6. DISCUSSION

CAD is spreading worldwide with significant ethnic and regional variations. Multiple management strategies have been developed to combat the disease. However, genetic differences are reported to govern the disease progression and severity. As the SNPs have the ability to influence the disease risk, drug efficiency and side-effects, the present study was designed to evaluate the impact of environmental as well as the genetic susceptibility of the SNPs in the genes involved in the process of atherosclerosis and inflammation. Gene-gene and gene-environment interactions were also studied to understand the relation between different entities existing within the disease. In this piece of work, a total of 1000 subjects, 500 CAD patients and 500 healthy controls belonging from different states of the North India were enrolled. While sampling, the exclusion as well as the inclusion criteria were strictly followed both in the cases as well as the controls to rule out any factor that could affect the study determinations.

6.1.1 Gene polymorphisms and their impact on CAD in the studied North Indian population

SNPs are considered to be markers of many complex diseases that have been preserved in the genomes of different populations and studying them would reveal the demographic histories as well as the management of health and diseases. In the past few years, multiple studies carried out worldwide have provided a catalogue of genetic variations existing among individuals of a population as well as within different populations worldwide. The GWA studies also have identified a plethora of genetic variants associations with CAD and multiple parameters involved in its etiology which can be used in developing new prevention and treatment strategies.

Keeping this in mind, the present study have been performed that targets the genetic aspects of CAD by studying the ALOX15 rs2619112 G/A and rs7217186 T/C, LOX1 rs11053646 G/C and rs1050283 C/T, ANRIL rs1333049 C/G, PCSK9 rs505151 A/G, IL-8 rs4073 A/T, IL-10 rs1800872 C/A, IFN-γ rs2430561 T/A and TLR4 rs4986790 and rs4986791 C/T gene polymorphisms in a North Indian population.

6.1.1 Genes involved in atherosclerosis

6.1.1.1. ALOX15 rs2619112 G/A and rs7217186 T/C

This is a pioneer study to document the allelic and genotypic frequency of ALOX15 rs2619112 and rs7217186 in context to a North Indian population. Allelic
frequency of G allele is higher (63.9%) in cases than the controls for rs2619112 whereas in rs7217186, frequency of the minor allele is higher in the control group (52.4%). Also, we are the first to report the genetic association of ALOX15 rs2619112 and rs7217186 gene polymorphisms and CAD risk in the Indian population. High risk association with CAD was observed for both the SNPs thereby confirming the atherogenic role of ALOX15 (Table 5-2).

For rs2619112 G/A, risk association was observed with age above 40 years, females, obesity, sedentary lifestyle, rural living, nil family history, non-vegetarian diet, smoking and diabetes. WHR, SBP, DBP, ApoB:ApoA-I, LDL, VLDL, TC and TG were significantly associated with GG, GA and AA, ApoA-I, ApoB, FBG and UA with GG and GA, hsCRP with GA and AA, CKMB with GG, HDL and TL with GA genotypes (Table 5-20). For rs7217186 T/C, risk association was observed with age (below and above 40 years), males, obesity, sedentary lifestyle, rural and urban living, nil and positive family history, vegetarian and non-vegetarian diet and smoking. Also, SBP, DBP, ApoB, ApoB:ApoA-I, LDL, VLDL, FBG, UA, TC and TG were significantly associated with TT, TC and CC, WHR, ApoA-I with TT and TC, HDL with TC and CC and TL with TT and CC genotypes (Table 5-21).

Till date, only two association studies have been done on ALOX15 rs2619112 G/A and rs7217186 T/C polymorphisms. The first study was conducted by (Zhang, Wang et al. 2010) on 519 patients having CAD and 608 healthy controls from the Chinese Han population. Their study reported significant risk association with CAD for both rs7217186 and rs2619112 (p=0.009, OR=3.2 and 95% CI (1.335–7.665) and p=0.011, OR=3.5 and 95% CI (1.343–9.330) respectively) which is in agreement to our results. The second study pertaining to these polymorphisms was done by (Zhao, He et al. 2012) in North Chinese Han population to see the association with ischaemic stroke (IS). They enrolled 396 IS patients and 360 healthy subjects. However, they could not find any significant association of the genotypes with both these polymorphisms and the disease after the statistical analysis.

The samples were also age stratified and observed for risk association for the CC and CT genotypes in rs7217186 in subjects in both below and above 40 years of age. (Zhang, Wang et al. 2010) in his study on Chinese population reported the risk association in subjects above 60 years (OR=5.7, 95% CI (1.557–21.097) and p=0.009). However in rs2619112, risk association was observed only for the minor allele A and
Discussion

under the dominant model. (Zhang, Wang et al. 2010) results are in conformity with ours. They stated that for rs2619112 G/A, the subjects carrying the minor allele and above 60 years of age have a higher predisposition to CAD (OR=4.9, 95% CI (1.215–19.547) and p=0.025).

Gender stratified analysis revealed the risk association with males for rs7217186 in both the heterozygous and mutant genotypes. However, (Zhang, Wang et al. 2010) reported the risk association in females (OR=9.3, 95% CI (1.048–82.213) and p=0.045) for rs7217186. The discrepancy in results might be due to the difference in the sample size for males (362 patients and 401 controls) and females (157 patients and 207 controls) enrolled in their study as compared to our data. (Zhao, He et al. 2012) in his study on Chinese population also reported higher risk association of IS in males. Similarly, gender stratified analysis for rs2619112 revealed risk association for female in the heterozygous state and in the dominant model. However in the study by (Zhang, Wang et al. 2010), risk association in males was reported (OR=3.5, 95% CI (1.136–11.006) and p=0.029). Therefore one can ascertain that both genetic and hormonal factors have a role in the CAD occurrence and progression.

The present study holds a significance level as first time the allelic and genotypic frequencies pertaining to these polymorphisms with respect to North Indian population are being documented and a strong positive correlation with disease risk is being reported and gender specific associations are also observed. No data pertaining to these polymorphisms is available in the Indian context, so this is the first documentation of the data. Both the SNPs locate in the regulatory region of the ALOX15 and are also tagSNPs (Zhang, Wang et al. 2010). They are present intronically and do not code for any protein but their strong association with CAD risk lead to the fact that they might be linked to a functional variant thereby affecting the gene expression (Zhang, Wang et al. 2010). Previous epidemiological studies have revealed the role of UTR genetic variations in ALOX genes with amplified risk towards bronchial asthma, renal dysfunction, insulin resistance, colorectal cancer, vascular inflammation, atherosclerosis and stroke (Werz and Steinhilber 2006; Girelli, Martinelli et al. 2007; Helgadottir, Thorleifsson et al. 2007; Assimes, Knowles et al. 2008; Crosslin, Shah et al. 2009; Burdon, Rudock et al. 2010; Hersberger 2010; Zhang, Wang et al. 2010; Camacho, Martinez-Perez et al. 2012; Zhao, He et al. 2012) thereby supporting the present study that intronic variants are also important and one cannot ignore them.
6.1.1.2 LOX1 rs11053646 G/C and rs1050283 C/T

In the present study, the LOX1 rs11053646 G/C and rs1050283 C/T polymorphisms were investigated and significant risk association towards CAD in a North Indian population was observed. PCR-RFLP was used to genotype polymorphisms and the results reported the mutant genotype in both the polymorphisms conferred risk towards CAD in the studied population with statistically significant p values and risk was also observed under the recessive model (Table 5-3). Additionally categorizing the data on the various selected phenotypic characteristics, the rs11053646 G/C polymorphism showed risk towards CAD for age above 40 years, males and females, obesity, rural and urban living, sedentary lifestyle, family history, non-vegetarian diet, smoking and diabetes. Also, WHR, SBP, ApoB:ApoA-I, LDL, UA and TC were significantly associated with GG, GC and CC, DBP, ApoA-I, ApoB, hsCRP, HDL, VLDL, FBG and TG with GG and GC genotypes (Table 5-22). The rs1050283 C/T revealed risk towards CAD for age above 40 years, males and non-obese, rural living, sedentary and active lifestyle, family history, non-vegetarian diet, smoking, Also, WHR, SBP, DBP, ApoB, ApoB:ApoA-I, LDL, UA, TC and TG were significantly associated with CC, CT and TT genotypes, ApoA-I, hsCRP, HDL, VLDL and FBG with CC and TT and TL with TT genotypes (Table 5-23).

During initiation of atherosclerosis, ECs majorly express LOX1 but as the atherosclerotic lesions build up, the expression could also be seen on SMCs and macrophages after being induced by oxLDL, pro-oxidative and biomechanical stimuli and pro-inflammatory cytokines (Kataoka, Kume et al. 2001; Catar, Müller et al. 2007; Morawietz 2007; Ogura, Kakino et al. 2009; Hofmann, Brunssen et al. 2017), LOX1 expression is upregulated in various disease conditions such as human atherosclerosis (Kataoka et al. 1999, Kume et al. 2001, Mitra et al. 2011), HTN (Nagase et al. 2001) and MI (Kataoka et al., 2003). LOX1 further promotes the secretion of various pro-inflammatory cytokines like IL-6, MCP-1, IL-8 or TNF-α by NF-κB pathway hence, promoting the plaque formation(Pirillo, Norata et al. 2013; Xu, Ogura et al. 2013).

