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Review Article

**Investigation of the Role of Single Nucleotide Polymorphisms (SNPs) in Premature Coronary Artery Disease**

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Cheras, Kuala Lumpur, Malaysia**\*Article History:****Received:** 09/06/2017**Revised:** 01/07/2017**Accepted:** 01/07/2017**DOI:** <https://doi.org/10.7439/ijbr.v8i7.4225>**Abstract**

Premature coronary artery disease is a concerning epidemic around the world. Therefore, there is a need to find useful biomarkers for a diagnostic tool to screen for the disease. This review aims to explore studies utilizing single nucleotide polymorphism (SNPs) to explain the heritable variation of bases in certain population that contribute to premature coronary artery disease. The studies have concluded the changes involved in lipid metabolism, oxidation of lipid and the significance of 9p locus. In recent years, many studies have done to reveal the gene-associated disease. The most robust genetic risk variant for CAD was identified on chromosome 9p21.3. This leads to the tremendous studies to explore the genetic variants underlie in the development of atherogenesis in early-onset CAD. Genome-wide association studies (GWAS) have enabled the discovery of 33 genetic risk variants for CAD by microarrays of SNPs. This includes 23 risk variants with unknown mechanism and only 10 associating with hypertension or lipids. This review will discuss on the association of SNPs studies with lipid metabolism, inflammation and oxidation.

**Keywords:** Premature coronary artery disease, atherosclerosis, single nucleotide polymorphisms, risk allele, GWAS.

**1. Introduction**

Coronary artery disease is an intricate disease influenced by various factors including genetic and environment [1]. Many factors are contributing to the development of CAD including tobacco use, decreases physical activity, and increases consumption of unhealthy foods which contains high fats and cholesterol [2,3]. Multiple combinations of gene-gene and gene-environment interactions play a key role in the development of CAD [4,5]. Family history of MI was shown to increase CAD risk [6,7]. Following this discovery, it has stimulated an intense research for novel genetic determinants of CAD. The rise in cardiovascular risk factors in younger population could lead to an increase in premature CAD cases [8]. Premature CAD is defined as the development of coronary artery disease that occurs at age 45 and below [9,10]. 10% of all heart attacks occur before age 45 and mostly strike men [11].

In recent years, many studies have done to reveal the gene-associated disease such as genome-wide association studies (GWAS). GWAS have enabled the discovery of 33 genetic risk variants for CAD by microarrays of SNPs. Single nucleotide polymorphisms (SNPs) are the most common genetic variation occurred in the human genome. SNPs involve in base-pair substitutions and in the non-coding region of genes and do not result in mutant phenotypes. Therefore, SNPs have proven to be valuable genetic markers. It provides crucial information about the relationship among different ethnic groups and human evolution. The study of SNPs also provides the information about genes involved in susceptibility to a disease that lead to the identification of the actual disease-causing gene [12]. This includes 23 risk variants with unknown mechanism and only 10 associating with hypertension or lipids [13]. The most robust genetic risk variant for CAD was identified on chromosome 9p21.3 [14].

This leads to the tremendous studies to explore the genetic variants underlie in the development of atherogenesis in early-onset coronary artery disease. SNPs may react with environmental factors to enhance the

development of atherosclerosis as in Figure 1. Therefore, this review will discuss on the SNPs studies and its interaction with lipid metabolism, inflammation and oxidation.

