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A Plausible Role of *5HT2A* gene SNPs on Chromatin and 5'UTR Related Genomic Architecture: Insights from Bioinformatics Methods

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Abstract

The neurobiological and signaling feature of the (5-hydroxytryptamine (5-HT2A)) receptor places it at the center of brain function. Several psychiatric diseases are implicated due to mal-functioning of the G protein-coupled receptors (GPCR) receptors, consequently GPCR are primarily the targets of drugs aimed at treating these disorders. While several disease-associated single-nucleotide polymorphisms (SNPs) have been identified and mapped in the human genome, the pathophysiological relevance for many synonymous remains largely anonymous. Gene expression is orchestrated by numerous *cis*control elements that may be located upstream or downstream of the Transcription start site (TSS) at several levels. Polymorphisms in this region can alter gene expression and lead to biological changes through alterations in chromatin, allelic and epigenetic marks. This class of polymorphism is attracting increased attention since they are responsible for a proportion of heritable phenotypic variations in several human diseases. In this study we explored plausible roles of *5-HT2A* gene SNP variations (rs6311 and rs6313) on genome architecture in the promoter flanking the TSS such as chromatin marksand mRNA secondary structure (folding) using bioinformatics methods.The results suggest differential chromatin marks such as binding sites for various transcription factors, alterations to TSS and enhancers. Further,Mold analysis suggested a plausible role of SNP (rs 6313) on RNA secondary structure with different thermodynamic properties for each of the alleles. In summary, the SNPs could impact gene expression directly at the transcriptional level or through mechanisms acting on the mRNA and therefore at transcriptional and translational levels.

Keywords: Nonsynonymous and Synonymous polymorphisms; Regulome; Chromatin; allele-specific expression (ASE); ChIP.

1.Introduction

1.1.*5HT2A* gene arrangement, function of the receptor GPCR-protein in the central nervous system, and human physiology.

The *5HT2A* gene spans 65 kb and is encoded from the antisense strand (Gene ID: 3356; Chr-13q14.2) (NCBI ref). According to Qin-shy Zhu (1995), the basal promoter is essential for the transcriptional regulation of the receptor. On the other hand, transcription can react to stimuli due to multiple transcription initiation (TSS). A silencer and enhancer sequence helps to facilitate the process of cell and drug-specific selective neuronal expression control through the DNA domain in the gene's 5' flanking region (Tooth, Miklos, 1996). The region contains hallmark sequences for a number of transcription factors, including E-boxes, PEA3, Spy, and cyclic AMP response element-like sequences. Both genetic (imprinting) and epigenetic mechanisms tightly regulate the actions enabling CNS functions. The receptor is found in multiple brain regions, including the forebrain, cortex and basal ganglia where it is found in high concentrations (Mengod G). In order to facilitate a variety of physiological processes, the receptor is expressed in different organs including the intestine, smooth muscles, endothelial cells, and platelets (I raote 2007). Learning, memory, and cognition are among the neurobiological functions of the receptor. At the cellular level, these include neurogenesis, maintenance of the neuronal microtubule, electron transfer through ETC (Electron-transport chain), ATP (Adenosine triphosphate) generation, and calcium level modulation. Numerous hallucinogenic substances function as receptor agonists to create psychoactive effects. The receptor is proposed to act on the central nervous system like a hallucinogen. There are various psychiatric disorders linked to the aberrant receptor activity.

1.2.Promoter polymorphisms in neurotransmitter genes and association in various neuropsychiatric diseases

Nucleotide polymorphisms can be silent (synonymous), or alter the encoded amino acids (nonsynonymous), or those thatappear in non-coding areas. They contribute to sizable percentage of heritable phenotypic variants and are mapping instruments used in human genetic and genomics (Chee Seng Ku2010). Further, several polymorphisms impact inheritance and genome stability and also contribute to genome evolution. ShwetaMalhotra, Vinod Goyal 2014; Yusuke Nakamura 2009).Through cellular and genomic techniques, the functional importance of promoter polymorphisms—a significant but largely unstudied class of genetic variation—have recently been identified (Ignatov EV, Matrosova EA 2021).Microsatellites, variable number tandem repeats (VNTRs), copy number polymorphisms (CNV), insertion/deletion (ID), and single-nucleotide

polymorphisms (SNPs) are some examples of these polymorphisms. Few methods by which polymorphism in the promoter region can modify gene expression and bring about biological changes include: alteration to messenger RNA (mRNA) conformation (stability); removal of natural binding sites from transcription factors; and subcellular localization of mRNAs and/or proteins (Chorley et al., 2008). Additionally, through modifications to the chromatin structure that affect regulatory sequences, through adjustments to the three-dimensional (3D) genome architecture that result in the rewiring of enhancers and promoters, and also through changes to the epigenetic mark. Positive or negative associations have been shown between these groups of polymorphisms and a number of psychiatric syndromes, including SCZ, BPAD, and autism. The preceding paragraph describes few associations to highlight the range of polymorphisms and disease associations. Microsatellite (GT)(n) repeats in the promoter of N-methyl-D-aspartate receptor 2A subunit (*GRIN2A*) with SCZ (Yoshimi Iwayama-Shigeno et al., 2005); also repeats with Cytohesin 4 gene (*D. CYTH4*)GTTT-Repeat (Ehteram Khademi et al., 2017). Further, variants in the VNTR variant that enhance heroin addiction in the Monoamine Oxidase a (*MAOA*) gene(Chia-Chang Chineet al., 2010): X-Ray Repair Cross Complementing 5(XRCC5) in BPAD susceptibility (Mostafa Sadat and MahboubehKazemi-Noughabiet al., 2016). Several insertion deletion(I/D) polymorphisms have been suggested to be associated with smoking behavior (Maria Angelica Ehara Watanabe et al., 2011) such as 5-HTTLPR in serotonin transporter(*SLC6A4*) and autism (Hongbing Wang et al., 2019); the -141C polymorphism of dopamine D2 receptor(*DRD2*) (Xiaofeng Zhao et al., 2016) and angiotensin-converting enzyme (*ACE*) gene in SCZ(Mohammadarian Akbari et al., 2022). Three synaptic gene (*DLG2, PCDH15, and ASTN2*) CNV are associated with autism spectrum disorder, BPAD, and SCZ (ItaruKushima et al., 2022). Catechol-O-Methyltransferase (*COMT*) gene with SCZ (Ryoko Higashiyama et al., 2016), Nuclear Receptor Subfamily 4 Group A Member 2 (*NR4A2*) gene with ASD (Virpi M Leppaet al., 2016). Due to the density, ease of detection through laboratory tests, SNP and correlation with a variety of phenotypes, increased in years (Barkur S Shastry2009; Gonzaga-Jauregui et al., 2012).

Numerous SNPs in genes that code for proteins involved in biochemical pathways and neurotransmitters has being examined for potential links to a variety of neuropsychiatric disorders. Few examples include SCZ is linked to promoter polymorphisms in the Matrix Metallopeptidase 9(*MMP-9*) (Luong Pan et al., 2022) gene and Synaptosome Associated Protein 29(*SNAP29*) (T Saito et al., 2001) gene. Promoter polymorphisms of *COMT* (Ancon, Inés 2011) and Tryptophan Hydroxylase 2 (*TPH2)* gene(Vincenzo De Luca and Daphne Voin2005) have been linked to BPAD. In Autism the genes Arginine Vasopressin Receptor 1A (*AVPR1A*) and Insulin Like Growth Factor 1(*IGF-1*) promoter polymorphism (KatriKantojärvi et al., 2015; Mahsa

Abedini et al.,2022). Two genes, acetyl serotonin methyltransferase (*ASMT*) (Daniel F Kripke et al., 2011) and *mir-17-92* (Peng Liang et al., 2024), have been linked to depression through promoter polymorphisms. A list of SNPs in genes related to neurotransmitter signaling and related disorders are described in table 1.

1.3.Promoter polymorphisms impact on gene expression through alteration of chromatin architecture, allelic imbalance and epigenetics.

