CHAPTER 2

REVIEW OF LITERATURE

In the pursuit to understand the extent and degree of genetic component for the development of depression, scientists have mulled over many pathways under the influence of several genes and genetic variants. There is a huge repertoire of genes conferring the risk of depression elucidated by gene association studies (Caspi et al. 2003, Garriock et al. 2006, Xiang et al. 2008, and Lopez Leon et al. 2005). Gene-environment studies deciphered the contribution of DRD4 and SLC6A4 genes and their interaction with environmental stressors. The first meta-analysis revealed that those subjects who are S allele carriers of SLC6A4 are at higher risk (OR 1.23) of developing MDD (Furlong et al. 1998) however, the other two meta-analyses (Anguelova et al. 2003, Lasky-Su et al. 2005) could not confirm this finding. Another meta-analysis comprising 183 papers, 393 polymorphisms of 102 genes confirmed statistical evidence for six genes i.e APOE, DRD4, GNB3, MTHFR, SLC6A3, and SLC6A4 as the genetic determinants for the risk of major depression (Lopez Leon et al. 2008). Almost 15 GWAS studies have been conducted on genetics of depression so far. Astonishingly, none of the SNPs out of these GWAS studies reached genome wide significance except rs1545843 of SLC6A15 gene.

Several studies investigated the association of DRD4 and in relation to depression. (Lopez Leon et al. 2005, Garriock et al. 2006, and Xiang et al. 2008). None of the study examined the relationship of DRD4 for the risk of type 2 diabetes except a study by Prasad et al. (2008) which revealed that DRD2 gene rather than DRD4 is associated with susceptibility to chronic renal insufficiency in Asian Indian population.

Similarly many studies have reported the association of SLC6A4 gene and genetic variants as the genetic determinants of depression (Lesch et al. 1996,
Gutierrez et al. 1998, Caspi et al. 2003). Only few studies have checked the role of SLC6A4 in relation to type 2 diabetes. One study on Greek population has revealed that S allele of SLC6A4 is strongly associated with T2DM after adjusting the effect of obesity (Iordanidou et al. 2010). Another study revealed that SLC6A4 polymorphism interacts with COMT gene to confer the risk of T2DM in Chinese population (Xiu et al. 2015).

After digging the scientific literature on the role and relevance of DRD4 and SLC6A4 gene polymorphism as genetic determinants of depression in T2DM, it is affirmed that there are plenty of studies, which have revealed the influence of DRD4 and SLC6A4 genes on depression but none of the study has checked the potential role of those gene polymorphisms in connection with the risk of depression in subjects having T2DM. Hence, the review of literature searched for the present study addresses the association of these two genes in relation to depression only.

**Solute carrier family 6 member 4 (SLC6A4) gene:**

Serotonin (5-hydroxytryptamine or 5-HT) is an important neurotransmitter in the central and peripheral nervous system. When it is released at the synaptic cleft, SLC6A4 a high affinity sodium (Na⁺) and chloride (Cl⁻) dependent transporter, which is localised in the presynaptic boutons, clears it up. This 5-HT site in the brain serves as a principal site of action for many tricyclic antidepressants and regulates behavioural or toxic effects of cocaine and amphetamines. SLC6A4 takes up the serotonin into the presynaptic neurons hence, terminates the action of serotonin by taking this neurotransmitter into its pool. Hence, the serotonin transporter protein encoded by 5-HTT gene (SLC6A4) plays a role in the regulation of serotonin in the central nervous system. This gene spans 31 kb, localises on chromosome 17q11.2 and contains 14 exons. In the scientific literature the names 5-HTTLPR, SERT and SLC6A4 genes are used synonymously.

The serotonin transporter encoded by SLC6A4 gene is considered to be the target for some antidepressant drugs i.e. selective serotonin reuptake inhibitors
(SSRIs). First report on the structure of serotonin transporter in unipolar and bipolar depression has been given by Lesch *et al.* (1995). The results of the study provided preliminary evidence that alterations in the structure of 5-HTT are associated with unipolar and bipolar depression. In 1996, this inference was supported by another study (Battersby *et al.* 1996), which revealed that allelic variation of variable number of tandem repeat (VNTR) region of SLC6A4 contributes to the risk of depression.

