CHAPTER 1

INTRODUCTION

Diabetes mellitus is becoming a huge threat in its global perspective especially in India where, highest number of diabetic population (40.9 million) of the world is living. A study by World Health Organization (WHO) projected that this number will increase further to 70.4 million by the year 2030, if it remains unchecked and untreated (Wild et al. 2004). In India, the prevalence of type 2 diabetes mellitus (T2DM) has been observed to be 18.6 percent in urban and 9.2 percent in rural population (Ramachandran et al. 2008). Government National Programme and Control of Cancer, Diabetes, Cardiovascular disease and Stroke (NPCDCS) reported that 6.3 percent of the population is suspected to be diabetic in Punjab (Pandian and Sudhan, 2013). The word ‘diabetes mellitus’ is a term in Greek which denotes “a siphon” (diabetes) and “honey or sweet” (mellitus). It is assumed that Greeks named it because the urine of diabetics attracted flies and bees because of excessive sugar in it.

Diabetes mellitus (DM) is a group of complex metabolic disorders with the primary defect of insulin secretion and insulin action or both in the target cells of muscle and liver (Sicree et al. 2006). Owing to this, the individuals have higher levels of sugar for longer durations in their blood. Normally what an individual eats (sugar, starch and other food components) gets converted into energy by the action of insulin. Insulin is secreted by the beta cells present on the pancreas which commands the cells to uptake glucose, so that the cells can utilize the energy produced by the sugar. The production or synthesis of insulin by the beta cells called ‘islets of langerhans’ is influenced by several genetic and environmental factors. In diabetic subjects, the insulin production is either reduced or completely stopped because of the degrading beta cells. Initially pancreatic beta cells respond and adopt to insulin resistance but gradually nutrient excess, higher free fatty acids
and hyperglycemia have negative influence on beta cell function. Several mechanisms including the production of reactive oxygen species (ROS), changes in metabolic pathway, increase of intracellular calcium and endoplasmic reticulum stress, lead to impaired insulin secretion, attenuation of insulin action, compensated gene expression and finally beta cell apoptosis (Chang et al. 2008). In such subjects adipose cells may become resistant to the effect of insulin that results in diminished ability of these cells to take up and metabolize glucose. As a consequence, when glucose levels are increased in the blood beyond the renal threshold (about 160 mg/dl) then reabsorption of glucose in the proximal tubule becomes defective and begins to excrete glucose in the urine (glycosuria). This condition of renal glycosuria increases the osmotic pressure of the urine and furthermore prevents the water reabsorption by the kidney, which results in excessive urine production (polyuria) and increased fluid loss. Water in the body cells and other compartments replaces the loss of blood volume osmotically causing dehydration and increased thirst (polydipsia).

Types of Diabetes Mellitus

Diabetes mellitus is broadly of two types. Type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), however some other forms are also relevant in relation to health and disease.

(i) Type 1 diabetes mellitus (TIDM)

This is an autoimmune condition where pancreatic cells are attacked by the body's own immune system, hence resulting in lack of insulin production or there is no secretion of insulin at all, which makes the body to depend upon the insulin therapy (Seino et al. 2010). There are 5-10 percent of cases of type 1 diabetes, which are continuously spreading worldwide and have short and long term effects. Management of type 1 diabetes can be done by taking care of many aspects such as,
insulin administration, blood glucose monitoring, meal planning and screening for diabetes related complications.

(ii) **Type 2 diabetes mellitus (T2DM)**

T2DM is the most common form of diabetes mellitus. Patients suffering from T2DM have high blood sugar (hyperglycemia) or their cells become resistant to insulin (insulin resistance) or having decreased insulin production. (Shoback, 2011). The most common symptoms in T2DM subjects include excessive thirst (polydipsia), excessive urination (polyuria), excessive hunger (polyphagia), weight loss and unhealing wounds. If it is not treated and managed effectively, it may lead to microvascular complications (diabetic retinopathy, nephropathy and neuropathy) and macrovascular complications (atherosclerosis, cardiovascular disease and cerebrovascular disease). Scientists have revealed several risk factors that are significantly associated for the development of T2DM. These risk factors include life style factors, obesity, sedentary life style, smoking, alcohol drinking, higher lipid levels and depression. Besides these risk covariates, genetics plays an important role in the pathophysiology of T2DM. Genome wide association studies (GWAS) have discovered over 200 single nucleotide polymorphisms (SNPs) associated with T2DM (Sun *et al.* 2016). All these studies have deciphered overlapping SNPs among different populations hence these reports are suggestive rather than conclusive. In a GWAS study comprising ancestry specific human phenotypes revealed that different pathways are involved in the pathogenesis of T2DM in different ancestries (Qiu *et al.* 2016). It has emerged as consensus among scientists that gene-gene and gene-environmental interactions contribute to the etiology of T2DM, however it may be influenced by epigenetic processes also (Ling and Groop, 2009). Other mechanisms such as genomic imprinting, fetal programming and microRNA (miRNA) expression are also involved in the development of this disease (Ferland *et al.* 2010). Lately, it has been reported that
evolutionary triangulation (ET) can filter the ‘link to T2DM’ for improving our understanding of discovering novel loci causing T2DM (Huang et al. 2016).

