatin alone had a mean reduction of 17% in their LDL-C, versus 72% reduction in LDL-C for those receiving 80mg atorvastatin plus the PCSK9 antibody***(58)***. Phase III clinical trials are currently ongoing. This is a very important development for prevention of heart disease, recognizing that cholesterol is a major culprit in the pathogenesis of CAD. It was recently confirmed in a UK study***(59)*** that only 28% of individuals receiving a statin reached the recommended target for plasma LDL-C. 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. 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 myocardial infarction with therapeutic implications

It is surprising the only genetic risk variant associated primarily with MI (see Table 1), rather than coronary atherosclerosis is that of blood groups A and B. In a CARDIoGRAM study***(34)***, a GWAS was performed in 4,372 patients with documented CAD by angiography and confirmed MI, and 2,739 patients with documented CAD without MI. There was a strong association between the ABO blood group locus at 9q34.2 and MI, however, no association with CAD *per se*. This was replicated in an independent population. Epidemiologists have claimed for decades that blood group O offers protection from MI. A, B and O blood groups are different forms of the same gene at 9q34.2. The A and B genes encode for a protein (alpha-1-3N-acetylgalactosaminyltransferase) which 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 which has been mutated and lacks any biochemical activity and thus does not transfer the carbohydrate moiety onto vWF. Thus, 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 over 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%***(60)***. It has also 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***(61).***
These results have important implications for people undergoing angioplasty, by-pass surgery and other such procedures. Individuals of blood group A or B should be considered for some form of antiplatelet therapy.

Plasma cholesterol, a known risk factor for CAD is under intense genetic regulation

Epidemiological and genetic studies have documented for decades that 80% of cholesterol is synthesized endogenously and plasma levels of LDL–C, HDL–C, triglycerides, and total cholesterol are 60% to 80% regulated by genetic variants***(62),(63),(64)***. The Lipid Consortium and the CARDIoGRAM consortium performed a GWAS on a sample size of over 100,000 individuals, genotyped for genes related to plasma levels of lipids. We identified a total of 95 associated genetic variants***(65)***, and in a follow-up study the Global Lipid Genetics consortium with a sample size of 188,577 individuals, identified an additional 62 genetic variants associated with plasma lipids***(66)***. The combination of the two studies discovered 157 genetic variants associated with plasma lipid levels [55 associated with plasma HDL–C; 37 associated with LDL–C; 54 associated with total plasma cholesterol levels; and 24 associated with plasma triglyceride levels]. The results of GWAS have repeatedly shown that triglyceride is a risk factor for coronary artery disease. These studies along with epidemiological studies strongly indicate that triglyceride levels must be managed along with the management of cholesterol. Interestingly, in a recent study of cardiogram we did not observe any association of genetic variants in HDL–C with MI, despite the life-long exposure to increased plasma levels of HDL–C. In the same studies, genetic variants associated with increased plasma levels of LDL–C were associated with a marked increase risk of MI with ratios of 2.1 as expected from previous studies. Previous studies involving niacin, alcohol, exercise, and statins showing plasma HDL-C protects against CAD are confounded because while these drugs increase HDL–C, they also decreased LDL-C and to some extent plasma triglyceride levels. In our study we selected genetic variants associated solely with an increased plasma HDL-C, without any change in plasma LDL–C or triglycerides. The indication that HDL–C, is not protective of MI is also in keeping with recent clinical trials showing increased plasma HDL–C is not associated with a decreased incidence of cardiac events, including mortality. There is, however, the concern that previous studies related protection to HDL mass, whereas studies for the past four decades have related protection to plasma HDL cholesterol.

Where do genetic risk variants fit in 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. Another 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 (ATPIII). Currently, the ATPIII recommends LDL-C≥190 mg/dl be reduced in individuals with one other risk factor and individuals with LDL-C≤160 mg/dl be reduced if they have 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, are independent of conventional risk factors. The ATP panel could then assess whether individuals positive for 9p21 and no other risk factors should have LDL-C reduced. It is important to note that for 9p21, in individuals with premature CAD, that it is associated with a two-fold increased risk—this is greater than the risk from smoking or that associated with a moderate increase in blood pressure or plasma LDL-C.

