SUMMARY

Type 2 diabetes (T2D) is a complex and polygenic metabolic disorder arising from a complex amalgamation of genes and the environment (Tuomi et al., 2014). International Diabetes Federation (IDF) 2014 predicted the 592 million people will be suffering from T2D worldwide by 2035. Asia has emerged as ‘diabetes epicentre’ in the world due to rapid economic development, urbanization and associated lifestyle changes (Mohan et al., 2007). Recent estimates have rated India at 2nd rank with the largest number of people predicted to have diabetes mellitus in 2035 with the projected figure of 109 million (International Diabetes Federation, 2013). India has been populated by diverse caste and tribal groups, with intergroup gene flow impeded by a hierarchical caste system, geographical dispersal, and subdivision of the country into different linguistic regions. This has led to significantly higher genetic diversity within India, compared with Europe and East Asia (Reich et al., 2009). Given high genetic differentiation of both Indian populations and T2D risk variants, studies within ethnically homogeneous Indian populations may provide novel insights into genetic effects underlying T2D susceptibility. To understand the role of genetic heterogeneity in T2D predisposition, mechanistic evaluation of pathogenesis of diabetes has to be studied in various ethnicities. With this background of diversity/endogamous nature of the population and the scanty information of the polymorphisms in CAPN10 (SNP-19, -43 and -63), PPARG (Pro12Ala) and PGC-1α (Thr612Met, Asp475Asp, Gly482Ser, Thr528Thr, Thr394Thr, IVS2+52C>A) genes, the present case control study was focused to screen these genetic variants in different endogamous groups, Bania, Brahmin and Jat Sikh groups of Punjab. Among them Jat Sikhs are predominantly agriculturists, Brahmins belonging to priestly class but now performing various occupations and Banias belong to a trader community (Sekhon, 2000). These groups were selected on the basis of their ethnicity and occupation, which influences their genetic susceptibility to lifestyle diseases like T2D. The association of various epidemiological, anthropometric and biochemical variables with T2D was also investigated. The impact of the haplotype profile and interaction between candidate
Chapter 6: Summary

genes for better understanding the role of these genes in the aetiology of T2D was furthermore examined.

To achieve the proposed objectives, the present case control study enrolled 1125 subjects (554 T2D cases and 571 controls) comprising of 554 T2D cases (202 Banias, 151 Brahmins and 201 Jat Sikhs) and 571 healthy unrelated age, gender and ethnicity matched controls (207 Banias, 158 Brahmins and 206 Jat Sikhs). This sample size was attained by calculating power of this study (>80) through Gaussian approximation with univariate set up. Ethical approval was taken for this case-control study from Institutional Ethics Committee (Guru Nanak Dev University). Anthropometric and clinical details were documented on proforma prepared after critical perusal of literature. With informed consent (as per guidelines of ICMR, New Delhi), 3ml of blood samples were collected from participants of various regions of Punjab in pre-labeled 0.5M EDTA vials. Cases were diagnosed according to the criteria of American Diabetes Association (2010), (Fasting Plasma Glucose ≥126 mg/dl and Random Plasma Glucose ≥200 mg/dl) belonging to studied groups. T2D subjects with other complications of diabetes and early age of onset were excluded from the study. Age, gender and ethnicity matched controls with no positive familial history of T2D were enrolled. Blood plasma was separated for biochemical analysis. Genomic DNA was isolated from blood samples using inorganic method given by Miller et al., 1988 with certain modifications. DNA samples were quantified by UV spectrophotometer and dilution of 20 ng/μl of each sample was prepared. The genotyping was done by either ARMS-PCR or PCR-RFLP methods and amplified PCR and RFLP products were checked by agarose gel electrophoresis. 10% of the samples were randomly picked and genotyped again to recheck the accuracy of the results. Data analysis was done by using appropriate statistical tools (SPSS v.20, Haploview v.4.2 and MDR v.3.0.2).

Genotypes and allele frequencies (represented as percentages) were calculated by gene counting method. Genotypes were tested for the Hardy Weinberg Equilibrium (HWE). The distribution of genotype and allele frequencies in cases and controls were compared by using chi-square analysis. The extent of association was determined by Odd’s ratio (OR) at 95% confidence interval (CI). Binary Logistic regression analysis was used for correction of confounding variables such as age, sex, body mass index (BMI) and waist
to hip ratio (WHR). The continuous data was compared using Student’s t-test. One-way ANOVA was used to compare the effect of genotypes of selected genes on the baseline parameters. Haplotype frequencies and pairwise linkage disequilibrium (LD) for the selected polymorphisms among both studied populations were estimated using Haploview software. SNP-SNP interaction was analysed using Multifactor Dimensionality Reduction (MDR) software. Meta-analyses were performed using Comprehensive Meta-Analysis (CMA) software (version 2). All results were considered significant at p<0.05.

The overall obesity was observed to be higher in all endogamous groups as well as in pooled population of Punjab. The higher mean values were seen in cases than controls as depicted by waist circumference (WC) in Banias (p=0.0001), Brahmins (p=1.06×10^{-6}) and no association of WC with T2D was found in Jat Sikhs but when analysis was carried out on pooled samples, a significantly higher mean values of WC were observed (p=1.49×10^{-9}). Higher mean values of systolic blood pressure (SBP) was also found in Banias (p=5.02×10^{-9}), Brahmins (p=6.06×10^{-5}) and total pooled population (p=1.49×10^{-9}) than controls except Jat Sikhs. Significant differences in mean values were noticed for diastolic blood pressure (DBP) in all endogamous groups except Jat Sikhs [Banias (p=3.15×10^{-6}), Brahmins (p=4.08×10^{-6}, Total pooled group (p=3.95×10^{-5})]. Fasting blood sugar (FBS) and random blood sugar (RBS) were found to be statistically significant between cases and controls in all groups.