(iii) Type 3 diabetes mellitus (T3DM)

Type 3 diabetes is the name given to Alzheimer’s disease (AD) because of the newly emerged theories regarding impaired insulin signaling, impairment in cerebral glucose utilization and energy metabolism which may lead to neuronal degradation—a precursor to the pathogenesis of AD. Earlier many molecular, biochemical and histological abnormalities comprising excessive neurofibrillary tangles, dystrophic neurites, amyloid precursor protein, deposition of amyloid beta, mitochondrial dysfunction, oxidative stress and DNA damage have been proposed. Although, several studies have pinpointed the above mentioned causes of AD but all this information is riddled with complex, conflicting and confusing concepts that failed to highlight the association of metabolic syndrome, obesity and insulin resistance with AD. Scientists mulled over and noticed that T2DM causes brain insulin resistance, cognitive decrements and oxidative stress, finally concluding that “Type 3 diabetes” perfectly exhibits the fact that AD represents a form of diabetes which corresponds to insulin resistant mediated AD neurodegeneration (de la Monte and Wands, 2008).

(iv) Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is a condition exhibited by a pregnant diabetic woman with high blood glucose levels especially during the third trimester. GDM is modifiable and if remains untreated, it may lead to delivery complications, seizures or still birth. The worsened form of GDM in pregnant women can lead to the development of T2DM and after pregnancy may pose higher risk of preeclampsia and caesarean sections (Donovan, 2010). It has been observed that in some pregnancies, hormones and fat deposits interfere with the insulin
action at the level of cell signaling pathway behind the insulin receptor, which because of insulin resistance do not allow the entry of glucose into the cells, resulting in increased glucose in the bloodstream (Carr and Gabbes, 1998). Although cortisol and progesterone are considered to be the main contributors, however, placental lactogen, prolactin and estradiol also participate. Besides autoimmunity, obesity and single gene mutations are also considered to be the reasons behind GDM (Buchanan and Xiang, 2005).

(v) Maturity onset diabetes of the young (MODY)

MODY often called as “monogenic diabetes” is a condition which is caused by the mutation in an autosomal dominant way. Unlike type 1 and type 2 diabetes, it involves single gene and some environmental factors. It has huge heritable component, whereby 50 percent of the first degree relatives inherit the same mutation giving the 95 percent life time risk of developing MODY. There are two different clinical presentations of this disease. In some patients of MODY, significant hyperglycemia along with polydipsia and polyuria are prevalent however, in some patients no clear signs of diabetes are present. Inappropriate and ineffective insulin secretion by pancreatic beta cells is the main reason for the development of MODY. Primarily, it happens because of the mutation of transcription factor gene. Different types of MODY (MODY1-MODY11) have been reported and observed to be linked with different genes (Neve et al. 2005).

(vi) Latent autoimmune diabetes in adults (LADA)

LADA often refers to ‘late onset autoimmune diabetes of adulthood’, ‘slow onset type 1 diabetes’ or ‘diabetes type 1.5’ is a clinical condition in which the autoimmune beta cells failure is slow despite the presence of islets antibodies at the diagnosis of diabetes. LADA patients do not require insulin during the first six months after the diagnosis (Stenstrom et al. 2005). According to the Immunology of Diabetes Society (IDC), its diagnosis is based on three features; (i) age over 35
years, (ii) presence of at least one of four circulating autoantibodies to islets cells antigens and (iii) lack of requirement of insulin at least up to six months after diagnosis (Otto-Buczkawska, 2013). In some prospective studies, it has been shown that those LADA patients who have multiple islets antibodies develop beta cell failure within five years of diabetes, whereas those subjects who have only glutamic acid decarboxylase (GAD) antibodies (GADAs) or only islets cell antibodies (ICA) develop beta cell failure after five years of the diabetes (Stenstrom et al. 2005). Although, LADA shows overlapping signs of both T1DM and T2DM but, it is considered as a clinical entity different from both of these disorders. Its preventive therapy is based on GADA and ICA, otherwise chances of misdiagnosis and ambiguous treatment may worsen its pathology.

Risk factors associated with depression in T2DM

Many studies have tried to delineate the risk factors and their association with the development of T2DM, but clear cut information regarding the causal factors is still wanting. Besides, unknown reasons of rising prevalence of T2DM, some risk factors are insulin resistance, advancing age, population growth, abdominal adiposity, urbanization, physical inactivity, sedentary life style and smoking. These risk factors vary significantly from one geographic region to the other (Zimmet et al. 1990). All these risk factors can cooperate and collaborate in the untreated diabetes, worsen the pathology of diabetes and increase the risk of complicated hypertension, cardiovascular disease, neuropathy, nephropathy, retinopathy, neurocognitive impairment and depression (Singh et al. 2015). In the pursuit of reducing the burden of diabetes, scientists and researchers have mulled over for the indepth understanding of *modus operandi* of these risky variables for the development and exacerbation of T2DM. Of these variables, depression has emerged as a significant concomitant for the risk of T2DM (Anderson et al. 2001).