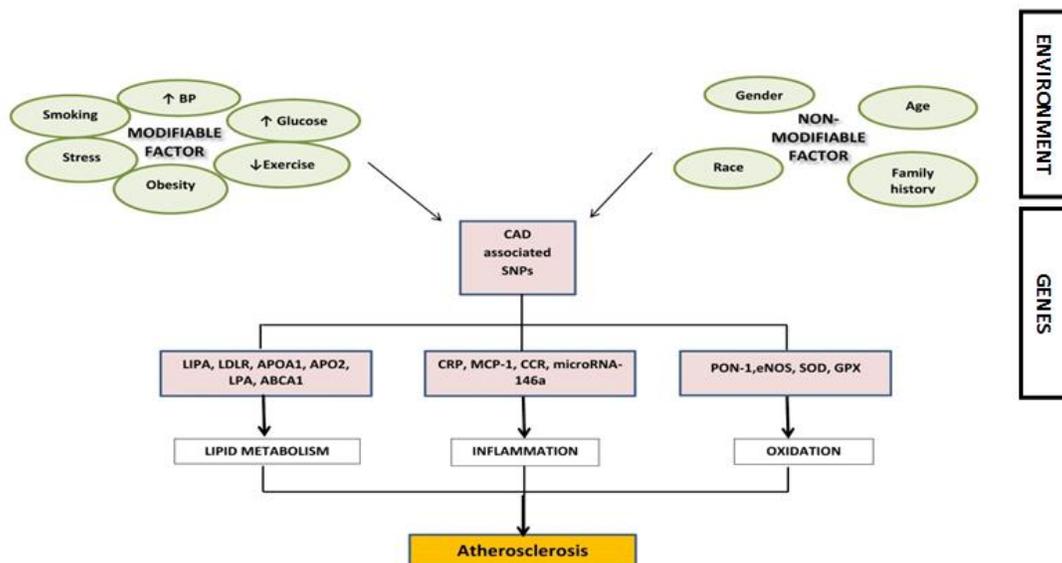


Figure 1: The interaction between gene and environment that leads to the development of atherosclerosis

## 2. Lipid metabolism

A prolonged high fat diet and physical inactivity play a role in the development of visceral obesity. This is a subsequent effect from dysregulation of adipocytokine and free fatty acids production thus; increase the proliferation of adipose tissue. The adipose tissue plays a critical function in the pathogenesis of the metabolic syndrome leading to the development of atherosclerosis [15]. The abdominal obesity enhances the occurrence of hypertension, atherogenic dyslipidemia and insulin resistance. These will accelerate metabolic syndrome that circulate low density lipoprotein (LDL) cholesterol level in blood. Increasing of reactive oxygen species (ROS) generation by oxidative stress due to excessive flux of free fatty acids (FFAs), which are known to affect insulin signal transduction pathway and induce endothelial dysfunction [16]. ROS will further oxidize LDL and accumulate the foam cells (lipid-containing intimal macrophages) along with the presence of inflammatory cells such as monocytes. Growth factors then induce the foam cells to proliferate causes the development of plaque – atherosclerosis [17].

One of the crucial metabolisms involved in the prevalence for CAD is lipid metabolism. High density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides are the most common risk factors for CAD and are targeted for therapeutic agent. Many studies revealing the association of lipid metabolism and SNPs which include two consortiums that focused on LIPA gene lipase A, lysosomal acid, cholesterol esterase located at chromosome

10q23.31 which encodes lysosomal acid lipase (LAL). LAL hydrolyzes cholesteryl esters and triglycerides (TG) in the lysosome of cells to generate free cholesterol and free fatty acid. The IBC 50 K CAD Consortiums and Consortium CAD [18,19] reported a significant CAD-association polymorphisms in LIPA gene(rs2246942, rs11203042). The Coronary Artery Disease (C4D) Genetics Consortium also found strong association between another variant of LIPA gene (rs1412444)with increased risk of CAD[20](**Table 1**). Similarly, a study in premature CAD for Mexican population, they found rs1412444 is significantly associated with increased LIPA expression level thus increase LAL activity producing increased cholesteryl ester hydrolysis [21].

Loss-of-function allele at R46L (G→T) (rs11591147) in PCSK9 (Proprotein convertase subtilisin/kexin type 9) gene was not only decreasing LDL cholesterol levels but also providing protection against myocardial infarction [22]. The minor L allele (2.4% frequency in controls) of R46L (Arginine→Leucine) was associated with a reduced risk of myocardial infarction [22]. This atheroprotective effect study was replicated in Italy population with 1880 patients of premature myocardial infarction and showed a protective effect of the L46 allele [23]. On the other hand, another variant in PCSK9 (rs11206510) which in the promoter region, however is associated with increase LDL cholesterol with T risk allele [24] suggesting that PCSK9 locus is related with LDL cholesterol or lipoprotein (A) concentration.

