Dosage effects of candidate genes in the 22q11.2 deletion syndrome ~~a mini review~~ and promoter analysis of *PRODH* gene

Abstract:

22q11.2 deletion syndrome (22q11.2DS), also known as Velo-Cardio-Facial Syndrome (VCFS) or DiGeorge Syndrome, is a genetic disorder due to a micro deletion on chromosome 22-q11.2. The clinical phenotype, which is complex and variable, includes specific congenital defects of the cardiovascular system, craniofacial and immune system. Further, dosage sensitivity of candidate genes in the region disrupts neurotransmitter signaling and is associated with neuro-behavioral symptoms. Unique repetitive elements DNA in the region are a source of copy number human genetic variation and contribute risk for complex disease like full name (SCZ). In this ~~mini review~~ chapter we review the topic covering anatomical, genetic and genomics perspectives. We conduct repetitive elements analysis of the regions flanking candidate genes. Additional, analysis include network analysis of the *PRODH* gene to infer interaction proteins and promoter analysis to gain insights in to regulatory and chromatin elements. The results indicate unique repetitive map flanking the genes which could serve as hotspots for chromosomal rearrangement. The network analysis indicates a hub of 11 genes with a full name (PPI) value-($4.59e^{-06}$). Unique promoter maps include a CpG island, insulator, gene enhancer and regulatory element (cluster). Chromatin analysis revealed deoxyribonuclease I (DNASE1) (DNase I hypersensitive) and Formaldehyde Assisted Isolation of Regulatory Elements (FAIRE) (~~Formaldehyde Assisted Isolation of Regulatory Elements~~) regions along with repetitive elements. Cumulatively, the results support the hypothesis of dosage effects of gene/s in the 22q11 region and associated syndrome. The results shed light on the complex phenotype observed at the locus which could be a result either from the overlapping regulation of several genes within this region or through a combinatorial participation in a regulated process such as ~~neutrotrasmission~~ neurotransmission or signal transduction.

Keywords- Velo-Cardio-Facial Syndrome (VCFS), 22q deletion syndrome, Schizophrenia (SCZ), Copy number variations (CNV).

1.Introduction:

The hemizygous microdeletion at 22q11 (3Mb) is the cause of Velo-Cardio-Facial Syndrome (VCFS) (Cirillo A et al.,2022). Variable phenotypic expression is linked to the 22q11.2 deletion disease which has an incidence of 1:4000 (Goodship et al., 1998). The condition affects males and females equally (Botto et al., 2003) with greater rate in Hispanics than other ethnic groups (~1:3800) (Kobrynski & Sullivan 2007). VCFS patients have an abnormally high prevalence of psychiatric diseases like psychosis and mood disorders, especially schizophrenia (SCZ) and bipolar disorder. In addition other neuropsychiatric conditions like attention deficit hyperactivity syndrome (ADHD), Autism spectrum disorder (ASD), and anxiety disorders are also reported. The International Consortium on Brain and Behavior, which examined the lifetime psychiatric diagnoses of 1,402 people of various ages supports this finding (Schneider et al., 2014). Parkinson's disease (PD) with early onset is another neurological sign observed (Booij et al., 2010). From an etiologic perspective, the deletion occurs as a *de novo* mutation in vast majority of the patients (~90-95%) (McDonald-McGinn et al., 2001)7, while it is inherited in the remaining patients (5-10%) (Scambler 2000). VCFS and the 22q11 deletion represent the highest known risk factor for SCZ aside from having either parents or a monozygotic twin with the disease (Drew et al., 2010).

Genetic architecture and physical position of genes:

The loss region covers nucleotides 18,658,219 -21,865,185bp (hg19) on chromosome 22, with four major locus control region (LCR)-LCR22 A, B, C, and D (Shaikh et al., 2000). A pictorial representation of the region is depicted in (Figure-1). About 90 genes are covered by the 3 Mb loss, and about 55 genes are covered by the smaller proximal ~1.5 Mb deletion (Guna et al., 2015). The majority of the 90 genes are expressed in the brain (n = 41, 89.1%), and more than half of them code for proteins (n = 46, 51.1%). Several genes are expressed in the developing and adult central nervous system and potentially contribute to VCFS neuropsychiatric phenotypes (Maynard et al., 2003-; Arinami, 2006). Several genes in the region have been studied as candidate genes for SCZ. The region contains 27 pseudogenes and one read through transcript, which is categorized as a non-coding RNA. Additionally, the area has seven microRNA (miRNA) genes and nine non-coding RNA genes. The 22q11.2 region spans over 40 protein genes, a number of

which are expressed in mouse and human brains (~~Daniel~~ Meechan DW et al. 2009) and have roles in brain development, neurotransmitter levels, and myelination. (Jungerius et al., 2008; Prasad et al., 2008).

3.Major brain expressed genes in the region and gene expression studies:

Of the several brain expressed genes in the region the Catechol-O-methyl transferase (*COMT*) gene encodes an enzyme with degrades catecholamines including dopamine in the synapse. Two main isoforms that display an altered structure, affinity, and capacity for their substrate are the shorter cytoplasmic soluble form (S-COMT), which accounts for ~95% of total enzymatic activity (Tunbridge et al., 2006). The other form is a longer membrane-bound form (MB-COMT) that is more prevalent in brain tissue and responsible for dopamine inactivation (Chen et al., 2011). The nonsynonymous functional polymorphism (G allele substituted by an A - rs4680) results in an amino acid substitution (valine>methionine) at codon 108 in S-COMT and 158 in MB-COMT transcripts (Lachman et al., 1996). Both isoforms' activities change as a result of the polymorphism, with the Val COMT form (G variation) with a greater activity than the Met form (A variant). In prefrontal cortex (PFC) tissues, the Met polymorphism reduces enzymatic activity by 40% (Chen et al., 2004). In contrast to the dopamine transporter and subsequent monoamine oxidase metabolism for neuronal synaptic uptake, COMT has a limited function in dopamine clearance, although it is expressed in all areas of the brain (Gogos et al., 1998). However, due to dopamine transporter expression is low in the PFC, the impact of active COMT is more significant. Accordingly, individuals with a 22q11.2 deletion who carry the less active Met allele are expected to have higher dopamine levels in the PFC brain region, which could lead to an increased risk for developing psychosis (Egan et al. 2001). Another gene the *PRODH* codes for proline dehydrogenase, a mitochondrial membrane enzyme that catalyzes the first step in the proline degradation pathway (Bender et al., 2005). Proline is a nonessential amino acid, which has roles as modulator/precursor of neuronal glutaminergic activity (Yao and Han 2022). The protein is mostly located in the cortex and hippocampus of the mouse brain and is expressed in the adult human brain (brain atlas). According to Gogos et al. (2006), homozygous *PRODH* mutation in mice results in significantly lower prepulse inhibition (PPI) and higher proline levels. Several research studies implicate equivocal correlation between SCZ and *PRODH* variations. Lack of association in Europe and Japanese populations (Williams et al., 2003; Glaser et al., 2006) and positive association in Chinese population (Ma X 2007). The neuronal voltage-activated L-type

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calcium channel's γ subunit, the *CACNG2* gene maintains the calcium channel while it is inactive (Morimoto-Tomita et al., 2009). The mouse stargazin protein (Bedoukian et al., 2006) which is also connected to alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking is the ortholog. The AMPA receptor is responsible for synaptic plasticity and mediates rapid excitatory synaptic transmission in the brain (Yamasaki et al., 2011). According to protein interaction networks, the glutamatergic signaling process may be influenced by the recruitment of AMPA receptors mediated by CACNG2 (Hsu 2008).

The zinc finger and DHHC domain-containing protein 8 (*ZDHHC8*) gene is expressed in the adult human brain and is found predominantly in the cortex and hippocampus of the mouse brain (brain atlas). Protein palmitoylation a post-translational modification of proteins requires a transmembrane palmitoyltransferase which is carried by the protein. Several brain development and signaling pathways are dependent on palmitoylation with the lipid palmitate (Peng 2024). *ZDHHC8* has been proposed as a plausible candidate gene contributing to the behavioral phenotype of 22q11.2.2DS given its functional role and its association with PPI (Karayiorgou 2010). Studies have found equivocal associations with ZDHHC8 variants and SCZ .Lack of association in Japanese and European populations (Ujike et al., 2005; Saito S et al., 2005) and moderate association in German population (Faul et al., 2005). Gamma-glutamyl transpeptidase 2 gene is involved in the degradation of glutathione *(GGTP2*)(ncbi-gene). The encoded enzyme acts as part of a full name GSH pumping gamma-glutamyl cycle in the brain and may also be involved in gamma-glutamyl amino acid formation. The protein encoded by the gene hypermethylated in cancer 2 (HIC2) (genecards). The protein enables transcription repressor activity, RNA polymerase II-specific and RNA polymerase II cis-regulatory region sequence-specific DNA binding activity. It regulates of cytokine production and regulation of immune system process. The ubiquitin-conjugating enzyme E2L 3 (UBE2L3) gene encodes a protein member of the E2 ubiquitin-conjugating enzyme which is part of the cellular mechanism for targeting abnormal or short-lived proteins for degradation (ncbi-gene).

Several studies demonstrate co-gene expression, patterns and interaction between genes in the region. Paterlini et al., (2005) reported an upregulation of COMT mRNA in the frontal cortex of ZDHHC8 mutant mice. In addition, when tolcapone (a COMT inhibitor) was administered to the ZDHHC8 mutants, there was a greater disruption in PPI and working memory compared with the non-treated ZDHHC8 mutants, suggesting a gene–gene interaction between ZDHHC8 and COMT.

This is consistent with the proposal that 22q DS is a contiguous gene syndrome, in which deficiency in more than one gene contributes to the increased risk. Wilson et al. (2008) reported two patients with interstitial deletion of the 22q13 region with intact SHANK3 indicating haploinsufficiency for other 22q13 genes could also have major effects on cognitive and language development. The above studies are consistent with the hypothesis that the phenotypic expression in VCFS in terms of cognition and co-morbidity can be affected by each gene alone and by interaction between genes that affecting the pathway (e.g., dopaminergic, glutamatergic). Cumulatively, all of these findings point to a potential connection between genes at the 22q locus and their role in the neuropsychiatric phenomenology seen in VCFS. Additionally simulation support to this premise through the Boolean network model of the biochemical route (neurotransmitters) demonstrates that networks are significantly perturbed when specific biologically critical nodes are deleted or knocked out (Gupta et al., 2007).