According to Ashley S. Doane and Olivier Elemento (2017), human regulatory/promoter DNA is made up of a range of cis-regulatory regions where the coordinated binding of transcription factors results in focal changes in chromatin structure. Genetic variations have a crucial influence on the population-level changes in the spatial chromatin architecture of human genomes. These changes often involve the formation of new regulatory networks, disruption of transcription factor recognition sequences, and changing of allelic chromatin states (Michal Sadowski et al., 2019).Further research suggest association of polymorphisms in diseases such as : drug addiction and depression (William Renthal, Eric J Nestler. 2009), structural variation in cancer genomes (Geoff Fudenberg et al., 2011), and neurodevelopmental illnesses such as autism (Li Yu et al., 2015). *Drosophila* models in neuropsychiatric diseases and intellectual disabilities support these investigations (Hiroaki Taniguchi, Adrian W Moore2014). Allelic changes are known to cause chromatin links to break, which can lead to transcriptional dysregulation and incorrect gene expression (Matthew T. Maurano et al., 2012). This is supported by chromatin immunoprecipitation investigations and DNA sequence motif analysis (Feng Yan et al.,2020).

Furthermore, a number of studies point to a regulatory role for many common variations that is particular to tissue and cell type. According to Symonds ME et al., 2009 these changes may have an impact on delicate and significant developmental processes such early prenatal exposures or environmental shocks; deviations from these processes may result in diseases.

Major Histocompatibility (MHC) locus on chromosome 6 has an open chromatin landscape of cell-type based gene promoters in prefrontal cortex (PFC) neurons in SCZ of epigenetic signals (histone H3-K4 trimethylation, or H3K4me3). Furthermore, as is widely known, the promoter's corresponding binding sites cause the chromatin landscape to change in response to cellular exposures such hormones and medications (Sam John et al., 2011). One of the theories explaining how promoter polymorphisms affect gene expression is allele specificity, which is the state in which two alleles of a gene express themselves differently in a particular cell (Siobhan Cleary, Cathal Seoighe.2021).The process could also be altered due to differential methylation of the alleles(epialleles)(Lucile Marion-Poll et al., 2021).

Correlation between allelic imbalances and gene expression profiles is supported by the various studies such as cancers (Rubén Ferrer-Luna et al., 2011); neuropsychiatric diseases(Xiang Xu et al., 2011); Retinal Diseases(Pablo Llavona et al., 2017).

Many studies have linked regulatory polymorphism mechanisms to an increased risk of developing a range of diseases. These include multiple myeloma (Molly Went et al., 2018), papillary kidney cancer (Shantao Li, et al., 2017), dopamine beta-hydroxylase (*DBH*) in blood pressure and autonomic functions (Yuqing Chen et al., 2010), suicide completers (Laura M Fiori and Gustavo Turecki2010), and type 2 diabetes (*HMG20A* gene). These studies emphasize the functions of chromatin and the special characteristics of chromatin changes in both healthy and diseased conditions.

2.Association studies of *5HT2A*gene SNPs rs6311 and rs6313.

We will concentrate on the polymorphisms of the serotonin receptor 2A (*5HT2A*) gene in this section. The Human Genome Project discovered that a high proportion of synonymous single nucleotide polymorphisms (suns) exist in the genome. Suns are found in both gene-coding and non-coding regions, occur more frequently than previously thought, and have a minor allele frequency of more than 1 %(EmilioCapriati et al., 2013). Since the protein's main sequence is preserved, suns have no bearing on the protein. Through processes influencing messenger RNA splicing, stability, and structure as well as protein folding, a number of suns modify the structure, function, and expression level of proteins (ZishuoZeng , Yana Bromberg 2019). They are also under pressure from evolution(Mikhail Gudkovet al., 2024). Research indicates that these variations in SNPs may have an impact on cellular response to therapeutic targets, which may account for the varying reactions of individual patients to a certain drug or combination of medications(Zuben E Sauna et al., 2007). It is unknown what direct consequences the silent mutations have on disease but, according to current theorysSNPs work in concert with other driver mutations to determine the course and etiology of the disease (James C Chen et al., 2014). This group includes a number of SNPs that impact complex illnesses like cancer, asthma, hematological disorders, and neuropsychiatric disorders. Table 2 provides a list of sSNPs linked to human diseases.

Among *5HT2A* SNPs, rs6311 (−1438 A/G) and rs6313 (−102 T/C) have received major attention in research. Sanders-Bush et al. (2003) report both SNPs are in perfect linkage disequilibrium (LD). Perhaps by using a different transcription start point that yields a longer 5' UTR, the (−1438 A/G) is translated in minor isoforms of *5HT2A* mRNA produced in the brain. Reduced expression of isoforms containing this 5' UTR extension is linked to the variation "A" allele. The expression of receptor mRNA and protein has also been linked to this SNP. The polymorphism is associated with

behavioral phenotypes such as human aggression (Marina L Butovskaya et al., 2015), tardive dyskinesia (C Ronpirin et al., 2007), and smoking behavior (Shin Narita 2013). Additionally, hand full of investigations suggests that rs6311 has an allele-specific impact on gene expression (Virginia R Falkenberg et al., 2011; Zoraida Verde et al., 2019).

The *5HT2A*-102 T/C polymorphism could be caused by either thymine (T) or cytosine (C) at nucleotide position 102. Since both alleles encode for a serine in codon 34, this mutation does not alter the amino acid sequence (J T Warren Jr et al., 1993). The 102 T/C polymorphism is thought to alter the transcript's secondary structure, which could therefore have an impact on the transcript's stability or translational activity, given that it is situated in exon 1 (Williams et al., 1996), close to the gene promoter (Kouzmenko et al., 1997).

Furthermore, compared to mRNA transcribed from the 102C allele, it has been proposed that mRNA transcribed from the 102T allele may display a distinct secondary structure (Arranz et al. 1995). Lower expression of post-synaptic serotonin receptors is linked to the C/C genotype. Furthermore, the SNP has the ability to influence promoter methylation and transcription factor binding (Manik Vohraet al., 2020). Numerous neuropsychiatric disorders, including the effectiveness of antipsychotic medications Wang (Yulong 2023), SCZ (Levinson DF 2005), BPAD (Tan J, 2014), Cocaine misuse (AyshehAlrfooh and Ryan M Smith.2022), and Suicidal conduct (Ali Bani-Fatemi et al., 2017), have been linked to the polymorphism.

In conclusion, the distinctive characteristics of the SNP, such as its proximity to the transcription hub and promoter, its impact on differential gene expression via imprinting and epigenetics, and its correlation with a variety of neuropsychiatric phenotypes, call for in-depth chromatin and mRNA level investigations.

3.Mold analysis of SNP variants to assess plausible roles in mRNA folding.

In metazoans, high-fidelity transcription and translation are supported by a close relationship between the structures of mRNA and the proteins it encodes(Guilhem Faure et al., 2017). Protein folding is generally regulated by highly structured mRNAs, according to a comparison of mRNA and protein structures. There appears to be 'fine tuning' of mRNA structure under selection for efficient protein folding, as this function is presumably carried out by separate structural components in the mRNA. In fact, mRNA reveals a second layer of structural information that is dictated by the sequence itself because of its inherent tendency to fold swiftly and dynamically into higher order structures. These properties confer fluidity in changing environmental conditions by efficiently tuning translation in response to diverse signals, such as ligands (drugs), regulatory proteins, or small RNAs (David Z Bushhouse et al., 2022). Several features of the mRNA can cause variations in mRNA

translation speed, including codon choice, adjacent codons or codon, context, the secondary structure of mRNAs, and repetitive sequences (e.g., repetitive stretches, or specific sequences of the nascent peptide(Junhong Choi 2019).SNPs can cause different structural folds of mRNA and alter the RNA structural ensemble(LX Shen et al., 1999). Different secondary structure conformations energetically are more or less favorable, this change impacts the affinity of RNA for an RNA binding protein or a microRNA, and evidences suggest that these effects could be under selective pressure in the human transcriptome and associated with diseases(Halvorsen M et al., 2010).

The present study we explored plausible of the role of *5HT2A* gene SNP variation on cis-chromatin, allelic and epigenetic marks, further their role in mRNA folding was assessed using various bioinformatics methods.