**Table 1: SNPs associating with lipid metabolism**

Author	Gene	Chromosome	SNPs	Sample Size	Population	Non-risk allele	Non-risk Frequency	Risk allele	Risk Allele Frequency	Result OR (95 % CI)	P-value
Kathiresan et al. (2009) [24]	PCSK9 LDLR WDR12 PHACTR1 MRPS6	1p32, 19p13, 2q33, 6p24, 21q22	rs11206510, rs1122608, rs6725887, rs12526453, rs9982601	2,967 cases of CAD early-onset, 3,075 controls	Boston, US, Finland, Spain, Italy	C T T G C		T G C C C T	0.81, 0.75, 0.14, 0.65, 0.13	1.15(1.10–1.21), 1.14 (1.09–1.19), 1.16 (1.10–1.22), 1.13 (1.09–1.17), 1.19 (1.13–1.27)	9.6 x10-9 1.9 x 10-9 1.3 x 10-8 1.3 x 10-9 6.4x 10-11
Clarke et al. (2009) [89]	LPA: lipoprotein(a) gene	6q25.3	rs3798220, rs10455872	3145 case subjects; 3352 control	UK, Italy, Sweden, Germany			C G	0.02, 0.07	1.92 (1.48-2.49), 1.70(1.49-1.95)	5.9×10–51, 3.6×10–166
Schunkert H et.al (2011) [90]	1)ABO 2)ZNF259/A POA5-A4-C3-A1 3)CYP17A1/CNNM2/NT5 C2 PPAP2B 4)ANKS1A 5)TCF21 6)ZC3HC1 7)COL4A1/COL4A2 8)HHIPL1 9)SMG6/SRR 10)RASD1/S MCR3/ 11)GIP/ATP5 G1/SNF8	1)9q34.2, 2)11q23.3, 3)10q24.32 4)1p32.3, 5)6p21.31, 6)6q23.2, 7)7q32.2, 8)13q34, 9)14q32.2, 10)17p13.3, 11)17p11.2, 12)17q21.32	rs579459, rs964184, rs12413409, rs17114036, rs17609940, rs12190287, rs11556924, rs4773144, rs2895811, rs216172, rs12936587, rs46522	22,233 individuals with CAD (cases) and 64,762 controls	European descent			C G G A G C C C G C C G T	1)0.21 2)0.13 3)0.89 4)0.91 5) 0.75 6)0.62 7)0.62 8)0.44 9)0.43 10)0.37 11)0.56 12)0.53	1.10 (1.07–1.13), 1.13 (1.10–1.16), 1.12 (1.08–1.16), 1.17 (1.13–1.22), 1.07 (1.05–1.10), 1.08 (1.06–1.10), 1.09 (1.07–1.12), 1.07 (1.05–1.09), 1.07 (1.05–1.10), 1.07 (1.05–1.09), 1.07 (1.05–1.09), 1.06 (1.04–1.08)	4.08 × 10–14, 1.02 × 10–17, 1.03 × 10–9, 3.81 × 10–19, 1.36 × 10–8, 1.07 × 10–12, 9.18 × 10–18, 3.84 × 10–9, 1.14 × 10–10, 1.15 × 10–9, 4.45 × 10–10, 1.81 × 10–8
IBC 50K CAD Consortium (2011) [18]	ABCG8 TRIB1 IL5 LIPA	2p21, 8q24.13, 5q31.1 10q23.31	rs4299376, rs17321515 rs2706399 rs2246942	European : 11,202 cases and 30,733 controls South Asian: 4,394 cases and 4,259 controls				G A G G	European : 1)0.33 2)0.54 3)0.52 4)0.35 S.Asian : 1)0.28 2)0.65 3)0.47 4)0.50	Per allele odds ratio: 1.06-1.09	1)7.4 x 10-5 2)1.3 x 10-4 3)8.0 x 10-5 4)4.8 x 10-7
The CARDIoGRAMplusC4D Consortium (2011) [19]	LIPA LDLR ZNF259/APOA5/APOA1	10q23.31 19p13,	LIPA: rs11203042 rs2246833 LDLR: rs1122608 ZNF259/APOA5/APOA1: rs9326246	63,746 cases and 130,681 controls	European and south Asian descent	C C T T G		T T G C	0.44 0.38 0.76 0.10	1.04 1.06 1.10 1.09	6.08 x 10-6 9.49 x 10-6 6.33 x 10-14 1.51 x 10-7
Coronary Artery Disease (CAD) Genetics Consortium. (2011) [20]	BCAP29: B-cell receptor-associated protein 29, PDGFD: platelet derived growth factor D LIPA : lipase A, lysosomal acid, cholesterol esterase	7q22, 11q22 10q23.31	rs10953541, rs974819 rs1412444	15,409 cases (European ancestry; 6,996 South Asian ancestry)/ 15,043 control		C		C T T	0.80 0.32 0.34	1.08 (1.05–1.11), 1.07 (1.04–1.09), 1.09 (1.07–1.12)	3.12 x 10-8 , 2.41 x 10-9, 2.76 × 10–13