A promising future for the prevention of CAD

The findings of genetic risk variants for CAD and other diseases are permanently incorporated into our genome. Their utilization to improve the prevention and treatment of CAD will undoubtedly occur in the future. It took 20 years from the time a genetic defect was discovered in the LDL-receptor was shown to be associated with hypercholesterolemia and premature MI until a drug was developed to lower plasma cholesterol. That drug of course is known today as a statin and is almost the sole treatment for prevention of CAD. It is less than 10 years since the first mutation was identified in PCSK9 and shown to be associated with plasma LDL-C. A monoclonal antibody to inhibit PCSK9 is already in Phase III Clinical Trials and shown to be a safe and potent therapy to lower LDL-C and decrease morbidity and mortality from CAD.

The challenge for the next decade will be to identify the molecular mechanisms mediating the risk of those genetic risk variants, particularly those that do not act through known conventional risk factors. There is considerable evidence***(41),(67)*** that several of these genetic risk variants predispose to CAD through inflammatory pathways. It is reasonable to assume that genetic risk variants will lead to further markers for earlier detection of CAD as well as other drug therapies to interrupt or attenuate the risk.

Table1: Genetic risk variants associated with coronary artery disease or myocardial infarction

Chromosonal Location	Single Nucleotide Polymorphism (SNP)	Nearby Genes	Frequency of Risk Variant in population	Odds Ratio (95% CI)
Risk Variant associated with LDL Cholesterol				
6q25.3	rs3798220	LPA		1.92 (1.48–2.49)
2p24.1	rs515135	APOB	2%	1.03
1p13.3	rs599839	SORT1	83%	1.29 (1.18–1.40)
19p13.2	rs1122608	LDLR	78%	1.14 (1.09–1.19)
19q13.32	rs2075650	APOE	77%	1.14 (1.09-1.19)
2p21	rs6544713	ABCG5-ABCG8	14%	1.07 (1.04-1.11)
1p32.3	rs11206510	PCSK9	29%	1.15 (1.10–1.21)
Risk Variant associated with HDL Cholesterol				
6p21.31	rs12205331	ANKS1A	81%	1.04
Risk Variants associated with Triglycerides				
8q24.13	rs10808546	TRIB1	65%	1.08 (1.04-1.12)
11q23.3	rs964184	ZNF259, APOA5-A4-C3-A1	13%	1.13 (1.10–1.16)
Risk Variants associated with Hypertension				
12q24.12	rs3184504	SH2B3	44%	1.13 (1.08–1.18)
10q24.32	rs12413409	CYP17A1, CNNM2, NT5C2	89%	1.12 (1.08–1.16)
4q31.1	rs7692387	GUCYA3	81%	1.13
15q26.1	rs17514846	FURIN-FES	44%	1.04
Risk Variant associated with increased coronary thrombosis and myocardial infarction				
9q34.2	rs579459	ABO	21%	1.10 (1.07–1.13)
Risk Variants whose mechanism for mediating risk for CAD is unknown				
9p21.3	rs4977574	CDKN2A,CDKN2B	46%	1.25 (1.18–1.31) to 1.37 (1.26–1.48)
1q41	rs17465637	MIA3	74%	1.20 (1.12–1.30)
10q11.21	rs1746048	CXCL12	87%	1.33 (1.20–1.48)
2q33.1	rs6725887	WDR12	15%	1.16 (1.10–1.22)
6p24.1	rs12526453	PHACTR1	67%	1.13 (1.09–1.17)
21q22.11	rs9982601	MRPS6	15%	1.19 (1.13–1.27)
3q22.3	rs2306374	MRAS	18%	1.15 (1.11–1.19)
10p11.23	rs2505083	KIAA1462	42%	1.07 (1.04–1.09)
1p32.2	rs17114036	PPAP2B	91%	1.17 (1.13–1.22)
5q31.1	rs2706399	IL5	48%	1.02 (1.01-1.03)
6q23.2	rs12190287	TCF21	62%	1.08 (1.06–1.10)
7q22.3	rs10953541	BCAP29	75%	1.08 (1.05–1.11)
7q32.2	rs11556924	ZC3HC1	62%	1.09 (1.07–1.12)
10q23.31	rs1412444	LIPA	34%	1.09 (1.07–1.12)
11q22.3	rs974819	PDGF	29%	1.07 (1.04–1.09)
13q34	rs4773144	COL4A1, COL4A2	44%	1.07 (1.05–1.09)
14q32.2	rs2895811	HHIPL1	43%	1.07 (1.05–1.10)
15q25.1	rs3825807	ADAMTS7	57%	1.08 (1.06-1.10)
17p13.3	rs216172	SMG6, SRR	37%	1.07 (1.05–1.09)
17p11.2	rs12936587	RASD1, SMCR3, PEMT	56%	1.07 (1.05–1.09)
17q21.32	rs46522	UBE2Z, GIP, ATP5G1, SNF8	53%	1.06 (1.04–1.08)
5p13.3	rs11748327	IRX1, ADAMTS16	76%	1.25 [1.18-1.33]
6p22.1	rs6929846	BTN2A1	6%	1.51 (1.28–1.77)
6p24.1	rs6903956	C6orf105	7%	1.65 (1.44–1.90)
6p21.3	rs3869109	HCG27 and HLA-C	60%	1.15
1q21	rs4845625	IL6R	47%	1.09
Chr4	rs1878406	EDNRA	15%	1.09
7p21.1	rs2023938	HDAC9	10%	1.13
2p11.2	rs1561198	VAMP5-VAMP8	45%	1.07
Chr2	rs2252641	ZEB2-AC074093.1	45%	1
Chr5	rs273909	SLC22A4-SLC22A5	14%	1.11
6p21	rs10947789	KCNK5	76%	1.01
6q26	rs4252120	PLG	73%	1.07
8p22	rs264	LPL	86%	1.06
13q12	rs9319428	FLT1	32%	1.1