(i) Age
Advancing age has been reported to be associated with higher risk of depression especially in the subjects with T2DM (Khullar et al. 2016). The relationship of age with the risk of depression is interesting to examine as onset of both of these disorders do not overlap. Depression has onset in young adulthood with highest rate of prevalence during 20 to 45 years of age (Eaton et al. 1997) whereas, T2DM typically begins after the age of 40 years. Their different ages of onset lead us to the dilemma that whether cross sectional relationship of diabetes and depression are independent of age or depression reoccurs in the presence of diabetic pathology (Eaton, 2002). Nonetheless, age is not intransigent variable and its impact on depression seems variable that interacts with other independent risk variables in T2DM.

(ii) Gender

Although it is clear that depression discriminates none and occurs irrespective of the gender however, higher prevalence of depression is evident in T2DM women subjects (Khullar et al. 2016). Being a woman, has been observed as an independent risk factor that doubles the risk of depression in T2DM than men. Many hormonal factors such as menstrual cycle changes, pregnancy, postpartum alterations, pre-menopausal, peri-menopausal and post- menopausal changes may collaborate to increase the risk of depression in women. Moreover, social responsibilities both at work and home, taking care of children and ageing parents may further increase this risk.

(ii) Obesity

Perusing the literature to understand the relationship of obesity with depression and diabetes, it is perplexing as some studies have shown that obesity is associated with increased risk of depression whereas, others with decreased risk of depression (Roberts et al. 2000). Some studies have shown that obesity has no
effect on the risk of depression (Friedman and Brownell, 1995). National Health and Nutritional Examination Survey (NHANES) observed that there is a weak association of body weight with elevated depression scale in women but not in men (Istvan et al. 1992). Similarly, Ross (1994) in his study employed a representative sample of 2020 adults of 18 years and observed no significant effect of overweight on depression. Interestingly, it has been found that obesity is not related to risk of depression in women aged between 50-89 years, suggesting that depression is inversely related to obesity confirming the "jolly fat hypothesis" (Palinkas et al. 1996). It seems that being overweight is not an independent predictor for the risk of depression however, some other variables such as education, exposure to dieting and health problems can contribute to the risk of depression in T2DM (Robert et al. 2000).

(iv) Education attainment

It has been suggested that risk of depression is higher in those T2DM subjects who have less than a high school education in relation to more educated groups (Carnethon et al. 2003). Age, gender, race, body mass index (BMI) and family history adjusted effects have shown that interaction between depression and education was significant statistically, which suggested that risk of depression was significantly diminished in T2DM subjects with each year of education attained (Mezuk et al. 2008). Furthermore, depression risk was found to be 73 percent increased in T2DM subjects when compared between high school versus graduation after adjusting the effect of age, race, gender, smoking status and alcohol use. Higher education attainment has been observed to be a protective factor for anxiety and depression in T2DM subjects (Collins et al. 2009). These studies suggest that education encourages the subjects towards proper learning and understanding of depression and its complications. It leads the subjects towards inculcating interest in own health, increased compliance and adherence to drug therapies for better health outcome.
Therefore, it is consensus among scientists that depression is significantly associated with lower education levels in subjects suffering from T2DM (Ganasegeran et al. 2014).

(v) Hypertension

It is a condition in which blood pressure levels are high i.e, systolic blood pressure (SBP) >120mm Hg and diastolic blood pressure (DBP) > 80mm Hg. The worldwide prevalence of hypertension is 26.5 percent which is three times higher in T2DM subjects when compared with age matched non diabetic subjects (Kearney et al. 2004). Hypertensive diabetic subjects are susceptible to the risk of cardiovascular disease and respond poorly to antihypertensive therapy. It has been observed that the risk of depression is accelerated when hypertension is present in diabetic subjects. It has been suggested that hypertensive depressed patients have increased sympathetic tone and increased synthesis of cortisol and adrenocorticotropin hormone (Meng et al. 2012). Therefore, pathophysiologically it is possible that depression affects hypertension and vice versa in type 2 diabetes.

(vi) Socioeconomic status

By digging out the literature highlighting the effect of socioeconomic status (SES) on health and disease, a social dose-response relationship between SES, depression and diabetes is evident (Everson et al. 2002). SES has not been proved as an independent risk factor however, higher prevalence of smoking, alcohol consumption, poorer diets, sedentary life style and lack of psychosocial support contribute for the risk of anxiety and depression in low SES group T2DM subjects. Higher level of stress amongst low socioeconomic individuals may impinge upon the psychological and physiological pathways, which trigger cascade of neuroendocrine alterations and can play significant role in depression, hyperlipidemia and insulin resistance. Therefore, the presence of several
underlying factors may contribute to the development of depression and depressive symptomatology in lower levels of SES in comparison to the subjects belonging to the higher SES (Bruce et al. 1991).