Vargas-Alarcon, G. et al. (2013) [21]	LIPA	10q23.31	rs1412444, rs2246833	899 pCAD, 270 subclinical atherosclerosis, 677 healthy control	Mexican-Mestizo ancestry	C C		T T	0.561 0.563	rs 1412444, recessive =1.53(1.18-1.99) ; additive = 1.34 (1.14-1.58) rs2246833, recessive= 1.45(1.12-1.87); Additive = 1.28(1.09-1.51)	rs 1412444, recessive P= 0.0013 ; additive P= 5 x10 <sup>-4</sup> rs2246833, recessive P= 0.0039; Additive P = 0.0023
Ferreira CN. Et al. (2012) [91]	APOA5 = apolipoprotein A5 gene	11q23	rs662799	109 dyslipidemic subjects; 107 nondyslipidemic healthy controls	Brazilian	T	0.18	C	0.278	1.726(1.085-2.721)	0.019
Lee et al (2013) [92]	SORT1 CDKN2A/2B PDGFD MYL2 FLT1	1p13.3, 9p21.3, 11q22.3, 12q24.11, 13q12.3	rs599839, rs4977574, rs974819, rs3782889, rs9508025	Korean; 2293 CAD 4302 healthy controls Japanese:3052 cases 4976 controls		- - - T G	- - - 0.24 0.41	A G T C C	0.94, 0.46, 0.63, 0.29, 0.43	1.34(1.12-1.61) 1.26(1.16-1.36) 1.15(1.06-1.24) 1.26(1.18-1.36) 1.11(1.04-1.18)	1.3x10 <sup>-3</sup> 1.36x10 <sup>-8</sup> 7.9x10 <sup>-4</sup> 1.65x10 <sup>-10</sup> 0.0023
Shanker J. et al. (2008) [93]	APOA1-C3-A5= apolipoprotein A-I	11q23	rs1799837(-75G>A) rs5069(+83C>T) rs5128(Sac1)	523 families comprising 2318 individuals Indian.		G C -		A T -	0.19 0.15 0.32	Identity by descent (IBD); Pi mean proportion of allele sharing, -75G>A;Pi=0.56 +83C>T;Pi=0.52 Sac1;Pi=0.59	Sac1&-75;P<0.0001 83C;P=0.0012
Gustavsson, J. et al (2012) [94]	APOE	19q13.2	Genotype ε2+ ε3 ε4+	1735 CHD cases and 4654 controls	Swedish					1.45 (1.00–2.10) 2.25 (1.90–2.68) 2.37 (1.85–3.04) (ORs for ever versus never smoking)	0.07
Grammer, T. B. et al (2013) [95]	APOE	19q13.2	E2; ε22 ε23 E3; ε33 E4; ε24 ε34 ε44	3279 (3263 coronary angiograms, 626 unstable angina, 114 MI(non-ST-elevation), 289(ST-elevation)) Controls: 697 CAD : 2555	Germany					E4 as reference; E2 0.67(0.48-0.96)	0.026
Junyent, M. et al (2009) [96]	ABCG5/G8	2p21	rs4148189 rs4131229 rs6720173 rs3806471 rs4148211 rs11887534 rs6709904 rs4148217	Nonsmokers = 640 Smokers = 205	Hispanics	G T C A A A G A C	-	A C G C G C G A			0.936 0.013 <0.001 <0.001 0.857 0.463 0.012  * Associations between ABCG5/G8 SNPs and fasting lipid profiles

ATP-binding cassette transporter A1 (ABCA1) gene encoded for protein that plays a crucial role in efflux of intracellular cholesterol and phospholipids by mediating transport of intracellular cholesterol and phospholipids across cell membranes. Excess lipid molecules are removed from cells by apolipoprotein AI and other apolipoproteins of incipient high-density lipoprotein (HDL) [25, 26]. 12 non-coding SNPs was found in regulatory regions of ABCA1 gene consisting of the promoter, the 5' untranslated region (UTR) and four SNPs were found to be associated with CAD [27]. The G-191C variant and the C69T were discovered in three-fold and two-fold increase respectively in coronary events with an increased risk due to family history of CAD. In contrast, the C-17G was associated with a decrease in coronary events and the InsG319 was associated with less atherosclerosis [27]. A variant effect on -565C>T *ABCA1* gene promoter on *ABCA1* was found correlated with the expression and severity of atherosclerosis in 1170 white patients with CAD from Southern England [28]. This variant displayed a pattern with higher number of diseased coronary arteries in T/T homozygotes, but the differences were not statistically significant. However, the amounts of *ABCA1* transcript were lowest in T/T homozygotes and highest in C/C homozygotes [28]. The study was extended by observing the influence of age of symptom onset in coronary artery disease patients in polymorphisms in *ABCA1* and found that patients of the G/G or G/C genotype of the 2407G>C polymorphism had significant coronary artery stenosis (>75%) at a younger age than those having C/C genotype [29].