First time in 1996, Ogilvie and co-authors identified three novel polymorphisms within SLC6A4 gene, namely VNTR in intron 2, which consists of 17 bp VNTR repeats. The alleles were named as STin2.9, STin2.10 and STin2.12. These alleles contain 9, 10 and 12 copies of the VNTR respectively. The STin2.9 was considered to be the significant risk marker for unipolar and major depressive disorder (MDD). 12 repeat allele polymorphism of VNTR in intron 2 (STin2.12) was found to be associated with the susceptibility of depression in British Caucasian population (Rees *et al.* 1997). In another study the short (S) allele of the 5-HTT transporter linked polymorphism (5-HTTLPR) showed association with season related depression (Rosenthal *et al.* 1998). It was suggested that deletion/insertion polymorphism in the promoter region of SLC6A4, short variant (allele 484) reduced the transcriptional efficacy of this gene (Gutierrez *et al.* 1998). This locus was found to be in linkage disequilibrium with the VNTR marker, hence, associated with the major depression in melancholia (MDDM). STin 2.12 was found to be significantly associated with depression in Japanese population (Ohara *et al.* 1999).

Sleep deprivation is an independent predictor for depression and functional polymorphism within the promoter region of SLC6A4 gene regulates the serotonin in the mechanism of sleep deprivation in depression (Benedetti *et al.* 1999). A study on Han Chinese patients deduced that VNTR polymorphism within SLC6A4 was observed to be risk factor for both schizophrenia and unipolar depression (Liu *et al.* 1999).

Depression has also been observed to be associated with increased platelet activation. It has been observed that platelet activation is increased in the carriers of l/l genotype of SCL6A4 gene polymorphism in elderly depressed subjects (Whyte
et al. 2001). A study investigated the link between depressive response during tryptophan depletion (TRP) and functional polymorphism of SLC6A4 gene (Moreno et al. 2002). Significant association was observed between 1/1 homozygotes and depressive response to TRP depletion. Gender differences in relation to SLC6A4 gene polymorphism and depression were observed in geriatric population whereby, short allele homozygosity was found to be associated severely in female Geriatric population but not in males (Steffen et al. 2002). Behavioral response to tryptophan depletion in healthy women with and without family history of depression in relation to 5-HTTLPR allele was investigated (Neumeister et al. 2002). The findings of the study suggested that short allele of this polymorphism interacted additively with family history of depression for the risk of depression during tryptophan depletion.

The serotonergic system has been observed to play role in the production of N1 and P2 components of Auditory Evoked Potentials (AEPs). While analysing whether N1 and P2 components of AEPs are associated with SLC6A4 gene polymorphism in Chinese depressed patients, it was revealed that shorter P2 latency was associated with the s/s genotype carriers especially in female patients (Chen et al. 2002). Rausch and co-authors (2003) hypothesized that depressed patients had higher body temperature during day time, which might be associated with the 5-HT transporter long promoter region effects. Consequently, it was found that subjects with 5-HTTLPR deletion (short allele) were warmer than those subjects who lack short allele. The findings suggested that subjects with corrected body temperature above 98.3°F were 2.6 fold more likely to be depressed than the subjects having lesser body temperature during day time (Rausch et al. 2003).

In 2003, it was revealed for the first time that promoter polymorphism within SLC6A4 gene influenced the stressful life events for the risk of depression (Caspi et al. 2003). It was revealed that carriers of one or two copies of short alleles exhibited severe depression in relation to stressful events than individuals who were homozygous for long allele. Nellissery et al. (2003) observed that the association of short allele of SLC6A4 gene polymorphism with major depression was independent of the alcohol drinking. Furthermore, another study revealed that
s/s genotype was significantly associated with the family history of depression and this risk was different for suicidality (Joiner et al. 2003). The findings of the research conducted by Sen et al. (2004) clarified that short allele of 5-HTTLPR in collaboration with Pro358Serallele of GABA(A) gene conferred risk for the depression related traits.

