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9P21.3 locus; An Important Region in Coronary Artery Disease: A Panel Approach to Investigation of the Coronary Artery Disease Etiology

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Abstract

Coronary artery disease (CAD) is a disease of major concern worldwide. It is the main cause of mortality in many societies and improving the understanding about the CAD mechanism, progression and treatment, is necessary. Recent discovery of genetic factors underlying CAD has improved our knowledge of the disease in support of well-known traditional risk factors. Genotype-environment interaction is known as the main risk factor. Loci on many different chromosomes have been identified as a risk factors that increase CAD susceptibility. Here we performed a comprehensive literature review pinpointing hotspot loci involved in CAD pathogenicity. The 9p21.3 locus is the most common region associated with CAD and its specific structure and function have been remarkable in many studies. Moreover, the variations in the 9p21.3 locus have been implicated in CAD patients in different populations around the world. According to conclusions from this the 9p21.3 locus can be the first point of focus in etiology investigations of CAD patients.

INTRODUCTION

Cardiovascular diseases (CVD) involve the heart and blood vessels. Coronary heart disease (CHD) is one of a subset of CVD and a consequence of coronary artery disease (CAD) [1]. CAD is due to plaque aggregation in coronary arteries that leads to decreased blood supply to the heart muscle. This can lead to a wide spectrum of clinical manifestations ranging from asymptomatic to disease symptoms such as angina, silent myocardial infarction (MI), acute MI, and/or sudden death. It is a complex multifactorial disease to which genetic and environmental factors contribute. According to World Health Organization (WHO) reports, ischemic heart disease and stroke have been the most common causes of mortality in the last 15 years [2], and CHD as an ischemic heart disease is a global problem for all communities. It seems that national health control decisions are critical for CAD prevention in all populations [3]. Family history as a traditional factor that is independent from other risk factors can be useful in prediction of common diseases, e.g., CAD susceptibility in family members [4]. New technologies in recent years have improved the diagnosis of genetic differences between individuals in interacting with environment factors, such as the increased level of fibrinogen, homocysteine, C-reactive protein, low density lipoproteins (LDL), very low density lipoproteins (VLDL), cholesterol, triglyceride, systolic blood pressure, diastolic blood pressure, body mass index (BMI), lipoprotein A, decreased levels of high density lipoprotein (HDL), type 2 diabetes (T2D), some vitamin deficiencies, cofactor Q10, and other factors that increase over time [5, 6].

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Genetic Factors in Cad Causality

According to twin and pedigree studies, genetic factors account for about 45% of the variation in CAD [7]. Swedish and Danish twin registry studies showed that genetic factors are important contributory causes of death of CHD patients, with the same frequency in males and females [8, 9]. Therefore, genetic-environment interactions have been known as the main risk factor. Linkage studies also helped to identify inherited genes in large families with affected and unaffected individuals in different generations [10]. A monogenic forms of CAD were observed in a few cases of this disease and familial linkage studies established they were due to mutations in genes involved in known pathways of CAD such as lipid metabolism. Some of these cases are associated with increased LDL level, such as mutation in genes LDL receptor, *ApoB-100*, *ARH* and *PCSK9* [11]. Low-density lipoprotein (LDL) consists of cholesteryl ester as the molecular which coated by phospholipid and Apolipoprotein B-100. About 600 mutations in the LDL receptor gene leading to increased level of plasma LDL have been related to familial hypercholesterolemia. Mutations in the *ApoB-100* and *ARH* genes are responsible for reduced LDL uptake from plasma and elevated LDL levels, leading to familial ligand-defective Apo lipoprotein B-100 and autosomal recessive hypercholesterolemia, respectively [12]. The *PCSK9* gene encodes a protease enzyme that is involved in degradation of LDL receptor, and affects LDL metabolism leading to CHD. Overexpression of the *PCSK9* gene, or gain of function mutations, is related to hypercholesterolemia versus loss-of-function mutations, or inactivation of the enzyme, is related to hypocholesterolemia which has a protective effect on CHD. Therefore, we can target the *PCSK9* gene as a way of treatment of CHD in the future [13].

Plasma HDL reduction as a risk factor for CAD is due to mutations in genes such as *ApoA1, ABCA1*, and *LCAT* that cause familial hypoalphalipoproteinemia, Tangier disease, and Norum disease, respectively. Reduction of HDL and increased risk of CAD are observed in these disorders [11]. CAD has also been associated with polymorphisms in genes such as ApoA1-75A, R219K in *ApoA1* and *ABCA1* [14, 15]. High level of plasma triglyceride as a risk factor for CAD is associated with mutations in genes such as *LPL* and *ApoCII* that lead to hyperlipoproteinemia type I and hyperlipoproteinemia type Ib, respectively [11]. Mutations in another group of genes without a direct effect on alteration of plasma lipid levels including *MEF2A*, *LRP6*, *CYP27A1, ST6GALNAC5, ABCG5*, and *ABCG8* also increase the risk of CAD.

Gene *MEF2A* encodes a transcription factor that is expressed in endothelial cells of coronary arteries. Deletion of 7 amino acids of exon 11 of this transcription factor is associated with CAD and MI [16]. In another study three missense mutations including N263S, P279L and G283D identified in *MEF2A* caused reductions in transcription factor activity and hence were associated with CAD [17].

Mutations p.Val99Met and p.*337Qext*20 in the gene *ST6GALNAC5* were associated with CAD in the Iranian population. The mutant alleles of *ST6GALNAC5*, which encodes the sialyltransferase 7e, causes increased activity of the enzyme [18, 19].

Iranloorahatloo et al. demonstrated that the mutation p.Arg225His in gene *CYP27A1* is associated with CAD in the Iranian population. *CYP27A1* encodes the enzyme sterol 27-hydroxylase that is involved in vitamin D metabolism and reverse transportation of cholesterol. The mutant enzyme likely affects the reverse transportation of cholesterol [20]. Gene *LRP6* encodes LDL receptor-related protein 6 and a missense mutation named p.R611C in an Iranian ancestry is associated with CAD. The mutation is associated with high levels of LDL and TG but has no direct effect on HDL level [21]. Mutations in genes *ABCG5* and *ABCG8*, which encode transporters that influence cholesterol absorption, are the most causes of sitosterolemia and involve hyper absorption of sterols and cholesterol, again leading to increased risk of CAD [12].

