7. CONCLUSION

The present study aimed to reveal the genetic aspects of CAD by genotyping eleven SNPs in eight candidate genes. One thousand subjects belonging from North Indian population were recruited for the study. Majority of the population belonged from Punjab, Haryana, Chandigarh and Himachal Pradesh. Angiographically confirmed individuals with more than 50% stenosis in at least one coronary artery were enrolled as patients and comprised of 397 males and 103 females. Five hundred healthy, disease free subjects were enrolled as controls and comprised of 370 males and 130 females. The study has been conducted strictly adhering to all the inclusion and exclusion criteria. Institutional Ethics Committee, Panjab University, Chandigarh, India, granted the ethical clearance vide approval memo no. IEC No. -120A-1-1 dated 01.12.2014 for conducting the research work on human blood samples. Genotyping was done using the RFLP-PCR or ARMS-PCR method. SPSS software version 20.0 (SPSS, Inc., Chicago, IL) and Epi Info version 3.4.7 (CDC, Atlanta, GA) were used for statistical analysis. Multivariate logistic regression was used to analyze the association of SNP and the susceptibility to CAD adjusted for age and gender. Additionally, dominant and recessive models of inheritance were used to perform the association study. Association with the various parameters was seen and \( p < 0.05 \) was considered as statistically significant for all tests.

The conclusions determined from the present study are as follows:

7.1 ASSOCIATION OF ALLELIC AND GENOTYPIC FREQUENCIES

- While analyzing the \textbf{allelic frequencies}, significant \textbf{risk} towards disease was observed for \textit{LOX1} C/T, \textit{PCSK9} A/G, \textit{IL-8} A/T, \textit{TLR4} A/G and C/T polymorphisms. \textbf{Protection} was seen for \textit{ALOX15} G/A and T/C polymorphisms while \textbf{non-significant} allelic association was seen for \textit{LOX1G/C}, \textit{ANRIL} C/G, \textit{IFN-\( \gamma \)} T/A and \textit{IL-10} C/A polymorphisms.

- Genotypic frequency analysis revealed \textbf{risk} association for \textit{ALOX15} G/A in the \textbf{heterozygous} genotype, \textit{ALOX15} T/C, \textit{ANRIL} C/G, \textit{IL-8} A/T, \textit{TLR4} A/G and TLR4 C/T for \textbf{both the heterozygous and the mutant genotype} and \textit{LOX1} G/C and C/T, \textit{IL-10} and \textit{IFN-\( \gamma \)} T/A for \textbf{mutant genotype}.

- Dominant and recessive models were also analyzed to see the mode of inheritance. Statistically significant \( p \) values and risk association under \textbf{dominant} model was observed in case of \textit{ALOX15} G/A and T/C, \textit{PCSK9} A/G, \textit{IL-8} A/T and
Conclusion

TLR4 A/G thereby implying that only a single copy of the polymorphic allele is sufficient to cause the disease. For the recessive model, significant risk association was seen for ALOX15 T/C, LOX1 G/C and C/T, ANRIL C/G, IL-8 A/T, IL-10 C/A, IFN-γ T/A, TLR4 A/G and C/T thereby telling that two copies of the allele are required for the disease manifestation.

7.2 INTERACTION OF PHENOTYPIC CHARACTERISTICS AND SNPs

- For subjects below 40 years, significant risk was associated with ALOX15 C/T, IL-8 A/T, TLR4 A/G and C/T polymorphisms. For subjects above 40 years, significant risk was seen for ALOX15 G/A and C/T, LOX1 G/C and C/T, ANRIL C/G, PCSK9 A/G, IL-8 A/T, IL-10 C/A, IFN-γ T/A, TLR4 A/G and C/T polymorphisms.
- For males, risk association was observed for ALOX15 C/T, LOX1 G/C and C/T, ANRIL C/G, PCSK9 A/G, IL-8 A/T, IL-10 C/A, IFN-γ T/A, TLR4 A/G and C/T polymorphisms. In case of females, statistically significant risk was documented in ALOX15 G/A, LOX1 G/C, ANRIL C/G, IL-8 A/T, TLR4 A/G and C/T polymorphisms.
- In case of obese individuals, risk was observed for ALOX15 G/A and C/T, LOX1 G/C and C/T, ANRIL C/G, PCSK9 A/G, IL-8 A/T, IL-10 C/A, IFN-γ T/A, TLR4 A/G and C/T polymorphisms. For non-obese individuals risk association was seen in ALOX15 C/T, LOX1 C/T, PCSK9 A/G, TLR4 A/G and C/T polymorphisms.
- In case of subjects with sedentary lifestyle, statistically significant risk association was observed for ALOX15 G/A and C/T, LOX1 G/C and C/T, ANRIL C/G, PCSK9 A/G, IL-8 A/T, IL-10 C/A, IFN-γ T/A, TLR4 A/G and C/T polymorphisms. Active lifestyle was associated with risk in IL-8 A/T, IFN-γ T/A, and TLR4 A/G polymorphisms.
- Family history of CAD in the studied population was associated with ALOX15 C/T, ANRIL C/G, PCSK9 A/G, IL-8 A/T, TLR4 A/G and C/T polymorphisms as
risk factor. Subjects having no family history of CAD were associated with ALOX15 G/A and C/T, LOX1 G/C and C/T, PCSK9 A/G, IL-8 A/T, IL-10 C/A, IFN-γ T/A, TLR4 A/G and C/T polymorphisms as risk factor.