Hippocampal volumes have been considered to be related with depression in many neurological studies (Frodl et al. 2004, Putzhammer et al. 2005, Heinz et al. 2005). Frodl et al. (2004) observed that l/l homozygous genotype was significantly associated with smaller hippocampus gray matter and white matter volumes. Regulation of mood, motor activity and sleep patterns were investigated in relation to indel polymorphism of SLC6A4 gene and it was noticed that homozygosity for the long variant was a predisposing factor for night time motor activity (Putzhammer et al. 2005). Heinz et al. (2005) observed that SLC6A4 short allele carriers showed stronger activation of the amygdala during the presentation of aversive behaviour in depressed subjects. The findings of their study demonstrated that s allele carriers exhibited greater coupling between amygdala and ventromedial prefrontal cortex on functional magnetic resonance imaging, which contributes significantly in major depression.

Chorbov et al. (2007) conducted a study on sample of 247 young adult female twins from Missouri, USA to examine whether SLC6A4 polymorphism and adverse effects of life interact for the development of depression. The results of the study demonstrated that long allele carriers rather than short allele carriers were associated with MDD in the presence of environmental trauma. In order to understand whether the genotype influence risk of depression with disaster exposure and social support, a study revealed that short allele of 5-HTT polymorphism mediates risk of post disaster, post traumatic disorder and depression under the condition of high hurricane exposure and low social support (Kilpatrick et al. 2007). The interaction analysis of the two genes SLC6A4 and Brain-derived neurotrophic factor (BDNF) provided an evidence of biological epistasis of the neural mechanism, linking serotonergic and neurotrophic signalling in the brain for the implications of depression (Pezawas et al. 2008). The
interaction of val66met (Valine to Methionine) of BDNF gene and s/s genotype of 5-HTTLPR moderated the effects of childhood adversity for the risk of depression in adulthood (Aguilera et al. 2009).

Noskova et al. (2009) studied the relationship of several polymorphic loci of serotonergic genes with unipolar depression in Russian population. The findings suggested that genotype 10/10 of SLC6A4 gene and G/G genotype of HTR2A gene interact for increasing the risk of unipolar depression in ethnic Russians. It was realised that peer victimisation correlates with internalising symptoms which were more pronounced in girls. To clarify the role of SLC6A4 gene polymorphism and its interaction with relational peer victimisation to influence the risk of depression in young Mexican girls, the findings of the study highlighted the diathesis-stress model of depression, whereby short s/s genotype was observed to pose a risk of depression in adolescents girls when they experienced relational peer victimisation (Benjet et al. 2010). Xie et al. (2009) conducted a study to examine the effects of childhood adversity, adult traumatic events, 5-HTTLPR genotypes and gene environment interactions for the risk of post traumatic stress disorder. The inferences suggested that those participants who had both childhood adversity and adult traumatic events were more likely to develop life time post traumatic stress disorder when compared with those who experienced either type of adversity. This risk was found to be increased in subjects with one or two copies of S allele compared as with l/l homozygotes. In association study Costas et al. (2010) involved 44 candidate genes to understand their influence for the risk of depression and anxiety, postpartum in women. Post-hoc analyses at the unphased haplotype level revealed contribution of three important SNPs of SLC6A4, dopa decarboxylase (DDC) and protein kinase C, beta (PRKCB) for the risk of postpartum depression. In an interesting study by Goodyer et al. (2010), it was discovered that BDNF and 5-HTTLPR genes modify the risk of new depressive episode associated with elevated morning salivary cortisol. Morning salivary cortisol is a biomarker for unipolar depression in adolescents. To understand the interaction of highly stressful life events and subsequent depression in relation to 5-HTTLPR polymorphism, a study was conducted on elderly French war repatriated subjects.
(Artero et al. 2011). The findings suggested that short allele carriers were at higher risk of depression when repatriation X 5-HTTLPR gene polymorphism was taken into account. Another study revealed that the risk of depression because of the loss of partner was influenced by genetic variation controlled by 5-HTT activity (Fandino et al. 2013). The interaction of 5-HTTLPR and hypothalamic pituitary adrenal axis (HPA) system predicts the pathogenesis of depression in German population (Welper et al. 2014). A study suggested that 5-HTTLPR polymorphism might not be the only reason for the development of depression but it could be related with the age at onset of MDD, because of the gene environmental interactions (G X E) in Japanese population (Watanabe et al. 2015). In the sequence analysis of SLC6A4 gene polymorphism in relation to antidepressant response to SSRI/SNRI in Japanese depressed subjects, rs3813034, rs140701, rs1042173, and rs7224199 SNPs were found to be associated with SSRI/SNRI efficacy independently from other clinical variables (Nonen et al. 2016).

