CHAPTER 5

DISCUSSION

The present cross-sectional study investigated the role and relevance of two potential candidate genes i.e SLC6A4 and DRD4 as the genetic determinants of depression in diabetic population of Punjab. The study revealed the prevalence of depression to be 47.87 percent amongst T2DM patients of Punjab. The findings corroborate with other studies that have been conducted in the population of Chandigarh (Raval et al. 2010), Uttar Pradesh (Bajaj et al. 2012), Kerala (Madhu et al. 2013), Madhya Pradesh (Singh et al. 2014) and Punjab (Khullar et al. 2016). Some other studies showing prevalence were either under powered or suffered ambiguity from false positive results hence, comparing their results with the present study would have entailed false inferences. The analysis of the effect of environmental variables on depression and diabetes has revealed that sedentary life style is hugely prevalent in Punjab which predisposes the Punjabi population to so many chronic diseases including diabetes (Khullar et al. 2016). Partially, it is due to the fact that Punjab is a flourished state having high per capita income, whereby people enjoy fast food and ghee enriched meals quite often. It is evident in the scientific literature that sedentary life style can contribute substantially for the development and risk of T2DM whereas, aerobic exercise or active life style balances the ill effects of it on T2DM (Hu et al. 2003). In corroboration to this, sedentary life style emerged as the strongest predictor conferring approximately 4 times higher risk of depression and 2.2 times higher risk of developing diabetes in the present study. Higher levels of Low density lipoproteins (LDL) and Triglycerides (TG) were also observed to be independently associated with depression and diabetes in the
population of Punjab. The relationship of lipid levels with depression is complex because some studies have shown a significant correlation between lower levels of TC and depression (Patra et al. 2014, Kalle et al. 2014), however, others have reported that higher levels of Total cholesterol (TC), Low density lipoproteins (LDL) and Triglycerides (TG) increase the risk of depression (Park et al. 2014). San Antonio Heart Study in its 8 year follow up, revealed that those prediabetic subjects who had higher levels of Total cholesterol (TC), Low density lipoproteins (LDL), Triglycerides (TG), Body mass index (BMI) and Blood pressure (BP) developed clinical diabetes in comparison to those subjects who had normal levels of these parameters (Bays et al. 2007). Similarly, it was put-forth by scientists that diabetic dyslipidemia and atherogenetic lipoproteins increased the risk of cardiovascular diseases in the pre-diabetic subjects even before the onset of clinical diabetes (Hummel et al. 2011). There are several possible explanations for such mixed inferences regarding the association of lipids with depression. First of all study designs varied widely. Secondly, different diagnostic methods were used which could not converge to unequivocal identification of the depression. Moreover, different ethnicities, varied age range, locally occurring environmental correlates and diverse lifestyle have confounded the results. Present study also exposed that being a woman is a risky proposition for the occurrence of depression and diabetes. Similar results were also evident in the meta-analysis conducted by Anderson et al. (2001) and Ali et al. (2006). Some studies have shown that longer duration of diabetes (≥10 years) is a significant factor for the risk of depression in T2DM (Joshi et al. 2011, Mathew et al. 2012) which is in line with the inference of present study, whereby duration of diabetes > 5 years has been revealed to be a significant risk marker that almost doubles the risk of depression in T2DM patients. Another study by Ravishanker et al. (2014) also demonstrated that rate
of depression increased with the increase of duration of diabetes especially after 5 years. Furthermore, present study also exposed that higher BMI levels increase the risk of depression independently. In the review on comorbidity of diabetes mellitus and depression (Katon, 2008), it was noticed that signs and symptoms of depression were more prevalent in those diabetic subjects who had higher BMI values and had sedentary life style. Framingham Offspring Study (Laman-Fava et al. 2011) in its age adjusted analysis revealed that higher BMI points were found to be significant risk factors for depression in diabetes.

