Summary and Conclusions
Homocysteine is an amino acid that is known as an independent risk factor for cardiovascular disorders. Majority of Indian population adheres to a vegetarian diet, a healthier alternative in the context of cardiovascular disorders. However, vegetarian diet is deficient in vitamin B12 which is required for homocysteine metabolism. Deficiency of vitamin B12 leads to increase in concentration of homocysteine and cysteine. Along with environmental factors (including diet), various genetic changes may also play a role in elevating homocysteine concentration. In this study, we have checked the impact of diet on CAD and concentration of biochemical metabolites. We have also tried to find out basal frequency of various polymorphisms from the genes involved in the genetics of cardiovascular disorders.

To fulfill the objectives of the thesis, we have done our study into two different study groups- 1) AIIMS case-control group and 2) Indian Genome Variation Consortium (IGVC) group. This study was divided into three parts.

The first part of the study was done in AIIMS case-control group where we have checked the impact of diet on CAD status and effect of various parameters like homocysteine, cysteine, folate and vitamin B12 in CAD patients and controls. We have also studied various non-synonymous single nucleotide polymorphisms (nsSNPs) and their association with these parameters.

To summarize the results obtained in this part of the study:

- Percentage of individuals adhering to vegetarian diet were found to be significantly higher in CAD patients than controls which is in contrast to the earlier reports suggesting the incidence of CAD is lower in vegetarians.
- We observed vegetarians were deficient for vitamin B12 concentration and the median concentration were below the normal range (<150 pmol/L).
- Factors like CAD status, vegetarian diet, homocysteine, female sex, milk drinking and BMI were found to be significantly associated with reduced concentration of vitamin B12.
- CAD patients were found to be having significantly low median vitamin B12 concentrations as compared to controls.
Homocysteine concentration were significantly positively correlated with cysteine concentration while significantly negatively correlated with folate and vitamin B12 concentration which are in concurrence with the earlier studies.

- CAD patients were found with significantly increased concentration of thiol (homocysteine+cysteine) as compared with controls.
- Two polymorphisms (MTHFR C677T and CHDH A119C) were found to be significantly associated with homocysteine concentrations which were independent of other confounding factors. MTHFR C677T was found to be associated with increased concentration of homocysteine while CHDH A119C was found to be associated with decreased concentration of homocysteine. This is the first report showing the association of CHDH A119C with homocysteine concentration.
- Significant three ways interaction effect of vegetarian diet with MTHFR C677T and CHDH A119C was observed explaining the variation of homocysteine concentration.

In the second part of the study, individuals from both the study groups were included. Three important genes (CBS, MTHFR and TCN2) involved in homocysteine metabolism were screened for novel mutations/polymorphisms. CBS gene was screened in both AIIMS and IGVC study group individuals while MTHFR and TCN2 genes were screened only in AIIMS study group. Few SNPs were selected from CBS and MTHFR genes and genotyped in IGVC study group consisting of more than 1800 individuals recruited from 55 sub-populations from various parts of the country. 15 nsSNPs that were found to be polymorphic in AIIMS study were genotyped in about 550 individuals from 24 populations and their frequencies were compared with HapMap data. Additionally, 350 SNPs were genotyped in these individuals from 62 genes selected that are relevant in genetics of CVD to generate the basal frequency data in India population.

To summarize the results obtained in this part of the study:

- Thirty three novel genetic variations were observed in CBS gene in individuals from both AIIMS and IGVC study groups. Out of thirty
three mutations, four were from untranslated regions, three were exonic while twenty six were from intronic part of gene.

- Twelve novel genetic variations were observed in MTHFR gene in AIIMS study individuals. Out of twelve variations, one was from untranslated region, one from Intron while other ten were from exonic regions.
- Six novel genetic variations were observed in TCN2 gene in AIIMS study. Out of six variations, one was intronic while five were exonic.
- Minor allele frequency for the polymorphisms MTHFR C677T was found to be lower than Chinese, Japanese and Caucasian populations while it was comparable with African population.
- MTHFR 677TT genotype was absent in a majority of the sub-populations (29 out of 55) studied. 677TT genotype was mostly present in Northern part of the country. This genotype was mainly present in Indo-European and Tibeto-Burman linguistic groups.
- MTHFR A1298C polymorphism was present at a higher frequency as compared to the HapMap populations. Three sub-populations were observed where minor allele was flipped and “C” became the major allele. Out of these three sub-populations, two were from Indo-European while one from Tibeto-Burman.
- When 15 nsSNPs studied in AIIMS group were compared in four linguistic groups, MAF of 2 nsSNPs- MTRR A1049G and CTH G1208T did not vary significantly amongst the four groups. MAF for other SNPs varied significantly between one or more linguistic groups.
- When the distribution of both MTHFR C677T and CHDH A119C was studied together in 24 sub-populations, only four populations showed more than average prevalence of risk genotypes for both the polymorphisms.
- Out of 334 SNPs studied from 62 genes in four linguistic groups, 314 were found to be polymorphic while 20 were non-polymorphic. When the MAF for the 314 polymorphic SNPs were compared among four linguistic groups, 70 SNPs did not show any difference in these groups. MAF for 21 SNPs was significantly different among four linguistic groups. 70 SNPs showed difference in MAF between two linguistic groups.
Summary and Conclusions

Keeping in view the genetic, linguistic and geographical diversity in India, in the third part of study, we studied one SNP rs7566605 present upstream of *INSIG2* gene that was found to be associated with obesity in a genome wide scan. Various studies have shown positive association of this SNP with obesity while others fail to find the same. Different genetic and environmental modifiers among these populations might be a possibility for observing differences in associations. We screened this SNP both in AIIMS and IGVC study group individuals.

To summarize the results obtained in this part of the study:

- The minor allele frequency for rs7566605 present upstream of *INSIG2* gene was found to be comparable to that reported by Herbert *et. al.*
- The frequency of CC genotype was significantly higher in non-obese individuals as compared to the obese individuals under a recessive model in our AIIMS study individuals. This distribution was not significantly different in IGVC study individuals.
- Under the assumption of a recessive model, BMI did not vary significantly in individuals with CC genotype as compared to those with GG or CG genotype regardless of age.
- When the individuals were segregated on the basis of sex, we didn’t find significant difference in BMI as a function of age.

In conclusion, CAD patients were found to be deficient in vitamin B12. Vitamin B12 deficiency in CAD patients lead to significant increases in thiol (homocysteine+cysteine) concentration as compared to controls. *MTHFR* C677T and *CHDH* A119C polymorphisms significantly alter the concentration of homocysteine. Three way diet genotype interactions among vegetarian diet with *MTHFR* C677T and *CHDH* A119C was observed that can explain the variation in homocysteine concentration. MAF for the SNPs present in genes relevant in the genetics of cardiovascular disorders that were studied in four linguistic groups were significantly different in many cases revealing the genetic heterogeneity among these groups. SNP rs7566605 upstream of *INSIG2* gene that was found to be associated with obesity is not relevant in Indian population.