3b.Gene expression studies

According to the findings by Meechan et al. (2006) and Stark et al. (2008), the 22q11.2 DS represent combinatorial effects of reduced dosage of several genes/miRNAs operating on common cellular mechanisms involved in neuronal development and neurotransmission. The salient features of few studies are listed in the following paragraph. Lin et al. (2016) 54 demonstrated that haploinsufficiency correlated with RNA expression of *in vitro* neurons through possible disturbances of canonical pathways such as MAPK signaling, cell cycle, and apoptosis in SCZ. A plausible inter-chromosomal association between 22q11.2 and 6p21 was also implicated pointing to a molecular connection between immune deficit. In another study integrating comprehensive maps of genome-wide gene expression in the human brain with neuroanatomic data from 22q11DS individuals with molecularly confirmed A-D deletions, Forsyth et al. (2021) prioritized DGCR8 and AIFM3 as potential contributors to cortical SA alterations and P2RX6 as a potential contributor to cortical thickness alterations. Implicating "microprocessor complex," which comprises DGCR8, is crucial for the synthesis of miRNAs, that regulate gene expression at the protein level (Rajman and Schratt, 2017). The study implicates miRNAs that are down-regulated in the mouse cortex as a consequence of DGCR8 loss are enriched for fetal-specific expression and cell cycle and proliferation regulation. Given that AIFM3 is highly expressed in the human brain (https://gtexportal.org/home/), it is hypothesized that AIFM3 haploinsufficiency could influence normal apoptotic processes during corticogenesis, thereby contributing to SA

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deficiencies. Mukai et al., 2008 report specific subset of neuronal proteins, such as PSD95 (also referred to as DLG4), an adaptor molecule that has been demonstrated to modify the quantity of dendritic spines and potentially dendritic branches, appear to be palmitoylated by *ZDHHC8.* The poor dendritic growth and spine formation seen in Del (Dgcr2–Hira) 2Aam mice are caused by the complimentary effects of diminished palmitoylation and impaired miRNA synthesis. The results implicate deficits of *Zdhhc8* could account for declines in spine density, while deficiencies of Dgcr8 may indicate decreases in spine size. HIRA (Histone cell cycle regulator [MIM: 600237]) haploinsufficiency generates aberrant abnormalities in both *in vitro* and *in vivo* models, according to findings published by Jeanne et al., (2021) suggesting that this gene could be crucial for the control of neuronal differentiation and maturation. HIRA encodes a histone chaperone and is essential for the epigenetic regulation of gene expression (Dilg et al., 2016; Valenzuela et al., 2017).Since its tissue-specific knock-out impacts numerous fundamental cellular functions, that include DNA damage, limited *de novo* methylation, and global aberrant transcription.

Expression analyses demonstrate dynamic Tbx1 expression in tissues that form the pharyngeal apparatus which gives rise to the heart and face (Yamagishi et al., 2003). Tbx1 expression during embryogenesis necessitates precise regulation (Xu et al., 2005). Overexpression analyses revealed that increases in Tbx1 transcript levels lead to malformations resembling those seen in patients with 22q11.2DS (Vitelli et al., 2009), and a dose-response study discovered that phenotype severity increases as Tbx1 transcript levels decrease (Zhang and Baldini, 2008). Mechanism of Tbx1 action is through interactions with an array of transcription factors, microRNAs, and signaling pathways which are necessary for Tbx1 to perform its essential roles in organogenesis in distinct tissues governing cell fate, proliferation, and differentiation (Gao et al., 2013).Co-gene expression studies research by Gass et al. (2021) implicate *COMT* and *TRMT2A* gene constitute a fundamental genetic element linked to variations in resting-state connectivity patterns in the deletion of 22q11.2. A possible explanation for the molecular abnormality of brain network connectivity in 22q11.2 deletion syndrome is made apparent by a disruption of their co-expression in SCZ patients. The function of epigenetic processes that control COMT expression is another potential explanation for discrepancies. It has been demonstrated that methylation of CpG sites in the COMT promoter region influences COMT expression in the brain and is linked to prefrontal cognitive function and schizophrenia risk (Murphy et al., 2005; Ursini et al., 2011).

3c.Mouse models studies:

Complex clinical phenotype such as the 22q11.2DS deletion is successfully modeled in the mouse to address several questions in histology, biochemistry, genetics, and gene expression. Studies in the mouse models suggest that several genes from the deleted region affect behavior and could contribute to disease burden in patients. To this end, a variety of mouse mutants that carry multigene and single gene mutations have been generated by researchers. Several researchers have reported single genes affecting the phenotypes such as the mutation in the Tbx1gene is responsible for most of the congenital defects (Paylor R and Lindsay E. 2006). Homozygous or heterozygous loss of function of single gene compromises craniofacial and/or oropharyngeal morphogenesis related to the 22q11DS phenotypes. Another gene *Ranbp1* mediates nucleocytoplasmic protein trafficking is a dosage-dependent modulator of craniofacial development through disruption of Bone morphogenetic protein (BMP) signaling (Paronett et al., 2023). Histological study of the embryonic and adult cerebral cortices of mouse model of Del (3.0Mb)/+, mimicking the 3.0 Mb deletion were indistinguishable from the wild type. However, the morphologies of neurons were slightly changed from the wild type counterparts in a region-specific manner. In specific, the dendritic branches and/or spine densities in the medial prefrontal cortex, nucleus accumbens, and primary somatosensory cortex were reduced (Meechan 2015). Also, reduced axon innervations of dopaminergic neurons suggest aberration of the dopamine system (Tabata et al., 2023). In a study by (Motahari et al., 2020) divergent modes of initial axon growth that prefigure disrupted differentiation of the trigeminal nerve (CN V) was observed. The cranial nerve is essential for suckling, feeding and swallowing (S/F/S) an innate behavior compromised in multiple genetic developmental disorders including 22q11.2 DS. Combination of *in vivo* labeling and 3D imaging suggested altered anterior-posterior hindbrain patterning and gross morphological disruption of CN V seen in mice genes LgDel+/-. and Tbx1+/-, Ranbp1-/-, Ranbp1+/- and LgDel+/-, Raldh2+/- with axon phenotypes. To discern thymic hypoplasia in 22q11.2DS comparison of embryonic thymuses from mouse models of 22q11.2DS (Tbx1neo2/neo2) revealed proportions of mesenchymal, epithelial, and hematopoietic cell types similar to those of control thymuses. Bhalla et al., 2022 using single-cell RNA-Seq of embryonic thymuses uncovered 17 distinct cell subsets with unique differences in 5 mesenchymal subsets from the Tbx1neo2/neo2 cell line. The major affected proteins were the extracellular matrix proteins and collagen cross-links consistent with the increased collagen deposition. Mitochondrial abnormalities have also been reported in the

region. Devaraju and Zakharenko 2017 report hemizygous deletion of several mitochondrial genes in the genomic region can lead to symptoms associated with neuropsychiatric disease implicating abnormal neuronal and synaptic function. Finally, contribution of the genes in 1.4-Mb region to psychiatric disorders is poorly understood. Behavioral tests of Del (1.4 Mb)/+ mice displayed decreased locomotor activity, reduced prepulse inhibition and impairment of contextual- and cued-dependent fear memory. Furthermore, intact social recognition suggests that the impaired social recognition mimics the human 3.0-Mb deletion (Saito R et al., 2020).

4.Unique Genomic features of the region:

Longer lengths of repetitive sequences together known as low copy repeats (LCRs) are linked to the molecular basis of the rearrangements that cause 22q11 microdeletions (Molina et al., 2011). The genomic structure includes short interspersed nuclear elements (SINEs) and long interspersed nuclear elements (LINEs). Chromosome rearrangements and diseases have been linked to these elements especially SINEs. Alu elements, which belong to the SINE family of transposable elements, have also been shown to play a part in facilitating gene rearrangements and altering the architecture of the human genome in relation to human illnesses (Devine SE. 2023). Additionally, it has been proposed that non-allelic homologous recombination (NAHR) is mediated by low-copy repeats (LCRs) on 22q11, leading to rearrangements (Vervoort and Vermeesch 2022). Chromosome crossover occurs during meiosis I, when two homologous chromosomes exchange genetic material (allelic homologous recombination) (Kuzminov 2014). When homologous chromosomes break and subsequently reunite to exchange genetic material; this is known as crossover. This observation is supported by earlier research on breakpoint association (Uddin 2006). Also, studies implicate segmental duplications (SDs) that are in close proximity to each other which can initiate non-allelic homologous recombination (NAHR) and the deletion of adjacent genomic regions. Whence they may suffer intra-genomic rearrangement and loss, is selectively disadvantageous (Abdullaev 2021). It's interesting to note that different LCRs have different sequences and don't seem to have the same effect on microdeletions. Such genomic rearrangements can result in Copy number variations (CNVs) by affecting region-specific LCRs, or repeat sequences. Further, they are vulnerable due to factors like size, orientation, percentage similarity, and separation between copies (Pös et al., 2021). CNVs might involve a contiguous group of genes or just one gene. CNVs can result in dosage-sensitive genes which may be

responsible for a substantial amount of human phenotypic variability and disease susceptibility (Auwerx 2022). While these structural variations are often benign, they can disrupt vital biological pathways/functions resulting in diseases (Nowakowska et al., 2017). Studies *in vitro* and engineered mice show that CNVs affect genes in the duplicated region and flanking gene stretching to several Mb (Orozco 2009).

The locus also contains antisense RNA the genes in this class are identified by their inability to code for proteins, presence of non-canonical splice site signals, independent transcriptional units, and capacity for alternative splicing. This is accomplished by chromatin modifications, RNA processing, and epigenetic changes in addition to direct interactions (RNA-RNA or RNA-DNA) (Werner et al., 2009) 87. Antisense transcription can "rewire" regulatory networks, modify the architecture for protein complexes, and act as a fast-evolving regulatory switch. MicroRNAs (miRNAs) are a family of small non-coding RNAs that regulate a wide array of biological processes such as development, physiology, and disease. Mechanisms of miRNA functions include RNA silencing and post-transcriptional regulation of gene expression.

~~7.Results~~

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The results of the copy number analysis of several studies (including Bhoge gowda et al., 2012) care summarized in the table-1. A ideogram depicting brain expressed genes and part of analysis in Bhoge gowda et al., 2012 is depicted in figure-3. Analysis of the repeat elements abutting the candidate genes suggests a constellation of repeats including the Short interspersed nuclear elements (SINE), which include Alu, Long terminal repeat elements (LTR), and L1 retrotransposons and repetitive DNA/RNA elements. As evidenced from table-1, loss of CNV was observed at several genes at the 22q locus, however; the CNV copy number varied for each gene. High preponderance of Alu family of repetitive DNA viz., AluY, Alujo, Alujr, Alujb, Alusc, Alujy around the candidate genes was observed. The presence of Alu repeats suggests proclivity for secondary-structure formation and serve as hotspots for chromosomal rearrangement. Different class of repeats viz., the endogenous retroviruses (*HERV-K* (also known as *ERVK*), L1 retrotransposons and family of low complexity repeats (GC, AT rich) were also enriched flanking the candidate genes. Repetitive elements DNA-(MER1B, hAT- Charlie) repeat-a class of transposable elements (TEs) which are interspersed repetitive DNA families and RNA (U3 family) repeats were found flanking the *ZDHHC8* gene providing further credence to the

hypothesis that these repeats could predispose genomes to CNV through replication slippage or unequal crossing-over.