4.Materials and Methods

The sequence flanking the SNP was obtained from dbSNP (www.ncbi.nlm.nih.gov).
a.Regulome analysis of SNPs variants was carried out using server regulomedb.org.
Since the observation that several variants are non-coding variants, their functional significance become problematic. The Regulome analysis can provide additional information on the DNA structure of the variant and chromatin states such as chromatin state,ChIP data, Motif and accessibility.
b.ChIP signaling was carried out using server chip-atlas.org
Signal transduction pathways describe functional interdependencies between distinct classes of molecules that collectively determine the response of a given cell to signal molecules.
c.Mfold analysis was carried out using server www.unafold.org.

5.Results

The *5HT2A* gene organization and loci of SNPs rs6311 and rs6313 are depicted in Figure-1.Regulome analysis of the SNPs rs6311 and rs6313 are depicted in (Figures2-a and b)respectively. As evident from Figure-2a, analysis of SNP rs6311 revealed a CTCF binding site, whereas, the ChIP–seqwas suggestive of binding site for a transcription factor motif (GTGAGTGTCC).High expression was seen in brain and chromatin state analysis of SNP revealed higher effect on the promoter and enhancer sequences(Associated with TSS,enhancers(weak and low). Finally, ATAC-seqrevealed a high region of open chromatin, accessibility in motor neuron(spinal cord, and brain).The analysis of SNP rs6313 revealed binding sites for proteins EZH2, CTCF,CREB1,and CEBPA respectively.ChIP–seq data indicated high expression in brain and chromatin state analysis revealed effect on the TSS. Finally,the ATAC-seq revealed a high region of open chromatin, accessibility in

spinal cord.ChIPatlas signaling is depicted in Figure-3.Cistromic signaling pathways enable identification of a network of TF at the nuclear level. The data suggested multiple TF(± 10 kb TSS) sites for proteins such as CTCF, TCF12, FOSL2, JUND, REST, PBX3, and GATA3 respectively.

Mfold analysis of Synonymous SNPs are depicted in (Figures-4a,b and Figures-5a,b).The analysis of rs6311 was not significant.However, the rs6313 analysis suggested a plausible different RNA secondary structure with free energy changes –48.13cal/mol and 49.70- kcal/mol for the T102 and C102 allele respectively.

6.Discussion

Cis promoter single nucleotide polymorphism (SNP) impact genetic, epigenetic and regulatory (chromatin) structure and they also constitute major determinants of differential gene expression via the loss or gain of transcription factor binding or DNA methylation sites(Catherine Do 2017). In the present study plausible roles of *5HT2A* gene SNP rs6311 (−1438 A/G) and rs6313 (−102 T/C) variation in altering these marks,alsoon mRNA folding was assessed using various bioinformatics methods.Higher-order chromatin structure is important for the regulation of genes through distal regulatory sequences (Ivana Jerković et al., 2020). sSNPs that alter three-dimensional (3D) genome organization can lead to enhancer-promoter rewiring and result in human diseases, particularly in several human neurodevelopmental neuropsychiatric diseases. It is well known that chromatin connections could be disrupted due to different allele, promoting transcriptional dysregulation and mis-expression of genes.

Regulome analysis of SNPs indicates they belong to the category of SNPs likely to affect binding and linked to expression of a gene target. Their potential roles in transcription either involving the TSS or the enhancer sequences is further augmented through regulomescores (rank 1d,score- 0.93857 and 1f,score- 0.55436) for rs6311 and rs6313 respectively.

Analysis of rs6311 revealed a CTCF binding site suggesting thatthe polymorphism disrupts the transcriptional circuit in the region by disrupting the promoter-enhancer connections or perturbation of Pol II to initiate transcription. The CCCTC-binding factor (CTCF), are the 11 tandem ZF (Zinc finger protein loop-forming factors in vertebrates. CTCF is considered a crucial, pleiotropic genome organizer that establishes a connection between intricate biological processes and higher-order chromatin structure (Jennifer E. Phillips and Victor G. Corces, 2009;Victor V. Lobanenkov and Gabriel E. Zentner, 2018). The ChIP–seq data was suggestive of binding site for a transcription factor motif (GTGAGTGTCC) for the Myb/SANT DNA Binding Domain Containing 3 protein. Also, high expression was seen in brain and chromatin state assay demonstrated that the polymorphism affects the TSS strongly

and enhancers in the regions (both weak and low) Finally, ATAC-seq revealed a high region of open chromatin, accessibility in motor neuron (spinal cord, and brain) suggesting that the polymorphism hasa probable role in chromatin dynamics and architecture in this locus.Analysis of SNP rs6313 revealed binding sites for proteins EZH2, CTCF, CREB1, and CEBPA.The EZH2 protein catalytic subunit of the polycomb repressive complex 2 (PRC2) Polycomb-group (PcG) family proteins form the multimeric protein complexes, which are involved in maintaining the transcriptional repressive state of genes. CREB1transcription factor protein is a member of the leucine zipper family of DNA binding protein.The protein binds as a homodimer to the cAMP-responsive element, an octameric palindrome. The protein is phosphorylated by several protein kinases, and induces transcription of genes in response to hormonal stimulation. Finally, the CEBPA basic leucine zipper (bZIP) domain proteins recognize the CCAAT motif in the promoters of target genes. The encoded protein functions as homo and heterodimers. Activity of this protein can modulate the expression of genes involved in cell cycle regulation.This observation is suggestive of the plausible chromatin and genomic effects of the polymorphism in gene regulation given its location in the exon1 close to the promoter.ChIP–seq data indicated high expression in brain and analysis of chromatin state revealed the polymorphism has strong effect on the TSS. Finally, ATAC-seq revealed a high region of open chromatin, accessibility in spinal cord.

ChIP-Seq(cistromic) signaling mining identified 8 classes of TFs in the promoter regions (starting from 180-1800 bp)from the TSS which has roles in gene expression. Among these several binding sites for the CTCF binding were distributed across the promoter.Further, 5 classes of transcription binding factor binding were observed, they are, a.Transcription factor basic helix-loop-helix (bHLH) factor-TCF12, b. Transcription factor Basic leucine zipper (BZIP)- Fos and JunD, c. Transcription factor (Homeodomain protein)- PBX3, d.Transcription factor (Zinc factor) GATA3 and e.unclassified class of TF- REST.Transfacannotation of these transcription factors (https://genexplain.com/transfac/) is described briefly. The TCF12 protein is a member of the basic helix-loop-helix (bHLH) E-protein family that recognizes the consensus binding site (E-box). The protein is expressed in many tissues- skeletal muscle, thymus, B- and T-cells, and participates in regulating lineage-specific gene expression. One binding sites for this TF was observed. The Fos and Jun are oncoproteins, function as transcriptional activators stimulate transcription through direct and/or in direct interactions with members of the basal transcriptional machinery. Two binding sites for JunD were observed.PBX3 (PBX Homeobox 3) is a DNA-binding transcription factor with RNA polymerase II-specific activity the protein is involved in animal organ development including the nervous system.A single binding sites for this TF was observed. The GATA3 transcription factor are transcription factors containing two GATA-type zinc fingers that drives the

differentiation of T helper (Th) 2 cells. One binding sites for this TF was observed. REST are RE1-silencing transcription factor also known as neuron-restrictive silencer factor a zinc-finger transcription factor function as nuclear negative regulator of differentiation.Only one binding sites for this TF was observed. The binding sites for the other TF were within the (180-420 bp) of TSS.