A G>C change in the coding region of the LOX1 at 501 position (rs11053646 G/C) has been identified which results in the substitution of lysine in the C terminal domain at 167 position to asparagine. Functional analysis studies report that this substitution leads to a reduced oxLDL binding and uptake in vitro. Also an altered oxLDL-induced LOX1
expression is also observed (Biocca, Falconi et al. 2009; Hofmann, Brunssen et al. 2017). (Mango, Clementi et al. 2003) examined the gene variants of the rs11053646 G/C polymorphism and reported minor allele frequency lower in the MI group than controls (9% vs. 18%) and indicated that the C allele may show a protective effect. (Kurnaz, AYDOĞAN et al. 2009) in their study on Turkish population showed that the frequency of GG and the G allele was higher in patients than controls (p<0.05), while frequency of the CC genotype was high in control group but in our results, the frequency of GG was high in controls but the G allele was more in controls. The results of the present study also show less C allele frequency in patients with CAD but no significant association could be observed. A study on the North Indian population (basically from Lucknow) also report a significant predisposition towards CAD (p<0.001, OR=1.99 and 95% CI (1.42-2.78))(Tripathi, Tewari et al. 2012). (Tatsuguchi, Furutani et al. 2003) in their study on 102 MI patients and 102 controls revealed significant risk association of rs11053646 with MI in the Japanese population with OR=2.89, 95% CI (1.51–5.53) and p=0.002 which is comparable to our findings (OR=3.07, 95% CI (1.765-5.347) and p<0.001). In contrast to the above reported studies, (Trabetti, Biscuola et al. 2006) was unable to find any association between rs11053646 polymorphism and acute MI in Italian population of 350 patients and 327 controls, (Hattori, Sonoda et al. 2006) also reported no association between this polymorphism in a Japanese population comprising of 235 patients with ischaemic cerebrovascular disease in 274 age and gender matched healthy controls. In this study, a significant correlation with the rs11053646 G/C polymorphism and plasma lipid levels was observed but (Kurnaz, AYDOĞAN et al. 2009) have failed to find any relationship with the lipid levels.

The LOX1 rs1050283 C/T resides 188 bp downstream to stop codon in LOX1 gene in the UTR and can affect transcription or modulate exon splicing or binding affinity to the putative regulatory element (Chen, Reis et al. 2003; Hattori, Sonoda et al. 2006). Thus, population studies have been conducted to uncover whether LOX1rs1050283 C/T has a role in CAD but associations remains controversial. (Brinkley, Kume et al. 2008) exhibited a significant risk association between LOX1 rs1050283 T allele and reduced plasma LOX1 levels in a Caucasian and Afro-Caribbean population. Two studies done on Italian population have reported the risk association of the polymorphism with CAD. (Mango, Clementi et al. 2003) found significant risk association of rs1050283 C/T in a study comprising of 150 acute MI patients and 103 controls from Italian population with
Discussion

an OR of 3.74 and the results were supported by (Novelli, Borgiani et al. 2007) in their study on 496 subjects revealing the minor allele T is related with increased risk towards the disease. The data was also in concordance to the study reported by (Chen, Reis et al. 2003) on the U.S. population. In the current study, risk association has been observed with mutant genotype and under recessive model indicating that two copies of the polymorphic allele are required for disease manifestation. Examining the allelic frequencies has also revealed a significant risk association towards CAD in the studied population (Table 5-3). However, in a study on North Indian population from Lucknow comprising 329 CAD patients and 331 controls, no association was observed for this polymorphism with CAD (OR=1.13, 95% CI (0.89-1.43) and p=0.30) (Tripathi, Tewari et al. 2012). Nevertheless, this association with the disease risk and any of the parameters has not been supported by different studies on the Italian population by (Sentinelli, Filippi et al. 2006; Trabetti, Biscuola et al. 2006). But (Kurnaz, Akadam-Teker et al. 2012) reported significant association of smoking with CAD in Turkish population which is in consensus to the results given by our study. (Kurnaz, Akadam-Teker et al. 2012) in their study enrolled 83 cases and 99 controls and showed significant association with the three genotypes and SBP, HDL, VLDL and TG which is similar to the results obtained in the present piece of work.

6.1.1.3 ANRIL rs1333049 C/G

This study aimed to understand the risk association of ANRIL rs1333049 C/G towards CAD in a North Indian population. ARMS-PCR was used for genotyping and results showed a considerable risk association towards CAD and the same was observed in the recessive model (Table 5-4). Moreover, the allelic frequencies also conferred a significant association with CAD (p<0.05). Also, this polymorphism showed risk towards CAD for age above 40 years, males and females, obesity, rural and urban living, sedentary lifestyle, family history, non-vegetarian diet and smoking. Furthermore, WHR, SBP, DBP, ApoB, ApoB:ApoA-I, FBG and TC were significantly associated with CC, CG and GG, hsCRP, HDL, LDL, VLDL, UA and TG with CC and GG genotypes and ApoA-I and TL with CG genotypes (Table 5-24).

Numerous studies document the association of ANRIL rs1333049 C/G polymorphism with CAD risk and progression (Consortium 2007; Samani, Erdmann et al. 2007; Samani and Schunkert 2008; Schunkert, Götz et al. 2008; Bressler, Folsom et al.
Discussion


A very few studies from India have tried to explore genetic polymorphisms at this selected locus. GWA study done in Chennai (South India) on 9p21 locus reported two SNPs (rs2383207 and rs10757278) conferring elevated risk to CAD (AshokKumar, Emmanuel et al. 2011). Also, the work done by (Kumar, Yumnam et al. 2011) on the North Indian population (Delhi), report three SNPs (rs2383206, rs1333040 and rs10116277) at 9p21 locus to be associated with CAD risk. The rs10757278 polymorphism at the same locus also correlates with CAD risk as shown by two studies by (Maitra, Shanker et al. 2008) and (Bhanushali, Parmar et al. 2011). However till date, two studies document data on rs1333049 C/G and CAD risk in Western and Northern Indian population. (Bhanushali, Contractor et al. 2013) recruited 229 CAD patients and 136 controls from Western India and revealed an association towards CAD with an OR=2.460, 95% CI (1.139–5.314) and p=0.022). (Kashyap, Kumar et al. 2018) in their study on North Indian population (Lucknow) reported risk for both the allelic and genotypic frequencies. This study also showed an association with CAD with an OR=6.717, 95% CI (3.444-13.102) and p<0.001. Thus, the results point towards the fact that both the North Indians as well as the Western Indians are susceptible to CAD due to this polymorphic change in ANRIL rs1333049.

(Bhanushali, Contractor et al. 2013) also reported the SNP to be the robustly associated with premature or the early onset CAD which is also supported by the results of (Meng, Hughes et al. 2008; Ellis, Pilbrow et al. 2010) and the association was supported by meta-analysis by (Palomaki, Melillo et al. 2010), however magnitude of the association was small. But in the present study, no mutant in the CAD patients was found
below 40 years of age. A positive risk association in the subjects above 40 years of age was seen with the mutant genotype having an OR=5.506 with a highly significant p<0.001. The discrepancy in results emphasize the need to genotype all the risk variants particularly at this locus, as this will help in delineating the varied risk associations in different populations to CAD. However, the impact of the polymorphism with disease extent and severity is disputable with (Ye, Willeit et al. 2008; Dandona, Stewart et al. 2010) stating it as a predictor of severity and (Anderson, Horne et al. 2008; Chen, Ballantyne et al. 2009) contradicting it.

Although 9p21 locus association with risk of CAD is very well recognized, relationship with the clinical outcomes yet remains unclear and unanswered. Also the present study results showed a strong association with the family history which is in accordance to the work done by (Scheffold, Kullmann et al. 2011)(Preuss, König et al. 2010). Gender stratified analysis depicted significant association in both the genders which is in harmony to the results reported by (Ahmed, Ali et al. 2013) on Northern Pakistani population.

6.1.1.4 PCSK9 rs505151 A/G

The present study reports the genetic association of PCSK9 E670G (rs505151 A/G) and CAD in North Indian population for the first time. Analyzing the allelic and the genotypic frequencies showed that rs505151 A/G conferred risk to the disease (Table 5-5). However, in the current study population comprising of 500 CAD patients and 500 controls, no individual with homozygous mutant GG genotype was observed. The analysis of the phenotypic characteristics also revealed risk with age above 40 years, males, obesity (nil and positive), rural and urban living, sedentary lifestyle, family history (nil and positive), non-vegetarian diet and smoking. Moreover, ApoB, ApoB:ApoA-I, LDL, VLDL, FBG, UA, TC and TG were significantly associated with AA and AG and ApoA-I, hsCRP, HDL and TL with AG genotypes (Table 5-25).