Network analysis of PRODH gene is depicted in figure-4A,B..As evident from the figure the network consisted of 11 genes in a hub with 11 nodes and PPI enrichment p-value of 4.59e-06.The GO-ontology molecular function (GO-0004657) indicated proline dehydrogenase activity. Further, co-gene expression analysis revealed interaction between COMT and PRODH (Score-0.50), COMT and ZDHH8 (Score-0.058) and PRODH and ZDHH8 (Score-0.074) respectively.

Genetic variants that inactivate protein-coding genes are a powerful source of information. Genes that are crucial for the function of an organism will be depleted of such variants in natural populations, whereas non-essential genes will tolerate their accumulation. A complementary approach is to estimate the impact on fitness (e.g., survival and reproduction) for individuals who harbor heterozygous LOF variants in the gene (selection coefficient of heterozygous LOF variants) (Agarwal I et al., 2023) . The convention used is pHaplo scores ≥0.55 indicate an odds ratio ≥2. As evident from the table-2 the pHaplo scores of genes were COMT(0.27), ZDHHC8(0.32) and PRODH(0.39) respectively.

Complexes of promoters and enhancers, insulators networks at gene promoter or at specific sets of genes in the transcription start site (TSS+/-) along with transcription factors interact with RNA polymerases to regulate gene expression dynamically (Bhogegowda KKH et al., 2024). Methylation signature could alter this step leading to differential expression. A CpG island -55 was observed near the promoter suggesting a probable role of methylation in gene expression (Figure5A). Lower signal of H3K27Ac was evident in promoter, however several peaks were observed near 2-intron (Figure-5A). Gene enhancer and regulatory element analysis revealed a peak with high intensity for promoter, medium peak for interaction and cluster (Figure-5A). The TSS analysis revealed high density peaks in the promoter region with a signal for insulator (Figure-5A). The enhancer/promoter map revealed 2 enhancer/promoter and 3 enhancers (Figure-5B). As evident from the table-3 the enhancers GH22J018923 and GH22J018969 are associated with craniofacial abnormalities (https://cotney.research.uchc.edu/data/). With enhancer GH22J018923 acting as a hub for 84 TF associated with 13 genes and enhancer GH22J018969acting as a hub for 23 TF associated with 6 genes. The chromatin analysis revealed peaks sites for DNASE1 and FAIRE. Promoter analysis of for repetitive elements revealed

constellation of peaks (Figure-5B). Enhancers are found in areas of the genome that are free of nucleosomes, or open chromatin, and they are activated when one or more sequence-specific transcription factors (TFs) and coactivators attach to them. They control transcription and are found either in trans (on a different chromosome) or in cis (on the same chromosome) proximity to the gene (Ray-Jones & Spivakov, 2021). Peaks of repetitive DNA such as simple, interrupted and segmental repeats were observed (Figure-5C)

5.Network analysis of PRODH gene

The human brain exhibits variations at the micro- and macro-scale organization (myelo-, cyto-, and chemo-architecture) levels (van den Heuvel and Yeo 2017). Entire brain is composed of several interconnected electro-chemical transmitting neurons operating like network with hubs and nodes. Neurons form complex biological neural networks through which nerve impulses (action potentials) are relayed. Several genes in the 22q region participate in neurotransmission hinting at a plausible network. This hypothesis could be assessed *in silico* methods such as STRING (Szklarczyk et al., 2023). The premise is. protein-protein interactions-both physical interactions as well as functional associations are computationally predicted through a pipe-line of mining of the scientific literature from co-expression, conserved genomic context, databases of interaction experiments and known complexes/pathways from curated sources. Several researchers have successfully demonstrated the use of this method in unravelling networks in brain disorders (Quinlan et al., 2020; Vilela et al., 2023).Hence, in the present study network analysis was carried out to infer probable interacting protein and common pathways they converge.

5a.Promoter elements and chromatin architecture of PRODH gene:

Active transcription foci contain clusters—hubs—of enhancer-promoter interactions, transcriptional activators, and facilitate stepwise assembly of RNA Polymerase II (Pol II). Further, CRE elements (promoter region, enhancers, insulators, and silencers) and chromatin markers (DNaseI hypersensitivity; FAIRE) determine the trajectories of transcription and translation. An integrated model encompassing genetic, epigenetic, and chromatin variations in the context of developmental and environmental responses is proposed to explain *in nuclei* mechanisms and

genetic associations with disease states (iran Bhogegowda KKH et al., 2024). Variations in one of these elements individually or combinatorial can result in gene-expression changes including those of the genes in proximity or anywhere in the genome (based on the 3D topology). It is well documented that genes are tightly bound to one another functionally and no gene is an island unto itself. Further, the gene may be part of larger gene regulatory networks not necessarily by physical location in the genome (Lieberman Aiden et al., 2009). Support to this observation is the long-range interactions of COMT gene using 4C-seq to investigate *cis* and *trans* profiles carried out by Zeitz et al., 2013 in deletion-cell lines. The study revealed possibility of variability at a locus is the status of its physical interaction profile with other genetic elements. Several HI induced mechanisms include physical dissociation of the transcription unit from its cis-acting regulator chromatin modification/positioning within the nucleus has been suggested to influence the expression of gene/s. This observation has implications in 22q11 region since several genes in the gene are brain expressed with unique regulatory element configuration. A CNV in the promoter, intron or the intergenic region can change the transcriptional machinery configuration. Additionally, chromatin marks (DNaseI hypersensitivity; FAIRE) and methylation marks add to the perturbation and deride the unit. The HI can also lead to confirmation change in the architecture of nucleus and re-range long range interactions between flanking and gene distal from the deletion. A probable pictorial plausible model to explain the afore mechanisms are depicted in figure-2.

2.Neuro-anatomical findings:

The neuroanatomical findings of the disorder have been investigated by several researchers sing a spectrum of methods to asseess brain anatomy, cytoarchitectural features and ~~behavioural~~ behavioral? abnormalities. The following paragraph ~~summarises~~ summarizes salient features of few studies.

da Silva Alves F et al., 2011 report using proton magnetic resonance spectroscopy ((1)H-MRS) increased concentrations of glutamate and myo-inositol in the dorsolateral prefrontal cortex (DLPFC) and hippocampus~~.~~. In another meta-analysis, Morgan et al., (2019) observed abnormalities in the bilateral inferior parietal lobe, right precuneus, right superior temporal gyrus, and posterior cingulate cortex. While Olszewski AK et al., 2017 report that white matter disruption, specifically axonal coherence in the right inferior fronto-occipital fasciculus a

biomarker for social cognitive difficulties and psychosis in individuals with 22q11.2DS. Using anisotropy (FA) and the trough of radial diffusivity (RD) Momtazmanesh et al., 2021 report altered structural connectivity and disrupted microstructural organization of cortical and subcortical structures and white matter tracts indicative of neurodevelopmental delay. Additionally, there is a decline in the volume of hippocampus, cerebellum, and various cortical regions, while the corpus callosum area was increased in brain, including Tan GM ~~2019~~ 2009. Several investigators report neuroanatomical anomalies in several brain regions. White matter microstructural abnormalities affecting neuroanatomical tracts were discovered using diffusion tensor imaging. Additionally, substantial correlation between positive prodromal symptoms of psychosis and longitudinal fasciculus (ILF) measures reported by ~~Daniel~~ Tylee DS et al., 2017. Bohm 2017 et al., report malformations in persistent cavum septi pellucidi, *cavum septi pellucidi,* cortical veins and cortical dysplasia with hypoplastic internal carotid artery and hypoplastic cerebellum. Finally perturbation of task-based imaging sensory processing, social cognition, and working memory are indicative of functional brain alterations (Larsen et al., ~~2019~~2018).

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The zinc finger and DHHC domain-containing protein 8 (*ZDHHC8*) gene is expressed in the adult human brain and is found predominantly in the cortex and hippocampus of the mouse brain (brain atlas). Protein palmitoylation a post-translational modification of proteins requires a transmembrane palmitoyltransferase which is carried by the protein. Several brain development and signaling pathways are dependent on palmitoylation with the lipid palmitate (Jiang 2024). *ZDHHC8* has been proposed as a plausible candidate gene contributing to the behavioral phenotype of 22q11.2.2DS given its functional role and its association with PPI (Karayiorgou 2010).Studies have found equivocal associations with ZDHHC8 variants and SCZ .Lack of association in Japanese and European populations (Ujike et al., 2005; Saito et al., 2005) and moderate association in German population (Faul et al., 2005).Gamma-glutamyl transpeptidase 2

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gene is involved in the degradation of glutathione(*GGTP2*)(ncbi-gene). The encoded enzyme acts as part of a GSH pumping gamma-glutamyl cycle in the brain and may also be involved in gamma-glutamyl amino acid formation. The protein encoded by the gene hypermethylated in cancer 2 (HIC2) (genecards). The protein enables transcription repressor activity, RNA polymerase II-specific and RNA polymerase II cis-regulatory region sequence-specific DNA binding activity. It regulates of cytokine production and regulation of immune system process. The ubiquitin-conjugating enzyme E2L 3 (UBE2L3) gene encodes a protein member of the E2 ubiquitin-conjugating enzyme which is part of the cellular mechanism for targeting abnormal or short-lived proteins for degradation (ncbi-gene).

Several studies demonstrate co-gene expression, patterns and interaction between genes in the region. Paterlini et al., (2005) reported an upregulation of COMT mRNA in the frontal cortex of ZDHHC8 mutant mice. In addition, when tolcapone (a COMT inhibitor) was administered to the ZDHHC8 mutants, there was a greater disruption in PPI and working memory compared with the non-treated ZDHHC8 mutants, suggesting a gene–gene interaction between ZDHHC8 and COMT. This is consistent with the proposal that 22q DS is a contiguous gene syndrome, in which deficiency in more than one gene contributes to the increased risk. Wilson et al. (2008) reported two patients with interstitial deletion of the 22q13 region with intact SHANK3 indicating haploinsufficiency for other 22q13 genes could also have major effects on cognitive and language development. The above studies are consistent with the hypothesis that the phenotypic expression in VCFS in terms of cognition and co-morbidity can be affected by each gene alone and by interaction between genes that affecting the pathway (e.g., dopaminergic, glutamatergic). Cumulatively, all of these findings point to a potential connection between genes at the 22q locus and their role in the neuropsychiatric phenomenology seen in VCFS. Additionally simulation support to this premise through the Boolean network model of the biochemical route (neurotransmitters) demonstrates that networks are significantly perturbed when specific biologically critical nodes are deleted or knocked out (Simone et al., 2007).