(vii) Lipid levels

Higher lipid levels or dyslipidemia is common in diabetic patients and significantly contributes to the risk of macrovascular complications and depression. The relationship of lipid levels with depression is intricate as lower cholesterol levels are found to be associated with depression in some studies (Patra et al. 2014, Kale et al. 2014), however, some reports have highlighted that higher lipid levels especially low density lipoprotein (LDL), triglycerides (TG) are associated with risk of depression (Van Reedt et al. 2010, Park et al. 2014). It has been suggested that the association of higher lipid levels with the risk of depression can be because of the high prevalence of obesity, as age adjusted analysis have shown that higher BMI is a significant risk factor for higher LDL and TG (Khullar et al. 2016). In order to understand the symptoms of late life depression and lipids, a seven year follow up study has revealed that lipids affect depression according to gender. Depression is associated with low levels of HDL in women whereas, low level of LDL influence depression in men (Ancelin et al. 2010). In diabetes the risk of depression increases by two times in subjects diagnosed with dyslipidemia. The relationship of dyslipidemia–depression link is multi-pronged including alterations of nitric oxide (NO) and cytokine production, neuropeptide release, insulin resistance, activation of rennin angiotensin system (RAS) and mitogenesis of the vascular smooth muscle cells.

(viii) Alcohol drinking

Information on the correlation of alcohol use, depression and diabetes is scarce. Some population based studies have observed consistently that high alcohol intake increases the risk of T2DM (Kao et al. 2001, Carlsson et al. 2000),
whereas, moderate alcohol intake has been observed to have no effect on T2DM, rather, suggested to bear some protective value (Anderson et al. 2001). For subjects having diabetes, high alcohol consumption can be detrimental, as it inhibits glucose metabolism. Whereby obesity interacts with alcohol abuse for the development of T2DM. Similarly, the correlation of depression and alcohol use has not been discerned in many studies however, it has been observed that 80 percent of alcohol users report depression in their lifetime (Hitzemann, 2000). None of the study has reported the causal relationship, as to whether alcohol abuse can cause depression or depression causes alcohol misuse. Studies report either the link between depression and diabetes or diabetes and alcohol abuse and depression and alcohol abuse rather than reporting the interplay of these three illnesses in relation to human health.

(ix) Smoking

The association between cigarette smoking and depression is detrimental as smoking has been observed to be associated with increased insulin resistance (Ronnemma et al. 1996). Glassman et al. (1990) have observed higher rates of smoking in patients with depression as compared with those without depression. Anda et al. (1990) in a nine year follow up study concluded that smokers with depression were 40 percent less likely to quit than non depressed smokers. Smoking cessation in diabetic patients improves health against the risk of depression (Katon et al. 2004). It has also been suggested that smoking in early life can increase the risk of depression which may lead to increased risk for development of T2DM. Overall conclusion of the scientists conveys that viscous cycle is at play in smokers or depressed subjects as nicotine negatively damages certain pathways in brain that regulate mood and because of the mood swings, depression encourages people to smoke. Epidemiological and observational data has remained suggestive and unequivocally provide evidence of causation.
Relationship of diabetes with depression

In recent years, it has been observed that psychological disturbances are commonly prevalent in the endocrinological disorder especially in T2DM, hence, it becomes paramount important that psychological and social well being of the patients should be preserved. In order to understand various risk factors involved in diabetes, many studies have been conducted in relation to micro and macrovascular complications, of which depression has emerged as a significant risk factor for the development and propagation of its pathology (Chew et al. 2008). Besides several clinical, environmental and genetic risk factors T2DM has been observed to be associated with an increased risk of depression (Heckbert et al. 2010) and depression related symptoms are higher in T2DM patients in comparison to non-diabetic individuals. Both diabetes and depression coexist and have bidirectional relationship. According to two meta analysis, diabetic subjects have 24 percent increased risk of developing depression and depressed adults have 37 percent higher risk of developing diabetes, which further is increased with the long term use of antidepressants (Knol et al. 2006, Nouwen et al. 2010). High levels of stress hormone cortisol are present in depressed subjects, which make the cells resistant to insulin action resulting in insulin resistance and hyperglycemia. Whereas, in diabetic subjects, poor glycaemic control impinge upon Hypothalamus Pituitary Adrenal Axis (HPA-axis) triggering the molecular biology of mood disorder resulting in depression.

