

ADDRESSING CRISPR/CAS9 CHALLENGES IN AUTISM GENE EDITING: NAVIGATING OFF-TARGET EFFECTS, IMMUNOGENICITY, AND APOPTOSIS

Abstract

Autism spectrum disorder is an array of neurodevelopmental disorders with wide range of phenotypic and genetic diversity. Autism is a complex, behaviourally well-defined syndrome associated with pleiotropic genes. Majority of neuropsychiatric disorders are characterized by cognitive and social impairments with restricted and stereotypic patterns of eyes, fingers and toes. The neuro atypical disorders present on the wide spectrum of autism are believed to have strong genetic contribution with high heterogeneity, complicating the discovery of complete aetiology involved in ASD. A multitude of risk factors associate with the severity of autism that may develop into irreversible, lifelong neurological syndrome suggesting the importance of premature prediction and treatment of ASD. The early onset diagnosis of behavioural phenotype in children will greatly progress the research on genetics and neuroscience to guide the discovery of precise medicine for the treatment of ASD. Currently, CRISPR/CAS9 is the most efficient and genetic engineering technique for correcting disease specific mutations with wider clinical applications. It is a promising gene editing tool to develop in vivo and in vitro models that mimics the human phenotype with fewer off targets effects. CRISPR/CAS9 is applied in the clinical research domain to promote the discovery of target therapeutics and treatment against ASD.

Keywords: Autism spectrum disorder, neuropsychiatric, CRISPR/CAS9, behavioural phenotype, heterogeneity, pleiotropic, cognitive.

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I. INTRODUCTION

Autism spectrum disorder (ASD) is a constellation of highly heritable neurodevelopmental disorders with permanent phenotypic and genetic heterogeneous conditions (Hayek et al.,2020). These complex disorders affect all areas of child development (Aishwriya et al.,2022). They are characterized by impairments in social interactions, repetitive and restricted stereotyped behaviours, sensory abnormalities (Cao et al.,2022), and stubborn behaviour patterns (Liu et al.,2022). Globally, it is estimated that the prevalence of autism is 0.62-0.7% (Choi et al.,2017), indicating that 6 in 1000 are associated with autism and believed to have unclear aetiology(Faras et al.,2010). A multitude of causative agents contribute to autism, accompanied by a large number of genetic and environmental factors (Muhle et al.,2004). Genome-wide linkage and association studies (GWAS) have recognized multiple mutations in hundreds of risk genes associated with ASD in all human chromosomes (Genovese & Butler,2020).

The pleiotropic trait of ASDs made it challenging to detect the ASD-connected genes with rare missense variants that enhance the progression of neurodevelopmental disorder autism. (Diarmid et al.,2020). The genetic diversity of autism is attributed to irreversible ASD-associated syndromes (Choi et al.,2017). Therefore, early prediction is essential to prevent the onset of ASD-associated phenotype. (Hulbert and Jiang et.al.,2017) The advancements in genome editing technologies have unlocked the door for early diagnosis (Sandhu et al.,2023). They can alter the genetic mutations that contribute to the development of neurological disorders like ASD. (Kareklas et al.,2023). CRISPR-CAS9 is a promising genome editing tool aimed at curing a spectrum of neurodevelopmental disorders, including autism (Kennedy& Henderson,2017). It is regarded as the most accurate and advanced technique with a low mutation rate, high target and cost efficiency. The rising era of CRISPR-CAS9 enables the understanding of disease-related phenotypes and can be the diversity underpinning the pathogenesis of ASD. (Sandhu et al.,2023) It has excellent therapeutic potential against incurable genetic disorders like ASD(Sharma et al.,2020). Globally, this technology is considered a triggering revolution in genome editing (Lopez et al., 2020) to mimic the ASD-associated phenotype in different systems like *in vitro* cell lines, *in vitro* 3D organoid models and *in vivo* animal models. (Sandhu et al.,2023) Therefore, CRISPR-CAS9 is a powerful and most accepted technique for altering ASD-associated genes (Shao et al.,2016).