Scientific data support the relationship between disturbed glucose metabolism and pathogenesis of depression (Detka et al. 2015). It has been reported that higher glucose and glycogen levels determine prenatal stress in animal models, which suggests that glycolysis speeds up and the krebs cycle is slowed down in the brain. A retrospective study showed that 21.12 percent of depressed patients had high fasting plasma glucose levels (>100mg/dl) and approximately 14 percent had fasting blood glucose levels between 90-100mg/dl (Cassels, 2009). It has also been suggested that depression along with deficits in functional capacity can reduce the effectiveness of diabetes self management (Egede, 2005). Similar association was evident in the present study, when PHQ-9 scores were regressed upon the increasing glucose levels in linear regression model. It was observed that increasing glucose levels were significantly correlated with depression. It clearly indicated that higher the glucose levels more are the chances of having depression.

It is the first study in India that has intended to examine the genetic consequences of depression in diabetes. In this regard, three SNPs of SLC6A4 gene and four SNPs of DRD4 gene were investigated in 580 subjects of Punjab. It was observed that T allele of rs2020936 of SLC6A4 gene strongly influenced the risk of depression whereby, the
carriers of this allele were found to be at 1.52 times higher risk of depression than those subjects who possessed C allele. This SNP, rs2020936 was also found to be linked with depression and anxiety in Australian population (Wray et al. 2009). Besides other SNPs within SLC6A4 gene, rs2020936 was observed to be significantly associated with increased depressive symptoms and elevated IL-6 plasma levels. It suggested a common pathophysiology pathway linking depression and inflammation through the genetic participation of SLC6A4 gene (Su et al. 2009). Ishii et al. (2011) have demonstrated that SLC6A4 gene may impinge upon nicotine dependent depression as inevitable comorbidity of chronic obstructive pulmonary disease (COPD). They have suggested that by modifying the effect of SLC6A4 gene, depressive symptoms in COPD can be lessened. Logistic regression analysis for rs2020936 in the present study revealed the expression of minor allele C in both dominant and recessive model whereby, it showed the protective effect when present in single (dominant) or two copies (homozygous). Another SNP i.e rs2020942 within SLC6A4 gene was not observed to be associated with depression in diabetic individuals in the present study however, in Hungarian population, Lazary et al. (2008) observed that haplotypes comprising rs2020942, rs140700, rs3794808, and rs1042173 influenced life events on depressive phenotype although, individually this SNP was not associated with depression. This SNP was remained insignificant for the risk of depression in diabetic individuals of the present study.

A twenty two year longitudinal gene-environment study investigated the similarities and differences in the serotonergic diathesis for depression and suicide attempts in the population of Quebec, Canada. It was revealed that three SNPs of SLC6A4 gene participated stringently in depression, rs16527268 through main effects and two SNPs rs878567 and rs3794808 through gene-
environmental interactions with childhood physical abuse (Brezo et al. 2010). Major allele T of rs3794808 correlated significantly with Hospital Anxiety and Depression Scale (HADS) score (P=0.016) in COPD patients (Ishii et al. 2011). Similarly, the present study also revealed that T allele was a significant risk marker which added 1.74 times the risk of depression in T2DM patients however, minor allele of this SNP emerged as a protector for the effects of depression. The minor allele of this SNP exhibited its dominance in additive (heterozygous), dominant and recessive models for protection against the effects of depression in T2DM.

The release of neurotransmitter dopamine further stimulates dopamine receptor D4 (DRD4) which correlates with many neurological and psychiatric diseases owing to its functional relationship with prefrontal cortex. A family based association study showed that rs752306 and rs3758653 of DRD4 gene influenced the risk of mental retardation trivially when examined individually however, strong correlation was evident, when haplotype based analysis was observed comprising these SNPs (Zhang et al. 2012). Another study found that T allele of this SNP rs3758653 had a strong association for the pathogenesis of Alzheimer’s disease in Taiwanese population, psychotic symptomatology and cognitive function in Chinese population (Zhao et al. 2015) and Attention Hyperactivity Disorder (ADHD) in Australian population. None of the study has demonstrated its role and relevance for the pathogenesis of depression in T2DM sofar. Present study has revealed that major allele T of rs3758653 is a significant risk factor for depression whereas, minor allele equally protects it. The genetic model analysis revealed that minor allele C influenced the risk of depression in both dominant and recessive model.