In animal studies, neuronal degradation has been observed in the streptozotocin diabetic rats which may induce the HPA axis dysregulation—a precursor or hallmark of depression (Kamal et al. 2000). The other implications of diabetes for the risk of depression include synaptic plasticity, neuro-anatomical changes and deficit in insulin signaling (Kopf and Baratti, 1994). Furthermore, uncontrolled diabetes may cause irreversible inhibition of neurogenesis and neuronal apoptosis along with insulin receptor suppression in rodent brain (Beauquis et al. 2006).
Neurotransmitters-The key mediators of depression

Neurotransmitters are endogenous chemical messengers that play an imperative role in transmitting the neuronal signals through a chemical synapse from one neuron to another neuron (Lodish et al. 2000). These are released at the synaptic site from the small vesicles into the synaptic cleft, where they are identified and taken by the receptors on the target cells. Out of several neurotransmitters identified so far, many chemical messengers are produced from precursors of amino acids which are available from our diet and do not require complex biosynthetic pathways for their secretion and conversion (Cherry and Kendra, 2014). A neuron sends information through nerve impulse called ‘action potential’ which when arrives at presynaptic terminal at the synapses triggers the release of neurotransmitters. Here they bind to the receptor of the post synaptic terminal and influence either in excitatory or inhibitory way. This neuron in action is connected to many other neurons and if the excitatory action potential is larger than the inhibitory potential then this neuron fires. Because of this, a piece of information is passed from one neuron to another neuron. The major neurotransmitters of the brain include serotonin, dopamine, noradrenalin (norepinephrine) and acetylcholine (Miller, 2011). Any form of stress encountered by the individual leads to the over utilization of these neurotransmitters in order to cope up with ensuing stress. When this stress is not relieved and surpassed beyond a limit, the exhausted nervous system reduces the supply of neurotransmitters causing imbalance. Such imbalance may cause number of disorders like Parkinson’s, Attention Deficit Hyperactivity Disorder (ADHD), memory loss and most importantly depression. Genetics plays a potent role in regulating this neurotransmitter imbalance (Leo and Lacasse, 2007).

The most important point of the research process is selecting and deciphering candidate genes for the risk of depression in T2DM. Since the neurotransmitter genes primarily address neurological functions hence,
interactions of nerve cells participate in regulating mood disorders especially depression. Some of these genes and genetic variants play important role in different neuronal pathways, for instance, membrane proteins, ion channels, synaptic vesicle proteins and neurotransmitter genes for receptors and transporters. The most important contribution and influence for the development of depression is by the neuronal machinery involved in neurotransmission, named as serotonergic and dopaminergic systems. Furthermore, serotonergic and dopaminergic systems are the targets for various antidepressants and antipsychotic drugs. Antidepressants and antipsychotics modify serotonergic and dopaminergic mechanisms thereby, reduce the availability of dopamine receptors (Varga et al. 2011).

(i) **Serotonergic system**

Last 35 years of research has confirmed the role of serotonergic and dopaminergic system in depression and suicidal ideation. The genes of serotonergic system comprising tryptophan hydroxylase-1 (TRH1), tryptophan hydroxylase-2 (TPH2), serotonin transporter (5-HTTLPR), 5-hydroxy tryptamine receptor-1B (HTR1B) and 5-hydroxy tryptamine receptor-2A (HTR-2A) are associated with low cerebrospinal fluid concentration of the serotonin, less binding of serotonin transporter in prefrontal cortex, inhibit neuronal synapse and upregulate response to reduce serotonin release in depressed patients (Du et al. 1999). The altered expression (mutations) of these genes controlling the production and neurotransmission of the serotonins are independently associated with depression.

Tryptophan hydroxylase (TH) is the rate limiting enzyme of serotonin synthesis which helps in the conversion of tryptophan to L- hydroxytryptophan. The two important isoforms of this enzyme are coded by two genes namely TPH1 and TPH2. Polymorphisms within tryptophan hydroxylase 1 (TPH1) have been investigated for their possible association with depression (Gizatullin et al. 2006). Two SNPs localised on intron 7 designated as A218C (rs1800532) and A779C (rs1799913), have been observed to be associated with depression. Tryptophan
hydroxylase-2 (TPH2) is the rate limiting enzyme in the pathway for brain serotonin. It is considered as an important player for maintaining serotonin transmission in the central neuron system (CNS). TPH2 gene is localised on chromosome 12q21.1 and has 13 exons. Haplotype association studies of different SNPs with in TPH2 gene suggest strong association with depression and suicidal ideation. The functional polymorphism within this gene (rs2171363, rs4760815, rs7305115, rs6582076, and rs932502) yielded a haplotype-TAAGA which was found to be associated with enhanced mRNA expression in human pons. A meta-analysis identified rs4570625 SNP within TPH2 gene as a strong candidate for major depression (Gao et al. 2012).

Serotonin receptors have seven types and 14 subtypes but the most researched one is type1A–receptor that is HTR1-A. This receptor expresses abundantly with in the brain. Both the receptors i.e HTR1B and HTR-2A have post synaptic receptors and somatodendritic auto receptors. A Meta analysis has confirmed the role of 5HTR-1A for the risk of depression (Kishi et al. 2009). The serotonin receptor HTR-2A has been examined in few studies and its contribution for the risk of depression is contradictory and controversial (Tsai et al. 1999).