Reference List

(1) Ku CS, Naidoo N, Pawitan Y. Revisiting Mendelian disorders through exome sequencing. Hum Genet 2011 Apr;129 (4):351 -70.

(2) Jarcho JA, McKenna W, Pare JA, et al. Mapping a gene for familial hypertrophic cardiomyopathy to chromosome 14q1. N Engl J Med 1989 Nov 16;321(20):1372-8.

(3) Hejtmancik JF, Brink PA, Towbin J, et al. Localization of gene for familial hypertrophic cardiomyopathy to chromosome 14q1 in a diverse US population. Circulation 1991 May;83(5):1592-7.

(4) Olson TM, Michels VV, Thibodeau SN, Tai Y-S, Keating MT. Actin mutations in dilated cardiomypathy, a heritable form of heart failure. Science 1998;280:750-2.

(5) Gollob MH, Green MS, Tang AS, et al. Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. N Engl J Med 2001 June 14;344(24):1823-31.

(6) Brugada R, Tapscott T, Czernuszewicz GZ, et al. Identification of a genetic locus for familial atrial fibrillation. N Engl J Med 1997 March 27;336(13):905-11.

(7) Wang Q, Shen J, Splawski I, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell 1995;80:805-11.

(8) Chen Q, Kirsch GE, Zhang D, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature 1998 March 19;392(6673):293-6.

(9) Chronic Disease Prevention Alliance of Canada. Investing in our Future: Preventing Chronic Diseases in Canada. 2003.

(10) American Heart Association Heart Disease and Stroke Statistics - 2005 Update. Dallas, Texas: American Heart Assocation; 2005.