The PCSK9 is a serine protease majorly synthesized and secreted from the liver and has a pivotal role in controlling the plasma LDL levels via a post-transcriptional mechanism.Binding of LDLR to PCSK9 leads to a disruption in the endocytic recycling of LDLR and exposes it to the degradation in the lysosomes (Ferri, Tibolla et al. 2012). Consequently a downregulated LDLR will lead to a reduced uptake of LDL hence leading tovascular lipid accumulation and oxidation, hypercholesterolemia, IS and CAD (Aung,
Yin et al. 2011; Zhang, Song et al. 2016). Experimental studies have identified a biological link between PCSK9 and the development of atherosclerosis. Inactivating PCSK9 protects wild type and apoE deficient mice models from atherosclerosis, whereas PCSK9 over-expression results in more severe atherosclerotic phenotypes (Jänis, Tarasov et al. 2013).

The PCSK9 rs505151 leads to a E670G change that resides in cysteine rich C-terminal domain that mediates PCSK9 binding to LDLR. This domain of PCSK9 which is positively charged, interacts with the negatively charged entities on LDLR (Holla, Cameron et al. 2011; Tveten, Holla et al. 2011). Thus the substitution to glycine is postulated to lead to charge modification ultimately increasing the PCSK9 affinity towards LDLR and reducing LDLR, which therefore explains the elevated LDL levels in plasma.

In the current study, the results showed an association with the heterozygous genotype of rs505151 A/G with an OR of 1.590 and is the first study pertaining to this polymorphism in North India. However, a study has been conducted on Bengali population by (Maiti, Biswas et al. 2017) who attempted to study the PCSK9 Eam1104I polymorphism. They recruited 155 CAD patients and 102 healthy controls and performed PCR-RFLP analysis. They found only 4 mutants in patients and 3 in controls for Eam1104I polymorphism. Therefore one can conclude that the homozygous mutants of this gene are rare in the Indian population. (ArulJothi, Whitthall et al. 2016) performed a study on 30 patients from Chennai to look out for novel variations in PCSK9 gene, exon 7 and reported no pathogenic variations in this gene in the Indian population.

Various association studies discerning the link between PCSK9 polymorphism with lipid profile disorders and CAD risk have been carried out, but the results are inconclusive (Chen, Ballantyne et al. 2005; Evans and Beil 2006; Scartezini, Hubbart et al. 2007; Huang, Fornage et al. 2009; Norata, Garlaschelli et al. 2010; Mo, Li et al. 2015; Tsai, North et al. 2015). Even in people from same ethnicity, the outcomes of the studies were also inconsistent (Hsu, Teng et al. 2009; Aung, Yin et al. 2011; Meng and Liu 2011). Multiple studies substantiate the vital role of PCSK9 in hypercholesterolemia and IS(Zhang, Song et al. 2016), polygenic hypercholesterolemia and significantly increased LDL in men but not in women in European men (Evans and Beil 2006), LDL levels and CAD severity in African-Americans and Whites (Chen, Ballantyne et al. 2005) and high
Discussion

levels of TC and LDL and risk of CAD in Tunisian population (Slimani, Harira et al. 2014), stroke risk in Belgian population (Abboud, Karhunen et al. 2007), heightened levels of LDL and rapid intima-media thickness progression (Norata, Garlaschelli et al. 2010) and higher serum lipid parameters (TC, LDL, HDL and ApoB) in the Chinese Han population (Aung, Yin et al. 2011; Meng and Liu 2011). In our results, we have also observed a significant association of the lipid profile parameters and the PCSK9 genotypes (Table 5-25). Even the meta-analysis done by (Adi, Xie et al. 2015) reports the involvement of this polymorphism in CAD. They report the risk association under allelic model with OR=1.56, 95% CI (1.21-2.01) and p=0.001), dominant model with OR=1.46, 95% CI (1.14-1.88) and p=0.003 and recessive model with OR=3.46, 95% CI (1.19-10.10) and p=0.001. Similar risk associations have been reported in our piece of work (Table 5-5). In another meta-analysis by (Au, Griffiths et al. 2015) the risk association were observed indicating this gene to be an important one w.r.t. CAD. However, certain studies report that there is no role of this SNP with lipid profile disorders and the severity of coronary atherosclerosis (Kotowski, Pertsemlidis et al. 2006; Scartezini, Hubbart et al. 2007; Polisecki, Peter et al. 2008; Hsu, Teng et al. 2009; Huang, Fornage et al. 2009).

6.1.1.5 IL-8 rs4073 A/T

In the present study, genotyping of IL-8 rs4073 A/T polymorphism revealed risk association of the polymorphism with CAD in a North Indian population. Results showed that both the allelic and the genotypic frequencies had strong risk association with CAD and the risk association was observed in the dominant and recessive model (Table 5-6). In addition to this, phenotypic characteristics like age (above and below 40 years), gender (males and females), obesity, living (rural and urban), lifestyle (sedentary and active), family history (nil and positive), non-vegetarian diet and diabetes also revealed a risk association with the disease. Furthermore, ApoB, ApoB:ApoA-I, LDL, VLDL, FBG, UA, TC and TG were significantly associated with TT, TA and AA genotypes, ApoA-I with TT and TA, HDL with TT and WHR, SBP, DBP and hsCRP with AA genotypes (Table 5-26).

Because of the pro-inflammatory characteristics of IL-8 and it upregulation in CAD, it is hypothesized that this protein plays a role in the plaque formation and atherosclerosis (Boisvert, Curnss et al. 2000; Apostolakis, Papadakis et al. 2006). TNF-α, IL-1, LPS, and phorbl myristate acetate stimulate the fibroblasts, ECs, monocyte and macrophages to
Discussion

synthesis and secrete IL-8 which further induces the release of more cytokines (Hebert, Luscinskas et al. 1990; Mielke, Bauman et al. 1990; Strieter, Chensue et al. 1990). Also the complement system induces the IL-8 expression in atherosclerotic vessel walls (Rus, Vlaicu et al. 1996). During acute inflammation, the IL-8 is elevated for a longer period as compared to other cytokines which clear off in a few hours (DeForge, Fantone et al. 1992; Shebuski and Kilgore 2002) thereby recruiting smooth muscle cells and T lymphocytes to the sub-endothelial space (Liu, Hultén et al. 1997) and is therefore said to be a key player in the atherosclerotic plaque formation.

The IL-8 rs4073 A/T locates in promoter region of gene and influence the binding of the transcription factors and thus, regulate the expression of the IL-8 cytokine (Hacking, Knight et al. 2004; Ohyauchi, Imatani et al. 2005). Theoretically, greater the transcription of the IL-8 gene, greater will be the concentration of IL-8 in the atherosclerotic plaque. This will therefore, result in inflammation caused by macrophages, infiltration of the neutrophils and will lead to the development of CAD (Zhang, Zhang et al. 2011). Many studies support it and it's been documented that this promoter region polymorphism regulates the transcription activity of IL-8 gene and hence, the levels of production of IL-8 in many diseases like respiratory syncytial virus bronchiolitis, Helicobacter pylori related gastric diseases, atrophic gastritis and gastric cancer and acute respiratory distress syndrome (Hull, Thomson et al. 2000; Ohyauchi, Imatani et al. 2005; Taguchi, Ohmiya et al. 2005; Hildebrand, Stuhrmann et al. 2007). But since in the present study, the expression levels have not been studied, so it cannot be commented upon in the present North Indian population.

Results revealed significant risk association of this polymorphism with CAD in the studied population. Two studies carried out on Chinese population states the association of this polymorphism with CAD. In study by (Zhang, Li et al. 2017) on 217 patients and 245 control subjects showed that the heterozygous and the mutant genotypes were at an elevated risk as compared to the wild ones (p=0.04, OR=1.59 and 95% CI (1.01-2.57) and p=0.005, OR=2.06 and 95% CI (1.21-3.52) respectively). Also they analyzed the different genetic models and reported the risk association under dominant model with OR=1.75, 95% CI (1.13-2.73) and p=0.008 and also under recessive model with a risk OR=1.54, 95% CI (1.02-2.37) and p=0.04. These results stand in support of the results of the present study which also report a risk association in both dominant and recessive models with significant p values. A study on Swedish population by
Discussion

(Velásquez, Frumento et al. 2014) also showed risk association under additive (OR=1.2) and recessive model (OR=1.3). (Zhang, Zhang et al. 2011) on 675 patients and 636 controls revealed a strong association with OR=1.30, 95% CI (1.12–1.53) and p=0.004. Also, they reported the correlation of increased IL-8 serum levels in MI subjects revealing that polymorphism might affect IL-8 expression.

However, (Vogiatzi, Apostolakis et al. 2008) in their study on Caucasian Greek population, enrolled 241 CAD patients and 157 controls and concluded that the polymorphism is linked to a reduced risk among patients with ACS. But (Yang, Wang et al. 2015) showed no association of the polymorphism and the disease risk in their study on 410 patients and 410 controls belonging from China. Similarly, (Ren and She 2015; Wang, Liu et al. 2015) also supported the above said observations and found no association in the Chinese population.