3b.Gene expression studies

According to the findings by Meechan et al. (2006) and Stark et al. (2008), the 22q11.2 DS represent combinatorial effects of reduced dosage of several genes/miRNAs operating on common cellular mechanisms involved in neuronal development and neurotransmission. The salient features of few studies are listed in the following paragraph. Lin et al. (2016) 54 demonstrated that

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haploinsufficiency correlated with RNA expression of *in vitro* neurons through possible disturbances of canonical pathways such as MAPK signaling, cell cycle, and apoptosis in SCZ. A plausible inter-chromosomal association between 22q11.2 and 6p21 was also implicated pointing to a molecular connection between immune deficit.In another study integrating comprehensive maps of genome-wide gene expression in the human brain with neuroanatomic data from 22q11DS individuals with molecularly confirmed A-D deletions, Forsyth et al. (2021) prioritized DGCR8 and AIFM3 as potential contributors to cortical SA alterations and P2RX6 as a potential contributor to cortical thickness alterations. Implicating "microprocessor complex," which comprises DGCR8, is crucial for the synthesis of miRNAs, that regulate gene expression at the protein level (Rajman and Schratt, 2017). The study implicate miRNAs that are down-regulated in the mouse cortex as a consequence of DGCR8 loss are enriched for fetal-specific expression and cell cycle and proliferation regulation. Given that AIFM3 is highly expressed in the human brain (https://gtexportal.org/home/), it is hypothesized that AIFM3 haploinsufficiency could influence normal apoptotic processes during corticogenesis, thereby contributing to SA deficiencies. Mukai et al., 2008 report specific subset of neuronal proteins, such as PSD95 (also referred to as DLG4), an adaptor molecule that has been demonstrated to modify the quantity of dendritic spines and potentially dendritic branches, appear to be palmitoylated by *ZDHHC8*. The poor dendritic growth and spine formation seen in Del (Dgcr2–Hira) 2Aam mice are caused by the complimentary effects of diminished palmitoylation and impaired miRNA synthesis. The results implicate deficits of *Zdhhc8* could account for declines in spine density, while deficiencies of Dgcr8 may indicate decreases in spine size. HIRA (Histone cell cycle regulator [MIM: 600237]) haploinsufficiency generates aberrant abnormalities in both *in vitro* and *in vivo* models, according to findings published by Jeanne et al., (2021) suggesting that this gene could be crucial for the control of neuronal differentiation and maturation. HIRA encodes a histone chaperone and is essential for the epigenetic regulation of gene expression (Dilg et al., 2016; Valenzuela et al., 2017).Since its tissue-specific knock-out impacts numerous fundamental cellular functions, that include DNA damage, limited *de novo* methylation, and global aberrant transcription.

Expression analyses demonstrate dynamic Tbx1 expression in tissues that form the pharyngeal apparatus which gives rise to the heart and face (Yamagishi et al., 2003). Tbx1 expression during embryogenesis necessitates precise regulation (Xu et al., 2005). Overexpression analyses revealed that increases in Tbx1 transcript levels lead to malformations resembling those seen in patients

with 22q11.2DS (Vitelli et al., 2009), and a dose-response study discovered that phenotype severity increases as Tbx1 transcript levels decrease (Zhang and Baldini, 2008). Mechanism of Tbx1 action is through interactions with an array of transcription factors, microRNAs, and signaling pathways which are necessary for Tbx1 to perform its essential roles in organogenesis in distinct tissues governing cell fate, proliferation, and differentiation (Gao et al., 2013).Co-gene expression studies research by Gass et al. (2021) implicate *COMT* and *TRMT2A* gene constitute a fundamental genetic element linked to variations in resting-state connectivity patterns in the deletion of 22q11.2. A possible explanation for the molecular abnormality of brain network connectivity in 22q11.2 deletion syndrome is made apparent by a disruption of their co-expression in SCZ patients. The function of epigenetic processes that control COMT expression is another potential explanation for discrepancies. It has been demonstrated that methylation of CpG sites in the COMT promoter region influences COMT expression in the brain and is linked to prefrontal cognitive function and schizophrenia risk (Murphy et al., 2005; Ursini et al., 2011).

3c.Mouse models studies:

Complex clinical phenotype such as the 22q11.2DS deletion is successfully modeled in the mouse to address several questions in histology, biochemistry, genetics, and gene expression. Studies in the mouse models suggest that several genes from the deleted region affect behavior and could contribute to disease burden in patients. To this end, a variety of mouse mutants that carry multigene and single gene mutations have been generated by researchers. Several researchers have reported single genes affecting the phenotypes such as the mutation in the Tbx1gene is responsible for most of the congenital defects (Paylor R and Lindsay E. 2006) .Homozygous or heterozygous loss-of-function of single gene compromises craniofacial and/or oropharyngeal morphogenesis related to the 22q11DS phenotypes. Another gene *Ranbp1* mediates nucleocytoplasmic protein trafficking is a dosage-dependent modulator of craniofacial development through disruption of Bone morphogenetic protein (BMP) signaling (Paronett et al., 2023). Histological study of the embryonic and adult cerebral cortices of mouse model of Del (3.0Mb)/+, mimicking the 3.0 Mb deletion were indistinguishable from the wild type. However, the morphologies of neurons were slightly changed from the wild type counterparts in a region-specific manner. In specific, the dendritic branches and/or spine densities in the medial prefrontal cortex, nucleus accumbens, and primary somatosensory cortex were reduced. Also, reduced axon innervations of dopaminergic

neurons suggest aberration of the dopamine system (Tabata et al., 2023). In a study by (Motahari et al., 2020) divergent modes of initial axon growth that prefigure disrupted differentiation of the trigeminal nerve (CN V) was observed. The cranial nerve is essential for suckling, feeding and swallowing (S/F/S) an innate behavior compromised in multiple genetic developmental disorders including 22q11.2 DS. Combination of *in vivo* labeling and 3D imaging suggested altered anterior-posterior hindbrain patterning and gross morphological disruption of CN V seen in mice genes LgDel+/- and Tbx1+/-, Ranbp1 -/-, Ranbp1+/- and LgDel+/-, Raldh2+/- with axon phenotypes. To discern thymic hypoplasia in 22q11.2DS comparison of embryonic thymuses from mouse models of 22q11.2DS (Tbx1neo2/neo2) revealed proportions of mesenchymal, epithelial, and hematopoietic cell types similar to those of control thymuses. Bhalla et al., 2022 using single-cell RNA-Seq of embryonic thymuses uncovered 17 distinct cell subsets with unique differences in 5 mesenchymal subsets from the Tbx1neo2/neo2 cell line. The major affected proteins were the extracellular matrix proteins and collagen cross-links consistent with the increased collagen deposition. Mitochondrial abnormalities have also been reported in the region. Devaraju and Zakharenko 2017 report hemizygous deletion of several mitochondrial genes in the genomic region can lead to symptoms associated with neuropsychiatric disease implicating abnormal neuronal and synaptic function. Finally, contribution of the genes in 1.4 Mb region to psychiatric disorders is poorly understood. Behavioral tests of Del (1.4 Mb)/+ mice displayed decreased locomotor activity, reduced prepulse inhibition and impairment of contextual- and cued-dependent fear memory. Furthermore, intact social recognition suggests that the impaired social recognition mimics the human 3.0 Mb deletion (Saito et al., 2021).

4.Unique Genomic features of the region:

Longer lengths of repetitive sequences together known as low copy repeats (LCRs) are linked to the molecular basis of the rearrangements that cause 22q11 microdeletions (Molina et al., 2011). The genomic structure includes short interspersed nuclear elements (SINEs) and long interspersed nuclear elements (LINEs). Chromosome rearrangements and diseases have been linked to these elements especially SINEs. Alu elements, which belong to the SINE family of transposable elements, have also been shown to play a part in facilitating gene rearrangements and altering the architecture of the human genome in relation to human illnesses (Devine SE. 2023). Additionally,

it has been proposed that non-allelic homologous recombination (NAHR) is mediated by low-copy repeats (LCRs) on 22q11, leading to rearrangements (Vervoort and Vermeesch 2022). Chromosome crossover occurs during meiosis I, when two homologous chromosomes exchange genetic material (allelic homologous recombination) (Kuzminov 2014). When homologous chromosomes break and subsequently reunite to exchange genetic material; this is known as crossover. This observation is supported by earlier research on breakpoint association (Uddin 2006). Also, studies implicate segmental duplications (SDs) that are in close proximity to each other which can initiate non-allelic homologous recombination (NAHR) and the deletion of adjacent genomic regions. Whence they may suffer intra genomic rearrangement and loss, is selectively disadvantageous (Abdullaev 2021). It's interesting to note that different LCRs have different sequences and don't seem to have the same effect on microdeletions. Such genomic rearrangements can result in Copy number variations (CNVs) by affecting region-specific LCRs, or repeat sequences. Further, they are vulnerable due to factors like size, orientation, percentage similarity, and separation between copies (Pös et al., 2021). CNVs might involve a contiguous group of genes or just one gene. CNVs can result in dosage-sensitive genes which may be responsible for a substantial amount of human phenotypic variability and disease susceptibility (Auwerx 2022). While these structural variations are often benign, they can disrupt vital biological pathways/functions resulting in diseases (Nowakowska et al., 2017). Studies *in vitro* and engineered mice show that CNVs affect genes in the duplicated region and flanking gene stretching to several Mb (Orozco 2009).

The locus also contains antisense RNA the genes in this class are identified by their inability to code for proteins, presence of non-canonical splice site signals, independent transcriptional units, and capacity for alternative splicing. This is accomplished by chromatin modifications, RNA processing, and epigenetic changes in addition to direct interactions (RNA-RNA or RNA-DNA) (Werner et al., 2009) 87. Antisense transcription can "rewire" regulatory networks, modify the architecture for protein complexes, and act as a fast evolving regulatory switch. MicroRNAs (miRNAs) are a family of small non coding RNAs that regulate a wide array of biological processes such as development, physiology, and disease. Mechanisms of miRNA functions include RNA silencing and post-transcriptional regulation of gene expression.

4a.Neuropsychiatric disease preponderance:

It is hypothesized that the deletion of 22q11 VCFS may predispose to psychotic disorders by either unmasking harmful polymorphisms in the intact copy or by haplo-insufficiency of important brain developmental genes in this region. These observations provide crucial hints on the neuro-genetic process of non-VCFS-related SCZ as well as the genesis of SCZ symptoms in VCFS, given the exceptionally high prevalence of comorbid diagnoses of VCFS and SCZ. Interest in VCFS has been reignited by recent publications linking copy number variations (i.e., deletions/insertions) to genetic predisposition to neuropsychiatric illnesses SCZ (Kushima 2022), Autism (Nomura 2021). Several studies of the molecular mechanisms causing the clinical manifestations of VCFS offers a unique opportunity to concentrate on a specific region in the genome that contains a limited number of genes, and to assess their relevance to chromosomal abnormalities as well as neuropsychiatric disorders. To this end linkage studies highlight this region of interest for psychosis (Yoon 2016). Further, meta-analyses point to chromosome 22q as harboring one or more genetic risk factors for scz susceptibility (Badner and Gershon, 2002 ; Levinson et al., 2003) .