(11) Murray CJ, Lopez AD. Measuring the global burden of disease. N Engl J Med 2013 August 1;369(5):448-57.

(12) Wald NJ, Law MR. A strategy to reduce cardiovascular disease by more than 80%. BMJ 2003;326:1419-23.

(13) Yusuf S, Hawken S, Ounpuu S, et al. INTERHEART Study Investigators. Lancet 2004;364:937-52.

(14) Shepherd J, Cobbe SM, Ford I, et al. Prevention of Coronary Heart Disease with Pravastatin in Men with Hypercholesterolemia. N Engl J Med 1995 November 16;333(20):1301-8.

(15) Downs JR, Clearfield M, Weis S, 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.

(16) Roberts R. Personalized Medicine: An Idea whose time is approaching. JMDHC 2007;III(1):3-7.

(17) Roberts R, Wells GA, Stewart AF, et al. The genome-wide association study--a new era for common polygenic disorders. J Cardiovasc Transl Res 2010 June;3(3):173-82.

(18) Morgan T, Carney S, Roberts R. Changes in plasma renin activity and blood pressure after acute and chronic administration of beta-adrenergic receptor-blocking drugs. Clin Sci Mol Med Suppl 1975 June;2:81s-3s.

(19) Anand S, Xie C, Pare G, et al. Genetic variants associated with Myocardial Infarction risk factors in over 8,000 individuals from five ethnic groups - INTERHEART Study. Circ Cardiovas Genet 2009;2:16-25.

(20) Roberts R. New Gains in Understanding Coronary Artery Disease. Interview with Dr. Robert Roberts. Affymetrix Microarray Bulletin 2007;3(2):1-4.

(21) LaFramboise T. Single nucleotide polymorphism arrays: a decade of biological, computational and technological advances. Nucleic Acids Res 2009;37(13):4181-93.

(22) Gibbs RA. The International HapMap Project. Nature 2003 December 18;426(6968):789-96.

(23) Risch N, Merikangas K. The Future of Genetic Studies of Complex Human Diseases. Science 1996 September 13;273(5281):1516-7.

(24) Petretto E, Liu ET, Aitman T. A gene harvest revealing the archeology and complexity of human disease. Nat Genet 2007;39(11):1299-301.

(25) Roberts R, Stewart AF, Wells GA, et al. Identifying genes for coronary artery disease: An idea whose time has come. Can J Cardiol 2007 August;23 Suppl A:7A-15A.

(26) McPherson R, Pertsemlidis A, Kavaslar N, et al. A common allele on chromosome 9 associated with coronary heart disease. Science 2007 June 8;316(5830):1488-91.

(27) Helgadottir A, Thorleifsson G, Manolescu A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science 2007 June 8;316(5830):1491-3.

(28) 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.

(29) 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 February;40(2):217-24.

(30) 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 July;13(3):306-19.

(31) Roberts R. Genetics of coronary artery disease. Circ Res 2014 June 6;114(12):1890-903.

(32) 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 August 3;56(6):479-86.

(33) Horne BD, Carlquist JF, Muhlestein JB, et al. Association of variation in the chromosome 9p21 locus with myocardial infarction versus chronic coronary artery disease. Circ Cardiovasc Genet 2008 December;1(2):85-92.

(34) 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 Jan29;377(9763):383-92.

(35) Ardissino D, Berzuini C, Merlini PA, et al. Influence of 9p21.3 Genetic Variants on Clinical and Angiographic Outcomes in Early-Onset Myocardial Infarction. JACC 2011;58(4):426-34.

(36) 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(4):357-9.

(37) Preuss M, Konig IR, Thompson JR, 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 October;3(5):475-83.

(38) Schunkert H, Konig IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 2011 April;43(4):333-8.

(39) 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 April;43(4):339-44.

(40) IBC 50K CAD Consortium. Large-scale gene-centric analysis identifies novel variants for coronary artery disease. PLoS Genet 2011 September;7(9):e1002260.