- Risk was seen in subjects with non-vegetarian diet for ALOX15 G/A and C/T, LOX1 G/C and C/T, ANRIL C/G, PCSK9 A/G, IL-8 A/T, IL-10 C/A, IFN-γ T/A, TLR4 A/G and C/T polymorphisms. For vegetarians, risk was observed for ALOX15 C/T and IL-8 A/T polymorphisms.

- Smoker CAD patients showed statistically significant associations with all the selected polymorphisms.

- Significant risk association with diabetes was found for ALOX15 G/A, LOX1 G/C and C/T, IL-8 A/T, IL-10 C/A, TLR4 A/G and C/T polymorphisms.

- Significant association was seen for ALOX15 rs2619112 G/A in homozygous wild (GG) genotype for WHR, SBP, DBP, CKMB, ApoA1, Apo B, ApoB:ApoA1, LDL, VLDL, FBG, UA, TC and TG, in heterozygous (GA) genotype for WHR, SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC, TG and TL and in mutant (AA) genotype for WHR, SBP, DBP, ApoB:ApoA1, hsCRP, LDL, VLDL, TC and TG.

- Significant association was documented for ALOX15 rs7217186 T/C in homozygous wild (TT) genotype for SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, hsCRP, LDL, VLDL, FBG, UA, TC, TG and TL, in heterozygous (TC) genotype for WHR, ApoA1, Apo B, ApoB:ApoA1, hsCRP, LDL, VLDL, FBG, UA, TC, TG and TL and in mutant (CC) genotype for WHR, Apo B, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC, TG and TL.

- Significant p value was reported in LOX1 rs11053646 G/C for homozygous wild (GG) genotype for WHR, SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC and TG, in heterozygous (GC) genotype for ApoA1, Apo B, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC and TG and in mutant (CC) genotype for DBP, ApoB:ApoA1, LDL, VLDL, FBG, UA and TG.

- Significant p value was seen in LOX1 rs1050283 C/T in homozygous wild (CC) genotype for WHR, SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC and TG, in heterozygous (CT) genotype for WHR, SBP, DBP, Apo B, ApoB:ApoA1, LDL, UA, TC and TG) and in mutant (TT)
Conclusion

- Significant p value was observed in ANRIL rs1333049 C/G in homozygous wild (CC) genotype for WHR, SBP, DBP, Apo B, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC, TG and TL.
- Significant association was reported for PCSK9 rs505151 A/G for homozygous wild (AA) genotype for WHR, SBP, DBP, Apo B, ApoB:ApoA1, LDL, VLDL, FBG, UA, TC and TG and for heterozygous (AG) genotype for WHR, SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC, TG and TL.
- Significant p value was documented for IL-8 rs4073 A/T in homozygous wild (AA) genotype for WHR, SBP, DBP, Apo A, ApoB:ApoA1, HDL, LDL, VLDL, FBG, UA, TC, TG, heterozygous (AT) genotype for ApoA1, Apo B, ApoB:ApoA1, LDL, VLDL, FBG, UA, TC and TG and mutant (TT) genotype for Apo B, ApoB:ApoA1, hsCRP, LDL, VLDL, FBG, UA, TC and TG.
- Significant p value was seen for IL-10 rs1800872 C/A for homozygous wild (CC) genotype for WHR, SBP, DBP, Apo B, ApoB:ApoA1, LDL, FBG, UA and TC, heterozygous (CA) genotype for WHR, SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, hsCRP, LDL, VLDL, FBG, UA, TC and TG and mutant (AA) genotype for WHR, SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC, TG and TL.
- Significant association was witnessed for IFN-γ rs2430561 T/A for homozygous wild (TT) genotype for WHR, SBP, DBP, Apo A, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC, TG and TL and heterozygous (TA) genotype for WHR, SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC and TG.
- Significant p value was observed in TLR4 rs4986790 A/G for homozygous wild (AA) genotype for WHR, SBP, DBP, Apo A, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC and TG, heterozygous (AG) genotype for WHR, SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL,
Conclusion

FBG, UA, TC and TG and mutant (GG) genotype for WHR, SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, HDL, LDL, FBG, UA, TC, TG and TL.