Recent Approaches in Assessing Diseases Etiology

The recent sequencing of the entire human genome by the Human Genome Project and linkage disequilibrium (LD) patterns revealed in the Hap Map project led to discovery of single nucleotide polymorphisms (SNP) using microarray technology approaches and to the ability to perform genome-wide association studies (GWAS) [10]. GWAS detects statistical associations between SNP as genome markers with the phenotype (disease) with no previous hypothesis [22, 23]. Candidate gene studies are another type of association analysis. This type of study has been used for complex diseases and is based on comparisons of selected loci or alleles identified in earlier studies [22]. Since candidate gene studies are based on previous hypotheses loci in unknown pathways of disease pathogenesis cannot be studied. Conflicting and difficult confirmation results are disadvantages of Candidate gene study [24], Whole-genome sequencing (WGS) is a technology based on sequencing entire genomes and the method has focused mainly on simply inherited disorders and identification of rare variants with large effects [22]. Due to lack of distinction between causal and non-causal variants in the pathogenesis of the disease, this approach is useful for identification of mutations or genes that were confirmed in previous studies [25].

In addition to genomics studies, proteomics studies are helpful in identification of new genes associated with CAD [26]. You et al. compared the proteomes of

coronary arteries of CAD patients and control individuals. Two-dimensional electrophoresis and mass spectrometry showed elevated expression of light chain ferritin in CAD patients that was possibly associated with CAD by oxidation of lipid components of coronary artery plaques [27]. Recently designed commercial microarrays consisting of many common SNP distributed throughout the human genome have been used in different populations to locate genome markers associated with CAD. GWAS are based on allele frequencies and statistical differences among thousands of disease cases and control populations. The required level of statistical significance for confirmation of associated variants with disease in GWAS is set at $P = 5\times10^{-8}$. Associated markers when identified should be confirmed in different ethnic populations around the world [28]. GWAS was first proposed in the mid-1990s and 2006 saw the beginning of data from such studies [10]. The first robustly associated locus with CAD was located at position 9p21.3 on the short arm of chromosome 9 three independent GWAS in 2007 [29-31].

Genetic Association Studies of CAD during the Last Decade

1. Highlights for the Year 2007

McPherson et al. reported a 58 kb region in locus 9p21 that was associated with CHD in six Caucasian individuals and identified homozygous form of risk alleles increased CHD susceptibility about 35% [29]. Helgadottir et al. described a risk variant in 9p21.3 that was associated with MI. They concluded that the risk of MI in individuals homozygous for this allele was 1.64-fold more than heterozygous carriers [30]. Another study, the Welcome Trust Case Control Consortium (WTCCC) reported a survey of common human diseases such as CAD in about 50 cohorts from different places of the United Kingdom, and nominated 9p21.3 as a strongest associated locus with CAD. Other loci were also implicated in this study and some of them were confirmed in further studies [31]. Samani et al, in another GWAS combining data from two significant studies of white European populations, WTCCC and a German MI family study, found that 9p21.3, 6p25.1 and 2q36.3 were associated with CAD. Moreover, their combined analysis identified four potentially novel loci with high probabilities of association with CAD: 1p13.3, 1q41, 10q11.21, and 15q22.33 [32].

2. Highlights for the Year 2009

Seven loci, including 9p21.3, 6p25.1, 2q36.3, 1p13.3, 1q41, 10q11.21, and 15q22.33, which discovered in 2007 in a GWAS of nine European populations; 9p21.3 was clearly associated with CAD, and there was also convincing evidence for associations of the *SORT1* (1p13.3), *MIA3* (1q41) and *CXCL12* (10q11.21) loci with CAD, but not 6p25.1, 2q36.3 and 15q22.33 [33]. Erdmann et al. in a three-stage GWA analysis of the German population confirmed two loci including 9p21.3 and 1q41 and reported a novel *MRAS* (3q22.3) locus [34]. In another three-stage meta-analysis eight genes were associated with early onset of MI, six of them were reported previously and the novel loci were *SLC5A3-MRPS6-KCNE2* (21q22.11) and *PHACTR1* (6p24.1). They also reported the *WDR12* (2q33.1) locus [35], which was confirmed as a locus associated with CAD by Schunkert et al. in 2011 [36]. From a genome-wide haplotype association (GWHA) study Tregouet et al. discovered a cluster of genes, including *SLC22A3-LPAL2-LPA* (6q26-q27), associated with CAD [37]. Gudbjartsson et al. demonstrated an association of gene *SH2B3* (12q24) with MI in six different populations [38]. In a different study Soranzo et al. found that locus 12q24 was associated with increased platelet counts and an increased risk of CAD. They discovered a large haplotype block of about 1.6 Mb in the 12q24 locus which is associated with CAD. This haplotype block included ten SNP, one of which was a missense mutation in gene *SH2B3*, seven were intronic polymorphisms in genes *ATXN2, C12orf30, C12orf51* and *PTPN11*, and the remaining two were intragenic polymorphisms [39].

3. Highlights for the Years 2010/2011

Schunkert et al. (2011) reported thirteen novel loci as risk factors for CAD in a meta-analysis of 14 GWAS. These loci were *PPAP2B* (1p32.2), *ANKS1A* (6p21.31), *TCF21* (6q23.2), *ZC3HC1* (7q32.2), *ABO* (9q34.2), *CYP17A1-CNNM2-NT5C2* (10q24.32), *ZNF259-APOA5-APOA1* (11q23.3), *COL4A1/A2* (13q34), *HHIPL1* (14q32.2), *ADAMTS7* (15q25.1), *RAI1-PEMT-RASD1* (17p11.20), *SMG6* (17p13.3), and *UBE2Z* (17q21.32) [36]. In another study of European and South Asian cohorts, Mehta reported five novel loci associated with CAD, including *LIPA* (10q23), *PDGFD* (11q22), *ADAMTS7-MORF4L1* (15q25), *BCAP29* (7q22), and *KIAA1462* (10p11) [40]. Locus 10p11.23 was also found by Erdmann et al. (2010) in a study of a German MI Family (GerMIFS) that finally detected a missense mutation in *KIAA1462*, a CAD related gene [41]. Four other novel genes in GWAS of European and South Asian populations included *LIPA* (10q23.31), *IL5* (5q31.1), *TRIB1* (8q24.13), and *ABCG5/ABCG8* (2p21) [42].