~~**5.Network analysis of PRODH gene**~~

~~The human brain exhibits variations at the micro- and macro-scale organization (myelo-, cyto-, and chemo-architecture) levels (van den Heuvel and Yeo 2017). Entire brain is composed of several interconnected electro-chemical transmitting neurons operating like network with hubs and nodes. Neurons form complex biological neural networks through which nerve impulses (action potentials) are relayed. Several genes in the 22q region participate in neurotransmission hinting at a plausible network. This hypothesis could be assessed *in silico* methods such as STRING (Szklarczyk et al., 2023). The premise is. protein-protein interactions both physical interactions as well as functional associations are computationally predicted through a pipe-line of mining of the scientific literature from co-expression, conserved genomic context, databases of interaction experiments and known complexes/pathways from curated sources. Several researchers have successfully demonstrated the use of this method in unravelling networks in brain disorders (Quinlan et al., 2020; Vilela et al., 2023).Hence, in the present study network analysis was carried out to infer probable interacting protein and common pathways they converge.~~

~~**5a.Promoter elements and chromatin architecture of PRODH gene:**~~

~~Active transcription foci contain clusters hubs of enhancer-promoter interactions, transcriptional activators, and facilitate stepwise assembly of RNA Polymerase II (Pol II). Further, CRE elements (promoter region, enhancers, insulators, and silencers) and chromatin markers (DNaseI hypersensitivity; FAIRE) determine the trajectories of transcription and translation. An integrated model encompassing genetic, epigenetic, and chromatin variations in the context of developmental and environmental responses is proposed to explain *in nuclei* mechanisms and genetic associations with disease states (kiran et al., 2024). Variations in one of these elements individually or combinatorial can result in gene expression changes including those of the genes in proximity or anywhere in the genome (based on the 3D topology). It is well documented that genes are tightly bound to one another functionally and no gene is an island unto itself. Further, the gene may be part of larger gene regulatory networks not necessarily by physical location in the genome (Lieberman Aiden et al., 2009). Support to this observation is the long range interactions of COMT gene using 4C-seq to investigate *cis* and *trans* profiles carried out by Zeitz et al., 2013 in deletion cell lines. The study revealed possibility of variability at a locus is the status of its physical interaction profile with other genetic elements. Several HI induced mechanisms include physical dissociation of the transcription unit from its cis-acting regulator chromatin modification/positioning within the nucleus has been suggested to influence the expression of gene/s. This observation has implications in 22q11 region since several genes in the gene are brain expressed with unique regulatory element configuration. A CNV in the promoter, intron or the intergenic region can change the transcriptional machinery configuration. Additionally, chromatin marks (DNaseI hypersensitivity; FAIRE) and methylation marks add to the pertubation and deride the unit. The HI can also lead to confirmation change in the architecture of nucleus and re-range long range interactions between flanking and gene distal from the deletion. A probable pictorial plausible model to explain the afore mechanisms are depicted in figure 2.~~

6.Materials and methods:

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To identify putative repeat elements in the flanking regions of CNVs the sequence features around the CNV which could promote break points analysis was carried out using the servers 1.http://genome.ucsc.edu/ (Build 37.1) and repeat masker http://www.repeatmasker.org/

2.Network analysis of genes in the region was carried out to deduce using the server(https://string-db.org/

3.Haplo scores (PHaplo) were obtained from(https://mastermind.genomenon.com/)

4.The Enhancer elements were mapped using the server genecards (https://www.genecards.org/)

5.Epigenetics and promoter analysis of *PRODH* gene to was carried out using web-based resource available at ENCODE (https://genome.ucsc.edu/encode/) (accessed on february 2025) to identify methylation, CRE elements The genomic sequence of the gene 2 kb 5' flanks to transcription start sites (TSSs) and regions around exon 1 were analyzed (around 1kb (-) upstream and (+) downstream (defines the ROI). Specific tracks representing were activated and images were acquired.

~~7.**Results:**~~

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~~The results of the copy number analysis of several studies (including Bhoge gowda et al., 2012) care summarized in the table 1. A ideogram depicting brain expressed genes and part of analysis in Bhoge gowda et al., 2012 is depicted in figure 3.Analysis of the repeat elements abutting the candidate genes suggests a constellation of repeats including the Short interspersed nuclear elements (SINE), which include Alu, Long terminal repeat elements (LTR), and L1 retrotransposons and repetitive DNA/RNA elements. As evidenced from table 1, loss of CNV was observed at several genes at the 22q locus, however; the CNV copy number varied for each gene. High preponderance of Alu family of repetitive DNA viz., AluY, Alujo, Alujr, Alujb, Alusc, Alujy around the candidate genes was observed. The presence of Alu repeats suggests proclivity for secondary structure formation and serve as hotspots for chromosomal rearrangement. Different class of repeats viz., the endogenous retroviruses (*HERV-K* (also known as *ERVK*), L1 retrotransposons and family of low complexity repeats (GC, AT rich) were also enriched flanking the candidate genes. Repetitive elements DNA (MER1B, hAT- Charlie) repeat a class of transposable elements (TEs) which are interspersed repetitive DNA families and RNA (U3 family) repeats were found flanking the *ZDHHC8* gene providing further credence to the hypothesis that these repeats could predispose genomes to CNV through replication slippage or unequal crossing over.~~

~~Network analysis of PRODH gene is depicted in figure 4A,B..As evident from the figure the network consisted of 11 genes in a hub with 11 nodes and PPI enrichment p-value of 4.59e-06.The~~

GO-ontology molecular function (GO-0004657) indicated proline dehydrogenase activity. Further, co-gene expression analysis revealed interaction between COMT and PRODH (Score-0.50), COMT and ZDHH8 (Score-0.058) and PRODH and ZDHH8 (Score-0.074) respectively.

Genetic variants that inactivate protein-coding genes are a powerful source of information. Genes that are crucial for the function of an organism will be depleted of such variants in natural populations, whereas non-essential genes will tolerate their accumulation. A complementary approach is to estimate the impact on fitness (e.g., survival and reproduction) for individuals who harbor heterozygous LOF variants in the gene (selection coefficient of heterozygous LOF variants) (Agarwal I et al., 2023) 108. The convention used is pHaplo scores ≥0.55 indicate an odds ratio ≥2. As evident from the table 2 the pHaplo scores of genes were COMT(0.27), ZDHHC8(0.32) and PRODH(0.39) respectively.

Complexes of promoters and enhancers, insulators networks at gene promoter or at specific sets of genes in the transcription start site (TSS+/-) along with transcription factors interact with RNA polymerases to regulate gene expression dynamically **(**Kiran Kumar et al., 2024**)**.Methylation signature could alter this step leading to differential expression. A CpG island -55 was observed near the promoter suggesting a probable role of methylation in gene expression (Figure5A). Lower signal of H3K27Ac was evident in promoter, however several peaks were observed near 2 intron (Figure 5A). Gene enhancer and regulatory element analysis revealed a peak with high intensity for promoter, medium peak for interaction and cluster (Figure 5A). The TSS analysis revealed high density peaks in the promoter region with a signal for insulator (Figure 5A). The enhancer/promoter map revealed 2 enhancer/promoter and 3 enhancers (Figure 5B). As evident from the table 3 the enhancers GH22J018923 and GH22J018969 are associated with craniofacial abnormalities (https://cotney.research.uchc.edu/data/). With enhancer GH22J018923 acting as a hub for 84 TF associated with 13 genes and enhancer GH22J018969acting as a hub for 23 TF associated with 6 genes. The chromatin analysis revealed peaks sites for DNASE1 and FAIRE. Promoter analysis of for repetitive elements revealed constellation of peaks (Figure 5B). Enhancers are found in areas of the genome that are free of nucleosomes, or open chromatin, and they are activated when one or more sequence-specific transcription factors (TFs) and coactivators attach to them. They control transcription and are found either in trans (on a different chromosome) or in cis (on the same chromosome) proximity to the gene (Ray-Jones & Spivakov, 2021). Peaks of repetitive DNA such as simple, interrupted and segmental repeats were observed

~~(Figure 5C)~~

8.Discussion:

The instability of 22q11 has been demonstrated by the high frequency of pathological intra-chromosomal-rearrangements of the region. There is overwhelming evidence that hemizygosity of a large region greatly increases the risk of SCZ, also there is evidence that VCFS is over represented in largely unselected SCZ populations. Since hemizygosity changes the dosage of the genes in the region, one hypothesis is that this factor is related to susceptibility to SCZ. In the absence of hemizygosity, either a null mutation or a sequence variant that lowers transcriptional activity would have similar effects. *PRODH* and *ZDHHC8* genes in the 1.5 Mb region of chromosome 22 encode mitochondrial proteins expressed in the brain and maximal expression coincides with peak forebrain synaptogenesis shortly after birth. Distinct expression patterns and dynamic expression levels of these genes in the developing and adult brain suggest they contribute to construction and maintenance of neural circuits across synapses. Diminished dosage of the genes in the critical deleted region in a mouse model demonstrates aberrations in neurogenesis and subsequent differentiation in the cerebral cortex. Such developmental disruption may alter cortical circuitry and establish vulnerability for developmental disorders, including SCZ and autism (Meechan et al., 2009). Boolean network model of the biochemical pathway (neuro- transmitters) shows that, deletion/ knockout of certain biologically important nodes cause significant perturbation in networks (~~Lisa M~~ Pham LM et al., 2016). As evident from Network analysis the GO-ontology molecular function (GO-0004657) indicated proline dehydrogenase activity supported from PPI enrichment p-value of $4.59e^{-06}$. The COMT mediates dopaminergic and PRODH glutamatergic neurotransmission and cross-talk of these pathways is also documented. Further, DA plays a major role in motor and cognitive functions as well as in reward processing by regulating glutamatergic inputs (Peng 2024). Hence, a perturbation of the nodes in this network could affect the pathways. Further, co-gene expression analysis revealed interaction between COMT and PRODH and ZDHH8 genes respectively. This is suggestive of the involvement of Posttranslational modifications (PTMs). PTMs are critical events that occur during and after protein synthesis in brain synapses. Neurotransmission to postsynaptic receptors is mediated by chemical neurotransmitters packaged in synaptic vesicles (SVs) many synaptic proteins (including ZDHHC8), presynaptic and

postsynaptic proteins form specialized structures and perform crucial functions. PTMs are ubiquitous in synaptic proteins and play significant roles in enabling protein function and determining protein localization (Gardoni and Bellone 2015). It could be inferred that the three genes are part of network involving neurotransmission and synaptogenesis. A plausible model demonstrating interaction of the three genes is depected in figure-6.