The next risk allele associated its higher LDL-C is ABCG5/ABCG8. This allele was associated with higher LDL-C in Europeans and South Africans [18]. This locus encodes a protein which acts as a cholesterol transporter protein from liver into bile. Polymorphisms of *APOA2* also have been proven to be a contributor to the obesity risk. It is proven that this polymorphism is consistently linked with saturated fat intake across different ethnicities [30]. In study of interaction between *APOA2* -265T<C polymorphism across Caucasian and Hispanic populations and obesity risk, they found a person who carries C allele homozygotes consuming high amounts of saturated fat increased odds for obesity, compared with odds in those consuming low amounts of saturated fat [31]. Providing further support across ethnicities, results also replicated in Asian and Mediterranean population showing there was significant interaction with coronary artery disease [32]. Recently, another apolipoprotein, *APOC4* (rs1132899) polymorphism was associated with an increased risk of premature CAD in Chinese subjects and the result was more significant among male subjects particularly smokers [33].

### 3. Inflammation

CAD-associated SNPs is related to inflammation (Table 2). Recruitment of monocyte into atherosclerotic plaque by monocyte chemoattractant protein-1 (MCP-1) leading to production of interleukin-6 which involve in the process of inflammation. Simultaneously, this process will generate more C-reactive protein (CRP). Increased CRP then upregulates molecule adhesion and MCP in the surface of endothelium cells hence, triggers the adhesion of molecules to the cells [34, 35]. The transition (A >G) at the -2518 locus (rs1024611) of MCP-1 promoter has atherosclerotic association by increasing transcriptional activity and up-regulating the expression of MCP-1 as reported by previous study [36]. Other study also showed that, the variant of the MCP-1 -2518, G/G homozygotes was significantly higher among CAD patients as compared to the controls [37]. However, it is found to be weak CAD-association in a meta-analysis by using random-effects model [38].

Acting in concert with MCP-1, CCR (C-C motif) receptor also has been studied to investigate the relationship in their mechanism in inflammation. MCP-1 which also known as CC- chemokine ligand 2 (CCL2) bind to CCR2 along with CCL7 and mediate monocyte recruitment [39]. However, studies of CCR variation – substitution (Val > Ile) at the 64 amino acid residue (rs1799864) did not show any strong evidence that can claim there was atherosclerotic association [38]. Surprisingly, there was a report showing the correlation between -2518 A>G polymorphism in the promoter of the MCP-1 and reduced C-reactive protein levels with MLN1202 treatment. MLN1202 is a human monoclonal antibody directed against CCR2 and is in clinical development for the treatment of various inflammatory disorders. MLN1202 inhibit the CCR2-binding site thus decrease CRP level while increase serum MCP-1 levels. Patients with A/G or G/G genotypes in the MCP-1 promoter had significantly greater reductions in high-sensitivity C-reactive protein levels than patients with the wild-type A/A genotype as much as 26.6% from baseline [40].

For premature CAD, there was a study of -2518 A>G polymorphism in Chinese population. The result was -2518 A>G polymorphism did not affect plasma levels of MCP-1 or susceptibility to premature stable CAD for 3 genotypes (AA, AG, GG) indicating there was no significant difference in plasma MCP-1 level between cases and controls [41]. It is consistent with the result in Turkish population. There was no significance between this polymorphism and premature CAD by showing no differences between genotype distribution and allele frequencies in the premature CAD and control groups [42].

**Table 2: SNPs associating with inflammation**

Author	Gene	Chromosome	SNPs	Sample Size	Population	Non-risk allele	Non-risk Frequency	Risk allele	Risk Allele Frequency	Result OR(95 % CI)	P-value
Ramkaran P. et al. (2013) [59]	miR-146a = MicroRNA-146a		rs2910164	106 CAD; 100 age-, race- and sex-matched controls	young Indian male	G	0.67	C	0.33	1.025(0.6782-1.550)	P< 0.0001
Abd El-Aziz TA & Mohamed R.H (2013) [43]	CRP = C-reactive protein	1q21-1q23	rs1800947	116 CAD cases, 119 controls;	Egyptian	G		C	RAF= 0.103	95% CI= 0.028-0.12	0.007
Koch, W. et al (2001) [97]	TNF- $\alpha$ – tumor necrosis factor alpha	6p21.3	-863C, -308G	CAD (n=998) MI (n=793) Controls n=340)	Germany		C CAD MI (%) CC 71.1 71.7 68.9 CA 26.5 26.3 28.6 AA 2.4 2.0 2.5 GG 71.8 70.3 71.3 GA 24.4 26.9 27.1 AA 3.8 2.8 1.6			1.07 (0.81–1.41) 1.09 (0.72–1.66)	P =not significant for all comparison
Chu, H. et al (2012) [98]	TNF- $\alpha$ – tumor necrosis factor alpha	6p21.3	-308 G/A (rs1800629)	CHD n=535 MI n=420 Controls n=420	Han Chinese		GA and AA Controls= 20.78 CHD= 20 MI=21.43			1.743(0.325 - 1.423)	0.721
Manginas, A. et al (2008) [99]	IL-10, TGF- $\beta$ 1, IFN- $\gamma$ , IL-6, TNF- $\alpha$	1q31-q32, 12q14, 19q13.1, 7p21-p15, 6p21.3	IL-10-1082 A/G, -819 C/T, -592 A/C, TGF- $\beta$ 1-10 T/C, -25 G/C, IFN- $\gamma$ -874 T/A, IL-6-174 G/C, TNF- $\alpha$ -308 G/A	26 stable angina patients, 45 unstable angina patients 58 nonfatal myocardial infarction patients	Greece					IL-6 -174 G/C, Genotype 0.271(0.1012–0.7292) Phenotype 0.4US=2.368 (1.262-4.444)	IL-6-174 G/C, Genotype SA= 0.024; US=0.022; Phenotype SA=0.0099; US=0.03 Haplotype SA=0.007; US=0.0096 Other loci – P value are nor significant
Cho, H. C. et al (2013) [100]	TNF- $\alpha$	6p21.3	rs361525 rs1799724 rs1800630	197 CAD, 404 controls	Korean	G C C		A T A		1.74 (1.04–2.92) 1.14 (0.84–1.56) 0.89 (0.64–1.25)	0.03 0.40 0.51
Wang et al (2011) [38]	MCP-1 CCR2	17q11.2-q12 3p21.31	rs1024611 rs1799864	9844 CAD 11,821 controls	Caucasian African West Asian East Asian					MCP-1 1.42 [1.06, 1.92] CCR2 recessive 1.27 (95%CI: 0.81–1.99) dominant 1.06 (95%CI: 0.95–1.19)	0.02 0.3-0.31