Several methods including *in silico*enable in predictionof the ensemble of possible structures that a given mRNA strand can adopt such as Mfold(Michael Zuker 2003). Essentially, the core algorithm predicts a minimum free energy (d G), as well as minimum free energies for folding that must contain any particular base pair. The single stranded mRNA molecule is capable of forming complex configurations largely by base-pairing with itself, yielding the ‘‘secondary structure’’, which further folds through covalent attractions to form the ‘‘tertiary structure’’(Silverman SK.2008). The unique hairpin secondary structure is a typical feature of mRNA.sSNP in the promoter and flanking the TSS can affect mRNAs secondary structures and their functions by reducing or enhancing the expression levels of mature mRNAs(Ambuj Kumar 2014).Mfold analysis rs6311 was not significant suggesting the SNP may not impact the secondary structure features of mRNA. However, the rs6313 analysis suggested a plausible different RNA secondary structure with free energy changes – 48.13kcal/mol and 49.70- kcal/mol for the T102 and C102 allele respectively.As evident the figure- 5a,b, the SNP variant changed the multiple branch loop (indicated in flower bracket) changing the conformation and thermodynamic free energy. This change could impact the half-life of mRNA and also perturb several process associated with transcription and translation. Computations of secondary structures in 5'-UTRs and their folding free energies of mRNA genes in *S. cerevisiae* suggest correlations between folding free energies and various transcript features (Ringnér M and Krogh M. 2005). The study also revealed that 5'-UTRs have significantly higher folding free energies than other genomic regions and randomized sequences. Correlation between transcript half-life and ribosome occupancy supports a picture of competition between translation, degradation and turnover on a genomic scale. Several studies support evidence for widespread bias for 5'-UTRs to be weakly folded (Adam Master et al., 2016).

7.Conclusion

Following the sequencing of human genome, a large number of sSNPs have been identified that don’t affect disease phenotypes, and their exact roles remain unclear.Human regulatory DNA encompasses a variety of *cis*-regulatory elements within which the cooperative binding of transcription factors creates focal alterations in chromatin structure. Prevailing thought in the field is that the disease-associated variants systematically perturb transcription factor recognition sequences, frequently alter allelic chromatin states, and impact regulatory networks. Therefore,

analysis of the plausible roles of the sSNP on cis-regulation, chromatin architecture and genome transcription could shed light in this direction.

The production, maturation and export of mRNA from the nucleus precede the translation into the gene products (proteins) but this process can also be regulated by a variety of transcriptional and translational control mechanisms. At the transcriptional level the region around TSS, 5'UTR open reading frame (ORF) internal ribosome entry site (IRES), response elements (RE) referred to as downstream have important functions in fine tuning protein expression and specific roles in response to cellular requirement (Andersson R et al., 2015). These regulatory sequences recruit transcription factors (TFs) in a DNA sequence-dependent fashion, allowing cells to precisely control the rates of chromatin de-compaction, transcription initiation, and the release of RNA polymerase II (RNAPII) into productive protein elongation phase(Matthew S Sachs, Adam P Geballe 2006). The region could also involve enhancers which recruit co-activator and enable transcription in the transcriptional circuit.They also interact with the broad epigenetic domain marks and can impact epigenetic inheritance. Genetic variations in the 5′-UTR are associated recognized tobe associated with susceptibility to diseases(Sangeeta Chatterjee and Jayanta K Pal 2009).The SNP rs6313 in the exon1 region close to TSS could affect several of afore regulatory mechanisms through disruptionsto the transcription toolkit in an allele specificpattern, it could also disrupt the enhancer functioning. Evidence for the longer 5'UTR was suggested by (Ryan M. Smith et al., 2012, Ryan M. Smith et al.,2014).

Analyis of rs6311 revealed a CTCF and binding site for a transcription factor motif (GTGAGTGTCC) for the Myb/SANT DNA Binding Domain Containing 3 protein. Since the SNP is in upstream it can disrupt the enhancer-promoter CTCF insulator loops. Myb proto-oncogene protein is a member of the MYB (myeloblastosis) family of transcription factors containing three domains, with different functions(N-terminal DNA-binding domain, a central transcriptional activation and C-terminal transcriptional repression). SANT domain is a protein domain that allows many chromatin remodeling proteins to interact with histones. They have roles in recombination, genome maintenance (telomere specific) and developmental roles in animal and plants (Asmara M Baker et al., 2009; Butut Zhu, et al., 2019). Recurrent rearrangements of My/SANT DNA gene are reported in salivary cancers(Nicholas Barasch et al., 2017).From the results of SNP rs6313 analysis it could be inferred that the SNP has roles in genome regulation given its close proximity to promoter since several TFs involved in gene regulation were observed- EZH2 (transcriptional repressor),CTCF(transcriptional activator, a repressor or an insulator protein) CEBPA(cell cycle regulation).The CREB1 sites validates its promoter roles since its RE(hormonal).The results of ChIP atlas analysis it could be inferred that the SNP has roles in genome integrity and regulation since several TFs belonging to this class

were found. Specifically, twonervous system specific TF the TCF12 protein a member of the basic helix-loop-helix (bHLH) containing the E-box and the PBX3 (PBX Home box 3) DNA-binding transcription factor containing PBX domain were found (Nour A Aljouda 2024; E Longobardi et al., 2014). Further, GATA3 transcription factor with conserved zinc finger (Ziff) domain enable development and differentiation of hematopoietic cell lineages. These classes of TF with specific conserved DNA response elements enable developmental gene expression in in neurons, muscles, and other tissues(Yisong Y. Wan 2024).The c-fos and c-junoncoproteins have regulation or synthesis of proteins roles linked to tumorigenic cell growth. These protein form preferentially heterodimer that binds to DNA and modulates transcription of a wide variety of genes in response to mitogenic stimuli(Manios K, et al., 2020). The region also contains a neuron-restrictive silencer RE1-silencing transcription factor, these zinc-finger domain containing TF function as nuclear negative regulator of differentiation. REST-dependent genes encode important targets for transcription factors, transmitter release proteins, voltage-dependent and receptor channels, and signaling proteins. Additional roles include miRNAs and splicing(Pietro Baldelli and Jacopo Meldolesi 2015).

Eukaryotic mRNA molecules have a tripartite structure, with a 5' untranslated region (UTR) preceding the principal open reading frame (ORF) and followed by a 3' UTR. UTRs are critical to the regulation of gene expression, mRNA export from the nucleus and affect translational efficiency, subcellular localization and mRNA stability(Melissa J Moore 2005). Furthermore, mutations in UTRs can lead to serious pathology, demonstrating their importance in proper functions in the cell. A schematic diagram of mRNA, 5'-3' regions and their role in gene regulation is depicted in Figure-6 and diseases associated with 5' UTR variations in table-3. An important biophysical property of an RNA structure is its stability, contingent on several factors such as ion-mediated electrostatic interaction, conformational entropy, base pairing and stacking, and other non-canonical interactions. These factors determine to which extent an RNA molecule retains its structural integrity. For a secondary structure, different base stacks and loops are correlated to additive free energies (Yakovchuk P et al.,2006). As evident from the Mfold diagrams the SNP 6313 impacted the multiple branch loop (indicated in flower bracket) and creating a change in the conformation and thermodynamic free energy change. This change also changed the loop in the region(140,160,180 bp respectively).

The allele specific mfold analysis and SNP specific regulome indicate that the alleles have role in gene expression. It is well known that chromatin connections may be disrupted due to allelic change, promoting transcriptional dysregulation and mis-expression expression of genes. Allelic imbalance has been well documented in many systems- *Drosophila*, mouse, (León-Novelo L 2018; Saito A 2024) suggesting

their evolutionary conservation and roles in development and disease. The emerging field of epi-genomics tries to assess the correlation of epi-genotypes with differential gene expression and ultimately phenotypic variation. Parent-of-origin effect (genomic imprinting) and methylation studies of the two polymorphisms have resulted in contradictory reports (De Luca, Vincenzo 2007;De Luca, Vincenzo 2007). Cytosine of the T102C polymorphic site was significantly hypo-methylated in SCZ, BD, and their first degree relatives compared to the controls and early age at onset (Ghadirivasfi, Mohammad 2011;Abdolmaleky, Hamid Mostafavi 2011). Perhaps this is suggestive of allelic specific effects on epigenetic and imprinting. Probably, these variables were not included these studies. Metastable epialleles(ME) and are sources of inter-individual variation suggesting that stochastic DNA methylation is established before germ layer differentiation(Maria Derakhshan et al., 2024). ME methylation has been associated with outcomes relating to several complex diseases such as cancer, glucose metabolism, and thyroid function and neuropsychiatric illness in later life. Hence, MEs are an interface through which environmental effects in early development can influence disease risk in later life via epigenetic mechanisms(Pui-Pik Law and Michelle Holland 2019). Since, neuropsychiatric illness are now proposed to have a significant environmental component, this supports the prevailing thought of dysregulation of methylation in these disorders.