Present study also revealed a gender specific association of IL-8 rs4073 A/T with CAD. In both the heterozygous and homozygous mutant genotypes, strong associations are observed in case of males in multiple logistic regression. In females also, an OR of 2.867 was observed in the homozygous mutant genotype and under recessive model after multiple logistic regression. (Velásquez, Frumento et al. 2014) conducted a study on Swedish population and found only the male specific increased risk of MI. Till date no study has been conducted to find a gender specific link between the IL-8 gene polymorphism and CAD and this is the first to report it. Previous studies document gender specific associations of the minor allele with increased risk of cystic and idiopathic pulmonary fibrosis in men (Hillian, Londono et al. 2008; Ahn, Park et al. 2011). This piece of work is the pioneer to state gender differences w.r.t.IL-8 rs4073A/T in CAD. The SHEEP (Stockholm Heart Epidemiology Program) study also states the involvement of gender specific associations with the CAD risk (Gigante, Vikström et al. 2009; Zotova, Lyrenäs et al. 2009; Leander, Gigante et al. 2012) and may point towards the role of some sex related entities or the gender specific hormones and their interaction with IL-8 which lead to CAD but the exact phenomenon yet need to be deciphered and a link needs to be established.

6.1.1.6 IL-10 rs1800872 C/A

The current study targeted to delineate the influence of IL-10 rs1800872 C/A polymorphism towards CAD in North Indian population. Genotyping was performed
Discussion

using the ARMS-PCR method. Results illustrated the mutant genotype to confer disease risk (Table 5-7). Risk association was documented for phenotypic characteristics like age above 40 years, males, obese, living (rural and urban), lifestyle (sedentary and active), family history (nil and positive), non-vegetarian diet and diabetes also revealed a risk association with the disease. Furthermore, WHR, DBP, SBP, ApoB, ApoB:ApoA-I, LDL, FBG, UA and TC were significantly associated with CC, CA and AA, ApoA-I, hsCRP, VLDL, TG with CA and AA, HDL and TL with AA genotype (Table 5-27).

Both the pro- and anti-inflammatory cytokines are implicated in CAD pathogenesis and the delicate balance is involved in maintaining the vascular homeostasis and vessel integrity (Heiskanen, Kähönen et al. 2010; Khan, Ansari et al. 2011; Assis, Marques et al. 2014). The IL-10 mediates down regulation of the cell mediated and the cytotoxic inflammatory responses. IL-10 downregulates synthesis of many pro-inflammatory cytokines such as IL-6, IL-1 and TNF-α. It also downregulates expression of human leukocyte antigen (HLA) II on APCs and also reported to restrain the proliferation of T cells and cytokine production by them (Madeshiya, Singh et al. 2017). Family studies and twin studies show that about 75% variation in the production of IL-10 production is determined by the genetic factors (Westendorp, Langermans et al. 1997). The IL-10 rs1800872 maps to -592C/A in the regulatory region and results in low IL-10 levels (Trompet, Pons et al. 2007). As CAD is associated with inflammation, low levels of IL-10 due to this polymorphism are a result of altered binding of transcription factors which ultimately affect the IL-10 production.

Studies have been conducted in subjects from diverse ethnicities suggesting varied role of IL-10 rs1800872 C/A polymorphism in CAD pathogenesis. This study discloses a significant risk association with the mutant AA genotype with an OR=4.106. PROSPER study conducted in Netherland, Ireland and Scotland on 5804 subjects aged 70–82 years revealed a significant risk association with the coronary events (OR=1.21, 95% CI (1.04–1.36) (Trompet, Pons et al. 2007). Similarly, in a study carried out on Mexican patients (389 ACS patients and 302 healthy controls) a highly significant p<0.001 with an OR=1.48 was observed (Fragoso, Vallejo et al. 2011). Another epidemiological study carried on 1652 Chinese individuals, reports (AA vs. AC+CC genotype, OR=1.60, 95% CI (1.06-2.39) to be associated with IS even after controlling for covariates (Xie, Myint et al. 2013). Another Chinese case-control study performed on 249 CAD patients and 132
Discussion

healthy controls also revealed risk associations in A carriers (AA+CA) (p=0.012) (Jin, Wang et al. 2013). (Yu, Cho et al. 2012) conducted a study in a Korean population (313 control and 173 patients), and reported this SNP linked to CAD. In context to North Indian population, (Madeshiya, Singh et al. 2017) carried out a study on 384 patients and 386 controls and found the mutant allele A to be higher among the cases (40.1%) when compared to controls (34.2%) and the dominant model showed an association with CAD (OR=1.35, 95% CI (1.01–1.80) and p=0.040). But the current results show only a marginal difference in the minor allele frequency among controls and cases (35.8% v/s 36.8%) and a protective association was seen in dominant model whereas risk association was reported in recessive model showing that two copies of the mutant allele are required for the disease manifestation.

In contrast to this study’s findings, no significant association with cardiovascular risk was observed in the meta-analysis done by (Xuan, Wang et al. 2016). Similarly, non-association was observed in the study of(Wang, Liu et al. 2015)(Yao, Li et al. 2016). Also, this IL-10 promoter polymorphism was not associated with CAD or MI in subjects having a Caucasian origin) (Koch, Kastrati et al. 2001). Earlier studies report that about 75% of the individual difference in the IL-10 secretion is basically determined by genetic factors and also having a control at transcriptional level (Zuo, Che et al. 2014). However, a study carried out in Kolkata, India (Biswas, Ghoshal et al. 2014) on 500 MI patients and 500 controls revealed no mean plasma difference in concentration of IL-10 for each genotype of rs1800872 but revealed a protective association with an OR=0.697 and p=0.014 (Biswas, Ghoshal et al. 2014).

6.1.1.7 IFN-γ rs2430561 T/A

This piece of work reports the influence of IFN-γ rs2430561 T/A polymorphism on CAD in North Indian population. Significant risk association was found for the mutant genotype and under the recessive model (Table 5-8). Statistically significant risk association was also observed in subjects above 40 years of age, males, obese, rural living, active and sedentary lifestyle, nil family history, non-vegetarian diet and smoking. Significant p value was also observed for homozygous wild (TT) and heterozygous (TA) genotype for WHR, DBP, SBP, ApoA-I, ApoB, ApoB:ApoA-I, hsCRP, HDL, LDL, VLDL, FBG, UA, TC, TG and TL (Table 5-28).
Several genetic studies have found the association between cytokine genes with heart disease and its severity (Tedgui and Mallat 2006; Garg, Saraswathy et al. 2013). Among them, IFN-γ has been observed to be significantly contributing to atherosclerosis development due to its central role in inflammation. IFN-γ plays a role in atherosclerosis via the effect on the superoxide radicals, endothelial damage and deposition and activation of cellular elements in the artery walls (Harvey and Ramji 2005; Tedgui and Mallat 2006; McLaren and Ramji 2009; Voloshyna, Littlefield et al. 2014). IFN-γ is a pro-inflammatory cytokine playing a vital role in host defense by governing the immune response towards pro-inflammatory players like IL-6 and TNF-α, promoting antigen presentation on APCs, enhancing cell adhesion and proliferation and migration of SMCs and stimulating the production of MMPs (Billiau 1996; Firestein 2005; Adamopoulos, Kolokathis et al. 2011; Kim, Kang et al. 2012; Srikanth Babu, Pulla Reddy et al. 2012).

In this study, it was found that the mutant AA genotype shows a strong association with CAD with p<0.001 and OR=6.383. In agreement with the present study, a study conducted on Iranian population has revealed association of this polymorphism with CAD (Shateri, Farazmandfar et al. 2017) with p=0.021, OR=1.81 and 95% CI (1.12-2.93). Also a study on North Indian Agrawal subjects (138 CAD cases and 187 controls) reports risk association (Garg, Saraswathy et al. 2013). (Kim, Kang et al. 2012) performed an association study on 635 Korean individuals and concluded the association of IFN-γ gene polymorphism with thrombosis. (Balci, Col-Araz et al. 2013) has also shown a significant correlation of this SNP with DCM in Turkish population. Similarly, a study on 129 Greek individuals by (Manginas, Tsiavou et al. 2008) showed association with MI or USA implicating the polymorphism to be linked with major cardiovascular events. The inheritance models \emph{i.e.} dominant and recessive models were also analyzed and the results revealed a strong risk association in recessive model with OR=3.594, 95% CI (1.823-7.087) and p<0.001 after adjusting for confounders. Similar results are documented by (Shateri, Farazmandfar et al. 2017) who has also shown risk association in recessive model. These results point towards the fact that two copies of the polymorphic allele A are required for the CAD risk in the current population.

Gender stratified analysis revealed the risk association with CAD in males and the association was observed in the recessive model. But no association was seen with the CAD risk in the female gender. The Iranian study done by (Shateri, Farazmandfar et al. 2017) stands in support of our results who enrolled 285 males and 316 females and
showed association of males with CAD risk (p= 0.000, OR=2.64 and 95% CI (1.09-3.67). Studies have demonstrated that men with CAD have lower testosterone levels as compared to non-CAD individuals. A positive correlation of testosterone levels with HDL and negative correlation with LDL and triglycerides has been documented thereby augmenting the fact of testosterone being cardio-protective in nature (Morris and Channer 2012). With age it’s been said that there is a decline in the testosterone levels in male thereby, leading to an increased risk towards CAD.