(41) Deloukas P, Kanoni S, Willenborg C, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet 2013 January;45(1):25-33.

(42) 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.

(43) Stranger BEFM, Dunning M, Ingle CE, et al. Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science 2007;315(5813):848-53.

(44) Cavalli-Sforza LL, Feldman MW. The application of molecular genetic approaches to the study of human evolution. Nat Genet %2003 Mar ;33 Suppl:266 -75 2003 March.

(45) McDougall I, Brown FH, Fleagle JG. Stratigraphic placement and age of modern humans from Kibish, Ethiopia. Nature %2005 Feb 17;433 (7027):733 -6(7027:733-6).

(46) Finlayson C. Biogeography and evolution of the genus Homo. Trends Ecol Evol %2005 Aug ;20 (8):457 -63 Epub 2005 Jun 13(8:457-63):Epub.

(47) Levin L, Zhidkov I, Gurman Y, et al. Functional recurrent mutations in the human mitochondrial phylogeny: dual roles in evolution and disease. Genome Biol Evol 2013 ;5(5):876 -90.

(48) Carlson Chris. Considerations for SNP Selection. In: Michael P.Winer SGJCS, editor. Genetic variation: a laboratory manual. Cold Spring Harbor Laboratory Press; 2007. p. 263-81.

(49) Bhangale TR, Rieder MJ, Livingston RJ, Nickerson DA. Comprehensive identification and characterization of diallelic insertion-deletion polymorphisms in 330 human candidate genes. Hum Mol Genet 2005 January 1;14(1):59-69.

(50) Sun JX, Helgason A, Masson G, et al. A direct characterization of human mutation based on microsatellites. Nat Genet 2012 October;44(10):1161-5.

(51) Brown MS, Goldstein JL. Familial hypercholesterolemia: A genetic defect in the low-density lipoprotein receptor. N Engl J Med 1976 June 17;294(25):1386-90.

(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 February 4;100(3):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 March 23;354(12):1264-72.

(54) Graham MJ, Lemonidis KM, Whipple CP, et al. Antisense inhibition of proprotein convertase subtilisin/kexin type 9 reduces serum LDL in hyperlipidemic mice. J Lipid Res 2007 April;48(4):763-7.

(55) 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.

(56) Sjouke B, Kusters DM, Kastelein JJ, Hovingh GK. Familial hypercholesterolemia: present and future management. Curr Cardiol Rep 2011 December;13(6):527-36.

(57) Ni YG, Di MS, Condra JH, 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 January;52(1):78-86.

(58) Stein EA, Mellis S, Yancopoulos GD, et al. Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. N Engl J Med 2012 March 22;366(12):1108-18.

(59) Hovingh GK, Davidson MH, Kastelein JJ, O'Connor AM. Diagnosis and treatment of familial hypercholesterolaemia. Eur Heart J 2013 February 14.

(60) 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 September;32(9):2314-20.

(61) Gill JC, Endres-Brooks J, Bauer PJ, et al. The effect of ABO blood group on the diagnosis of von Willebrand disease. Blood 1987 June;69(6):1691-5.

(62) Roberts R. Genetics of premature myocardial infarction. Curr Atheroscler Rep 2008 June;10(3):186-93.

(63) Rader DJ, Maugeais C. Genes influencing HDL metabolism: new perspectives and implications for atherosclerosis prevention . Mole Med Today 2000;6:170-5.

(64) Cohen JC, Kiss RS, Pertsemlidis A, et al. Multiple rare alleles contribute to low plasma levels of HDL cholesterol. Science 2004 August 6;305(5685):869-72.

(65) Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 2010 August 5;466(7307):707-13.

(66) Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. Nat Genet 13 A.D. November 11;2013 Nov;45(11):1274-83..

(67) Huan T, Zhang B, Wang Z, et al. A Systems Biology Framework Identifies Molecular Underpinnings of Coronary Heart Disease. Arterioscler Thromb Vasc Biol 2013 March 28.