**SLC6A4 gene and influence of antidepressants**

Depression symptomatology has been observed to respond to antidepressant drugs which are called selective serotonin receptor inhibitors (SSRIs). The serotonin transporter (5-HTT) is a primary target for SSRIs. In order to understand the pharmacological treatment of depression, the 5-HTTLPR polymorphism and Fluvoxamine response was checked which suggested that long variant (l/l) homozygotes and (l/s) heterozygotes showed better response to Fluvoxamine than homozygote for the short allele (s/s) variant (Smeraldi et al. 1998). Zanardi et al. (2000) revealed that the functional polymorphism in the promoter region of SLC6A4 gene regulated the Paroxetine response in the subjects having depression. Another study analysed SLC6A4 gene polymorphism and antidepressant response to 6 weeks treatment with SSRI drug (Fluxetine) in Korean patients. The results of the study found that those depressed subjects who were homozygous (l/l) or (s/s) in intron 2, showed better response than the others (P< 0.001) (Kim et al. 2000).
The relationship of SLC6A4 gene polymorphism and antidepressant response was examined in 95 elderly depressed patients with Paroxetine or Nortriptyline (Pollock et al. 2000). The findings suggested that genetic variation within this gene influenced the variable response of patients with SSRI (Paroxetine). The indel polymorphism within SLC6A4 was found to be associated with better and faster response to SSRIIs with or without Pindolol augmentation in depressed patients (Weizman and Weizman, 2000). In an Italian study, 155 patients were treated with Fluvoxamine 300mg/d and either placebo or Pindolol in a double blind design for 6 weeks and the severity of the depression was checked with Hamilton Rating Scale for Depression (HAMD). Short variant was observed to be associated with poor response to Fluvoxamine treatment adjusted with the effects of clinical variables (Zanardi et al. 2001). In an interesting study on Japanese depressed patients, they concluded that Fluvoxamine was not less effective in depressed patients carrying S allele than those who carry L allele. Further they also suggested that this inference was not less effective in Japanese than in Caucasians (Yoshida et al. 2002). Dose response relationship of Fluoxetine in relation to 5-HTTLPR promoter polymorphism was analysed, which revealed that long allele carriers were more responsive to placebo as well as drug dose, in comparison to short allele carriers (Rausch et al. 2002). L allele was found to be associated with better SSRI (Fluoxetine) response in Taiwanese depressed patients (Yu et al. 2002). Arias et al. (2003) concluded that s/s genotype of SLC6A4 gene polymorphism was associated with non remission condition at 12 week of major depression.

With the aim to compare two selective serotonin receptors inhibitors in Japanese depressed subjects taking 5-HTTLPR polymorphism into account, the study revealed that Paroxetine was more effective than Fluvoxamine in s/s carriers after four weeks of medication but not in l/s carriers (Kato et al. 2005). Another study advocated that 5-HTTLPR polymorphism was associated with Citalopram adverse effects, where long allele conferred increased SLC6A4 transcription and increased serotonin transporter levels in brain (Hu et al. 2007).