4. Highlights for the Year 2013

Deloukas et al. identified thirteen novel loci associated with CAD in a large-scale analysis. They combined data from 14 GWAS (CARDIoGRAM Consortium) with data from 34 additional European and South Asian populations and validate the SNP in four independent populations. In addition to confirming many loci from previous studies they reported a number of new loci associated with CAD, including *IL6R* (1q21), *APOB* (2p24.1), *VAMP5-VAMP8-GGCX* (2p11.2), *SLC22A4-SLC22A5* (Chr5), *ZEB2-AC074093.1* (Chr2), *GUCY1A3* (4q31.1), *KCNK5* (6p21), *LPL* (8p22),

PLG (6q26), *FURIN-FES* (15q26.1), *FLT1* (13q12), *EDNRA* (Chr4) and *HDAC9* (7p21), and *AK097927* (chr2) [43].

5. Highlights for the Year 2015

Nikpay et al. reported ten novel loci associated with CAD, including *REST-NOA1* (4q12), *NOS3* (7q36), *SWAP70* (11p15), *SMAD3* (15q22), *MFGE8-ABHD2* (15q26), *BCAS3* (17q23), *PMAIP1-MC4R* (18q21), *POM121L9P-ADORA2A* (22q11), *KSR2* (12q24), and *ZNF507-LOC400684* (19q13) [44] .

6. Highlights for the Year 2017

Webb et al. added six new loci associated with CAD, including *KCNJ13-GIGYF2* (2q37), *C2* (6p21), *MRVI1-CTR9* (11p15), *LRP1* (12q13), *SCARB1* (12q24), and *CETP* (16q13) [45]. Verweij et al. reported fifteen novel loci, among which there were genes involved in angiogenesis. These were: *TDRKH* (1q21.3), *RHOA-AMT-TCTA* (3p21.31), *UMPS-ITGB5* (3q21.2), *SGEF* (3q25.2), *PRDM8-FGF5* (4q21.21), *MAD2L1* (4q27), *ZNF827* (4q31.21), *HDGFL1* (6p22.3), *ARNTL* (11p15.2), *HOXC4* (12q13.13), *HNF1A* (12q24.31), *TMED10* (14q24.3), *BCAR1* (16q23.1), *CDH13* (16q23.3), and *HNRNPUL1-TGFB1-B9D2* (19q13.2) [46]. In addition, there fifteen additional novel loci reported by Howson et al. identified several genes with various functions such as cell adhesion, leucocyte migration, coagulation, inflammation, VSMC differentiation, and atherosclerosis, these novel loci were *ATP1B1* (chr1), *DX59/CAMSAP2* (chr1), *LMOD1* (chr1), *TNS1* (chr2), ARHGAP26 (chr5), *PARP12* (chr7), *PCNX3* (chr11), *SERPINH1* (chr11), *C12orf43/HNF1A* (chr12), *SCARB1* (chr12), *OAZ2-RBPMS2* (chr15), *DHX38* (chr16), *GOSR2* (chr17), *PECAM1* (chr17), and *PROCR* (chr20) [47].

7. Highlights for the Year 2018

van der Harst and Verweij (2018) upgraded our insight and knowledge of the genetic architecture of CAD by detection of sixty four new loci [48]. All reported loci and their variants are summarized in Table 1.

Table 1. Recent Reports of CAD/MI-Associated loci

No.	Chromosomal location	Gene	Year	Reference
1	9p21.3	CDKN2/AB	2007	[29-31]
2	1p13.3	SORT1	2007-2009	[33]
3	1q41	MIA3	2007-2009	
4	10q11.21	CXCL12	2007-2009	
5	3q22.3	MRAS	2009	[34]
6	21q22.11	SLC5A3-MRPS6-KCNE2	2009	[35]
7	6p24.1	PHACTR1	2009	
8	2q33.1	WDR12	2009-2011	
9	6q26-q27	SLC22A3-LPAL2-LPA	2009	[37]
10	12q24	SH2B3	2009	[38, 39]
11	1p32.2	PPAP2B	2011	[36]
12	6p21.31	ANKS1A	2011	
13	6q23.2	TCF21	2011	
14	7q32.2	ZC3HC1	2011	
15	9q34.2	ABO	2011	
16	10q24.32	CYP17A1-CNNM2-NT5C2	2011	
17	11q23.3	ZNF259-APOA5-APOA1	2011	
18	13q34	COL4A1/A2	2011	
19	14q32.2	HHIPL1	2011	
20	15q25.1	ADAMTS7	2011	[36, 40]
21	17p11.2	RAI1-PEMT-RASD1	2011	
22	17p13.3	SMG6	2011	
23	17q21.32	UBE2Z	2011	
24	10q23	LIPA	2011	[40, 42]
25	11q22	PDGFD	2011	
26	7q22	BCAP29	2011	
27	10p11.23	KIAA1462	2011/2010	[40, 41]
28	5q31.1	IL5	2011	[42]
29	8q24.13	TRIB1	2011	
30	2p21	ABCG5/ABCG8	2011	
31	1q21	IL6R	2013	[43]
32	2p24.1	APOB	2013	
33	2p11.2	VAMP5-VAMP8-GGCX	2013	
34	Chr5	SLC22A4-SLC22A5	2013	
35	Chr2	ZEB2-AC074093.1	2013	
36	4q31.1	GUCY1A3	2013	
37	6p21	KCNK5	2013	
38	8p22	LPL	2013	
39	15q26.1	FURIN-FES	2013	
40	6q26	PLG	2013	
41	13q12	FLT1	2013	