In the vistas of depression research, serotonin transporters are of unique importance as they are the common targets for several antidepressants. The most studied polymorphism is the serotonin transporter (5-HTT). It is the protein responsible for removing extra serotonin back into the presynaptic boutons and has been suggested to play a key role in the pathophysiology of several psychiatric disorders including depression and suicidality. The gene coding for 5-HTT is solute carrier family 6 member 4 (SLC6A4) which is localised on chromosome 17q11.2, spans 31 kb and has 14 exons. An insertion deletion of 44bp is present in the promoter region approximately 1kb upstream from the coding region of SLC6A4 (Heils et al. 1996) resulting into two major alleles, long variant (L) comprises of 16 repeats and short variant (S) comprises of 14 repeats. The short allele has been suggested to decrease transcriptional activity of this gene by 50 percent (Lesch et al. 1996). Some studies have suggested that 5-HTTLPR polymorphism with in this gene is significantly associated with depression and respond to serotonin
associated with reuptake inhibitor (Hariri et al. 2002). Another common polymorphism with in this gene is VNTR (STin2), which is situated in the second intron comprising of 12 repeat alleles which are associated with the increase of transcriptional efficacy. Low levels of 5-HTT availability was found to be associated with S allele of 5-HTTLPR and 12 repeat alleles of STin2 influence the expression of brain serotonin transporters in acute depressed subjects (Bah et al. 2008).

(ii) **Dopaminergic system**

Dopaminergic system comprises neural pathways that transmit/transport dopamine from one region of the brain to another. In the neural transmission, receptors provide the surface on both sides of the synapse where transmitter binds and transmit stimuli whereas, transporters are responsible for reuptake of these transmitters to the presynaptic neurons. Dopaminergic system genes are responsible for both receptor and transporter activity (Vereczkei et al. 2009). The genes of dopaminergic system comprise of Dopamine transporter (DAT), Dopamine receptor D1 (DRD1), Dopamine receptor D2 (DRD2), Dopamine receptor D3 (DRD3), Dopamine receptor D4 (DRD4), Dopamine receptor D5 (DRD5), Catechol-o-methyl transferase (COMT) and tyrosine hydroxylase (TH).

The rate limiting step for dopamine synthesis is regulated by tyrosine hydroxylase, an enzyme which is coded by TH gene. Mutations with in this gene can attenuate tyrosine hydroxylase which participates for increasing the risk of depression. Five different dopamine receptors have been identified that are DRD1, DRD2, DRD3, DRD4, DRD5. DRD1 and DRD5 control the excitatory neurotransmission whereas DRD2, DRD3, DRD4 control inhibitory neurotransmission. 48 bp VNTR in 3’ untranslated region (UTR) of DAT1 (SLC6A3) gene has been examined for its role in ADHD, substance abuse and depression (Yang et al. 2007). An important enzyme COMT which breaks down dopamine in the prefrontal cortex is encoded by COMT gene. A valine to methionine (val to met) amino acid change at 158th codon of COMT gene has been examined and observed to be associated with obsessive compulsive disorder (OCD) (Azzam and Mathews, 2007).
2003), ADHD (Cheuk and Wong, 2006), bipolar disorder (Zhang et al. 2009),
cognitive impairment and depression (Barnette et al. 2008). Dopamine transporter
(DAT) is an intermembralal protein which removes dopamine from the synaptic
cleft and terminates the dopamine signal. -48 A/G SNP in the 5'UTR of the DRD1
gene is the most frequently studied polymorphism for the risk of depression.
-141C indel, Ser 311/Cys 311 and Taq1A polymorphisms of DRD2 gene are
observed to be associated with the risk of depression in different studies (Tsai et al.
2002, Hou and Li. 2009). rs6280 SNP within DRD3 gene which encodes serine to
glycine amino acid change has been studied in various populations for
understanding its role in depression.

DRD4 gene has been examined for the psychiatric disorders especially
depression. 48 base pair variable number tandem repeat (VNTR) in the third exon
has been examined for its association with novelty seeking (Reist et al. 2007),
ADHD, cognitive impairment and depression (Johnson et al. 2008).

**Neurobiology of Depression**

Within the realm of hypothalamic-pituitary-target endocrine gland axis,
hypothalamus secretes corticotropin releasing factor (CRF) and release inhibitory
hormone somatostatin. These peptides further trigger the release of
adrenocorticotropin hormone (ACTH) and growth hormone (GH) via stimulation of
the pituitary. These peptides and hormones are submitted to the peripheral
circulation which affects the adrenal cortex. This neuroendochemistry of
neurotransmitter activity in depression is altered by the perturbations of three axis
namely Hypothalamus Pituitary Adrenal axis (HPA), Hypothalamic Pituitary
Thyroid axis (HPT) and Human Growth Hormone (HGH).