Chen, Z. et al (2010) [41]	MCP-1		rs1024611	132 patients with premature stable CAD (cases) and 153 controls	Chinese	G	Cases: 0.561 Control 0.572	A	0.43 (cases) 0.42 (controls)	0.751–1.460	0.786
Gilbert, J. et al (2011) [40]	MCP-1	17q11.2-q12	rs102461	243 subjects 112- drug (MLN1202) 108- BASELINE	Caucasian	Patients with A/G or G/G genotypes in the MCP-1 promoter had significantly greater reductions in high-sensitivity C-reactive protein levels than patients with the wild-type A/A genotype.  The median percentage change in CRP from baseline to day 57 in A/G +G/G subjects was also significantly different (p = 0.0085) between subjects receiving placebo (+8.7%) and those receiving MLN1202 (26.6%)					
Grammer, T. B. et al (2009) [45]	CRP	1q21-1q23	rs2794521(-717) rs1800947(+1059) rs1130864(+1444)	3279(3252 coronary angiograms, 626 unstable angina, 114 MI(non-ST-elevation), 289(ST-elevation)) Controls: 697 CAD : 2555	German ancestry	C G C		T C T	2.7 (2.5–2.9) 2.3 (2.0–2.6) 2.9 (2.7–3.1)  *effect of CRP genotype on CRP concentration  *no significance found between CAD and CRP genotype	0.718 (1.9 % increase) 0.005 (18.7 % increase) <0.001 (20.2 % increase)	
Kaur, R. et al (2013) [46]	CRP	1q21-1q23	rs1800947(+1059)	266 T2D 109 T2D+CAD 206 CAD	Punjab	G		C	*Recessive model  4.19 (1.62–10.80)	0.003	

C-reactive gene located at 1q21-1q23 chromosome is associated with circulating CRP concentrations. CRP is believed to be the potential independent biomarker for coronary artery disease. Several studies found there was no association of CRP gene and coronary artery disease in both premature CAD and CAD [43-45]. However, in the presence of + 1059 G>C (rs1800947) which is silent at the amino acid level (CTG→CTC, Leu→Leu), hsCRP level is increased resulting to the development of metabolic syndrome in young age especially if associated with smoking. Elevation of hsCRP levels along with LDL level will lead to the emergence of premature coronary artery disease especially in male patients [43]. Interestingly, in North-West Indian population of Punjab, recessive model of GG genotype of CRP + 1059 G>C increases the risk of CAD by four fold [46].