6.1.1.8 TLR4 rs4986790 A/G and rs4986791 C/T polymorphisms

This is the first report on the evaluation of the genetic association of TLR4 rs4986790 A/G and rs4986791 C/T polymorphisms and CAD in a North Indian population and allelic and genotypic frequencies for both the SNPs revealed risk for the disease. Further categorizing the data to look up for the associations with other parameters, rs4986790 A/G showed risk towards CAD with age (above and below 40 years), gender (males and females), obesity, living (rural and urban), lifestyle (sedentary and active), family history (nil and positive), non-vegetarian diet, smoking and diabetes. Furthermore, WHR, SBP, DBP, Apo A1, ApoB, ApoB:ApoA-I, HDL, LDL, VLDL, FBG, UA, TC and TG were significantly associated with AA, AG and GG, hsCRP, VLDL with AA and AG and TL with GG genotype. Similarly, in case of rs4986791 C/T, risk was reported for age (above and below 40 years), gender (males and females), obesity, living (rural and urban), sedentary lifestyle, family history, non-vegetarian diet, smoking and diabetes. Also, WHR, SBP, DBP, ApoA-I, ApoB, ApoB:ApoA-I, LDL, VLDL, FBG, UA, TC and TG were significantly associated with CC, CT and TT, hsCRP with CT and TT, HDL with CT and TL with TT genotypes.

When the lipids and the lipoproteins undergo oxidative changes, DAMPs are formed which share structural motifs to the microbial PAMPs and therefore leads to the activation of same PRRs on the vascular and immune cells (Binder, Papac-Milicevic et al. 2016; Miller and Shyy 2017). This triggers the immune response in cardiomyocytes through TLR4 activation and the downstream signaling leads to the synthesis of pro-inflammatory cytokines, lipid uptake, foam cell formation and even activate adaptive immune system (Seneviratne, Sivagarunathan et al. 2012). This result in inappropriate activation of the immune system and thus atherosclerosis (Wijnand, Cheng et al. 2010; Miller, Choi et al. 2012).
Discussion

TLR4 function and activity appears to be influenced by the genetic variations (Balistreri, Candore et al. 2004). Two TLR4 polymorphisms viz. TLR4 (Asp299Gly) rs4986790 A/G and rs4986791 C/T have been shown to change the amino acid sequence in the ligand binding site of the receptor and exist in strong linkage disequilibrium (Rallabhandi, Bell et al. 2006).

Various studies report association between TLR4 rs4986790 A/G and CAD but the results are inconclusive. Certain studies document the polymorphic change to exhibit protection to CAD (Ameziane, Beillat et al. 2003; Balistreri, Candore et al. 2004; Holloway, Yang et al. 2005; Incalcaterra, Caruso et al. 2010) while many studies reported lack of correlation between the two (Reismann, Lichy et al. 2004; Heresiemi, Lehtimäki et al. 2006; Koch, Hoppmann et al. 2006; Nebel, Flachsbart et al. 2007; Lima-Neto, Hirata et al. 2013; Golovkin, Ponasenko et al. 2014; Guven, İsmailoğlu et al. 2015; Zhou, Zheng et al. 2016). Meta-analysis study revealed the polymorphism not linked with MI (Yin, Sun et al. 2014) and CAD (Chen, Gu et al. 2015; Wu, Zhu et al. 2017). But in this study, a significant risk association was observed in both the heterozygous and mutant genotypes. Also the G allele was found to confer risk to CAD and the risk association was seen in the recessive model. In concordance to this work, the studies by (Boekholdt, Agema et al. 2003) in the Netherland population and (Edfeldt, Bennet et al. 2004) on Swedish population stand in support of these results and show risk associations. This discrepancy in findings can be explained on the basis of population differences. The study on German population by (Reismann, Lichy et al. 2004; Koch, Hoppmann et al. 2006; Nebel, Flachsbart et al. 2007) exhibited non-associations whereas two studies on Italians revealed protective associations (Balistreri, Candore et al. 2004; Incalcaterra, Caruso et al. 2010) which was also supported in the UK study (Holloway, Yang et al. 2005). The present study reported association of polymorphism with CAD in both the genders but (Edfeldt, Bennet et al. 2004) reported that men with the polymorphic change for both the SNPs had more predisposition towards MI with OR=1.4 but for women, no association could be ruled out. Also, an association with BP was revealed by (Schneider, Koch et al. 2015) in their study on 2679 CAD patients aged 50-80 years.

The association of the rs4986791 C/T polymorphism was also investigated and risk association with the heterozygous and mutant genotypes and also with the minor allele T was seen. Upon analyzing the recessive model, significant odds ratio was
Discussion

reported that signified that the two copies of the mutant allele are required for the CAD risk. However, the studies carried out worldwide report a disparity in results. (Boekholdt, Agema et al. 2003; Reismann, Lichy et al. 2004; Norata, Garlaschelli et al. 2005; Koch, Hoppmann et al. 2006; Lalouschek, Schillinger et al. 2006; Lima-Neto, Hirata et al. 2013; Golovkin, Ponasenko et al. 2014; Guven, İsmailoğlu et al. 2015) revealed non-association of this polymorphism with CAD. In DCM patients, both these genetic variants impaired the cardiac recovery, independently of the medical treatment and the presence of other cardiac risk factors.

To sum up, the results stated by this piece of work are in concordance with many studies while many state opposite results to its findings. Such discrepancies in the genetic association studies are quiet common and there are multiple reasons for these divergent observations. There might be certain confounding host and environmental factors prevailing in the diverse populations worldwide that have the tendency to modulate penetrance of the variant allele. Also, the variability in the sample size of the study and multiple other selected clinic-pathological characteristics, disparity in diagnostic measures and different genotyping methods employed for genotyping might have led to production of variable results. Moreover, in certain studies where the negative results are obtained, many of them might have never been published, known as “file drawer effect”. Such unreported findings therefore, cause significant bias and thus, distorting the picture which we are observing at this moment. Additionally, it is a well-accepted fact that CAD has multifactorial nature and involves the polygenic interactions along with the lifestyle and environmental factors. Consequently, the small contribution by the polymorphic change towards the disease may get masked by presence of other dominant and commanding risk factors (Edfeldt, Bennet et al. 2004; Incalcaterra, Caruso et al. 2010).

Also in the different studies conducted worldwide, there would be variability in the selected populations on the basis of age, gender and lifestyle habits, which will have a strong influence on the different patho-physiological characteristics. This surely will lead to inconsistent and divergent results. People belonging from different ethnicities have a different predisposition to CAD. Also, even the subjects from same population, same ethnicity and same geographical area are studied, still “genetic heterogeneity” is observed. These all factors therefore, strongly influence the results observed in the genetic association studies.
6.2 COMBINED EFFECTS OF STUDIED GENE POLYMORPHISMS AND THEIR GENOTYPES TOWARDS CAD RISK

6.2.1 Combine effects of ALOX15 rs2619112 G/A polymorphism and other genotypes

Protection towards CAD was confirmed by interaction of ALOX15 rs2619112 G/A and ALOX15 rs7217186 T/C genotypes in rs2619112 (GG) and rs7217186 (TT) with OR=0.449 and p=0.041, rs2619112 (GG) and rs7217186 (CC) with OR=0.169 and p<0.001, rs2619112 (GA) and rs7217186 (TT) with OR=0.287 and p<0.001, rs2619112 (GA) and rs7217186 (TC) with OR=0.223 and p<0.001, rs2619112 (GA) and rs7217186 (CC) with OR=0.066 and p<0.001, rs2619112 (AA) and rs7217186 (TT) with OR=0.214 and p=0.001, rs2619112 (AA) and rs7217186 (TC) with OR=0.214 and p=0.001, rs2619112 (AA) and rs7217186 (CC) with OR=0.032 and p<0.001. High protective associations were observed between combinations of ALOX15 rs2619112 G/A and LOX111053646 G/C inrs2619112 (GG) and LOX1 11053646 (CC) with OR=0.394 and p=0.016, rs2619112 (GA) and LOX1 11053646 (GG) with OR=0.479 and p=0.001, rs2619112 (GA) and LOX1 11053646 (CC) with OR=0.371 and p<0.001, rs2619112 (AA) and LOX1 11053646 (GC) with OR=0.391 and p=0.026 and rs2619112 (AA) and LOX1 11053646 (CC) with OR=0.048 and p=0.004. Resistance towards CAD was contributed by interaction of ALOX15 rs2619112 G/A and IL-8 rs4073 A/T genotypes in rs2619112 (GG) and rs4073 (TT) with OR=0.253 and p<0.001, rs2619112 (GA) and rs4073 (AA) with OR=0.372 and p=0.002, rs2619112 (GA) and rs4073 (AT) with OR=0.326 and p<0.001, rs2619112 (GA) and rs4073 (TT) with OR=0.098 and p<0.001, rs2619112 (GA) and rs4073 (TT) with OR=0.270 and p=0.002, rs2619112 (AA) and rs4073 (AT) with OR=0.109 and p<0.001 and rs2619112 (AA) and rs4073 (TT) with OR=0.048 and p<0.001. The combinations of ALOX15 rs2619112 G/A and IL-10 rs1800872 C/A impose protective association in rs2619112 (GG) and rs1800872 (TT) with OR=0.328 and p=0.006, rs2619112 (GA) and rs1800872 (CC) with OR=0.364 and p<0.001, rs2619112 (GA) and rs1800872 (AA) with OR=0.284 and p<0.001 and rs2619112 (AA) and rs1800872 (CA) with OR=0.358 and p=0.016. Protective associations were contributed by genotypic interactions between ALOX15 rs2619112 G/A and IFN-γ rs2430561 T/A inrs2619112 (GG) and rs2430561 (AA) with OR=0.151 and p<0.001, rs2619112 (GA) and rs2430561 (TT) with OR=0.345 and p<0.001, rs2619112 (GA) and rs2430561 (AA) with OR=0.187 and p<0.001 and rs2619112 (AA) and rs2430561 (TA) with OR=0.329 and p=0.009. Protection to CAD was proven by
interaction of ALOX15 rs2619112 G/A and TLR4 rs4986790 A/G in rs2619112 (GG) and rs4986790 (GG) with OR=0.254 and p<0.001, rs2619112 (GA) and rs4986790 (AA) with OR=0.410 and p=0.006, rs2619112 (GA) and rs4986790 (AG) with OR=0.300 and p<0.001, rs2619112 (GA) and rs4986790 (GG) with OR=0.152 and p<0.001, rs2619112 (AA) and rs4986790 (AA) with OR=0.252 and p=0.002, rs2619112 (AA) and rs4986790 (AG) with OR=0.109 and p<0.001 and rs2619112 (AA) and rs4986790 (GG) with OR=0.109 and p<0.001. ALOX15 rs2619112 G/A and TLR4 rs4986791 C/T showed resistance towards disease in rs2619112 (GG) and rs4986791 (TT) with OR=0.411 and p=0.008, rs2619112 (GA) and rs4986791 (CC) with OR=0.476 and p=0.013, rs2619112 (GA) and rs4986791 (CT) with OR=0.481 and p=0.013, rs2619112 (AA) and rs4986791 (CC) with OR=0.339 and p=0.011, rs2619112 (AA) and rs4986791 (CT) with OR=0.164 and p<0.001 and rs2619112 (AA) and rs4986791 (TT) with OR=0.148 and p=0.001.