**HPA axis and Depression**

It has been well understood in the clinical chapters that hyperactivity of the
HPA axis is highly prevalent during stress and depression (Nemeroff, 1996).
years of research suggests that alterations within the hypothalamus in depression is significantly associated with increased number of CRF containing neurons and mRNA in the parvocellular neuroendocrine cells that triggers the increased secretion of hypothalamic CRF (Raadsheer et al. 1995). This increased synthesis and secretion of CRF when released at the median eminence from secretory terminals of neurons into the primary capillary plexus of the hypothalamus, hypophyseal portal system, CRF is carried to the anterior lobe of the pituitary. Here, it triggers the chain of events including stimulation of corticotrops to secrete ACTH and beta endorphins. Furthermore, ACTH helps in the synthesis of cortisol, glucocorticoids, mineralocorticoids and dehydroepiandrosterone (DHEA). These events translate into the enlargement of pituitary gland (Axelson et al. 1992), enlarged adrenal cortex (Rubin et al. 1996) and increased cortisol levels (Beck Friis et al. 1985). Hypercortisolemia sends the feedback to the pituitary, hypothalamus and hippocampus to attenuate the release of CRF and ACTH. It is believed that higher levels of cortisol downregulate the glucocorticoid receptors causing glucocorticoid resistance in depressed patients (Hansen et al. 1998).

It has been observed that variations in glucocorticoid receptors in some regions of the brain such as hippocampus, causes depression. The normal inhibitory effect on the HPA axis by hippocampus is impaired which leads to the continuous secretion of cortisol. It has been hypothesized that hypercortisolemia increases the risk of neurotoxicity and neurogenesis in the hippocampus. All these events including reduced hippocampal volumes lead to the depressive symptomatology. Although, increased levels of CRF, ACTH and cortisol are considered to be the culprits for major depression, but there is an emerging evidence that in some depressed patients, low HPA axis activity has also been observed especially in non psychotic depression, atypical depression and post traumatic stress disorder (Posener et al. 2000). Hence, both increased and decreased HPA axis activity contributes to the depression.
HPT axis and Depression

The concentration of pituitary glycoprotein-thyroid stimulatory hormone (TSH) mediates the activity of thyroid gland. The major regulation of TSH production is maintained by the inhibitory effect of thyroid hormone and stimulatory effect of thyrotropin releasing hormone (TRH). TRH is released from hypothalamus and encourages the receptors of TRH in the pituitary to release TSH. TSH induces specific receptors of the pituitary to release triiodothyronine (T3) and thyroxin (T4). These thyroid hormones send feedback to hypothalamus and pituitary to regulate HPT-axis (Kirkegaard et al. 1979). Research on HPT axis has enriched our understanding that increased levels of central TRH activity and hypothyroidism play pertinent role in depression.

Alterations within HPT axis owing to the imbalance of TRH and TSH are commonly exhibited by depressed patients (Reichlin and Utiger, 1967). The implications of HPT axis alterations include elevated cerebrospinal fluid (CSF) concentrations (Banki et al. 1988), blunting of TSH secretion because of the circadian rhythm perturbations (Weeke and Weeke, 1978) and blunted TSH response to exogenous TRH (Hansen et al. 1988).

Human Growth Hormone and Depression

Growth Hormone (GH), a 191 amino acid single chain peptide is secreted by the anterior somatotropic cells of anterior pituitary that plays an important role in growth, cell production and cell regeneration in humans. Growth hormone releasing factor (GRF) (stimulatory) and somatostatin (inhibitory) regulate its release from pituitary. During the night time peak, blunting of diurnal rhythm of GH secretion has been observed in depressed patients (Jarret et al. 1994). The nocturnal GH is elevated in depressed patients whereas day light GH are increased in unipolar and bipolar depression (Mendlewicz et al. 1985). It has also been shown that somatostatin levels within cerebrospinal fluid (CSF) are reduced in depressed subjects which may reflect and associate with hyper cortical activity.
(Rubinow, 1986). Low levels of CSF somatostatins are not only associated with type 2 diabetes but with type 3 diabetes also, as it inhibits insulin release from beta cells when somatostatin is released from delta cells of the pancreas (Holly et al. 1988).

**Molecular genetics of depression**

Genetic epidemiology and twin studies have shown that depression is influenced by genetic factors with considerable heritability component (Sullivan et al. 2000). Influence of genes and genetic variants that predispose diabetic subjects to the risk of depression has been done finitely. Although several genome wide association studies (GWAS) have been published recently, but all these studies failed to identify a single locus that accede to genome wide significance for major depression (Collins, 2013). These studies are suggestive rather than conclusive and missed important information regarding the collective contribution of multiple loci of small effects. Flint and Kindler (2014), have reviewed 200 candidate genes for major depressive disorder (MDD) from different studies, identified seven genes yielding significant P value. The genes were Solute Carrier Family 6 Member 4 (SLC6A4), Apolipoprotein E (APOE), Dopamine receptor D4 (DRD4), Guanine Nucleotide-Binding Protein, Beta-3 (GNB3), 5-hydroxytryptamine receptor 1A (HTRIA), Methylene tetrahydrofolate reductase (MTHFR) and Solute Carrier Family 6 Member 3 (SLC6A3). From this knowledge based inferences, the role and relevance of SLC6A4 and DRD4 as the genetic determinants of depression has been examined in the present research. Some studies have independently confirmed that SLC6A4 and DRD4 play a significance role in the development of depression (Ueno et al. 2003, Lopez et al. 2005).
The role of Serotonin transporter gene (SERT) or SLC6A4 in Depression