Genetic analysis revealed that minor A-allele of CAPN10 SNP-43 polymorphism conferred increased risk towards T2D progression in Brahmin, Jat Sikh and total pooled groups. Interestingly, D-allele of CAPN10 SNP-19 polymorphism played protective role against T2D in Brahmin group whereas, it provided risk towards T2D susceptibility in Jat Sikh group. Minor T-allele of CAPN10 SNP-63 polymorphism was observed to increase the risk of T2D in Bania, Jat Sikh and total pooled groups. Minor allele of PGC-1α Thr612Met and Thr394Thr polymorphisms were observed to provide risk towards T2D susceptibility in Jat Sikh and total pooled group. Allelic distribution of PGC-1α Thr528Thr polymorphism did not confirm any association with T2D susceptibility in all studied endogamous groups. However, AA genotype was found to provide 2.7 fold and 1.47 fold increased risk towards T2D susceptibility in Jat Sikh and
total pooled group, respectively. Intriguingly, A-allele of *PGC-1α* Gly482Ser polymorphism played protective role against T2D in Bania group whereas, it provided risk towards T2D progression in Jat Sikh group. However, *PGC-1α* IVS2+52C>A polymorphism, CA+AA genotype was found to provide 1.72 fold and CA genotype 1.97 fold increased risk towards T2D susceptibility in Brahmin group. *PGC-1α* Asp475Asp and *PPARG* Pro12Ala polymorphisms failed to replicate association with T2D susceptibility in all studied endogamous groups. Meta-analysis further provide the cumulative impact of particular SNPs towards T2D etiology showing some inconsistency with regard to present results among different endogamous groups, as there was high population heterogeneity among the studies. Therefore, in the present study we noticed that association of genetic polymorphisms may be modulated through ethnic diversity.

LD and haplotype analysis showed no significant LD in *CAPN10* gene polymorphisms. Haplotype 212 offered 4-fold risk of T2D in Bania group which is a novel one, showing role of minor alleles of SNP-43 and -63 towards T2D risk. The major allele combinations of haplotype 111 have been found to be play protective role against T2D in Brahmin and pooled group. An increased risk of diabetes (1.5-2 folds) has been detected for haplotype 221 in all groups except Banias. In context to *PGC-1α* gene, modest LD for Thr528Thr and Gly482Ser polymorphisms was seen in Jat Sikh T2D group indicating their combined role towards T2D predisposition. However, Thr612Met and Thr394Thr polymorphisms also displayed strong LD in Bania and total pooled group. Differences in the *PGC-1α* gene haplotype combinations associated with T2D risk among studied groups was inferred from this analysis. Gene-Gene interaction analysis was also performed between all studied variants of *CAPN10, PPARG, PGC-1α* genes. Strong synergistic interaction between *PGC-1α* Thr528Thr and Gly482Ser polymorphisms was observed in both Brahmin and total pooled groups. However, no strong gene interaction was observed in Bania and Jat Sikh group, implicating the role of different polymorphisms towards T2D association among studied groups.

Meta-analyses conducted on various polymorphisms undertaken in the present study illustrated the overall effect of these variants. The minor allele (A-allele) of *CAPN10* SNP-43 polymorphism was validated to confer increased risk by 1.33-fold towards T2D.
predisposition. Meta-analysis of \textit{CAPN10} SNP-19 polymorphism delineated the role of D-allele towards risk of T2D by one-fold, while differential pattern of association of D-allele was reported to confer similar increased diabetes risk in Jat Sikh cohort, whereas, this allele played protective role against T2D pathogenesis. Similarly, minor alleles of \textit{PGC-1}\textalpha\textit{Gly482Ser} and \textit{Thr394Thr} also posed a higher risk for the development of T2D. However, \textit{CAPN10} SNP-63 and \textit{PPARG} Pro12Ala variants did not reveal any overall association with T2D after combining results of various studies. The contradiction in some results observed between different endogamous groups under study and meta-analysis, there is need to unveil the role of ethnicity in outcome of complex disease like T2D.

In conclusion, the present study highlighted the role of \textit{CAPN10} and \textit{PGC-1}\textalpha\ genetic variants with the increased susceptibility to T2D in combination with obesity and their possible association with hypertension. This is the first study for the search of the genetic link in T2D with which provided a new baseline data for the selected variants in T2D. these genes among endogamous groups in population of Punjab. Further, the interaction results also unveiled the role of multigenic interaction in providing increased risk against T2D. Differential pattern of genetic association is observed in different endogamous groups reflecting the role of ethnicity in progression of disease. Given the complexity of Indian population, the studies hitherto focussed on molecular genetic association with T2D are few to draw any representative picture of India. A number of populations from different regions and ethnicities need to be screened for different candidate genes, not only to validate already known variants but also to see if any new variants that are unique to our population can be found. Therefore, further studies are required to cross-validate our findings in relatively larger samples as well as to explore novel SNPS of these genes that might be associated with T2D among various endogamous groups of Punjab. Genetic heterogeneity makes the understanding of complex diseases like T2D challenging. It is anticipated that sub-categorization of sample sets by social groupings like religion, caste, etc. and studies on larger data sets will help us better understand the genetic heterogeneity in T2D, especially in Indian populations.