No.	Chromosomal location	Gene	Year	Reference
42	Chr4	EDNRA	2013	
43	7p21.1	HDAC9	2013	
44	4q12	REST-NOA1	2015	[44]
45	7q36	NOS3	2015	
46	11p15	SWAP70	2015	
47	15q22	SMAD3	2015	
48	15q26	MFGE8-ABHD2	2015	
49	17q23	BCAS3	2015	
50	18q21	PMAIP1-MC4R	2015	
51	22q11	POM121L9P-ADORA2A	2015	
52	12q24	KSR2	2015	
53	19q13	ZNF507-LOC400684	2015	
54	2q37	KCNJ13-GIGYF2	2017	[45]
55	6p21	C2	2017	
56	11p15	MRVI1-CTR9	2017	
57	12q13	LRP1	2017	
58	12q24	SCARB1	2017	[45, 47]
59	16q13	CETP	2017	
60	1q21.3	TDRKH	2017	[46]
61	3p21.31	RHOA-AMT-TCTA	2017	
62	3q21.2	UMPS-ITGB5	2017	
63	3q25.2	SGEF	2017	
64	4q21.21	PRDM8-FGF5	2017	
65	4q27	MAD2L1	2017	
66	4q31.21	ZNF827	2017	
67	6p22.3	HDGFL1	2017	
68	11p15.2	ARNTL	2017	
69	12q13.13	HOXC4	2017	
70	12q24.31	HNF1A	2017	
71	14q24.3	TMED10	2017	
72	16q23.1	BCAR1	2017	
73	16q23.3	CDH13	2017	
74	19q13.2	HNRNPUL1-TGFB1-B9D2	2017	
75	chr1	ATP1B1	2017	[47]
76	chr1	DX59/CAMSAP2	2017	
77	chr1	LMOD1	2017	
78	chr2	TNS1	2017	
79	chr5	ARHGAP26	2017	
80	chr7	PARP12	2017	
81	chr11	PCNX3	2017	
82	chr11	SERPINH1	2017	
83	chr12	C12orf43/HNF1A	2017	
84	chr15	OAZ2-RBPMS2	2017	
85	chr16	DHX38	2017	
86	chr17	GOSR2	2017	
87	chr17	PECAM1	2017	
88	chr20	PROCR	2017	
89	1p36.33	MORN1	2018	[48]
90	1p36.32	PRDM16	2018	
91	1p34.3	FHL3	2018	
92	1p13.2	NGF	2018	
93	1q32.2	HHAT	2018	
94	1q42.2	AGT	2018	
95	2p21	PRKCE	2018	
96	2q24.3	FIGN	2018	
97	2q32.1	CALCRL	2018	
98	2q37.3	COL6A3	2018	
99	3p21.31	ALS2CL	2018	
100	3p21.31	CDC25A	2018	
101	3q22.1	DNAJC13	2018	
102	3q22.3	STAG1	2018	
103	3q25.31	CCNL1	2018	
104	3q26.31	FNDC3B	2018	
105	4p16.3	HGFAC-RGS12	2018	
106	4q21.1	SHROOM3	2018	
107	4q21.22	HNRNPD	2018	
108	4q22.3	UNC5C	2018	
109	4q32.3	PALLD	2018	
110	5p15.31	SEMA5A	2018	
111	5q11.2	MAP3K1	2018	

No.	Chromosomal location	Gene	Year	Reference
112	6p25.3	FOXC1	2018	
113	6p21.2	CDKN1A	2018	
114	6p21.1	VEGFA	2018	
115	6p11.2	PRIM2	2018	
116	6q14.1	FAM46A	2018	
117	6q22.32	CENPW	2018	
118	6q25.1	PLEKHG1	2018	
119	7p22.3	MAD1L1	2018	
120	7p22.1	DAGLB	2018	
121	7p21.3	TMEM106B	2018	
123	7p13	CCM2	2018	
124	7q31.2	CTTNBP2	2018	
125	8p22	NAT2	2018	
126	8p21.3	BMP1	2018	
127	8q23.1	ZFPM2	2018	
128	9q31.2	KLF4	2018	
129	9q33.2	DAB2IP	2018	
130	10p13	CDC123	2018	
131	10q23.1	TSPAN14	2018	
132	10q24.33	STN1	2018	
133	10q26.13	HTRA1	2018	
134	11p15.4	TRIM5-TRIM22	2018	
135	11p11.2	HSD17B12	2018	
136	11q22.1	ARHGAP42	2018	
137	12p13.31	C1S	2018	
138	12q22	NDUFA12	2018	
139	13q13.1	N4BP2L2-PDS5B	2018	
140	13q34	MCF2L	2018	
141	14q23.1	ARID4A	2018	
142	14q32.13	SERPINA2	2018	
143	15q26.2		2018	
144	16q23.3	PLCG2	2018	
145	17q11.2	CORO6-ANKRD13B	2018	
146	17q11.2	COPRS	2018	
147	17q21.2	DHX58-KAT2A	2018	
148	18q21.1	ACAA2	2018	
149	19p13.11	MAP1S-FCHO1	2018	
150	20q12	ZHX3	2018	
151	20q13.12	PCIF1-ZNF335	2018	
152	20q13.32	ZNF831	2018	
153	21q21.3	MAP3K7CL	2018	