Alu elements, part of the SINE family of transposable elements have been shown to modulate the architecture of the human genome, gene rearrangements and associated with human disorders (Kolomietz et al.,2002). On the other hand, class I transposable elements (TEs) with long terminal repeats (LTRs) immediately flanking an internal coding region are known as LTR retrotransposons (Cordaux & Batzer, 2009). Their functions in the promoter and enhancer regions involve chromatin structural modification, gene splicing, and epigenetic modifications that impact regulation of gene expression (Liao et al., 2023). However, genetic instability has been linked to aberrant LINE-1 and LTR element activity (Kazazian & Moran, 2017). In the brain, they are implicated in neurodevelopmental defects, neuroinflammation, and neurodegeneration, as well as neurological diseases (Blaudin de Thé et al., 2018). From the repetitive element analysis it could be summarized that both micro-homology based non-allelic homologous recombination (NAHR) and micro-homology mediated break induced replication (MMBIR) and fork stalling and template switching (FoSTeS) mechanisms as contributors for CNV and dosage effects. Bottom-up approaches to define gene– brain relationship investigating the relationship between genes and the neural substrates have provided essential insight into the pathophysiology of mental disorders in VCFS. Gene expression is orchestrated by numerous control elements that may be located anywhere in the gene/genome through the cis/trans effect and can regulate distal genes by physically interacting with them. Identification of active gene regulatory elements is the key to understanding transcriptional control. Further it is documented that CNVs can also result in a position effect either through gain or loss (Kleinjan and Heyningen 2005). Functional analysis of polymorphisms in the promoter regions of genes on 22q11 suggests that the *ZDHHC8* gene showed activity differences between haplotypes of greater than 1.5-fold. This implies that structural variations that modulate the rate of transcription of a gene may be found in the upstream of the gene (Hoogendoorn et al., 2004). Few previous studies which add support to this observation are the COMT haploinsufficiency-related dopaminergic dysfunction in ADHD proposed as a

endophenotype (~~Andreas J.~~ Fallgatter AJ 2007) 123. Another is the genomic aberration due to dosage-dependent copy-number gain resulting in a discrete clinical phenotype of MECP2 duplication syndrome (Vandewalle et al., 2009) .

From the pHaploscore it could be inferred that the PRODH gene has probability of exhibiting haploinsufficiency followed by ZDHHC8 and the COMT. Natural selection is a central component of modern evolutionary theory also is the process responsible for the evolution of adaptive features (Cerca José et al., 2023). Most mutations that arise in a constrained gene are not passed to subsequent generations as they are detrimental, therefore over time there are few mutations within constrained genes. Mutations in an unconstrained gene are tolerated and, therefore accumulate over time. Viewing the results from a selection lens it appears that the mutations in the genes are tolerated and unconstrained, since they are involved in important biological functions in the brain. It is reported that genes expressed in the human brain are slowed down in their evolution given the complexity of the biochemical network in the brain, with multiple gene-gene interactions this places strong constraints on the ability of most brain-related genes to change (Hu G~~anlu~~ et al., 2020).

Enhancers are the promoters of the promoter that allow the spatiotemporal tissue and cell type-specific gene transcription of the promoter; found close to a gene's TSS and serve as hubs for the assembly of the transcription machinery. The majority of the gene's regulatory elements will reside in the zone defined by insulators, also known as border elements. It is suggested that CRE enable this either by looping to their target promoter, creating repressive chromatin marks, and not allowing the transcription factor binding to non-cognate genes (Bansal et al., 2014). CRE plays essential roles in the precision spatiotemporal patterning of gene expression necessary during development ensuring cell specificity (Chatterjee & Ahituv, 2017). A wide range of cellular and functional variety arises in many neuronal classes from the precise control of these complex expression programs involving CRE (Carullo & Day, 2019). It has been documented that several neuropsychiatric illness are a result of aberrant regulatory regions (Douglas & Hill, 2014). The enhancers GH22J018923 and GH22J018969 are associated with craniofacial abnormalities (https://cotney.research.uchc.edu/data/). Craniofacial abnormalities are some of the most common birth defects in the world. This category includes cleft lips or palates and misshapen skulls. Genetic

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factors play an important role in these abnormalities which lie in the regulatory elements such as the enhancers.

They are also hubs for TF binding associated with genes in the vicinity. The arrangement of insulators, enhancers along with ncRNA and miRNA ensure faithful splicing, recombination and transcription and translocation events. However, due to HI abberations in one or several of these elements can lead to altered trajectories. A probable cartoon to demonstrate these mechanisms is depicted in figure-7. The model is support through the additive or synergistic enhancer roles of cis-enhancers in the control of the locus in the brain. Differential regulation of cells and their response to stimuli (in this case genomic re-arrangement) is mediated by enhancers which exhibit region-specific gene expression (~~Devika~~ Singh D and ~~Soojin V~~ Yi SV 2021). The identification of ehancers associated with Cranofacial abnormities is an evidence of abberant enhancer activity. Several research studies report these abnormalities are common features observed in patients with the deletion.

Additional unique features of the region include the antisense RNA and miRNA. Antisense RNA can contribute to chromosomal abnormalities by disrupting the normal regulation of gene expression, potentially leading to improper chromosome structure, replication errors, and epigenetic changes (Xu et al., 2018). It has been shown that various RNAs play key roles in the regulation of the structure of chromosomes and formation of dynamic chromatin structures (Bader A.S., 2020). Also, the formation of chromosomal rearrangements is also linked to the activity of several RNAs (Ding D.-Q., 2012). The study of aberrant expression of HOX ncRNAs such as HOXB derived ncRNAs in NPM1 mutated Acute myeloid leukemia (AML) AML suggests molecular leveraging of HOX-embedded non-coding RNAs (Wilson et al., 2024).Another example is the abnormal expression of ncRNAs in pathogenesis of including multiple myeloma (MM) a type of cancer (Leng et al., 2022). The role of microRNAs (miRNAs) in the pathogenesis of rare genetic disorders is increasingly been recognized. The ability to target multiple mRNAs and influence on wide range of cellular processes highlights their importance in both normal physiology and disease pathology. Mutations in miRNA genes and their associated regulatory pathways can lead to the dysregulation of critical genes involved in neurodevelopmental disorders.

Previous studies in Down syndrome (trisomy 21) has shown a subset of miRNAs, miR-99a, miR-125b-2, and miR-155, contribute to hematopoietic state and preleukemia development in which children. microRNA profiles supports the premise that HGBCL-11q is a distinct subtype of B-cell lymphoma (Zajdel et al., 2024) .

It is not clear why a deletion from only one chromosome would lead to complete loss of certain long-range DNA interactions. Few plausible explanations include a. allele-specific interactions. b. parent of origin (maternally inherited allele) specific interactions. The DiGeorge 6 (DGCR6) a gene in the region is an example. Alternatively, few interactions that occur when both alleles are present may occur relatively infrequently, and thus the loss of one allele may reduce the interaction frequency below the level of detection (c). Another possibility is the three-dimensional structure of the genome which explains some of this variability (d). e. physical interaction profile of a given gene locus with other genetic elements, such as enhancers and co-regulated genes contributing to its regulation. f. further possibility are the elements outside the deletion region and regulatory interactions. The associated symptom of congenital heart disease, including tetralogy of Fallot observed in patients with atypical deletions is an indication in this direction. Finally, proximal and distal genes flanking the deletion could act as modifiers of phenotype. An outcome of such interaction studies (Biological and *in silico*) could have implication in treatment and management. For example the study by Fallgatter 2007 139 of COMT haploinsufficency-related dopaminergic dysfunction in ADHD is proposed as a endophenotype.

9.Conclusions

In summary, the ~~review~~ sheds light on the prenatal, anatomical, genetic and genomic insights into the 22q11.2 deletion. Cumulative results of multiple analysis validate the complex phenotypes observed at the locus with preponderance for brain diseases. At the genome architecture the unique features of the region LCR, repetitive elements along with the distal and proximal genes could result in HI and varied genetic mechanism such as dominance, incomplete penetrance and expressivity. Discrete and common CRE and chromatin features of genes could explain the overlapping regulation within this region and resulting in allelic and parent of origin effects.

Additional contributors to the complexity include ncRNA and miRNA which have regulatory roles.

Ethics and Human consent/animal care declaration not necessary since a ~~review~~ and in silico methods.

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Figures and tables:

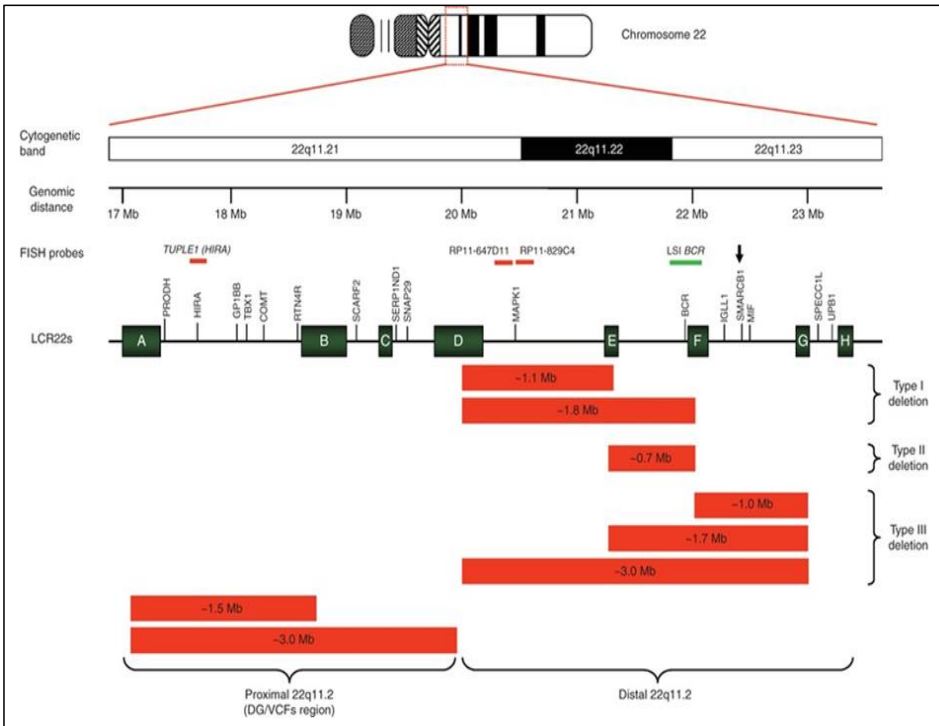
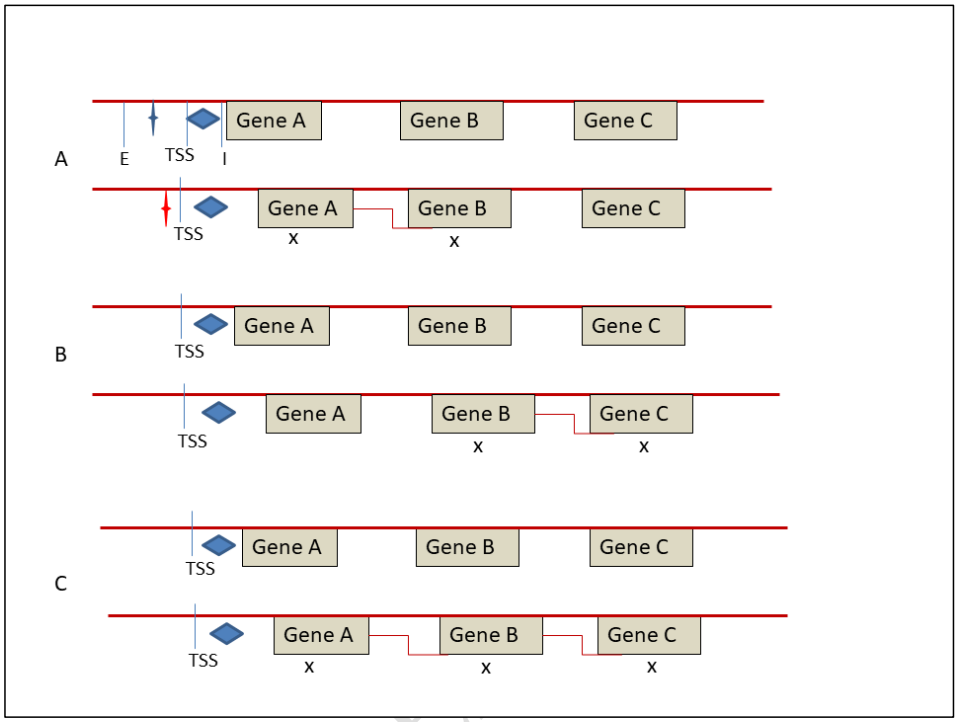