**Mixed association** was verified by genotypic interaction of ALOX15 rs2619112 G/A and LOX1 rs1050283 C/T. Risk for CAD was observed in rs2619112 (GG) and LOX1 rs1050283 (CT) with OR=1.803 and p=0.036 while high protection was contributed by genotypic interaction between the rs2619112 (GG) and LOX1 rs1050283 (TT) with OR=0.324 and p=0.003, rs2619112 (GA) and LOX1 rs1050283 (CC) with OR=0.419 and p<0.001, rs2619112 (GA) and LOX1 rs1050283 (TT) with OR=0.341 and p<0.001, rs2619112 (AA) and LOX1 rs1050283 (CT) with OR=0.416 and p=0.035 and rs2619112 (AA) and LOX1 rs1050283 (TT) with OR=0.046 and p=0.003. Similarly, varied association towards CAD was confirmed by the combinations of ALOX15 rs2619112 G/A and ANRIL rs1333049 C/G. Risk was imposed by rs2619112 (GG) and rs1333049 (CG) with OR=2.755 and p<0.001 and rs2619112 (GA) and rs1333049 (CG) with OR=1.731 and p=0.011 whereas protection was observed by genotypic interaction between rs2619112 (GA) and rs1333049 (CC) with OR=0.600 and p=0.021, rs2619112 (GA) and rs1333049 (GG) with OR=0.164 and p<0.001 and rs2619112 (AA) and rs1333049 (CC) with OR=0.265 and p=0.004. Mixed associations were confirmed by genotypic interactions of the ALOX15 rs2619112 G/A and PCSK9 rs505151 A/G genotypes. Risk towards CAD was imposed by genotypic interactions between rs2619112 (GA) and rs505151 (AA) with OR=1.988 and p=0.037 whereas protection was observed by genotypic interactions between rs2619112 (GA) and rs505151 (AG) with OR=0.300 and p=0.001 and rs2619112 (AA) and rs505151 (AA) with OR=0.160 and p=0.024.
6.2.2 Combine effects of ALOX15 rs7217186 T/C polymorphism and other genotypes

Significant risk was inflicted only by interaction of ALOX15 rs7217186 T/C and TLR4 rs4986790 A/G in rs7217186 (TT) and rs4986790 (GG) with OR=4.514 and p=0.050, rs7217186 (CC) and rs4986790 (AA) with OR=2.260 and p=0.050 and rs7217186 (CC) and rs4986790 (GG) with OR=3.305 and p<0.001.

Significant protection was observed between the combinations of ALOX15 rs7217186 T/C and TLR4 rs4986790 A/G in rs7217186 (TT) and rs505151 (AG) with OR=0.401 and p<0.001, rs7217186 (CC) and rs505151 (AA) with OR=0.280 and p<0.001 and rs7217186 (CC) and rs4986790 (GG) with OR=3.305 and p<0.001.

Resistance was again confirmed by the interaction between ALOX15 rs7217186 T/C and IL-8 rs4073 A/T in rs7217186 (TC) and rs4073 (AA) with OR=0.136 and p=0.011 and rs7217186 (CC) and rs4073 (TT) with OR=0.312 and p<0.001. High protection was noticed by genotypic interaction between ALOX15 rs7217186 T/C and TLR4 rs4986791 C/T in rs7217186 (TC) and rs4986791 (CC) with OR=0.172 and p<0.001, rs7217186 (TT) and rs4986791 (TT) with OR=0.510 and p=0.034, rs7217186 (CC) and rs4986791 (CC) with OR=0.177 and p<0.001, rs7217186 (CC) and rs4986791 (GT) with OR=0.225 and p<0.001.

Mixed association was substantiated by genotypic interaction of ALOX15 rs7217186 T/C and LOX1 rs11053646 G/C genotypes. Risk for CAD was enforced by genotypic interactions between rs7217186 (TT) and LOX1 11053646 (GC) with OR=2.019 and p=0.005 whereas protection was observed for rs7217186 (TT) and LOX1 11053646 (GC) with OR=0.347 and p<0.001 and rs7217186 (CC) and LOX1 11053646 (CC) with OR=0.078 and p<0.001. For ALOX15 rs7217186 T/C and LOX1 rs1050283 C/T, risk was observed in rs7217186 (TT) and LOX1 rs1050283 (CT) with OR=2.500 and p<0.001, rs7217186 (TC) and LOX1 rs1050283 (CT) with OR=1.751 and p=0.019 whereas protection was observed for rs7217186 (CC) and LOX1 rs1050283 (GC) with OR=0.398 and p=0.001 and rs7217186 (CC) and LOX1 rs1050283 (GT) with OR=0.108 and p<0.001.

ALOX15 rs7217186 T/C and ANRIL rs1333049 C/G showed significant risk for CAD in rs7217186 (TT) and rs1333049 (CG) with OR=3.528 and p<0.001, rs7217186 (TC) and rs1333049 (CG) with OR=2.305 and p<0.001, rs7217186 (CC) and rs1333049 (GG) with OR=1.276 and p=0.005 whereas protection was observed for rs7217186 (CC) and rs1333049 (CC) with
Discussion

OR=0.418 and p=0.003 and rs71217186 (CC) and rs1333049 (GG) with OR=0.105 and p<0.001. Mixed associations were confirmed by combination of ALOX1 rs7217186 T/C and IL-10 rs1800872 C/A. Considerable risk for CAD was inflicted by genotypic interaction between rs7217186 (TT) and rs1800872 (CA) with OR=2.372 and p<0.001, rs7217186 (TC) and rs1800872 (CA) with OR=1.833 and p=0.011 whereas protection was observed for rs7217186 (TT) and rs1800872 (AA) with OR=0.443 and p=0.046, rs7217186 (TC) and rs1800872 (AA) with OR=0.470 and p=0.030, rs7217186 (CC) and rs1800872 (CC) with OR=0.322 and p<0.001 and rs7217186 (CC) and rs1800872 (AA) with OR=0.181 and p<0.001. Similarly, assorted result was associated with ALOX1 rs7217186 T/C and IFN-γ rs2430561 T/A. Risk was confirmed by the genotypic interaction between rs7217186 (TT) and rs2430561 (TA) with OR=2.312 and p=0.001, rs7217186 (TC) and rs2430561 (TA) with OR=1.822 and p=0.010 whereas protection association was observed for rs7217186 (TT) and rs2430561 (AA) with OR=0.318 and p=0.015, rs7217186 (TC) and rs2430561 (AA) with OR=0.308 and p=0.003, rs7217186 (CC) and rs2430561 (TT) with OR=0.357 and p<0.001 and rs7217186 (CC) and rs2430561 (AA) with OR=0.064 and p<0.001.