CDKN2/AB, Cyclin Dependent Kinase Inhibitor 2/AB; *SORT1*, Sortilin 1; *MIA3*, MIA SH3 domain ER export factor 3; *CXCL12*, C-X-C motif chemokine ligand 12; *MRAS*, muscle RAS oncogene homolog; *SLC5A3*, solute carrier family 5 member 3; *MRPS6*, mitochondrial ribosomal protein S6; *KCNE2*, potassium voltage-gated channel subfamily E regulatory subunit 2; *PHACTR1*, phosphatase and actin regulator 1; *WDR12*, WD repeat domain 12; *SLC22A3*, solute carrier family 22 member 3; *LPAL2*, lipoprotein(a) like 2, pseudogene; *LPA*, lipoprotein(a); *SH2B3*, SH2B adaptor protein 3; *PPAP2B*, phospholipid phosphatase 3; *ANKS1A*, ankyrin repeat and sterile alpha motif domain containing 1A; *TCF21*, transcription factor 21; *ZC3HC1*, zinc finger C3HC-type containing 1; *ABO*, ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase; *CYP17A1*, cytochrome P450 family 17 subfamily A member 1; *CNNM2*, cyclin and CBS domain divalent metal cation transport mediator 2; *NT5C2*, 5'-nucleotidase, cytosolic II; *ZNF259*, zinc finger protein 259; *APOA5*, apolipoprotein A5; *APOA1*, apolipoprotein A1; *COL4A1*, collagen type IV alpha 1 chain; *COL4A2*, collagen type IV alpha 2 chain; *HHIPL1*, HHIP like 1; *ADAMTS7*, ADAM metallopeptidase with thrombospondin type 1 motif 7; *RAI1*, retinoic acid induced 1; *PEMT*, phosphatidylethanolamine N-methyltransferase; *RASD1*, ras related dexamethasone induced 1; *SMG6*, SMG6, nonsense mediated mRNA decay factor; *UBE2Z*, ubiquitin conjugating enzyme E2 Z; *LIPA*, lipase A, lysosomal acid type; *PDGFD*, platelet derived growth factor D; *MORF4L*1, mortality factor 4 like 1; *BCAP29*, B cell receptor associated protein 29; *KIAA1462*, JCAD junctional cadherin 5 associated; *IL5*, interleukin 5; *TRIB1*, tribbles pseudokinase 1; *ABCG5*, ATP binding cassette subfamily G member 5; *ABCG8*, ATP binding cassette subfamily G member 8; *IL6R*, interleukin 6 receptor; *APOB*, apolipoprotein B; *VAMP5*, vesicle associated membrane protein 5 *VAMP8*, vesicle associated membrane protein 8; *GGCX*, gamma-glutamyl carboxylase; *SLC22A4*, solute carrier family 22 member 4; *SLC22A5*, solute carrier family 22 member 5; *ZEB2*, zinc finger E-box binding homeobox 2.; *GUCY1A3*, guanylate cyclase 1 soluble subunit alpha 1; *KCNK5*, potassium two pore domain channel subfamily K member 5; *LPL*, lipoprotein lipase; *FURIN*, furin, paired basic amino acid cleaving enzyme; *FES*, FES proto-oncogene, tyrosine kinase; *PLG*, plasminogen; *FLT1*, fms related tyrosine kinase 1; *EDNRA*, endothelin receptor type A; *HDAC9*, histone deacetylase 9; *REST*, RE1 silencing transcription factor; *NOA1*, nitric oxide associated 1; *NOS3*, nitric oxide synthase 3; *SWAP70*, switching B cell complex subunit SWAP70; *SMAD3*, SMAD family member 3; *MFGE8*, milk fat globule-EGF factor 8 protein; *ABHD2*, abhydrolase domain containing 2; *BCAS3*, BCAS3, microtubule associated cell migration factor; *PMAIP1*, phorbol-12-myristate-13-acetate-induced protein 1; *MC4R*, melanocortin 4 receptor; *POM121L9P*, POM121 transmembrane nucleoporin like 9, pseudogene; *ADORA2A*, adenosine A2a receptor; *KSR2*, kinase suppressor of ras 2; *ZNF507*, zinc finger protein 507; *KCNJ13*, potassium voltage-gated channel subfamily J member 13; *GIGYF2*, GRB10 interacting GYF protein 2; *C2*, complement C2; *MRVI1*, murine retrovirus integration site 1 homolog; *CTR9*, CTR9 homolog, Paf1/RNA polymerase II complex component; *LRP1*, LDL receptor related protein 1; *CETP*, cholesteryl ester transfer protein; *TDRKH*, tudor and KH domain containing; *RHOA*, ras homolog family member A; *AMT*, aminomethyltransferase; *TCTA*, T cell leukemia translocation altered; *UMPS*, uridine monophosphate synthetase; *ITGB5*, integrin subunit beta 5; *SGEF*, Rho guanine nucleotide exchange factor (GEF) 26; *PRDM8*, PR/SET domain 8; *FGF5*, fibroblast growth factor 5; *MAD2L1*, mitotic arrest deficient 2 like 1; *ZNF827*, zinc finger protein 827; *HDGFL1*, HDGF like 1; *ARNTL*, aryl hydrocarbon receptor nuclear translocator like; *HOXC4*, homeobox C4; *HNF1A*, HNF1 homeobox A; *TMED10*, transmembrane p24 trafficking protein 10; *BCAR1*, breast cancer anti-estrogen resistance 1; *CDH13*, cadherin 13; *HNRNPUL1*, heterogeneous nuclear ribonucleoprotein U like 1; *TGFB1*, transforming growth factor beta 1; *B9D2*, B9 domain containing 2; *ATP1B1*, ATPase Na+/K+ transporting subunit beta 1; *DX59/CAMSAP2*, calmodulin regulated spectrin associated protein family member 2; *LMOD1*, leiomodin 1; *TNS1*, tensin 1; *ARHGAP26*, Rho GTPase activating protein 26; *PARP12*, poly(ADP-ribose) polymerase family member 12; *PCNX3*, pecanex 3; *SERPINH1*, serpin family H member 1; *C12orf43*, chromosome 12 open reading frame 43; *HNF1A*, HNF1 homeobox A; *SCARB1*, scavenger receptor class B member 1; *OAZ2*, ornithine decarboxylase antizyme 2; *RBPMS2*, RNA binding protein, mRNA processing factor 2; *DHX38*, DEAH-box helicase 38; *GOSR2*, golgi SNAP receptor complex member 2; *PECAM1*, platelet and endothelial cell adhesion molecule 1; *PROCR*, protein C receptor; *MORN1*, MORN repeat containing 1; *PRDM16*, PR/SET

domain 16; *FHL3*, four and a half LIM domains 3; *NGF*, nerve growth factor; *HHAT*, hedgehog acyltransferase; *AGT*, angiotensinogen; *PRKCE*, protein kinase C epsilon; *FIGN*, fidgetin, microtubule severing factor; *CALCRL*, calcitonin receptor like receptor; *COL6A3*, collagen type VI alpha 3 chain; *ALS2CL*, ALS2 C-terminal like; *CDC25A*, cell division cycle 25A; *DNAJC13*, DnaJ heat shock protein family (Hsp40) member C13; *STAG1*, stromal antigen 1; *CCNL1*, cyclin L1; *FNDC3B*, fibronectin type III domain containing 3B; *RGS12*, regulator of G protein signaling 12; *HGFAC*, HGF activator; *SHROOM3*, shroom family member 3; *HNRNPD*, heterogeneous nuclear ribonucleoprotein D; *UNC5C*, unc-5 netrin receptor C; *PALLD*, palladin, cytoskeletal associated protein; *SEMA5A*, semaphorin 5A; *MAP3K1*, mitogen-activated protein kinase kinase kinase 1; *FOXC1*, forkhead box C1; *CDKN1A*, cyclin dependent kinase inhibitor 1A; *VEGFA*, vascular endothelial growth factor A; *PRIM2*, DNA primase subunit 2; *FAM46A*, family with sequence similarity 46 member A; *CENPW*, centromere protein W; *PLEKHG1*, pleckstrin homology and RhoGEF domain containing G1; *MAD1L1*, mitotic arrest deficient 1 like 1; *DAGLB*, diacylglycerol lipase beta; *TMEM106B*, transmembrane protein 106B; *CCM2*, CCM2 scaffold protein; *CTTNBP2*, cortactin binding protein 2; *NAT2*, N-acetyltransferase 2; *BMP1*, bone morphogenetic protein 1; *ZFPM2*, zinc finger protein, FOG family member 2; *KLF4*, Kruppel like factor 4; *DAB2IP*, DAB2 interacting protein; *CDC123*, cell division cycle 123; *TSPAN14*, tetraspanin 14; *STN1*, STN1, CST complex subunit; *HTRA1*, HtrA serine peptidase 1; *TRIM22*, tripartite motif containing 22; *TRIM5*, tripartite motif containing 5; *HSD17B12*, hydroxysteroid 17-beta dehydrogenase 12; *ARHGAP42*, Rho GTPase activating protein 42; *C1S*, complement C1s; *NDUFA12*, NADH:ubiquinone oxidoreductase subunit A12; *PDS5B*, PDS5 cohesin associated factor B; *N4BP2L2*, NEDD4 binding protein 2 like 2; *MCF2L*, MCF.2 cell line derived transforming sequence like; *ARID4A*, AT-rich interaction domain 4A; *SERPINA2*, serpin family A member 2 (gene/pseudogene); *PLCG2*, phospholipase C gamma 2; *ANKRD13B*, ankyrin repeat domain 13B; *CORO6*, coronin 6; *COPRS*, coordinator of PRMT5 and differentiation stimulator; *KAT2A*, lysine acetyltransferase 2A; *DHX58*, DExH-box helicase 58; *ACAA2*, acetyl-CoA acyltransferase 2; *FCHO1*, FCH domain only 1; *MAP1S*, microtubule associated protein 1S; *ZHX3*, zinc fingers and homeoboxes 3; *ZNF335*, zinc finger protein 335; *PCIF1*, PDX1 C-terminal inhibiting factor 1; *ZNF831*, zinc finger protein 831; *MAP3K7CL*, MAP3K7 C-terminal like.