The human serotonin transporter protein is encoded by a single gene (SLC6A4) located on chromosome 17q 11.1 - 17 q12, which spans 31 kilobases (kb) and consists of 14 exons (Lesch et al. 1994). Recent studies have reported association between the serotonin transporter gene regulatory region polymorphism and many psychiatric disorders, like depression (Ueno et al. 2003), obsessive compulsive disorder (McDougle et al. 1998) and posttraumatic stress disorder (Lee et al. 2005). Besides polymorphisms at 5' and 3'UTR alongwith intronic regions, functionally two common alleles of serotonin transporter promoter region include short (s) allele of 448 bp and the long (L) allele of 528 bp (rs2020942). An attenuated promoter segment of (s) allele is associated with reduced transcription and functional capacity of serotonin transporter relative to (L) allele.

A prospective longitudinal study (Caspi et al. 2003) investigated functional polymorphism in the promoter region of SLC6A4 gene (rs25531) and showed that 1 or 2 copies of the short allele exhibited more depressive symptoms and suicidal tendency than individuals homozygous for the long allele. Another study involving German population tested this polymorphism in relation to depression and supported the gene-environmental interaction of short allele indicating a higher mental vulnerability to social stress and depression (Grabe et al. 2005). A functional MRI study suggested that S allele carriers of this gene have increased chances of having depression in response to adverse events than L allele carriers (Heinz et al. 2005). Taylor et al. (2005) studied the influence of this polymorphism on hippocampal volumes in late life depression. Their analysis showed that later age of depression onset was associated with smaller hippocampal volumes in subjects with L/L genotype but earlier age of onset was associated with smaller hippocampal volumes in subjects having S/S genotype. Similarly, another study exposed that carriers of S allele had significantly reduced gray matter volume compared to those with L allele (Selvaraj et al. 2011). The role and relevance of this...
gene as the genetic determinant of depression in T2DM remains to be examined and hence investigated in the present study.

**The role of Dopamine D4 receptor gene (DRD4) in depression**

DRD4 is the most important gene in the psychiatric genetics because of its involvement in the pathophysiology of depression. This gene contains 4 exons and localized on chromosome 11p15.5 (Van et al. 1991). At the cellular level, DRD4 mRNA acts as a peripheral marker of central dopaminergic function in major depression (Rocc et al. 2002). DRD4 is expressed in human and rodent amygdaloid nuclei, with the highest levels observed in the basal and central nuclei. In the basal nucleus, the levels of the DRD4 mRNA are significantly higher in patients with major depression compared with control Subjects (Xiang et al. 2008). Three polymorphisms within DRD4 engrossed attention in dopamine related major depressive disease or depression. These are 120 bp duplication at 5’ untranslated region, -521 C/T (rs1800955) and 48 bp VNTR in exon 3. Besides depression, these polymorphisms have been studied extensively in relation to illnesses like schizophrenia, attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), bipolar manic-depressive disorder and behavioural traits such as novelty seeking.

A statistically significant difference in the genotype frequencies and distribution of DRD4 exon III variable number tandem repeats was found to be associated with patients having depression (Garriock et al. 2006). A recent meta-analysis of 12 studies demonstrated a significant association between a 48bp repeat polymorphism in DRD4 and the occurrence of depression where, DRD4.2 allele was significantly associated after correcting for multiple comparisons (Lopez et al. 2005). Another meta-analysis of 183 papers containing 20 polymorphisms of 18 genes revealed that DRD4 is amongst six others being; SLC6A3- 40bp VNTR, SLC6A4-44 BP Indel, APOE, GNB3 825T and MTHFR 677T) most significantly associated genes for depression (Lopez et al. 2008). Promoter polymorphism contributes largely to the expression as they regulate gene function. Lately, it has
been revealed that promoter polymorphisms i.e. rs936460 (-1106T/C), rs3758653 (-906T/C), rs747302 (-809G/A) and rs1800955 (-521C/T) are associated with the risk of schizophrenia, depression and mental retardation (Zheng et al. 2012). Whether these dopaminergic gene variants influence diabetes for the risk of depression has not been addressed hitherto.

In order to understand the extent and degree of participation and contribution of SLC6A4 and DRD4 gene in depression, the present study was designed to achieve the following objectives

Objectives:
1. To examine the role and relevance of two candidate genes (DRD4 and SLC6A4) as the genetic determinants of depression risk in diabetes.
2. To assess their gene-gene, gene-environmental interactions both individually and at haplotype level.
3. SNP-SNP interactions will be analysed for their epistatic or hypostatic effect on the risk of depression.