Addition to contradict result of CRP polymorphisms, an additive gene-gene interaction was found in subjects with GG genotype at IL-6-572 and allele variant at CRP SNPs [47]. There is elevation of serum CRP level with IL-6 polymorphisms rather than genetic variant of CRP alone [47]. Many populations had been scrutinized to find the interaction between IL-6 polymorphisms and CAD. South Asians are found to be independently associated with both premature CAD and CAD [48-50]. The relationship however was not found in Middle East

population [51-53]. Even though IL-6 polymorphisms also have controversial findings, it is noteworthy to observe that variant at the location -174G>C and -572G>C is working together with other polymorphisms to develop atherosclerosis. IL-6 -174G>C polymorphism also evidently associated with risk of coronary heart disease by influencing the increased systolic blood pressure [54]. IL-6 acts as pro-inflammatory cytokine which can regulate the progression of atherosclerosis by stimulate proliferation of smooth muscle cells and facilitate the migration of leukocytes by activating endothelium cell into the vessel [55, 56]. Polymorphism of IL-6 -174G>C is reported to elevate the serum IL-6 levels significantly in patients with CC genotype [50, 57] thus induces the predisposition of atherosclerosis.

In premature coronary artery disease studies, there was a protective gene which is microRNA-146a that targets interleukin-1 receptor associated kinase-1 (IRAK-1) and tumour necrosis factor receptor associated factor 6 (TRAF-6) which results in inhibition of Nuclear factor KappaB (NF-κB) via the Toll-like receptor (TLR) pathway. NF-κB activation may play role in regulating inflammatory conditions associated with coronary artery disease [58]. The polymorphism of microRNA-146a (rs2910164) in young South African Indians did influence CAD association when compared to controls and CAD patient with GG genotype, CC genotype patients expressed significantly higher levels

of miR-146a (6.25-fold increase) and significantly lower levels of IRAK-1 and TRAF-6. Thus it is suggested the CC genotype serves a protective function by increasing miR-146a levels and reducing inflammation [59].

#### 4. Oxidation

The endothelium plays an essential role in maintaining vascular tone and blood pressure and one of the most important products of endothelial cells is nitric oxide (NO). It is a major mediator of endothelium dependent vasodilation made in the endothelial cells from L-arginine through the action of the homodimeric enzyme endothelial nitric oxide synthase (eNOS). In addition to vasodilation, NO inhibits platelet aggregation, proliferation of vascular smooth muscle cells, and leukocyte adhesion to endothelial cells [60]. Therefore, eNOS may have an important atheroprotective role by these actions and then protects against the pathogenesis of atherosclerosis. The gene encoding eNOS is located on chromosome 7 (7q35-36) [61, 62]. Several polymorphisms of the eNOS gene have been reported so far but their impact on eNOS production and expression remain unclear. However, the G894T(Glu298Asp) and T786C (a mutation located in the 5-flanking region of the eNOS gene) genetic polymorphisms seem to be implicated in the development of coronary heart disease [63-66].

The premature coronary artery disease is associated with the Glu298Asp polymorphism of the endothelial nitric oxide synthase gene in Turkish population. The patients with eNOS TT genotype had 15 fold risk of coronary artery disease compared with the control [67]. It is similar in Greece population, a significantly higher frequency of homozygosity for the 786C (32%) and the 894T (21%) alleles of the eNOS gene in patients who develop early MI [68]. In Korean study, the eNOS polymorphisms were not an independent predisposition factor to coronary artery disease. Nevertheless, there is significant association in 786TNC polymorphism in CAD patients when adjusted with various cardiovascular risk factors [69].

Paraoxonase 1 (PON1) is a member of a three-gene family (PON1, PON2, and PON3). PON1 activity dominates in human plasma. PON1 is an enzyme responsible as antioxidant as it can inhibit oxidation lipoproteins by reactive oxygen species formed during oxidative. Atherogenic products of oxidative lipid modification such as phospholipid peroxides and cholesterol ester hydroperoxides also can be hydrolyzed by PON1 according to the report done by Mackness *et al*[70]. Many studies have been done to discover the relationship of polymorphism of PON1 on the development of atherosclerosis[71]. It is found that the M55L PON1 polymorphism is independently associated with the early formation of atherosclerotic plaques, at the common carotid artery and to the total number of plaques at any site ( $P<0.05$ ) [72]. Similar association was found, suggesting that the PON1 LL genotype is an excellent predictor of CAD [73].

Another variants of PON1, Q192R involves a mutation from glutamine (Q, wild type) to arginine (R, variant) at amino acid position 192 of the protein sequence as reported by Bhattacharyya *et al.* comparing with participants with either the PON1 RR192 or QR192 genotype, subjects with the QQ192 genotype demonstrated an increased risk of all-cause mortality of PON1 polymorphism (43/681 deaths [6.75%] in RR192 and QR192 and 62/584 deaths [11.1%] in QQ192 and an increased prevalence of coronary artery disease was observed in subjects with the PON1 QQ192 genotype (461/962 [47.9%] with disease and 169/405 [41.7%] without coronary artery disease) (Table 3)[74]. Balcerzyk *et al* showed there is significant association between Q allele carriers of PON1 gene and premature CAD. They also demonstrated presence of synergistic effect between Q allele, smoking and increased level of total cholesterol [75]. In early onset of myocardial infarction, a first MI occurred 1.8 years earlier in patients with PON1 QQ and QR genotypes as compared with RR carriers [76].