8. Other Reports

In addition to the foregoing studies of European populations, other studies evaluated CAD-associated loci in East Asian populations. Some of these studies are reviewed below. Several GWAS studies tried to identify novel loci involved in lipid metabolism in association with CAD; for example, Willer et al. (2008) confirmed strong associations between previously identified genes and reported new loci involved in lipid metabolism. Those confirmed in later studies included genes *PCSK9* (1p32.3), *LDLR* (19p13.2), and *APOE* (19q13.32) [49]. By 2013 157 loci were associated with lipid metabolism 157 [50]. Siewert et al. (2018) discovered six previously unreported loci associated with TG, LDL and total cholesterol and with CAD. These researchers performed a bivariate GWAS by combining data from a meta-analysis of CAD and the Global Lipid Genetics Consortium and detected variants causing increased levels of triglyceride, LDL and cholesterol associated with CAD [51]. Yamada et al. (2011) performed a GWAS study that identified locus *BTN2A1* (6p22.1) associated with MI [52]. This gene caused hypertension in the Japanese population [53] and was probably associated with MI as a consequence. In the same year, Wang et al. (2011) discovered a *C6orf105* (6p24.1) variant in a Chinese population. They reported SNP rs6903956 polymorphism that was associated with a reduced level C6orf105 mRNA and CAD susceptibility [54]. In a meta-analysis by combination of two GWAS Lu et al. (2012) discovered and later confirmed four loci in Chinese populations, including *TTC32-WDR35* (2p24.1), *GUCY1A3* (4q32.1), *C6orf10-BTNL2* (6p21.32), and *ATP2B1* (12q21.33) and confirmed SNP in the 9p21.3, *PHACTR1*, *TCF21*, *C12orf51* loci that were identified previously in European populations [55]. Takeuchi et al. (2018) reported the association of three loci with CAD including *BRAP* and *ALDH2* (12q24) and *MHC* (6p21) and confirmation of 9p21.3 in a Japanese population [56]. Lee et al. (2013) performed a GWAS in a Korean and Japanese population and loci which previously identified in European populations including 9p21.3, 1p13.3 and 11q22.3 were confirmed. They found a strong association of an rs3782889 variant in gene *MYL2* (12q24.11) with CAD [57]. A recent study of the Japanese population identified twenty-six novel loci associated with early onset of CAD [58].

Although GWAS identified many loci or genes associated with CAD providing new insights on CAD pathophysiology, the specific clinical manifestations of those variants were not clear because very few of the variants were confirmed in other populations [59]. The best confirmed locus from all GWAS is 9p21.3. Different variants in this important locus were identified in GWAS confirmation many different ethnic groups emphasize the importance role of the region in CAD.

Importance of Locus 9p21.3 in CAD

The locus most often implicated in GWAS and in confirmation replication studies worldwide was 9p21.3. Clearly, this locus has an important role in CAD. The frequency of the risk alleles of 9p21.3 in different populations was variable, with the highest frequency of risk variants found in Europeans population (50%) and least in the African American population (24%) [60]. Surprisingly, some variants in this locus apparently protect the African American population from CAD [61]. The risk of CAD in homozygous mutant individuals is twofold greater than in heterozygous individuals [29, 62] indicating gene dosage effects of at least some SNPs in this locus on the disease. Gene dosage of 9p21.3 variants is also related to disease severity [63]. There is an association of particular 9p21.3 variants with disease severity and mortality frequency [64] but quantitative factors in different populations produce confounding results. For example, the quantitative criteria for disease severity provided by quantitative coronary angiograms (QCA) based on vessel lumen diameter and numbers of lesions in coronary arteries in Caucasian individuals in a Lipoprotein and Coronary Atherosclerosis Study (LCAS) did not confirm any association of 9p21.3 variation and CAD severity, the Gensini scoring system based on the rate of stenosis in coronary arteries confirmed the association of 9p21.3 variation and CAD severity in the Chinese population [65]. Also, two semi-quantitative systems for scoring the severity and extent

of CAD confirmed the effect of 9p21.3 on the severity and progression of CAD [66].

In addition to possible effects of 9p21.3 on CAD severity, there is some evidence that shows an association of 9p21.3 variants with age at the time of disease onset [67]. Although there is an association between these variants and early onset MI [30] there does not seem to be an association with worsening clinical symptoms or mortality [68]. However, more serious clinical outcomes such as recurrent MI and mortality of patients with the acute coronary syndrome (ACS) has been associated with 9p21.3 [69]. There have been reports of some clinical signs and symptoms that lead to ACS, symptoms that are also dangerous manifestations of atherosclerosis in coronary arteries. Thus CAD patients are at the risk of ACS [70].

In summary, 9p21.3 variants are common among angiographic CAD cases and such variants can predict CAD prevalence independently of traditional risk factors among angiographical CAD patients [71].