Thus, the combinatorial toolkit of cis-regulation, chromatin architecture and associated allelic and methylation differences due to sSNP in the upstream and downstream of TSS could plausibly regulate the gene at the spatio-temporal, also at cell and conditional level. Diverse sequence features of 5' UTRs impact mRNA translatability, and also the ribosome-dependent and ribosome-independent mRNA-surveillance pathways(Jia, Longfei 2020). Regulation of Ataxin-1 in spinocerebellar ataxia type 1 (SCA1) supports this observation in brain(Manek, Rachna 2020). The study needs *in vivo* validation in suitable cell-lines. Also, we have not tested the roles of SNP on ORF, IRES or REs. Further,variables such as salts, base-pair secondary features are also known to impact mRNA folding. Future studies focused on these lines will enable a better understanding of the SNPs in gene expression of *5HT2A*.

In summary the study implicates plausible role of *5-HT2A* gene SNP variations (rs6311 and rs6313) on genome architecture in the promoter flanking the TSS such as chromatin marks and mRNA secondary structure and folding. Since, sSNPs form appreciable portion of heritable phenotypic variations in neuro-psychiatric human diseases such bioinformatics methods can help assign and delineate their plausible roles.

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Author contribution
Design, literature collation analysis and drafting Kiran Kumar H.B, design, literature collation, drafting Sajeda Niketh, literature review, collation of data, generation of tables Souwmyashree B.S., literature and data collation, drafting, Rajiv Ramachandra Kolgi, literature review, proofing drafting Kiran Kumar D.J.

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Table-1.Promoter SNPs in neurotransmitters coding genes associated with neuropsychiatric diseases.

No	Gene	SNP/Variant	Description of variant	Associated conditions	References
1.	*5HT2C*	rs3813929	Promoter	1. Association with Attention Deficit Hyperactivity Disorder. 2. Risperidone-Induced Adverse Drug Reactions in Schizophrenia. 3. Pharmacogenetics in bipolar disorder. 4. Multivariate permutation analysis associates multiple polymorphisms with sub phenotypes of major depression.	Hou YW et al.,2018 Alladi CG et al.,2017 Salloum NC et al.,2014 Hahn MK et al.,2008
2.	*5HT2C*	rs518147	Promoter	1. Treatment-resistant schizophrenia: current insights on the pharmacogenomics of antipsychotics. 2.Metabolic Syndrome in Patients with Schizophrenia Taking Clozapine. 3. attention deficit hyperactivity disorder in UK samples. 4. sub phenotypes of major depression.	Lally J et al.,2016 Kang SH et al.,2011 Mulder H et al.,2009 Xu X et al.,2009

3.	*5HT1A*	rs6295	Promoter	1. Parameters of Frontal Lobe Dysfunction in Schizophrenia. 2. Social anxiety, and risk-taking behavior. 3. Association between genetic polymorphism and antidepressants in major depression: a network meta-analysis. 4. Depression.	Morozova A et al. Stamatis CA et al., 2020 Du D et al.,2020 Kautzky A et al.
4.	*DRD4*	*rs3758653*	Promoter	1. A pharmacogenetics of schizophrenia 2. Pharmacogenetics of attention-deficit/hyperactivity disorder: long-term effects. 3. Autism spectrum disorders. 4. Schizophrenia in Han Chinese.	Osmanova DZ et al.,2019 Gomez-Sanchez CI et al.,2017 McCracken JT et al.,2014 Zheng C et al.,2012
5.	*DRD4*	*rs1800955*	Promoter	1. Schizophrenia Spectrum Disorders. 2. Opioid Use Disorder (OUD) 3. Schizophrenia 4. Schizophrenia in a Korean population	Frydecka D et al.,2021 Abijo T et al.,2020 Lee KY et al.,2011 Kiyohara C et al.,2011

6.	*DRD2*	rs1799732	Promoter	1. Metabolic Syndrome in Patients with Schizophrenia. 2. Cannabis Dependency. 3. Schizophrenia. 4.Parkinson's Disease.	Paderina DZ et al.,2022 Chmielowiec J et al.,2022 Michalczyk A et al.,2020 Redenšek S et al.,2020
7.	*DRD2*	rs6276	promoter	1. Substance Use Disorder. 2. Deficit syndrome in schizophrenia. 3. Major Depression and Bipolar Disorder. 4. Alcohol addiction	Boroń A et al.,2022 Michalczyk A et al.,2020 Calabrò M et al.,2018 Lucht M et al.,2007
8.	*HTR3B*	-100_-102delAAG	Promoter	1.Treatment-resistant schizophrenia 2.Bipolar affective and schizophrenic patients	Xiaofei Ji 2008 Bernd Frank et al.,2004
9.	*GAD2*	rs2236418	Promoter	1.Methamphetamine dependence 2.Heroin addiction 3.Alcoholism	SirilukVeerasakul et al.,2017 Yuhui Shi et al.,2020 Jaakko Lappalainen et al.,2007

Table-2.sSNPs implicated in several human diseases

Sl.no	**variant**	**Gene affected**	**Associated disease**	**References**
1	(c.1584G>A)	*CFTR*	Cystic fibrosis (CF)	Giovana B. Bampi et al., 2020
2	c.261G>C	*ERα*	Cancer	SudipaSaha Roy and Ratna K Vadlamudi . 2012
3	c.135T>C	*SFTPD*	COPD	Marilyn G Foreman et al., 2011
4	-44G→A	*Cx40*	Atrial fibrillation	Robert C Wirka et al., 2011
5	rs12966547	*TCF4*	Schizophrenia	K V Wirgenes et al., 2012
6	rs2816881	*Zfp326*	Depression	Ying-Jay Liou et al., 2012
7	rs356168	*SNCA*	Parkinson's disease	Omolara-Chinue Glenn et al., 2017

Table - 3. List of spectrum of human diseases caused to variations in the 5'UTR.

Sl.no	**Disease**	**Gene**	**References**
1	Ovarian cancer	Chemokine ligand 1 (*CXCL1*)	Guo M et al., 2020
2	Cardiovascular	Protein S (*PROS1)*	Soukarieh O, et al.,2022
3	Retinal disease	Retinol Dehydrogenase 12(*RDH12)*	Alfredo Dueñas Rey et al., 2024
4	Charcot-Marie-tooth disease	Gap Junction Protein Beta 1 (*GJB1*)	MeiYi Li et al., 2023
5	Parkinson's disease	Dopamine transporter gene (*DAT1, SLC6A3*)	XhensinaTafani et al., 2020
6	Type 2 diabetes	Phosphatase And Tensin Homolog(*PTEN*)	Hajime Ishihara et al., 2003
7	Schizophrenia	X-linked zinc finger MYM-type containing 3 (*ZMYM3*)	F Alizadeh et al.,2018
8	Bipolar disorder	X-linked zinc finger MYM-type containing 3 (*ZMYM3*)	F Alizadeh et al., 2019

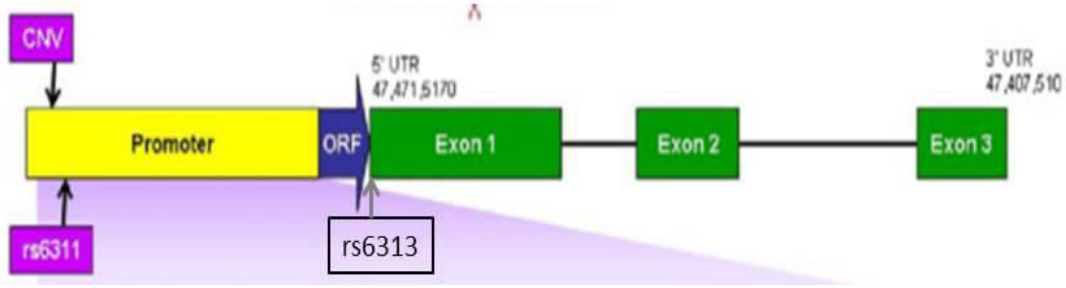

Figure-1.HTR2A gene depicting gene organisation and loci of SNPs rs6311 and rs6313 .