Figure- 1. Schematic representation of the 22q11.2 region and the recurrent microdeletions reported in this region.(hg18). FISH probes used are illustrated by the horizontal bars. LCR22 clusters are illustrated by boxes. The red horizontal bars below the map depict the reported deletions at both the proximal and distal 22q11.2 regions. Ref- PhD thesis Afnan Salah Salaka 2017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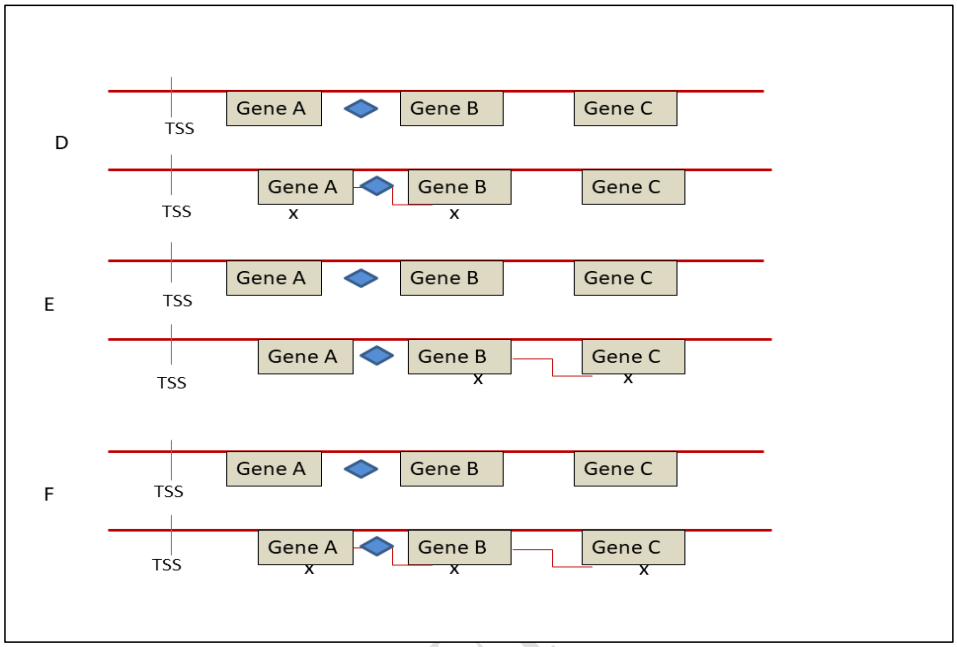

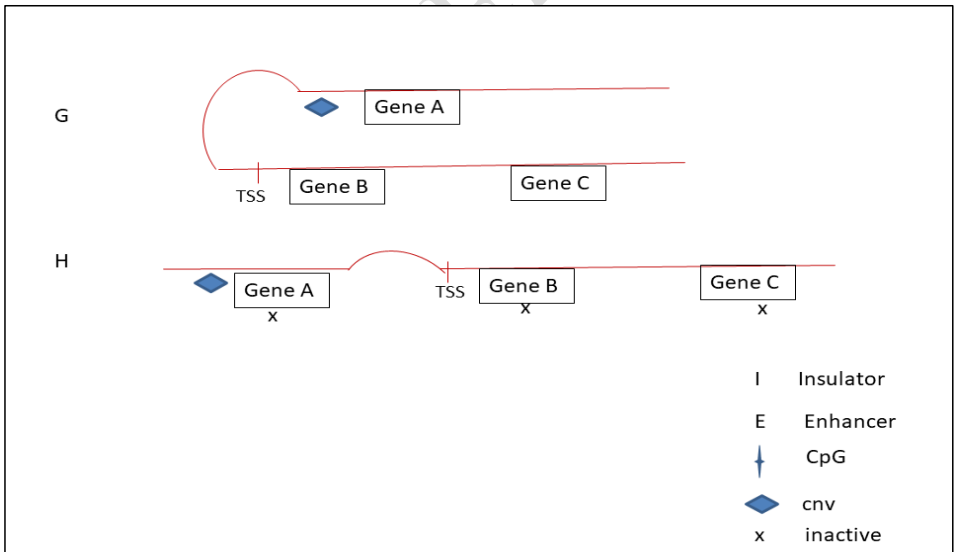

Figure- 2. plausible models to illustrate CNV and position effects
A,B,C- CNV in promoter. D,E,F-Intergenic,.G,H –CNV altering chromatin loop.

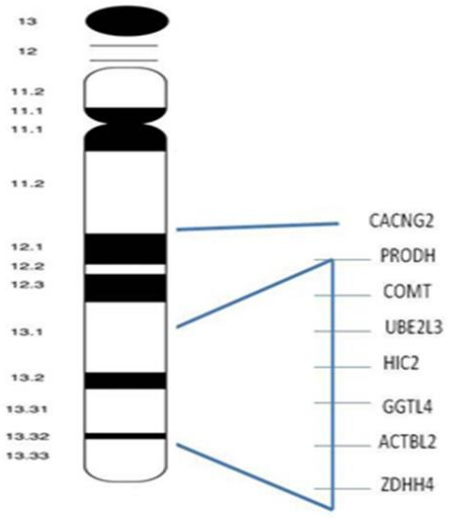

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Figure -3. Ideogram of 22q11 deletion with few candidate genes analysed for CNV
Ref- Kiran et al., 2012

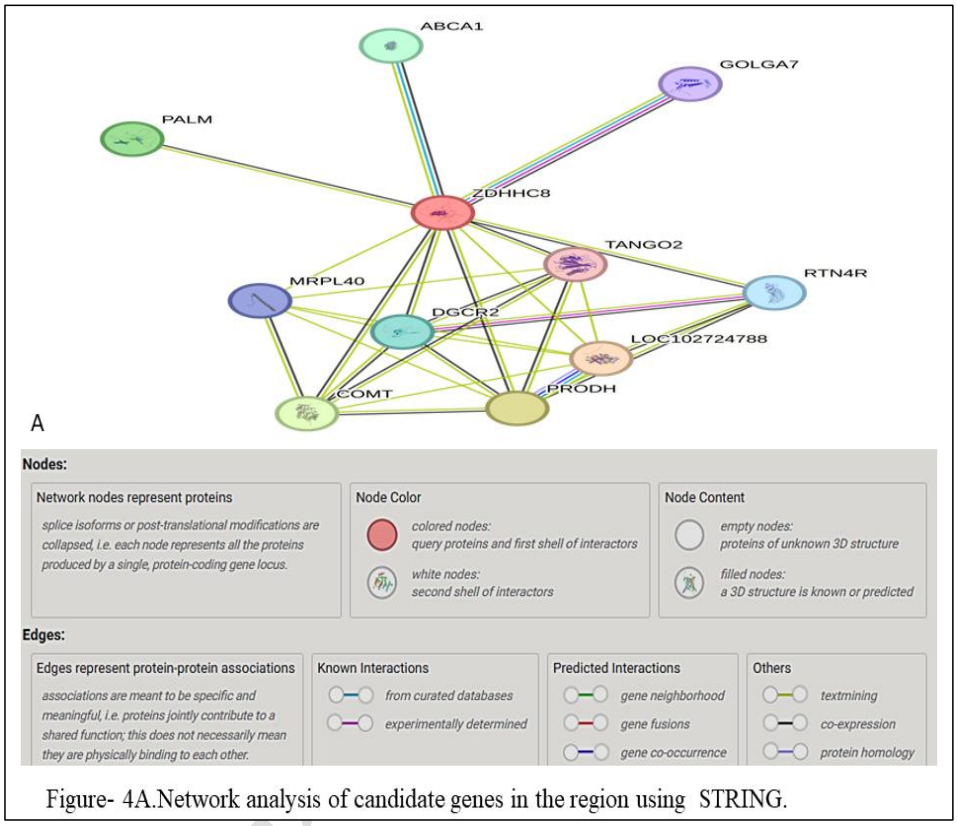


Figure- 4A.Network analysis of candidate genes in the region using STRING.

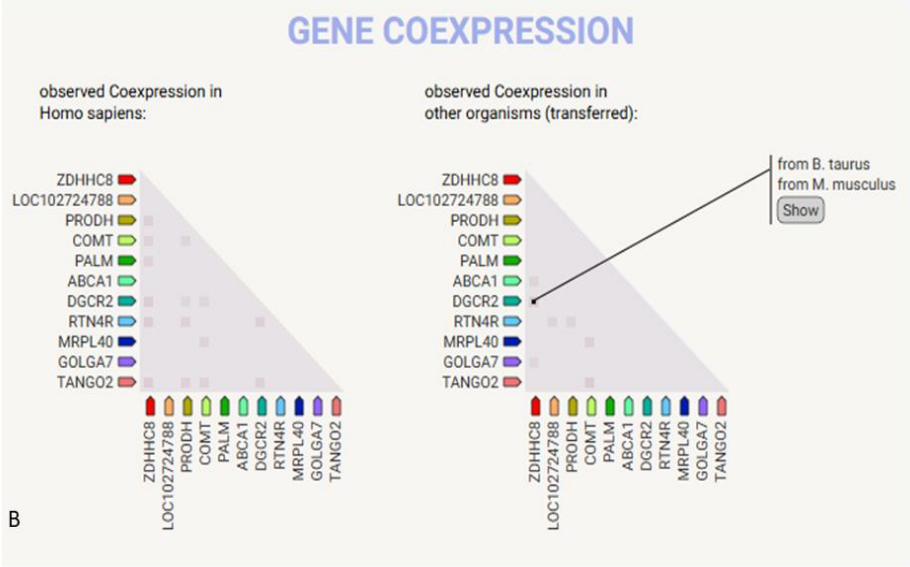


Figure- 4B.Co-Gene expression of candidate genes in the region using ProteomeHD.