For the treatment of depression some scientists consider repetitive transcranial magnetic stimulation (RTMS) to be a painless and safe brain
stimulation technique. The improved depression symptomatology was observed in l/l homozygotes of 5-HTTLPR and val/val homozygotes of BDNF gene in drug resistant subjects (Bocchio-chiavetto et al. 2008). To examine whether interferon-alpha treatment is genetically modified in depressed subjects, a cohort of 71 non-depressed hepatitis-C patients about to receive interferon alpha therapy was checked. It was found that long allele was associated with decreased rate of developing MDD with l/l genotype being the most resilient (Lotrilech et al. 2009).

SLC6A4 gene polymorphism and response to treatment in adult depressed patients and symptoms severity rating ≥8 were tested for 8 weeks with open label Sertraline (100-200mg/d). It was observed that s/s carriers achieved remission under combined Sertraline/Atomoxetine treatment relative to non s/s carriers. This study suggested that patients with poor response to Sertraline should be given Atomoxetine also especially in s/s carriers (Reimherr et al. 2010).

Lee et al. (2010) tested response of Venlafaxine XR (extended release) in Korean depressed subjects in relation to SLC6A4 polymorphism. Response to Venlafaxine at week 4 was observed to be significantly associated with l/l and l/s genotype. Studies comprising neuroimaging technology in MDD have suggested that dysregulation in prefrontal cortex, subgenual cingulate and amygdale is controlled by serotonergic system (Brockman et al. 2011). The findings of the study showed that SLC6A4 gene polymorphism mediated the resting state perfusion in mood processing. Both serum concentrations of SSRI and 5-HTTLPR polymorphism influence the response to SSRIs. In this regard, a study revealed that those MDD patients, who had high antidepressant serum concentrations and are L allele carriers, responded better to the treatment than patients with low serum concentrations (Dreimuller et al. 2012). Matseumoto et al. (2014) investigated five genetic variants of 5-HTR2A, BDNF, SLC6A4, CREBB1 and TPH2 as the genetic determinants for the pharmacological treatment outcomes. The findings of the study exhibited rs18076005 of SLC6A4 gene as the significant modulator of antidepressant response in depressed subjects. Another study concluded that norepinephrine transporter (NET) 182C and 5-HTT polymorphism interact to cause treatment resistant depression and electroconvulsive therapy treatment
response in Finnish depressed subjects (Kautto et al. 2015). Similarly Phillips et al. (2015) showed that NET and 5-HT-A polymorphism affected the hippocampal volumes in the Canadian depressed subjects.

**Dopamine D4 receptor (DRD4) gene:**

Clinical, epidemiological and association studies have highlighted the relationship of dopamine D4 receptor (DRD4) with neuropsychiatric disorders especially depression (Lopez Leon et al. 2005, Lauzon and Laviolette, 2010, Lai et al. 2010). DRD4 is a G-protein coupled receptor of the dopamine D2 like receptor family. Functionally, it inhibits adenylyl cyclase. Adenylyl cyclase is an enzyme which plays key role in the catalyses and conversion of adenosine triphosphate (ATP) to 3', 5'-cyclic AMP (cAMP) and pyrophosphate. DRD4 gene is localised near telomeric region on 11p15.5 and contains 4 exons. A 48 bp sequence is identified in the exon III of DRD4 gene which contains variable number of tandem repeat (VNTR). This 48 bp VNTR in exon III ranges from 2 repeats allele (2R) to 11 repeats allele (11R). DRD4 gene encodes 387 amino acid protein with seven transmembrane domains, a potential N-linked glycosylation site and several phosphorylation sites. The other three genes i.e. DRD1, DRD2 and DRD3 share 28 percent, 41 percent and 39 percent sequence homology respectively with DRD4. DRD4 has been observed to be abundantly present in frontal cortex, mid brain area, amygdala and medulla.