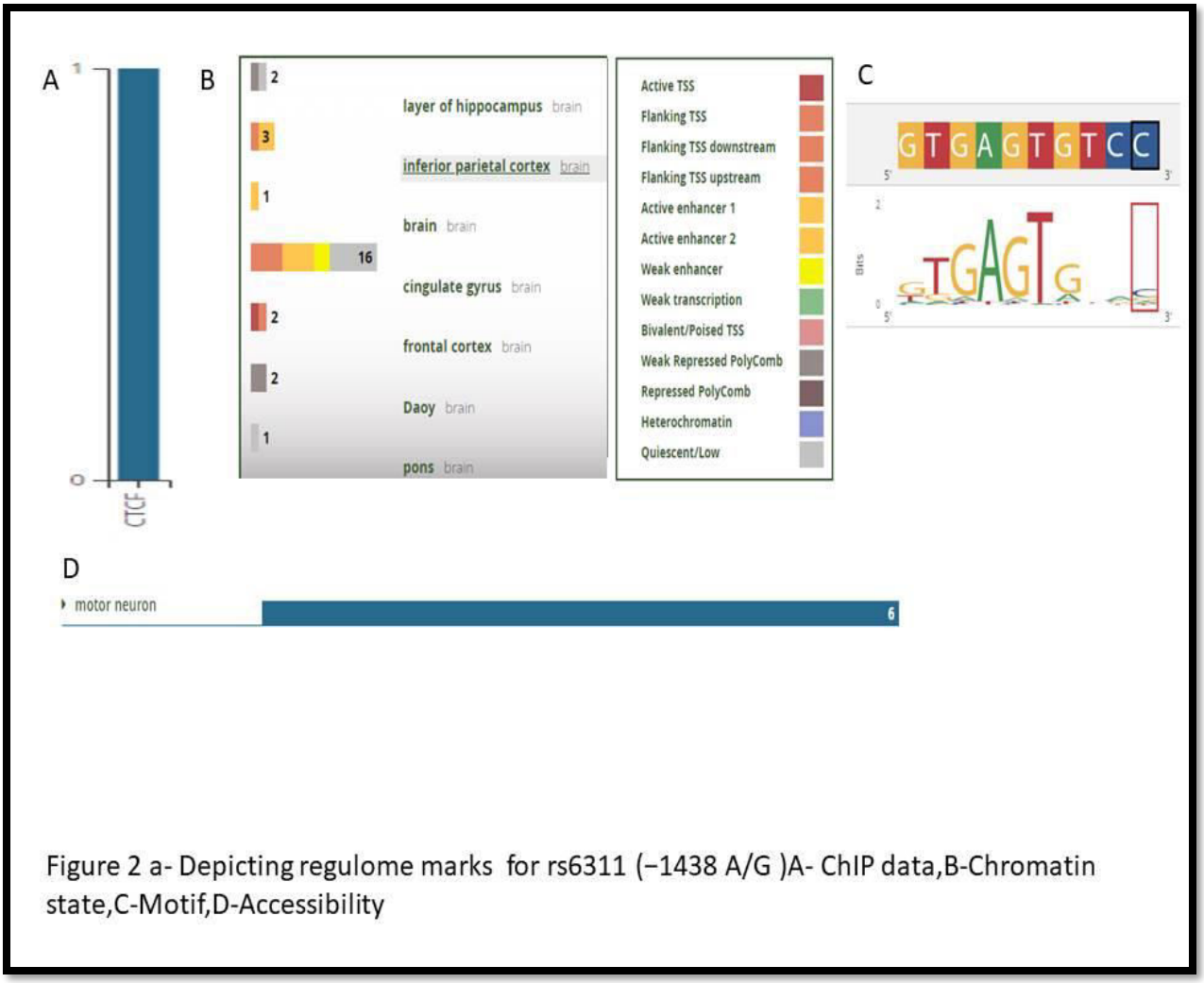

Figure 2 a- Depicting regulome marks for rs6311 (−1438 A/G)A- ChIP data,B-Chromatin state,C-Motif,D-Accessibility

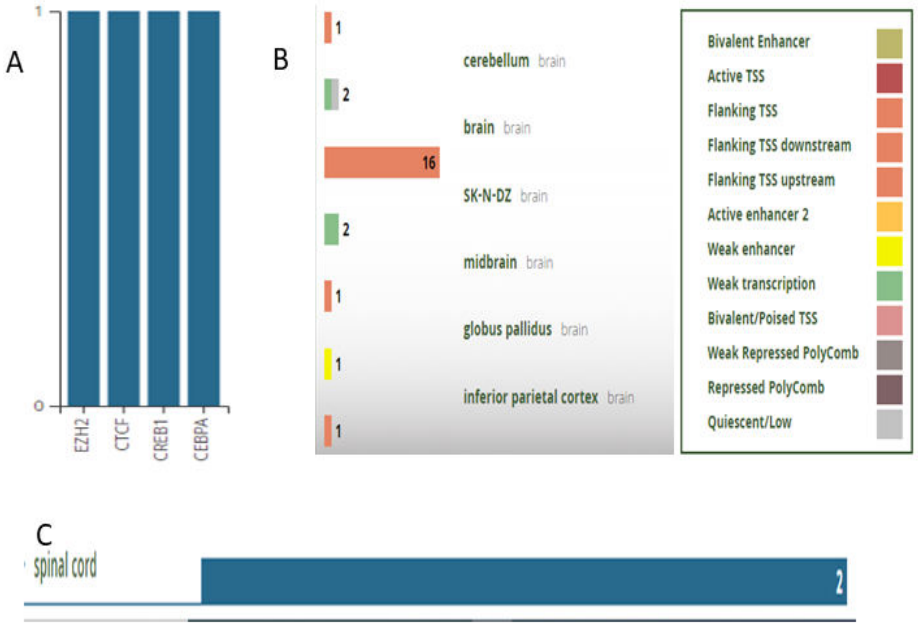

Figure 2 b Depicting regulome marks rs 6313(T102C)A- ChIP data,B-Chromatin state,C-Accessibility