None of the study has investigated the contribution of SNP rs747302 of the DRD4 gene in the pathophysiology of depression sofar
except few studies which reported this SNP to be a risk factor for heroin dependence (Vereczkei et al. 2013) and Parkinson’s disease (Ferrari et al. 2016). The present study observed that minor allele of this SNP conferred approximately 1.5 times the risk of depression. This allele expressed only in recessive mode of inheritance whereby two copies are required for its expression. Another SNP of DRD4 gene i.e rs1800955 has also not been investigated within the realm of depression so far. Few studies reported its association with drug response in schizophrenia (Tsutsumi et al. 2009), ADHD (Das et al. 2011), idiopathic intellectuality disability (Bhowmik et al. 2011), Parkinson’s disease (Kiyohara et al. 2011) and heroin dependence (Vereczkei et al. 2013). However, the present study observed that this SNP did not contribute to depression. SNP rs916455 of DRD4 gene was observed to be implicated in the pathogenesis of Alzheimer’s disease (AD) and ADHD (Lin et al. 2012, Li et al. 2012). The present study revealed that major allele C was a risky allele which conferred 1.44 times the risk of depression however, minor allele showed protective effect when present in double dose (recessive model).

This is the first time that SNPs rs2020936, rs2020942, and rs3794808 of SLC6A4 gene have been examined for their possible contribution in the manifestation of diabetes in population of Punjab however, earlier these SNPs have been investigated in relation to irritable bowel syndrome, COPD, obsessive compulsive disorder, migraine and depression. (Wray et al. 2009, Liu et al. 2011, Ishi et al. 2011, Lei et al. 2012, Yuan et al. 2014). Major allele T of rs2020936 and rs3794808 were observed to influence the development of T2DM in the present population whereas, their minor alleles remained protective.

DRD4 gene has been investigated in relation to several disorders such as ADHD, Alzheimer’s disease, schizophrenia, Parkinson’s disease and heroin dependence (Lin et al. 2012, Zheng et al. 2012, Vereczkei et
al. 2013, Dadds et al. 2016, Ferrari et al. 2016). However, this is the first study which determined the role and relevance of DRD4 SNPs as the genetic determinants of T2DM. C allele of rs916455 and T allele of rs3758653 were observed to influence the risk of diabetes whereas, rs1800955 and rs747302 did not show any effect.

It has been reasoned in the scientific literature that inferences derived from the single gene association studies are spurious owing to the absence of their synergistic interactions hence, the impact of individual SNPs on diabetes or depression risk may vary if other SNPs within SLC6A4 or DRD4 gene also participate especially, when they are non-randomly associated with each other. To overcome this discrepancy, haplotype analysis was done which revealed that TAT haplotype within SLC6A4 gene conferred 2.19 times the risk of depression in T2DM. One may argue that this association is miscalculated because of the obvious presence of type I and type II errors in the present study. It is noteworthy, that if it may so, then change in the allele pattern in this haplotype would not alter the risk of depression. To examine this, statistical testing was designed by considering that subjects with depression and without depression have the same frequencies (null hypothesis) and secondly, that subjects carrying TAT haplotype have higher frequencies in subjects with depression than subjects without depression (alternative hypothesis). While rejecting null, it was observed that influence of haplotype TAT on depression risk disappeared significantly with the replacement of T allele on the first SNP to C as in the case of haplotype CAT (OR 0.84, 95% CI 0.39-1.80, P>0.05).

Furthermore, haplotype TCTC within DRD4 gene contributed 4.1 times more to the risk of depression in T2DM subjects. The in-depth analysis for this haplotype also revealed that by replacing C allele of the fourth SNP with T allele in TCTC haplotype forming haplotype TCTT, its
influence for the risk of depression disappeared outrightly (OR 0.94, 95% CI 0.44-1.99, P>0.05). Amazingly, this haplotype TCTT which was observed to be insignificant for depression influenced substantially for the risk of diabetes, which suggests that C allele of rs916455 showed epistatic effect on the alleles T, C and T of rs3758653, rs747302 and rs1800955 respectively, for the risk of depression whereas, T allele of rs916455 showed the epistatic effect on the alleles of these three SNPs for the risk of diabetes. We could not compare our results to other studies which investigated various SNPs within SLC6A4 and DRD4 genes primarily because of the different disease conditions, differences in the selected SNPs or estimated haplotypes.