6.2.3 Combine effects of LOXI 11053646 G/C gene polymorphism and other genotypes

The combinations of LOXI 11053646 G/C and LOXI rs1050283 C/T genotypes conferred increased risk towards CAD. Risk for CAD was observed in the genotypic interaction between LOXI 11053646 (GG) and LOXI rs1050283 (CT) with OR=1.246 and p=0.001, LOXI 11053646 (GC) and LOXI rs1050283 (CT) with OR=2.990 and p<0.001 and LOXI 11053646 (CC) and LOXI rs1050283 (TT) with OR=3.517 and p=0.003. Increased risk was observed for CAD due to the combinations of LOXI 11053646G/C and ANRIL rs1333049 C/G in LOXI 11053646 (GG) and rs1333049 (CG) with OR=1.416 and p=0.006, LOXI 11053646 (GG) and rs1333049 (GG) with OR=1.566 and p<0.001, LOXI 11053646 (CC) and rs1333049 (CC) with OR=3.535 and p=0.001 and LOXI 11053646 (CC) and rs1333049 (GG) with OR=6.978 and p<0.001. Significant risk was inflicted by interaction of LOXI 11053646G/C and PCSK9 rs505151 A/G. Risk towards CAD was imposed by genotypic interactions between LOXI 11053646 (GG) and rs505151 (AG) with OR=3.664 and p=0.001, LOXI 11053646 (GC) and rs505151 (AG) with OR=5.201 and p<0.001, LOXI 11053646 (CC) and rs505151 (AA) with OR=5.871 and p<0.001 and LOXI 11053646 (CC) and rs505151 (AG) with
OR=3.011 and p=0.021. Risk was again attributed by the interaction of \( \text{LOX1} \) 11053646G/C and \( \text{IL-8 rs4073 A/T} \) in \( \text{LOX1} \) 11053646G/C (CC) and rs4073 (AA) with OR=2.163 and p<0.001, \( \text{LOX1} \) 11053646G/C (CC) and rs4073 (AT) with OR=3.054 and p=0.023 and \( \text{LOX1} \) 11053646G/C (CC) and rs4073 (TT) with OR=4.070 and p=0.002. Elevated risk for CAD was observed in combinations of \( \text{LOX1} \) 11053646G/C and \( \text{IL-10 rs1800872 C/A} \) in \( \text{LOX1} \) 11053646 (GC) and rs1800872 (CA) with OR=2.931 and p=0.001, \( \text{LOX1} \) 11053646 (GC) and rs1800872 (AA) with OR=2.342 and p<0.001, \( \text{LOX1} \) 11053646 (CC) and rs1800872 (CC) with OR=3.736 and p=0.003 and \( \text{LOX1} \) 11053646 (CC) and rs1800872 (CA) with OR=3.127 and p=0.018. Significant risk associations were seen in interaction of \( \text{LOX1} \) 11053646G/C and \( \text{IFN-\gamma rs2430561 T/A} \) in \( \text{LOX1} \) 11053646 (GC) and rs2430561 (TA) with OR=1.310 and p=0.001, \( \text{LOX1} \) 11053646 (GC) and rs2430561 (AA) with OR=2.525 and p<0.001 and \( \text{LOX1} \) 11053646 (CC) and rs2430561 (TT) with OR=3.030 and p=0.002. Significant risk associations were seen in interaction of \( \text{LOX1} \) 11053646G/C and \( \text{TLR4 rs4986791 C/T} \) in \( \text{LOX1} \) 11053646 (GC) and rs4986791 (CT) with OR=2.121 and p=0.002 and \( \text{LOX1} \) 11053646 (CC) and rs4986791 (TT) with OR=1.251 and p=0.002.

**Mixed associations** were confirmed by genotypic interactions of \( \text{LOX1} \) 11053646G/C and \( \text{TLR4 rs4986790 A/G} \). Risk for CAD was imposed by genotypic interaction between \( \text{LOX1} \) 11053646 (GC) and rs4986790 (AA) with OR=1.667 and p=0.040, \( \text{LOX1} \) 11053646 (CC) and rs4986790 (GG) with OR=2.198 and p<0.001 whereas protection was seen in genotypic interactions between \( \text{LOX1} \) 11053646 (GG) and rs4986790 (GG) with OR=0.386 and p<0.001, \( \text{LOX1} \) 11053646 (CC) and rs4986790 (AA) with OR=0.11053646 and p=0.042 and \( \text{LOX1} \) 11053646 (CC) and rs4986790 (AG) with OR=0.490 and p=0.018.

### 6.2.4 Combine effects of \( \text{LOX1 rs1050283 C/T} \) gene polymorphism and other genotypes

**Risk** for CAD was imposed by genotypic interaction between \( \text{LOX1 rs1050283 C/T} \) and \( \text{ANRIL rs1333049 C/G} \) in \( \text{LOX1 rs1050283} \) (CC) and rs1333049 (CC) with OR=3.371 and p<0.001, \( \text{LOX1 rs1050283} \) (CC) and rs1333049 (GG) with OR=4.479 and p<0.001, \( \text{LOX1 rs1050283} \) (CT) and rs1333049 (CC) with OR=4.599 and p<0.001, \( \text{LOX1 rs1050283} \) (TT) and rs1333049 (GG) with OR=3.371 and p<0.001. Risk for CAD was
also observed in the genotypic interaction between \textit{LOX1} rs1050283 C/T and \textit{PCSK9} rs505151 A/G in \textit{LOX1} rs1050283 (CC) and rs505151 (AG) with OR=2.729 and p=0.018 and \textit{LOX1} rs1050283 (CT) and rs505151 (AG) with OR=5.040 and p<0.001. The combinations of \textit{LOX1} rs1050283 C/T and \textit{IL-8} rs4073 A/T conferred increased risk towards CAD. The rs1050283 (CT) and rs4073 (AT) imposed risk with OR=2.616 and p=0.001, \textit{LOX1} rs1050283 (CT) and rs1800872 (AA) with OR=6.820 and p<0.001 and \textit{LOX1} rs1050283 (TT) and rs1800872 (CC) with OR=6.535 and p=0.001, \textit{LOX1} rs1050283 (CT) and rs1800872 (CA) with OR=4.591 and p=0.002. High risk association was contributed by genotypic interaction of \textit{LOX1} rs1050283 C/T and \textit{IL-10} rs1800872 C/A in \textit{LOX1} rs1050283 (CT) and rs1800872 (CA) with OR=4.691 and p=0.015, \textit{LOX1} rs1050283 (CT) and rs4986790 (AG) with OR=4.053 and p=0.001 and \textit{LOX1} rs1050283 (CT) and rs4986790 (GG) with OR=3.522 and p<0.001. Considerable risk towards CAD was confirmed by genotypic interactions between \textit{LOX1} rs1050283 C/T and \textit{TLR4} rs4986791 C/T in \textit{LOX1} rs1050283 (CT) and rs4986791 (TT) with OR=5.900 and p<0.001, \textit{LOX1} rs1050283 (TT) and rs4986791 (CC) with OR=6.535 and p=0.001, \textit{LOX1} rs1050283 (CT) and rs4986791 (AT) with OR=4.875 and p=0.022 and \textit{LOX1} rs1050283 (TT) and rs4986791 (TT) with OR=9.978 and p<0.001.

6.2.5 Combine effects of \textit{ANRIL} rs1333049 C/G gene polymorphism and other genotypes

The combinations of \textit{ANRIL} rs1333049 C/G and \textit{PCSK9} rs505151 A/G polymorphisms conferred increased risk towards CAD. rs1333049 (CC) and rs505151 (AG) conferred risk with OR=3.679 and p=0.012, rs1333049 (CG) and rs505151 (AG) with OR=5.060 and p<0.001 and rs1333049 (GG) and rs505151 (AA) with OR=4.837 and p=0.002. Risk to CAD was confirmed by genotypic interaction between \textit{ANRIL} rs1333049 C/G and \textit{IL-8} rs4073 A/T in rs2619112 (CC) and rs7217186 (AT) with OR=1.071 and p=0.001, rs2619112 (CC) and rs7217186 (TT) with OR=2.666 and
Discussion

p<0.001, rs2619112 (CG) and rs7217186 (AA) with OR=4.884 and p=0.043, rs2619112 (CG) and rs7217186 (AT) with OR=3.684 and p<0.001, rs2619112 (CG) and rs7217186 (TT) with OR=2.281 and p<0.001, rs2619112 (GG) and rs7217186 (AT) with OR=3.721 and p=0.046 and rs2619112 (GG) and rs7217186 (TT) with OR=7.550 and p=0.002. Risk towards CAD was confirmed by the genotypic interaction between ANRIL rs1333049 C/G and IL-10 rs1800872 C/A in rs1333049 (CC) and rs1800872 (CA) with OR=3.106 and p<0.001, rs1333049 (CG) and rs1800872 (CC) with OR=3.733 and p<0.001 and rs1333049 (CG) and rs1800872 (CA) with OR=8.633 and p<0.001. Significant risk towards CAD was contributed by genotypic interactions between ANRIL rs1333049 C/G and IFN-γ rs2430561 T/A in rs1333049 (CG) and rs2430561(TT) with OR=2.700 and p=0.002 and rs1333049 (CG) and rs2430561(TA) with OR=5.919 and p<0.001. Significant risk for CAD was was seen in genotypic interactions between ANRIL rs1333049 C/G and TLR4 rs4986791 C/T in rs1333049 (GG) and rs4986791 (CC) with OR=3.432 and p=0.048, rs1333049 (GG) and rs4986791 (CT) with OR=2.576 and p<0.001 and rs1333049 (GG) and rs4986791 (TT) with OR=3.623 and p<0.001.