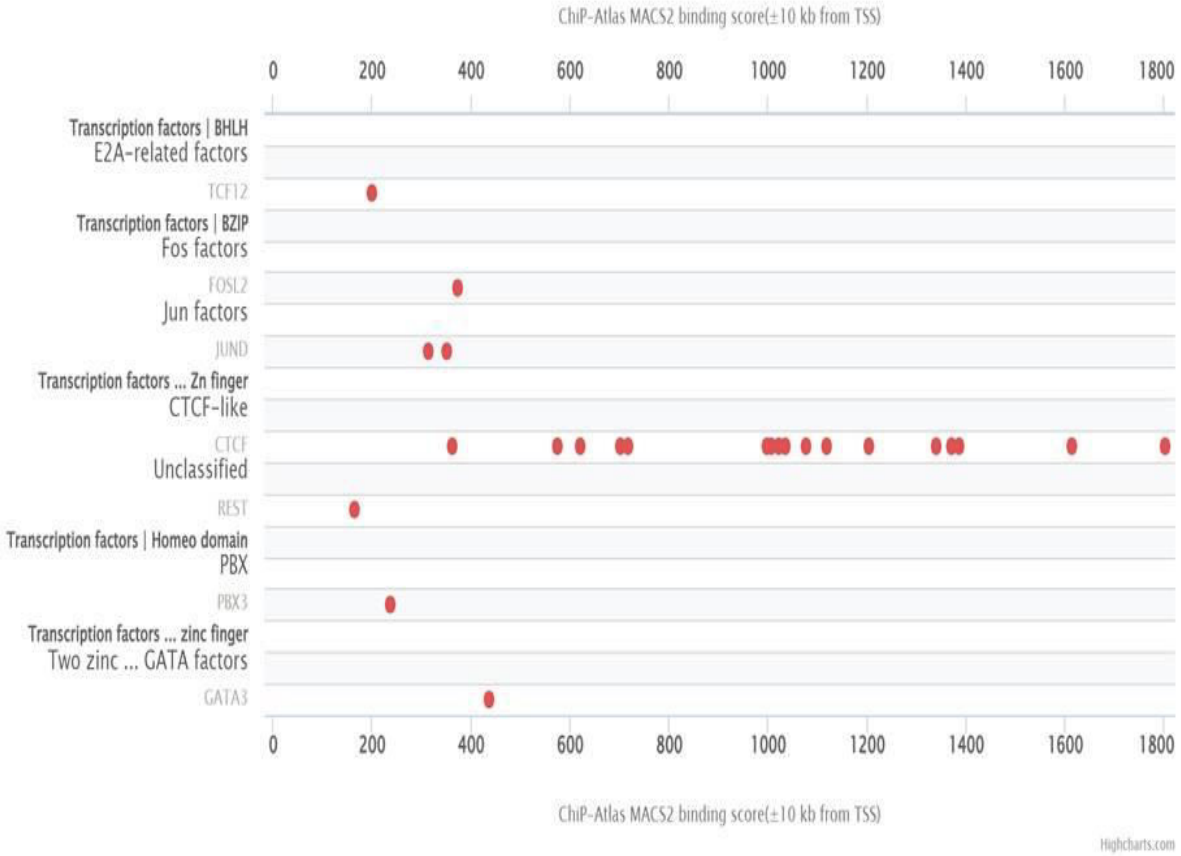

Figure 3- ChIP atlas Transcription factor signaling analysis.

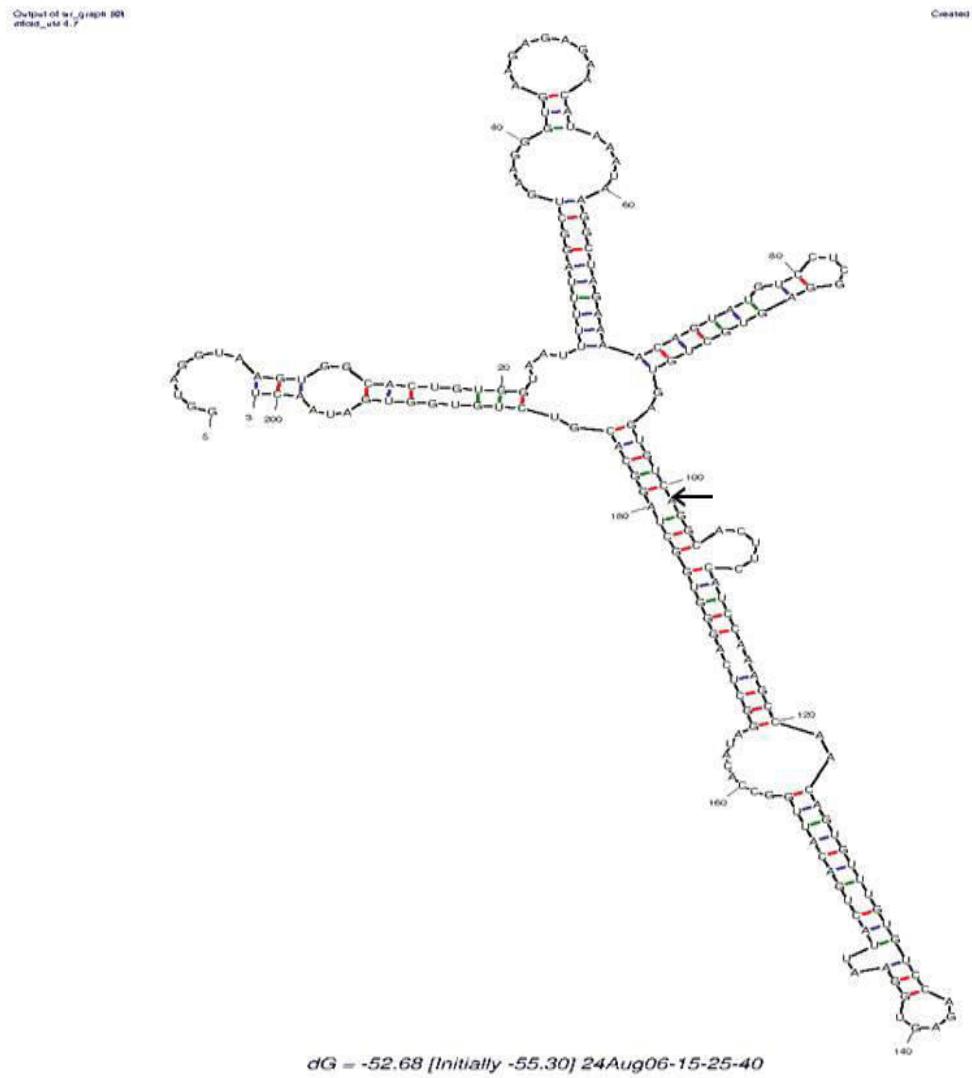

Figure 4a- Mfold result of rs6311(A).Arrow indicates site of variant.

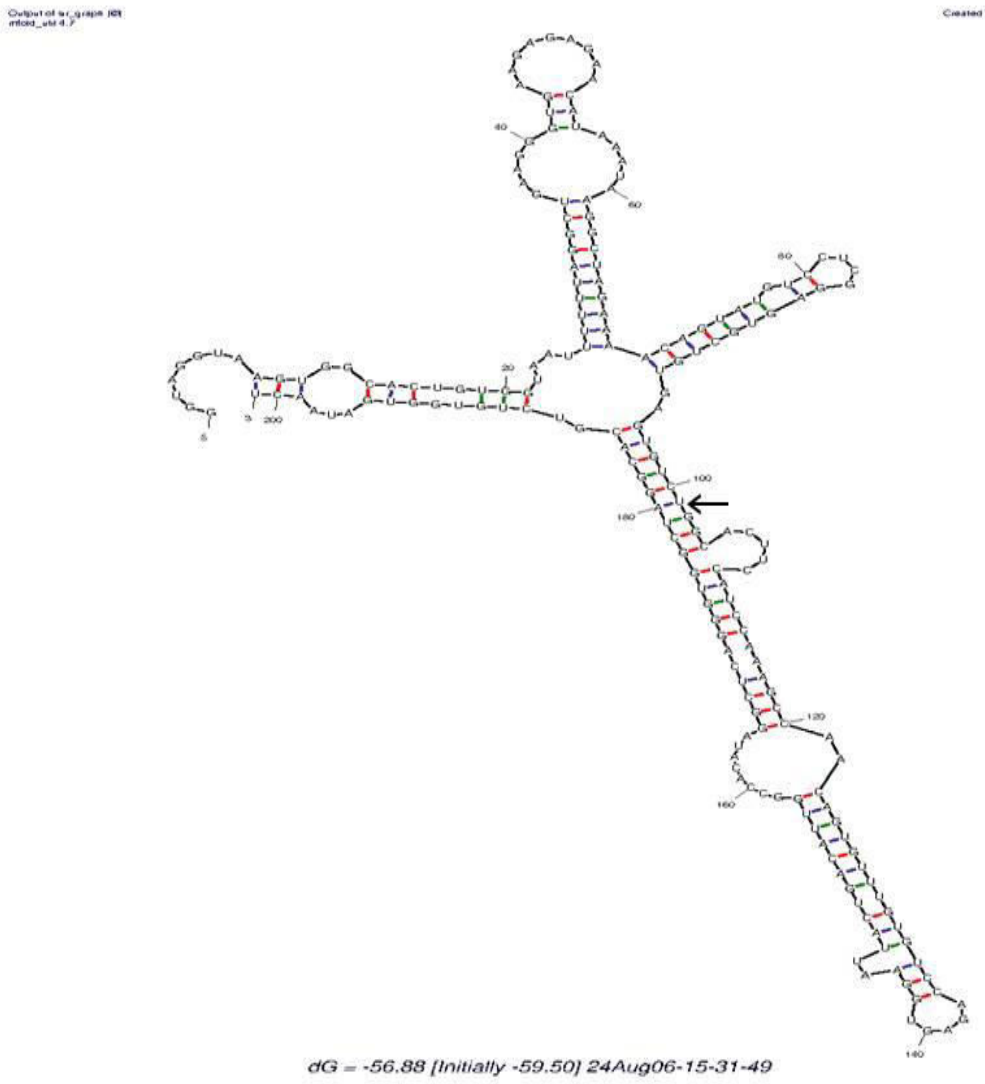

Figure 4b- Mfold result of rs6311(G). Arrow indicates site of variant.