The Structure of the 9p21.3 Locus in CAD

The 9p21.3 sequence of about 55 Kb encompasses both coding and non-coding regions. There are four genes at the location, i.e., cyclin-dependent kinase inhibitors genes *CDNK2A* and *CDNA2B*, methylthioadenosine phosphorylase (*MTAP*) and *ANRIL* (antisense noncoding RNA in the INK4 locus). The first three are involved in proliferation of cells such as vessel smooth muscle cells (VSMCs) and inflammatory cells that are important in atherosclerosis [72-76]. The alternative splicing products of gene *CDKN2A* are $p16^{ink4a}$ and $p14^{ARF}$ and of gene *CDKN2B* is $p15^{ink4b}$. This tumor suppressor region in 9p21 was identified by Kamb et al. as a multiple tumor suppressor (*MTS*) region [77]; the *MTS1* region is identical to the p16 coding region or *CDKN2A*, which contains three exons and two transcripts from two different promoters. The $p16^{ink4a}$ and $p14^{ARF}$ proteins encoded by *CDKN2A* gene that has a different exon 1 sequence. $p16^{ink4a}$ is encoded by exon1α and $p14^{ARF}$ is encoded by a smaller transcript including exone1β [78, 79]. These inhibitors bind and inactivate CDK4/6 as well as stopping the signaling pathway that controls progression from cell division phase G1 to S and hence regulates cell proliferation [80, 81] . These proteins are involved in tumor suppressor pathways, and contribute to essential cell processes such as the cell cycle, cell aging and apoptosis [82].

MTAP encodes an enzyme involved in the methionine salvage pathway. Initially, the *MTPA* gene was identified to contain eight exons with a single transcript, but in 2012 three additional exons and six additional transcripts (v1-v6) were discovered by Camacho-Vanegas et al. [83]. Deficiencies of *MTAP* cause changes in the degree of genome methylation, reductions in methionine pathway metabolites and reduced CD4+ T-cell counts [84]. *MTAP* is another gene involved in regulation of atherosclerosis and is known as a tumor suppressor gene [84, 85]. Generally, there is an association of this locus with susceptibility to atherosclerosis [86] accompanied by abnormal changes in intrinsic characteristics of arterial wall and susceptibility to vascular diseases [87, 88]. SNPs in this region are associated with various cardiovascular diseases such as CAD, carotid artery plaque, stroke, aneurysms, peripheral artery disease, and heart failure [89], and also other diseases including type 2 diabetes (T2D) [90], glaucoma [91, 92], several types of cancer [93], Alzheimer's [94], endometriosis [95], and periodontitis [96].

Structural analysis of 9p21.3 includes the enhancer elements in the region [97, 98]. These enhancer elements physically interact with surrounding genes *CDKN2A, CDKN2B,* and *MTAP* and participate in long-range interaction with the interferon-α21 gene [98], but an effect of the 9p21.3 locus on CAD with modulation from type 1 interferon's like interferon-α21 has not been proved [99]. Risk alleles identified by GWAS can alter the activity of these enhancer elements; for example, SNP rs1333045 disrupts the Smad binding site with effects on cell proliferation by the TGF-β signaling pathway [97]; and rs10811656 and rs10757278 can disrupt the STAT1 binding site. STAT1 mediates cellular responses to interferon's (INF). INFγ led to a 2-fold decrease in CDKN2B expression and a 4-fold increase in ANRIL expression. Thus, failure of STAT1 failed to bind to its binding site in this region there was an alteration in gene expression [98]. However, the relationship of 9p21.3 genotype and CAD is not clear since in another study expression of *CDKN2A* and *CDKN2B* was increased by the effects of INFγ treatment independently of risk genotype, therefore indicating that this locus plays a more complicated role in CAD [100]. The rs10811656 and rs4977757 variants disrupt a binding site of TEAD transcription factors, and affect p16 which is involved in regulation of gene expression and cell proliferation. This control process is likely to be damaged in individuals carrying risk alleles [101].

Noncoding RNA is another mechanism for regulation of gene expression. The complex structure of 9p21.3 is due to noncoding regions. A transcripts of these regions is a long noncoding RNA named *ANRIL* (antisense noncoding RNA in the INK4 locus) [102]. This non-coding RNA was first identified in a French family with melanoma-NST syndrome and harboring a germ-line deletion that included the *ANRIL* and *INK4b-ARF-INK4a* cluster. The length of the *ANRIL* gene is 126.3 Kb and it overlaps with the *CDKN2B* gene [103]. *ANRIL* has 20 exons and produces a long transcript of 3,834 bp (DQ485353) and two shorter transcripts of 2,659 bp and 688 bp (DQ485454 and EU741058, respectively) [97]. It has a complex regulatory role in different tissues and conditions due to its multiple transcripts. Variants in the region are associated with atherosclerosis [104, 105] and the linear/circular

structure of ANRIL affects the regulation of gene expression [106]. An animal model study indicated that homozygous deletions of *ANRIL* led to decreased *Cdkn2a* and *Cdkn2b* gene expression and an increased rate of mortality [75]. A study of association of polymorphisms in this locus with changes in gene expression indicated that *ANRIL* expression is altered to a greater extent than *CDKN2A* and *CDKN2B*; therefore, changes in *ANRIL* expression have important roles as causes of susceptibility to different diseases [107]. *ANRIL*, like other noncoding RNAs regulates gene expression by different mechanisms, including epigenetic regulation where it participates in chromatin remodeling and DNA methylation [108, 109]. Methylation of DNA in this region is associated with CAD [110]

ANRIL as a non-coding RNA also plays a significant role in regulation of *cis* and *trans* genes [111]. A gene expression analysis study concluded that the expression of 46 genes in heart tissue was associated with common risk alleles of 9p21.3. The majority of these genes were involved in the transition of the cellular state from the G1 phase which is associated with the regulatory roles of the *CDKN2A/B* genes. Therefore, individuals carrying risk alleles are susceptible to increased cell proliferation and CAD risk. Transcription analysis showed risk variation at 9p21.3 is associated with *ANRIL*, *CDKN2A/B*, and *C9orf53* (open reading frame) expression [111]. *ABCA1* encodes a transporter protein that regulates the flow of cellular cholesterol from the cell membrane and HDL formation, hence this gene is associated with HDL level [113]. In another study of ANRIL, shRNA (short hairpin RNA) interference and knock-down of two transcripts demonstrated that the *ADIPOR1*, *VAMP3* and *C11ORF10* genes are regulated by ANRIL, meaning that ANRIL is involved in glucose and lipid metabolism regulated by these genes [114]. Furthermore, *CARD8*, another gene regulated by ANRIL expression is involved in ischemic stroke [115]. According to recent reports *DUT, EIF1AY, CASP14, DHRS9, ABCA1, ADIPOR1, VAMP3, C11ORF10* and *CARD8* are all regulated by ANRIL [116].