Last 20 years of medical literature have revealed that DRD4 is associated with the regulation of processing and execution of memory. Neuronal activity in both medial pre-frontal cortex and basolateral amygdala is significantly mediated by the dopamine signals from the ventral tegmental area (VTA) through both dopamine D2 and D1 like receptors. Genetic association studies have reported a strong association of DRD4 gene polymorphism with attention deficit hyperactivity disorder (ADHD) (Li et al. 2006), anorexia nervosa (Bachner-Melman et al. 2007), Schizophrenia (Okuyama et al. 1999), obesity (Levitan et al. 2006), drug addiction (John John McGeeary, 2009) and personality disorder (Nemoda et al. 2010). Besides
that, it is also evident from the previous reports that DRD4 gene plays an important role in the development and risk of depression (Rocc et al. 2002, Lopez Leon et al. 2005, Garriock et al. 2006, and Xiang et al. 2008). It has been revealed that DRD4 mRNA at the cellular level acts as a peripheral marker of central dopaminergic function in depression. Rodent studies have also suggested that DRD4 is highly expressed in rodent’s basal and central nuclei (Xiang et al. 2008). In case control comparison, it has been observed that levels of DRD4 mRNA in basal nuclei are substantially higher in depressed patients in comparison to control subjects. It has been documented that DRD4 gene plays a role in dopamine signal transmission within neural emotional processing centres and also regulates the signal flow in amygdala-prefrontal cortex in depressed subjects (Mrzljak et al. 1996). This viewpoint has been endorsed by inferring of preferential distribution of DRD4 in GABAergic neurons within several brain regions including hippocampus, prefrontal cortex and thalamus.

Manki et al. (1996) investigated the association of dopamine D2, D3 and D4 gene polymorphism in relation to mood disorder in Japanese population. Their results suggested a significant association of DRD4 gene polymorphism with mood disorders especially major depression. A study by Lerman et al. (1988) examined the presence of genetic subgroups of depressed individuals who were more or less predisposed to engage in self medication of smoking in relation to DRD4 gene polymorphism. The inferences of the study suggested that rewarding effects of smoking and beneficial effects of nicotine replacement therapy for depressed smokers depended upon the dopamine transmission mediated by DRD4 gene. To understand whether the alterations of dopaminergic system in major depression was regulated by DRD4 mRNA expression, DRD4 mRNA expression in peripheral blood mononuclear cells (PBMC) from depressed subjects was examined before and after 8 weeks of treatment with Paroxetine at 20-50 mg/d. The results suggested that DRD4 mRNA was expressed as a peripheral marker of the central dopaminergic function in major depression (Rocc et al. 2002). In order to understand the behavioural components in adolescents of Israel in relation to DRD4 gene, assessment was made by structural interview and rating scales for.
detailed clinical history, diagnosis, suicidal intent, impulsivity, violence and depression (Zalsman et al. 2004). It has been observed that the DRD4 allele was significantly associated with depression in suicidal adolescents. Szantai et al. (2005) identified a novel 27 bp deletion, 524bp upstream of the initiation code of DRD4 gene near to -521 C/T SNP. They highlighted that 27 bp deleted region contained consensus sequences of binding sites for various transcriptional factors, which suggested that this might play role in transcriptional regulation of DRD4. A meta-analysis comprising 12 studies examined the role of DRD4, 48 bp repeat polymorphism in mood disorder (Lopez Leon et al. 2005). Their findings suggested that DRD4, 2 repeat allele was a risk marker for depression and depression related symptomatology. It was also suggested that not only DRD4 exon III VNTR correlated with depression but number of risk genotypes added up the risk of major depressive disorder (Garriock et al. 2006). Garriock et al. (2006) conducted candidate gene study to examine the genetic basis of major depressive disorder (MDD) and the capacity to respond to antidepressant treatment. Statistical significant differences were evident between control and depressed subjects for DRD4 exon III VNTR genotype frequencies. A meta-analysis comprising 183 papers, 393 polymorphisms in 102 genes was conducted (Lopez Leon et al. 2008). It was revealed that DRD4 gene along with other five candidate genes (APOE, GNB3, MTHFR, SLC6A3, and SLC6A4) proved to be significant genetic markers for depression. Trajectory of depression symptoms in adolescents and young adulthood was tested in relation to dopamine D2 and D4 genes. Secondly, the association between receptors and depression in relation to socioeconomic disparity, child-parent ties and social support was checked (Guo and Tilliman, 2009). The findings of this research yielded an interaction of genetic predisposition and social environment for the risk of depression. Pritchard et al. (2009) investigated dopamine receptor polymorphisms (DRD1, DRD2, DRD3 and DRD4) in relation to depression in Australian Alzheimer’s patients. The findings of the study revealed a potential role of VNTR variants of DRD4 gene in the development of depression in Alzheimer’s patients. To understand whether self medicating smoking was genetically modified in depressed patients, promoter polymorphism
of DRD4 gene in relation to depression was assessed in Hungarian population (Kotyuk et al. 2009). It was discerned that repeat polymorphism of exon III, -521 C/T and -616 C/G polymorphism within DRD4 gene influenced the nicotine addiction in depressed patients. A study by Opmeer et al. (2010) suggested that interaction between VNTR of dopamine D4 receptor (DRD4) and dopamine transporter (DAT) genes were potential candidates that influenced the susceptibility to major depressive disorder (MDD). An individual’s risk to depression has many underlying components that may accumulate and exacerbate its risk in childhood. It has been reported that 7 repeat allele of DRD4 gene x environmental (Childhood adversity) interaction play significant role in depression in later stages of life (Couney Landers, 2011). It is a general consensus among researchers that depressive symptoms exhibit a normative inverted ‘U’ shaped trajectory before and during transition of adolescents to adulthood. The role and relevance of five monoamine candidate genes on depression in adolescence and young adults was investigated and they found that carriers of 5 repeat allele of DRD4 gene showed distinct depression symptom trajectories from adolescents to adulthood, however, for males, 3.5 repeat allele of monoamine oxidase-A (MAOA) gene was found to be correlated to depression (Adkins et al. 2012).