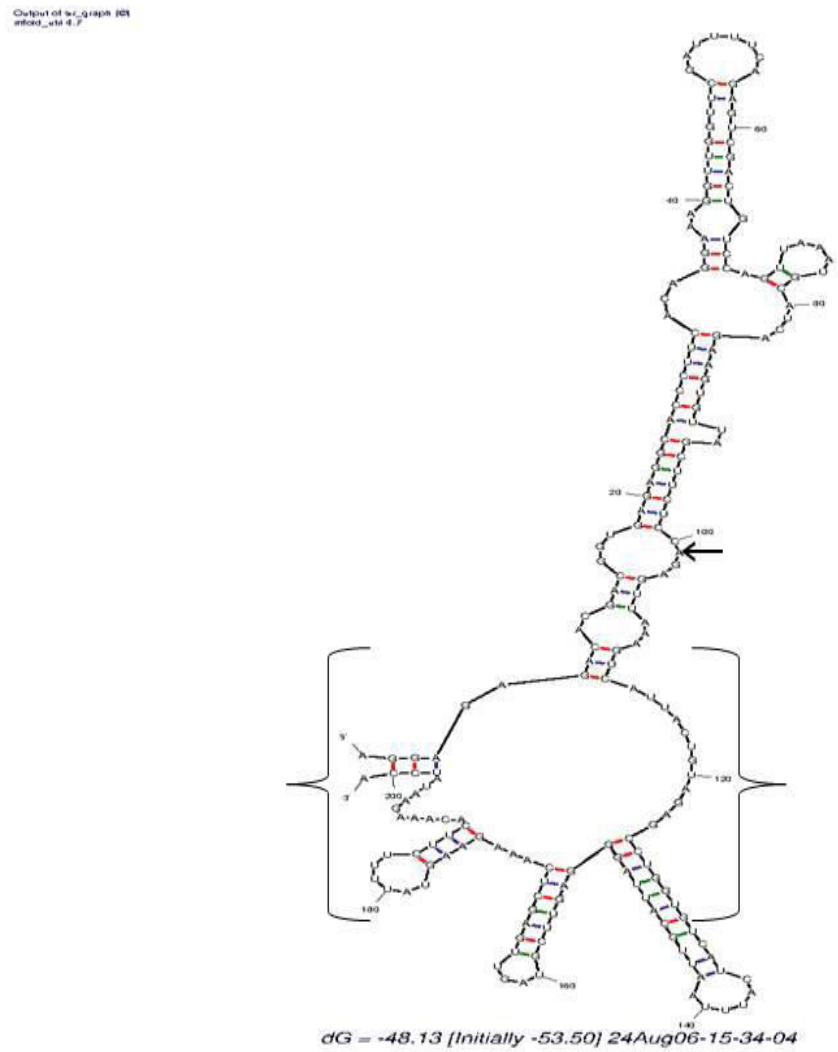

Figure 5a- Mfold result of rs6313(T). Arrow indicates site of variant

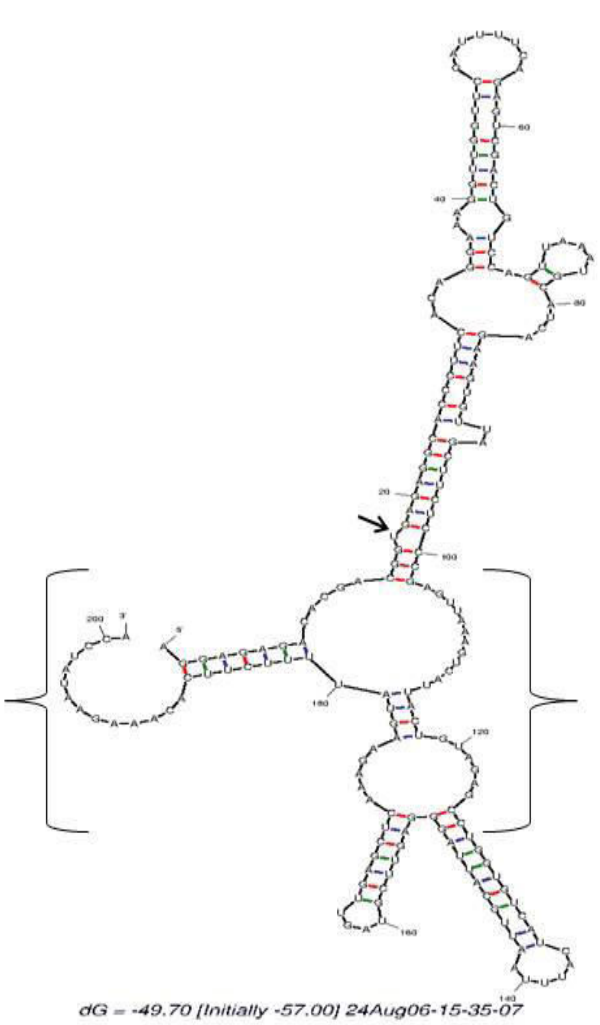

Figure 5b- Mfold result of rs6313(C). Arrow indicates site of variant

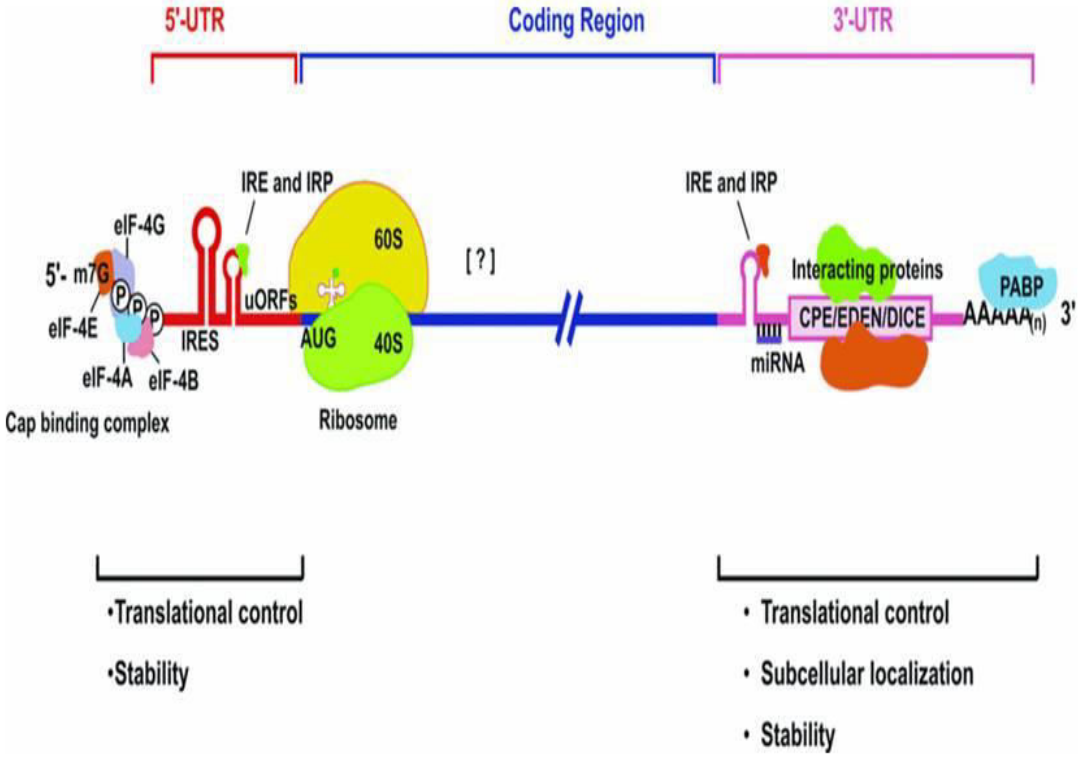

Figure – 6. Structural organization of eukaryotic mRNA and roles of 5′- and 3′-untranslated regions in gene regulation. Adapted from Sangeeta Chatterjee, Jayanta K Pal .Biol Cell. 2009 May;101(5):251-62.