Mixed associations were substantiated by genotypic interactions of ANRIL rs1333049 C/G and TLR4 rs4986790 A/G. Risk to CAD was confirmed by genotypic interaction between rs1333049 (CG) and rs4986790 (AA) with OR=2.887 and p<0.001 and rs1333049 (CG) and rs4986790 (AG) with OR=2.314 and p<0.001 whereas protection was observed for rs1333049 (CC) and rs4986790 (GG) with OR=0.425 and p=0.003, rs1333049 (GG) and rs4986790 (AG) with OR=0.234 and p=0.003 and rs1333049 (GG) and rs4986790 (GG) with OR=0.168 and p<0.001.

6.2.6 Combine effects of PCSK9 rs505151 A/G gene polymorphism and other genotypes

Risk was observed in interactions of PCSK9 rs505151 A/G and IL-8 rs4073 A/T in rs505151 (AA) and rs4073 (TT) with OR=3.471 and p<0.001 and rs505151 (AG) and rs4073 (AT) with OR=2.822 and p=0.001. Risk towards CAD was proven by genotypic interaction between PCSK9 rs505151 A/G and TLR4 rs4986790 A/G in rs505151 (AA) and rs4986790 (AG) with OR=2.913 and p=0.001, rs505151 (AA) and rs4986790 (GG) with OR=2.813 and p=0.003, rs505151 (AG) and rs4986790 (AA) with OR=4.147 and p<0.001, rs505151 (AG) and rs4986790 (AG) with OR=1.94 and p=0.031. Risk to CAD was imposed by genotypic interactions between PCSK9 rs505151 A/G and TLR4
Discussion

rs4986791 C/T in rs505151 (AA) and rs4986791 (CT) with OR = 3.145 and p < 0.001, rs505151 (AA) and rs4986791 (TT) with OR = 2.278 and p = 0.013, rs505151 (AG) and rs4986791 (CC) with OR = 4.450 and p < 0.001, rs505151 (AG) and rs4986791 (CT) with OR = 2.704 and p = 0.002.

Mixed associations were seen in genotypic interaction between PCSK9 rs505151 A/G and IL-10 rs1800872 C/A. rs505151 (AA) and rs1800872 (AA) conferred risk with OR = 1.652 and p = 0.013 whereas high protection was attributed by genotypic interactions between rs505151 (AA) and rs1800872 (CA) with OR = 0.429 and p < 0.001, rs505151 (AG) and rs1800872 (CA) with OR = 0.452 and p = 0.010 and rs505151 (AG) and rs1800872 (AA) with OR = 0.212 and p < 0.001. Speckled associations toward CAD was substantiated by combinations of rs505151 A/G and IFN-γ rs2430561 T/A. Risk was confirmed by genotypic interaction between rs505151 (AA) and rs2430561 (AA) with OR = 1.586 and p = 0.021 while protection was contributed by genotypic interaction between rs505151 (AA) and rs2430561 (TA) with OR = 0.440 and p < 0.001, rs505151 (AG) and rs2430561 (TA) with OR = 0.308 and p = 0.001, rs505151 (AG) and rs2430561 (AA) with OR = 0.107 and p < 0.001.

6.2.7 Combine effects of IL-8 rs4073 A/T gene polymorphism and other genotypes

Highly protective associations were contributed by genotypic interaction between IL-8 rs4073 A/T and TLR4 rs4986790 A/G genotypes in rs4073 (AA) and rs4986790 (AG) with OR = 0.368 and p = 0.014 and rs4073 (TT) and rs4986790 (GG) with OR = 0.307 and p < 0.001. Highly protective association was observed in the genotypic interaction between the IL-8 rs4073 A/T and TLR4 rs4986791 C/T in rs4073 (AT) and rs4986791 (CC) with OR = 0.213 and p = 0.008, rs4073 (AT) and rs4986791 (TT) with OR = 0.503 and p = 0.049, rs4073 (TT) and rs4986791 (CC) with OR = 0.165 and p < 0.001, rs4073 (TT) and rs4986791 (CT) with OR = 0.252 and p = 0.021, rs4073 (TT) and rs4986791 (TT) with OR = 0.371 and p < 0.001.

Mixed associations were confirmed by genotypic interactions of IL-8 rs4073 A/T and IL-10 rs1800872 C/A genotypes. Significant risk was verified by genotypic interactions between rs4073 (AA) and rs1800872 (CA) with OR = 2.811 and p < 0.001, rs4073 (AT) and rs1800872 (CA) with OR = 2.241 and p < 0.001 whereas protective association was observed in the genotypic interaction between rs4073 (TT) and rs1800872 (CC) with OR = 0.455 and p = 0.006 and rs4073 (TT) and rs1800872 (AA) with
Discussion

OR=0.218 and p=0.001. Similarly, assorted result was associated with *IL-8* rs4073 A/T and *IFN-γ* rs2430561 T/A genotypes. Significant risk to CAD was imposed by genotypic interactions between rs4073 (AA) and rs2430561 (TA) with OR=3.991 and p<0.001, rs4073 (AT) and rs2430561 (TA) with OR=2.667 and p<0.001 whereas protection was observed in rs4073 (AT) and rs2430561 (AA) with OR=0.294 and p=0.011, rs4073 (TT) and rs2430561 (TT) with OR=0.533 and p=0.027 and with OR=0.162 and p=0.001.

6.2.8 Combine effects of *IL-10* rs1800872 C/A gene polymorphism and other genotypes

*Varied associations* towards CAD were confirmed by combinations of *IL-10* rs1800872 C/A and *IFN-γ* rs2430561 T/A genotypes. Risk was proven by genotypic interactions between rs1800872 (CC) and rs2430561 (TA) with OR=6.316 and p=0.019, rs1800872 (CA) and rs2430561 (TA) with OR=2.393 and p<0.001, whereas protection was observed for rs1800872 (AA) and rs2430561 (AA) with OR=0.330 and p<0.001. Similarly, assorted result was associated with *IL-10* rs1800872 C/A and *TLR4* rs4986790 A/G genotypes. Risk was verified by genotypic interactions between rs1800872 (CA) and rs4986790 (AA) with OR=2.196 and p=0.001, rs1800872 (CA) and rs4986790 (AG) with OR=1.942 and p=0.006 whereas protection was observed for rs1800872 (CC) and rs4986790 (GG) with OR=0.421 and p=0.002, rs1800872 (AA) and rs4986790 (AG) with OR=0.452 and p=0.025 and rs1800872 (AA) and rs4986790 (GG) with OR=0.230 and p=0.001. Mixed associations were confirmed by genotypic interactions of *IL-10* rs1800872 C/A and *TLR4* rs4986791 C/T genotypes. Significant risk to CAD was established by genotypic interaction between rs1800872 (CC) and rs4986791 (CT) with OR=1.266 and p=0.025, rs1800872 (CA) and rs4986791 (CT) with OR=2.780 and p<0.001, rs1800872 (CA) and rs4986791 (TT) with OR=1.333 and p<0.001 whereas protection was observed for rs1800872 (CC) and rs4986791 (TT) with OR=0.706 and p=0.005, rs1800872 (AA) and rs4986791 (CC) with OR=0.529 and p=0.002 and rs1800872 (AA) and rs4986791 (TT) with OR=0.342 and p=0.008.

6.2.9 Combine effects of *IFN-γ* rs2430561 T/A gene polymorphism and other genotypes

*Mixed associations* were confirmed by genotypic interactions of *IFN-γ* rs2430561 T/A and *TLR4* rs4986790 A/G genotypes. Risk for CAD was imposed by genotypic interactions between rs2430561 (TA) and rs4986790 (AA) with OR=2.192 and p=0.001,
Discussion

rs2430561 (TA) and rs4986790 (AG) with OR=1.913 and p=0.007 whereas protection was observed for rs2430561 (TT) and rs4986790 (GG) with OR=0.475 and p=0.007, rs2430561 (AA) and rs4986790 (AA) with OR=0.339 and p=0.023, rs2430561 (AA) and rs4986790 (AG) with OR=0.329 and p=0.005 and rs2430561 (AA) and rs4986790 (GG) with OR=0.068 and p=0.005. Varied association towards CAD was confirmed by the combinations of IFN-γ rs2430561 T/A and TLR4 rs4986791 C/T genotypes. Risk association was observed by genotypic interaction between rs2430561 (TA) and rs4986791 (CC) with OR=2.993 and p<0.001, rs2430561 (TA) and rs4986791 (CT) with OR=2.803 and p<0.001 while protection was seen for rs2430561 (AA) and rs4986791 (TT) with OR=0.092 and p=0.001.

6.2.10 Combine effects of TLR4 rs4986790 A/G gene polymorphism and other genotypes

Highly protective associations were contributed by genotypic interactions between TLR4 rs4986790 A/G and TLR4 rs4986791 C/T genotypes in rs4986790 (AA) and rs4986791 (TT) with OR=0.474 and p=0.034, rs4986790 (AG) and rs4986791 (CC) with OR=0.314 and p=0.001, rs4986790 (AG) and rs4986791 (TT) with OR=0.512 and p=0.032, rs4986790 (GG) and rs4986791 (CC) with OR=0.391 and p=0.007, rs4986790 (GG) and rs4986791 (CT) with OR=0.488 and p=0.034 and rs4986790 (GG) and rs4986791 (TT) with OR=0.340 and p<0.001.