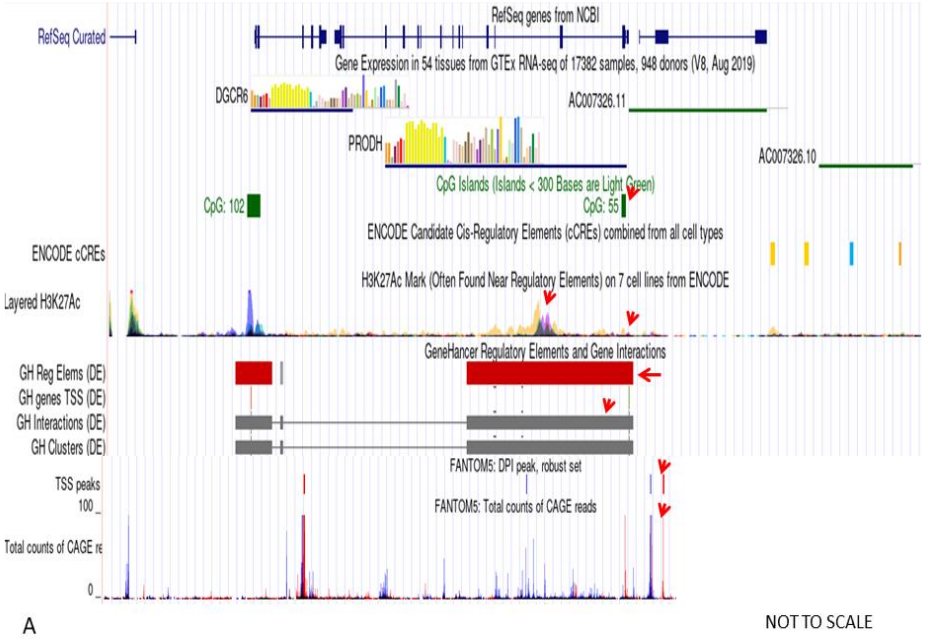
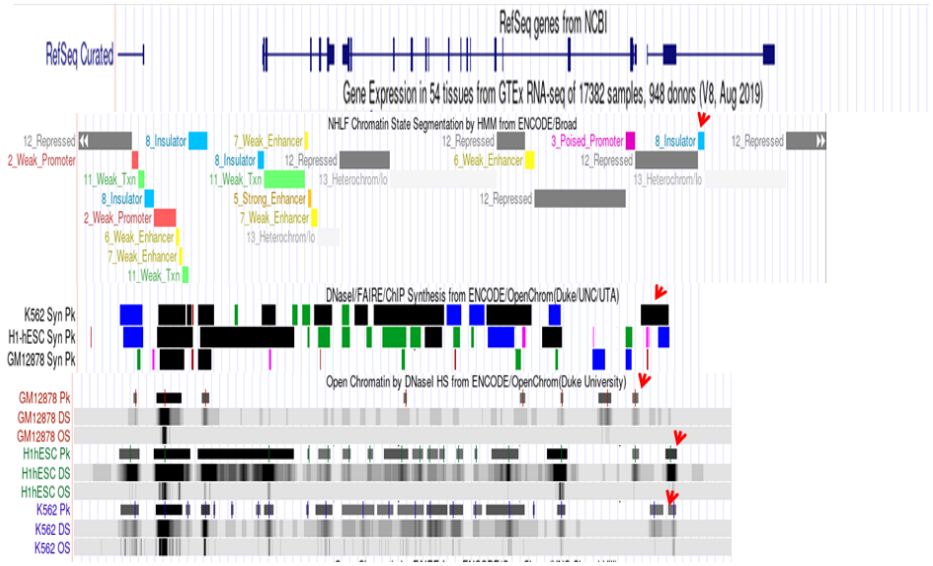


Figure- 5A.Promoter analysis of PRODH gene(ENCODE) depicting CpG island, regulatory elements, clusters, interactions and Transcription start site(TSS). Arrow head – highlights the specific peak. Ref-https://genome.ucsc.edu/encode/



B

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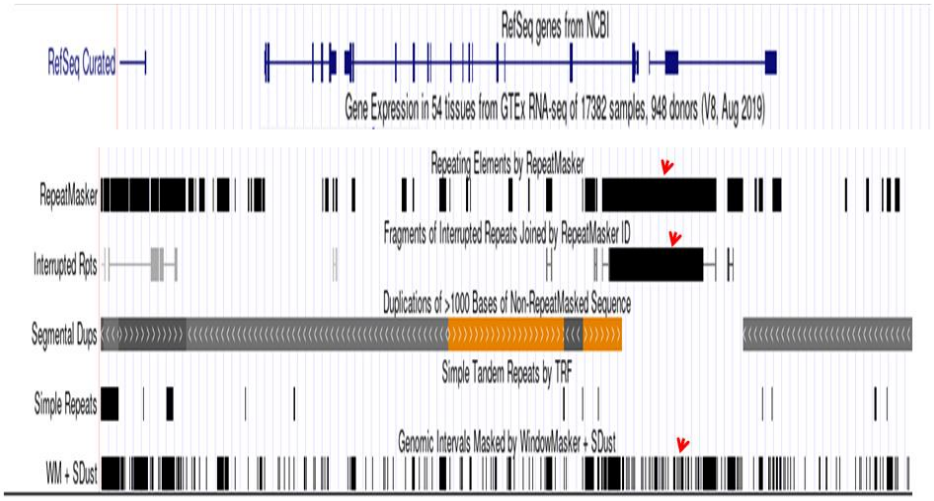
Figure- 5B.Promoter analysis of PRODH gene(ENCODE) for Chromatin elements- DNaSe1 and FAIRE. Arrow head –highlights the specific peak.



C

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Figure- 5C.Promoter analysis of PRODH gene(ENCODE) for repetitive elements. Arrow head –highlights the specific peak.

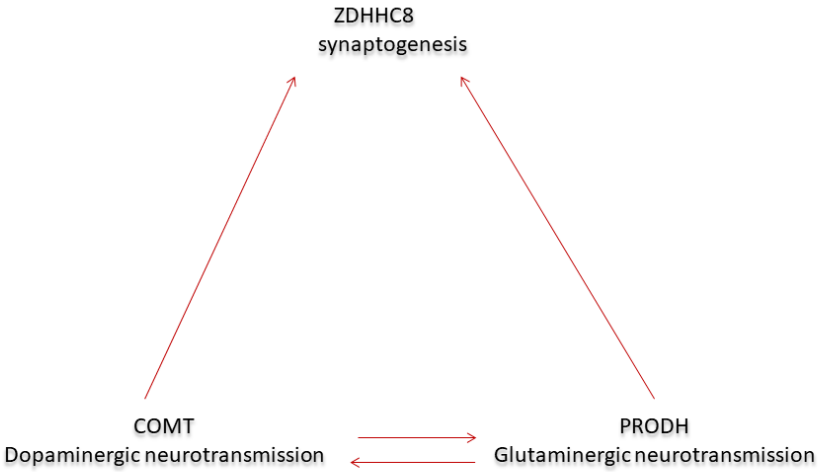

Figure- 6.Plausible model to illustrate the network of COMT,PRODH and ZDHHC8 genes.

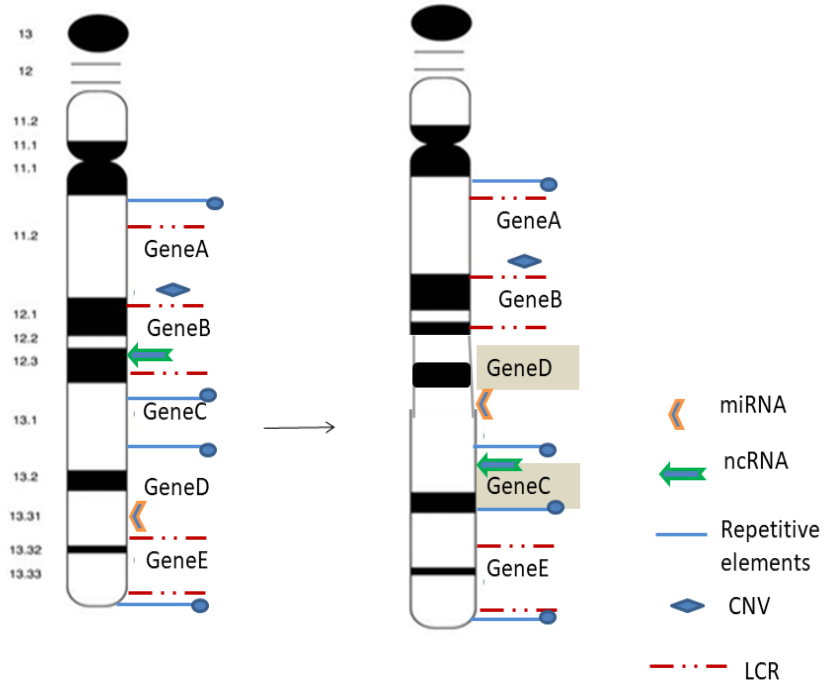

Figure- 7. A probable model illustrating HI mediated intra-chromosomal rearrangement mediated through CNV, LCR, and Repettitve elements

Table- 1.Common dosage sensitive genes identified in few studies.

Genes identified by	Dosage sensitive genes		
	COMT	**ZDHH8**	**PRODH**
Van berevan 2012	+		
Sivagnanasundaram 2007		+	+
Salaka 2017	+		
Jalbrzikowski 2015	+	+	+
Kiran et al 2012	+	+	+

Table-2.Haploinsufficiency scores of genes

no	Gene	Haploinsufficiency score(pHap score)
1	COMT	0.27
2	PRODH	0.32
3	ZDHH8	0.39

Table-3.Details of the enhancers associated with PRODH gene.

No	Enhancer (SCORE)	Distance from TSS (Kb)	TF binding
GH22J018923	2.8	+50	83
GH22J018969	1.6	-33.7	23
GH22J018965	0.4	-29.0	4
GH22J018953	0.7	-17.9	22
GH22J018950	0.4	-14.2	3

Dosage sensitive genes identified in study(Van berevan 2012)	Dosage sensitive genes identified in study(sivagnanasundaram 2007)	Dosage sensitive genes identified in study(Salaka 2017)	Dosage sensitive genes identified in study(jalbrzikowski 2015)	Dosage sensitive genes identified in study(kiran et al 2012)
COMT	ZDHH8	COMT	COMT	PRODH
	PRODH		PRODH	COMT
			ZDHH8	ZDHH8

no	Gene	Haploinsufficiency score(pHap score)
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