PRC1 and PRC2 proteins participate in epigenetic regulation and histone remodeling [117]. The presence of Alu elements in the *ANRIL* region is necessary for *trans* regulation of ANRIL. These elements help ANRIL and the PRC1/2 complex to recognize their target genes [118]. A study of CAD patients in comparison with normal individuals showed the presence of four tandem duplications of about 50 kb in CAD patients [119].

DISCUSSION

9p21.3 and its variants represent a key candidate locus that is associated with CAD. In 2007, 9p21.3 was first recognized as a genomic region associated with CAD; SNP rs1333049 and rs6475606 were identified as the strongest CAD-related variants [31]. Later, rs10757274, rs2383206 [29], rs2383207, rs10116277, rs1333040 and rs10757278 [30] were reported from two independent studies. These variants except for rs1333040 (excluded from the study because of erroneous application of the Hardy–Weinberg equilibrium in the control population) were confirmed in studies of European populations from the United Kingdom, Germany, Italy, and Sweden. All of these variants were confirmed with similar odds ratios ranging from 1.29 to 1.26 [120]. In another GWAS of the German population three variants, rs4977574, rs2891168 and rs1333042, were associated variants with CAD/MI [41]. Moreover, in separate GWAS, rs4977574 variant was implicated as a risk factor of MI [35] and CAD [36]. Variants rs3217992 [43] and rs1333042 were identified in another GWAS of European and South Asian populations [42].

The strongest indicator in most GWAS was the rs1333049 variant which was confirmed in other populations [32, 33]. An analysis of seven loci identified as risk loci for CAD provided clear evidence of a strong association of rs1333049 and CAD [33]. In another study of two large cohorts genotyping of three variants in three different loci established that rs1333049 was significantly associated with increased risk of CAD [121]. A meta-analysis of 9p21.3 variants showed there was a 29% increased risk of MI for individuals carrying each risk allele of a rs1333049 variant [122].

Various studies of Asian populations indicated a similar relationship of 9p21.3 variants with CAD; for example, rs9632884, rs10757274, rs1333042, rs1333049 were detected in a Chinese population [55], rs4977574 and rs1333049 in a Korean population [57], and rs1333049 in a Japanese population [56]. The rs1333049 was the strongest signal in East Asian population being detected in Korean, Japanese, and Chinese as well as European populations. The present study also demonstrates the significant association of the rs6475606, rs4977574, rs29891168, rs1333042, rs1333048 and rs1333049 variants with CAD [123].

The rs10811656 and rs10757278 variants are involved in disruption of transcription factors at the STAT1 binding site [98]. These variants were more frequent in disease cases than in controls in a Polish population study [124]. rs10811656 and rs4977757 disrupt TEAD binding site [101] and the association of these risk alleles with CAD was confirmed in a Chinese population [125].

Association of three most frequently detected SNP, rs1333049, rs2383206 and rs10757278, in European populations was confirmed in a large meta-analysis of an East Asian population [126]. Among these variants, rs2383206 and rs10757278 displayed the closest association with CAD in an Indian population [127, 128]. Investigation of CAD in a Saudi population identified four SNPs including rs564398, rs4977574, rs2891168, and rs1333042 associated with CAD/MI [129]. The first large case-control study (PROMIS) of a Pakistan population concluded that six (rs1333049,

rs10757274, rs4977574, rs2891168, rs1537372 and rs9632885) of eighty-nine investigated SNP in the 9p21.3 locus were associated with MI [130]. In an analysis of an American Caucasian population with a familial history of CAD/MI, rs10757274, rs2383206, rs2383207, and rs10757278 were associated with the premature/familial form [131]. Moreover, these variants were confirmed in MI patients in German families with a high frequency of CAD/MI patients compared with families having no history of the condition [132].

Studies of African American populations showed no association of 9p21.3 variants with CAD. For example, genotyping of rs10757274, rs2383206 and rs10757278 variants showed no association variants in the African American population with CAD, but a haplotype analysis of African American patients found that they carried more risk alleles more than unaffected individuals [133]. Another surprising result was a study of an Iranian population where rs1333049 had no significant association with CAD whereas rs10757274 was associated [134-137]. However, investigation of the rs10757274 and rs1333042 variants showed that the risk alleles of these SNP in a haplotype form did constitute a risk factor for CAD [137]. This demonstrated that some haplotype arrangements have protective effects against CAD, such as a GAAAA haplotype for five common variants, rs1333049, rs10757278, rs2383206, rs4977574 and rs10757274 [127].

The majority of SNP in the *ANRIL* gene were located in intronic regions, but recently two exonic variants were reported to be associated with MI. Sequencing of the promoter region and UTR upstream of *ANRIL* showed no variant significantly associated with MI [138]. Some other studies investigating the effects of 9p21.3 variants on expression and regulation of 9p21.3 genes indicated that rs1333049 altered the expression of *CDKN2A/B* and *ANRIL*. This alteration caused in proliferation of vascular smooth muscle cells (VSMC), and thereby an association with CAD [139, 140]. Moreover, rs10757278 reduced transcription of the *p15, p16, p14* and *ANRIL* genes leading to an increased risk of CAD [141].

CONCLUSION

CAD is a complex multifactorial disease that causes plaque aggregation in coronary arteries leading to a decreased of blood supply to the heart muscles. Different studies showed a significant component of the variation in CAD was heritable. Mutations in genes involved in known pathways of CAD such as lipid metabolism including genes LDL receptor, *ApoB-100, ARH* and *PCSK9* cause monogenic forms of CAD. The locus most frequently implicated in GWAS was 9p21.3. Different variants in this locus associated with CAD were discovered and confirmed in many ethnic populations. Clearly, variation in 9p21.3 is strongly associated CAD and has a significant role in atherosclerosis. It seems that variants confirmed in different populations, including rs1333049, rs10757278, rs10757274, rs2383206, rs6475606, rs2383207, rs10116277, rs1333040, rs4977574, rs2891168, rs1333042, rs1333048 and 1333045, can be used in etiology and research on cardiovascular disease.

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Conflict of Interest

The authors have no conflict of interest to declare.

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