Bobadilla et al. (2013) conducted a study to evaluate the genetic link of DRD4 gene polymorphism with substance (marijuana) use. The study showed that 48 bp VNTR of DRD4 gene correlated significantly with marijuana use in depressed population. A study was conducted in Han population of China to examine the antidepressant outcome in response to selective serotonin reuptake inhibitors (SSIs) and DRD4 gene polymorphism in relation to depression (Yin et al. 2015). It was highlighted that clinical characteristics, neuroendocrine factors and DRD4 gene polymorphism (rs1800544) influenced the SSRI response in Chinese Han major depressive disorder (MDD). Furthermore, this inference was confirmed by another study conducted by Yin et al. (2016).
None of the study is complete without analyzing the contribution of environmental components influencing the disease condition. In this way GxE studies identify and analyse the degree to which genetic variants modify the association between environmental factors and depression. The environmental factors are stressful life events that may influence the risk of depression.

The first GxE study was published in 2002 in Science (Caspi et al. 2003). This study conducted longitudinal design using data from 26 years in Newzealand population for examining the role of functional polymorphism in the promoter region of SLC6A4 and its interaction with the stressful life events. Results of this study suggested that atleast one copy of short (S) allele (S/S or S/L) genotypes was associated with depression in response to stressful events when compared to the subjects who did not carry S allele.

After publication of Caspi et al. (2003) several studies were conducted on 5-HTTLPR and its interaction with environmental variables. Some other gene variants such as brain derived neurotropic factor (BDNF), monoamine oxidase-A (MAOA), FK506 binding protein 51 (FKBP5), corticotropin releasing hormone receptor1 (CRHR1), catechol-o-methyltransferase (COMT) and cAMP response element binding protein 1 (CREB1) have also been studied in relation to the environmental correlates. Many replication studies have been done on stressful life events interacting with the genetic endowment of the subject. In continuation to understand the GxE of depression some studies found consistent GxE effects and others reported contradictory results (Uher et al. 2008, Caspi et al. 2010). Similarly, two meta-analyses (Risch et al. 2009, Munafo et al. 2009) supported the potential role of GxE in depression however, meta-analysis by Karg et al. (2011) failed to find it. An animal study reported that loss of function mutation in the serotonin gene was associated with the depression in rodents and this genetic variation has been linked to depression in non human primates (Caspi et al. 2010). Some studies suggested that the results would be more appealing, if experimental images of amygdala activity and 5-HTTLPR variation alongwith treatment response were
evaluated (Caspi et al. 2010, Munafo et al. 2012). Although G×E studies are very important in elucidating the environmental and genetic component for the risk of depression, but G×E clarifying the extent of contribution by genetic or environmental variables in the risk of depression is still an unexplored area.