Gene-Gene interactions have been recognised as important indicators of underlying genetic mechanisms for complex traits. Present study elucidated five types of gene-gene interaction effects of two candidate genes SNPs: additive x additive, additive x dominance, dominance x additive, dominance x dominance and SNP x SNP. The extended Kempthorne genetic model used in the epiSNP software has been considered highly accurate, as its removes false negatives and positives from the analysis (Mao et al. 2006). The important feature of the analysis by this software is that it only displays the significant SNP-SNP interactions within the adjusted threshold of P value. It has emerged that both the genes are quite important as the genetic determinants of depression in the population of Punjab as SNPs within these genes interact significantly showing all the five different effects. While examining the SNP-SNP collaborations for their role in the development of diabetes, it has been revealed that rs2020936 of SLC6A4 gene interacted with rs916455 of DRD4 genes and rs3794808 interacted with rs3758653 through dominance x dominance and additive x dominance modes respectively in the control population. The results in the present study also brought out an important feature that
some SNPs within these genes interact to show the effect in diabetic subjects without depression as well as in controls. These effects further open avenues for the gene-gene interaction analysis in the diabetic population without depression and even controls. Further studies will need to look into this fact that why these interactions are there in the control population and which will enrich the knowledge regarding the functional concert of these genes in the manifestation of T2DM in the population of Punjab.

If a genetic effect is considered to be important on the manifestation of some complex disease, particularly when other genes are also contributing then gene-gene interactions are considered to display the complete picture. Nonetheless, in the multifactorial complex disorders like T2DM, the effects are usually missed and picture remains unclear, if the potential interactions of genes with environmental factors are not investigated. For the first time, present study revealed that SNPs rs2020936, rs2020942, rs3794808 of SLC6A4 gene interacted with environmental variables such as Duration of diabetes (DOD) >5 years, Body mass index (BMI) ≥30 kg.m^{-2}, higher levels of Total cholesterol (TC), Triglycerides (TG), Low density lipoprotein (LDL) and lower levels of High density lipoprotein (HDL) supporting the significant gene x environmental interactions in the manifestation of depression and T2DM in the population of Punjab. Similarly, SNPs within DRD4 gene also interacted significantly with quantitative environmental traits through marker, additive and dominant effects. The inference that these SNPs of both the genes did not correlate with systolic and diastolic blood pressure for the manifestation of depression and T2DM is also noteworthy.

Epistasis depicts the expression of an allele at one locus masked by an allele at another locus. This was explained in a statistical manner as the deviation of genetic influence from additivity in a linear model.
One locus effect only determines that which gene (SNP) is epistatic and which gene is hypostatic whereas, two locus epistatic effect delivers a complete picture by all possible forms of epistasis including the interactions between additive effects of two loci, dominant effect at one locus and additive effect at the second locus, additive effect of first locus and dominant effect of second locus as well as dominant effects at the two loci. All these different types of epistasis contribute differently to the overall genetic value of two locus genotype. The results in the present study gave the first evidence that rs202936 of SLC6A4 gene and rs747302 of DRD4 gene show two way epistatic effects through dominance x additive model for the risk of depression among diabetic subjects. Some other epistatic effects such as between rs3794808 and rs1800955 through additive x additive and between rs202942 and rs747302 through interactive effect were exposed. SNP-SNP cross talks were also deduced for the two epistatic effects in the manifestation of diabetes which suggested that SNPs within these genes do communicate to aggravate the overall effect on diabetes. More studies among different populations on large sample size are required to reach unequivocal conclusions of the gene-gene and gene-environmental interactions which may lead to develop the predictive abilities of these genes in the manifestation of depression among diabetes and the development of diabetes as well.