**Table 3: SNPs associating with oxidation**

Author	Gene	Chromosome	SNPs	Sample Size	Population	Non-risk allele	Non-risk Frequency	Risk allele	Risk Allele Frequency	Result OR(95 % CI)	P-value
Chen, Y.H et al (2002) [101]	HO-1	22q12		<b>Control</b> 259= without diabetes 63= with diabetes <b>Patient</b> 323= CAD without diabetes 151= CAD+diabetes	Chinese	(GT) <sub>n</sub> = S (shorter repeats)		(GT) <sub>n</sub> = longer repeat (L)	16.6% P=0.004	*genotypic frequency related to CAD (at least one L allele) 4.7(1.9-12.0)	0.001
Chen, Y. H. et al (2008) [102]	HO-1	22q12		322= Subjects without CAD including 63 diabetic subjects 664= with CAD including 200 diabetic subjects	Chinese	(GT) <sub>n</sub> = S (shorter repeats)		(GT) <sub>n</sub> = longer repeat (L)		2.81 (1.22-6.47) *association of L/L genotype with CAD among diabetic subjects	0.015
Bhattacharyya, T. et al (2008) [74]	PON-1	7q21.3	rs662	Baseline with CVD = 1116 Baseline without CVD= 283	American		QQ192 = 11.1% QR192 = 6.75% RR192 = 6.75%			2.05(1.32-3.18) *increased risk of all-cause mortality	0.001
Manfredi, S. et al (2007) [103]	GSTM GSTT CYP1A1	1p13.3 22q11.23 15q24.1	Polymorphism Wild Variant Null	222 CAD smoker patients	Italian					GSTM1 <sup>null</sup> GSTT1 <sup>null</sup> genotypes 3.9 (1.3–11.4) *for CAD interaction  Others are not significant	0.01

## 5. 9p21 locus

Over the last decades, polymorphisms in 9p21 locus have become a huge interest in SNPs studies and thus were placed under scrutiny. 9p21 in a dose-dependent relationship was found to be strongly associated with the severity of CAD as determined by the number of coronary vessels involved [14]. Similar findings were reported to confirm 9p21 (rs1333049, rs10757278) as a predictor of the severity of CAD [77-79] across racial lines. As pertinent to premature CAD, a meta-analysis study was conducted to investigate the association in 7123 subjects from 7 case-control studies and there were premature CAD association in rs2383206, rs10757278, and rs10757274 [80]. In parallel study, rs10757274, rs2383206, rs2383207, and rs10757278 in 9p21 are significantly associated with premature and familial MI and CAD in the GeneQuest Caucasian

population [81]. The variant rs10757278 is located in a region adjacent to genes encoding the cyclin-dependent kinase inhibitors CDKN2A and CDKN2B [82]. CDKN2A and CDKN2B gene products are believed to have vital regulatory roles in cell proliferation, ageing, and apoptosis processes therefore, they are also important in the pathogenesis of CAD and atherosclerosis [83]. The risk allele is contained in a long noncoding RNA (lncRNA) of 126,000 bps, referred to as ANRIL which is an antisense RNA hypothesized to be a part of the cellular transcriptional machinery [84]. To date, there is no detail explanation of polymorphisms of 9p21 locus which confers CAD development.

Several studies reported that 9p21.3 specifically rs1333049 significantly associated with CAD only in the presence of coronary calcification. This result proposes the polymorphism was related to post-revascularization in

atherogenesis [85, 86]. Other polymorphism, rs10965219 is strongly associated with platelet reactivity and coronary artery calcification (CAC) suggested that 9p21 locus have pleiotropic effect on CAD [87]. Most recent study showed presence of association between diastolic blood pressure and coronary artery calcification quantity modified by 9p21.3 region (rs2069416) in CDKN2B-AS1 which CAC quantity is higher with lower diastolic blood pressure [88].

## 6. Conclusion

Genetic variation in the chromosomes which associated with development of coronary artery disease may contribute to the etiology of early-onset CAD. Many studies demonstrated the polymorphisms in certain gene that confer strong association with CAD. However, the real underlying mechanisms in each genes remain unclear. Thus, studies to correlate the mechanisms should be done to reveal the effect of polymorphisms especially in premature coronary artery disease in order to invent therapeutic agent. Moreover, replicated and further studies are needed to provide a more definitive and comprehensive conclusion across ethnicities.

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## Disclosure statement

The authors declare that there is no conflict of interest.

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