- Significant association was reported for TLR4 rs4986791 C/T for homozygous wild (CC) genotype for WHR, SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, LDL, VLDL, FBG, UA, TC, TG, heterozygous (CT) genotype for WHR, SBP, DBP, ApoA1, Apo B, ApoB:ApoA1, hsCRP, HDL, LDL, VLDL, FBG, UA, TC and TG and mutant (TT) genotype for WHR, DBP, ApoA1, Apo B, ApoB:ApoA1, hsCRP, LDL, VLDL, FBG, UA, TC, TG and TL.

7.3 IMPACT OF COMBINATION OF GENOTYPES


- ALOX15 rs7217186 T/C gene polymorphism showed risk only with TLR4 A/G gene polymorphism. Significant protective association was found with ALOX15 G/A, PCSK9 A/G, IL-8 A/T and TLR4 C/T gene polymorphisms. However varied association was observed with LOX1 G/C and C/T, ANRIL C/G, IFN-γ T/A and IL-10 C/A gene polymorphisms.

- LOX1 rs11053646 G/C gene polymorphism found significant risk with LOX1 C/T, ANRIL C/G, PCSK9 A/G, IL-8 A/T, IL-10 C/A, IFN-γ T/A and TLR4 C/T gene polymorphisms. Resistance from CAD was found only with ALOX15 G/A gene polymorphism. Mixed association was imposed by ALOX15 T/C and TLR4 A/G gene polymorphisms.

- LOX1 rs1050283 C/T gene polymorphism displayed risk association with LOX1 G/C, ANRIL C/G, PCSK9 A/G, IL-8 A/T, IL-10 C/A, IFN-γ T/A, TLR4 A/G and C/T gene polymorphisms. However, mixed association was imposed with ALOX15 G/A and ALOX15 T/C gene polymorphisms.

- ANRIL rs1333049 C/G gene polymorphism revealed risk association with LOX1 G/C and C/T, PCSK9 A/G, IL-8 A/T, IL-10 C/A, IFN-γ T/A and TLR4 C/T gene polymorphisms. Varied association was observed with ALOX15 G/A and T/C and TLR4 A/G gene polymorphisms.
Conclusion

- **PCSK9 rs505151 A/G** gene polymorphism showed risk with LOX1 G/C and C/T, ANRIL C/G, IL-8 A/T, TLR4 A/G and C/T gene polymorphisms. Significant protective association was found with ALOX15 T/C gene polymorphism only. However, mixed association was seen with ALOX15 G/A, IFN-γ T/A and IL-10 C/A gene polymorphisms.

- **IL-8 rs4073 A/T** gene polymorphism revealed significant risk with LOX1 G/C and LOX1 C/T, ANRIL C/G and PCSK9 A/G gene polymorphisms. Resistance to CAD was seen with ALOX15 G/A and T/C, TLR4 A/G and C/T gene polymorphisms. Varied association was observed with IFN-γ T/A and IL-10 C/A gene polymorphisms.

- **IL-10 rs1800872 C/A** gene polymorphism displayed risk association with LOX1 G/C and LOX1 C/T, ANRIL C/G and PCSK9 A/G gene polymorphisms. However, protection was observed only with ALOX15 G/A gene polymorphisms. Mixed associations were seen with ALOX15 T/C, IL-8 A/T, IFN-γ T/A, TLR4 A/G and C/T gene polymorphisms.

- **IFN-γ rs2430561 T/A** gene polymorphism found significant risk with LOX1 G/C and C/T and ANRIL C/G gene polymorphisms. Protection towards CAD was seen only with ALOX15 G/A gene polymorphism. Mixed associations were observed with ALOX15 T/C, PCSK9 A/G, IL-8 A/T, IL-10 C/A, TLR4 A/G and C/T gene polymorphisms.

- **TLR4 rs4986790 A/G** gene polymorphism showed risk with ALOX15 T/C, LOX1 C/T and PCSK9 A/G gene polymorphisms. Resistance for CAD was observed with ALOX15 G/A, IL-8 A/T and TLR4 C/T gene polymorphisms. However, mixed associations were documented with LOX1 G/C, ANRIL C/G, IFN-γ T/A and IL-10 C/A gene polymorphisms.

- **TLR4 rs4986791 C/T** gene polymorphisms displayed risk association with LOX1 G/C and C/T, ANRIL C/G and PCSK9 A/G gene polymorphisms. Protection towards CAD was observed with ALOX15 G/A and T/C, IL-8 A/T and TLR4 A/G gene polymorphisms. Mixed associations were seen only with IFN-γ T/A and IL-10 C/A gene polymorphisms.