**Genome Wide Association Studies (GWAS)**

In the last couple of years the advances of human genetics has paved the way for modern and updated techniques for the understanding of genetic underpinning of the disease. With the initiation of Human Genome Project, HapMap project, 1000 Genomes Project, the field of human genetics has ushered in indentifying and introducing the causal variants for their direct effect on the diseases. It has been realized that most of the candidate gene studies have underpowered inferences and their replications rarely produce the appropriate result. Recently the availability of DNA microarrays has enabled genome wide association studies (GWAS) that do not need any prior hypothesis. In GWAS studies one million or more single nucleotide polymorphisms (SNPs) can be examined for their association with disease, however, the threshold for declaring genome wide significance in GWAS, a P value of less than $5 \times 10^{-8}$ is required which is equivalent to P value of 0.05 corrected for million independent test.

In the GWAS data base more than 2000 GWAS studies have been published so far. Out of which 14 GWAS have been conducted on major depressive disorder or depression. Besides these, one study on age at onset of depression has also been conducted. All these studies are based on either a sample of European countries or represent a combination of population and clinical sample. The first GWAS on depression was conducted by Sullivan et al. (2009). This study included 1738 cases and 1802 controls. None of the SNP reached GWAS significance however, 11 of the 200 SNPs were observed in 1607 kb of the gene Piccolo Presynaptic Cytomatrix Proteins (PCLO). This gene plays a role in the establishment of active synaptic zones and synaptic vesicles tracking.
Kohli and colleagues (Kohli et al. 2011) reported a genome wide significant association for depression whereby, they found the recessive effect of rs1545843 SNP of SLC6A15 (solute carrier family 6, neutral amino acid transporter, member 15) to be the genetic variant for depression. This gene is associated with the transportation of neutral amino acids and this allele is found to be associated with reduced SLC6A15 expression in hippocampal tissue.

It has been realized by the researchers that the influence of these GWAS studies on depression is usually less in terms of allelic odds ratio hence, considerably larger samples are required to identify the genetic loci and their effects associated with depression. Consequently, Psychiatric Genomics Consortium was established in 2007 as an international collaborative forum. The consortium published a mega analysis of MDD comprising 9240 cases and 9519 controls of European ancestry (Ripke et al. 2013). Although the sample was quite large, but none of the SNP reached genome wide significance. Two most significant SNPs in the discovery panel were rs11579964 and rs7647854. These SNPs could not be confirmed in replication studies.

Luciano et al. (2012) conducted GWAS of depressive symptoms with no SNP reaching the genome wide significance. One SNP rs7582472 reached modest association (P=1.59×10⁻⁶) however, this SNP was also not confirmed in independent studies. Similarly, another intronic SNP, rs12912233 of retinoidrelated orphan receptor alpha (RORA) gene reached modest association (P=6.3×10⁻⁷) in relation to depression (Terracciano et al. 2010).

The largest meta-analysis comprised 17 populations with 34549 individuals but none of the SNP reached genome wide significance (Hek et al. 2010), however, when discovery panel and replication samples were combined, SNP rs40465 was found to be associated with depression at genome wide significance. Interestingly, this gene variant is located within the gene desert, an area of the genome where there are long regions without protein coding sequences having no biological function.

Prior to GWAS, meta-analysis of candidate genes has confirmed that 6 candidate genes (APOE, DRD4, GNB3, MTHFR, SLC6A3 and SLC6A4) are potential
markers for the risk of depression. Ironically, none of these genes have been confirmed at GWAS platform. Another interesting fallacy is that GWAS studies do not analyse the effect of environmental variable, hence, failed to capture G×E interactions. Moreover, these studies are suggestive rather than conclusive.