II. AUTISM SPECTRUM DISORDER (ASD)

Autism spectrum disorder (ASD) includes a wide range of early-onset neurodevelopmental disorders (Hoffmann et al.,2021), with an estimated prevalence of 1 68 in children across the world (Choi et al.,2017). Neurodevelopmental disorders like autism affect the mental, physical and social lives of ASD-affected individuals and their families. Early studies of autism were started in 1910 by a Swiss psychiatrist, Eugen Bleuler, who described patients diagnosed with schizophrenia (Kennedy& Henderson,2017). Later, in 1943, Leo Kanner developed a study on a group of Asperger's syndrome child patients experiencing social impairments in communication as a moderate form of autism. In 1977, Folstein and Rutter studied abnormal neural development in twins possessing behavioural anomalies due to the presence of causative genes of autism (Domadenik et al.,2018). Recent

studies suggest that the outbreak rate of autism in siblings is approximately 2% to 8%, estimated to be about 60% in monozygotic twins (Hong & Lakoucheva,2023).

Autism is not a disease but a syndrome with unclear aetiology (Muhle et al.,2004). Recent studies have suggested that genetic factors contribute to 50% - 60% of ASD cases (Wang et al.,2022). Most ASD-associated gene loci are present on chromosome X (Deneault et al.,2018); this proves that the ASD ratio of male to female is about 4:1 (Lopez et al.,2020). MRI studies identified that autism patients are accompanied by increased brain weight and size (Bailey et al.,1998), which regulates the impairment of neural and synaptic development and leads to dysfunctions in the regions of the brain that control high cognitive functions (Gordon & Geschwind,2020). The Research Domain Criteria guide advancements in genetics and neuroscience that help develop precision medicine against ASD(Canitano&Bozzi,2017). Research on these genetic mutations helps in the clinical discoveries for affected individuals (Kennedy& Henderson,2017). Therefore, these ASD-connected genes provide a therapeutic target for treating ASD(Sandhu et al.,2023).

III. BEHAVIOURAL PHENOTYPE

Autism is a subgroup of a broad spectrum of neurodevelopmental disorders referred to as pervasive developmental disorders (PDD) (Faras et al.,2010). It is clinically characterized by deficits in 3 behavioural domains, including social interaction, language communication and range of interests (Muhle et al.,2004). The manifestations of social impairment in autism are complex and are accompanied by social dysfunction, lack of emotions, and stubbornness (Bailey et al.,1998). The hallmarks of language problems include delayed speech, poor defensiveness, preserved eye contact, hyperactivity and repetitive hand and finger patterns (Clifford et al.,2007). A study on pleiotropic genes of ASD revealed that the specific skills, interests and characteristics of relatives will contribute to the phenotypic behaviour of autistic individuals (Bailey et al.,1998) associated with a broad spectrum of other characteristics such as intellectual disability(ID), anxiety, motor problems and developmental deficits (Gabellini et al.,2022)All these behaviours are long-lasting and declining quality of life (Hoffmann et al.,2021) .

IV. CLASSIFICATION

Depending on the origin of ASD, it is broadly categorized into two groups: Syndromic ASD and Non-Syndromic ASD. Syndromic ASD has a clear aetiology (Lopez et al.,2020) and is accompanied by severe symptoms contributed by monogenic syndromic forms of ASD like Fragile X, Rett, *MECP2* duplication, and Timothy syndromes. These Syndromes are attributed to about 5% of ASD studies (Ayhan& Konopka,2018). It is estimated that 80% of ASD cases do not possess a clear aetiology categorized under non-syndromic ASD. It is also called an idiopathic syndrome, caused by multiple risk factors, including environmental and epigenetic factors (Santos et al.,2023).

1. Aetiology: Even though the prevalence of ASD is increasing globally, the principal cause of ASD is not yet known (Choi et al.,2017), and there is a poor understanding of the mechanisms and pathogenesis involved in autism (Diarmid et al.,2020). ASD is caused by multiple prenatal, perinatal and postnatal factors that may be either genetic or environmental (Lopez et al.,2020). More than 100 genes contribute to the aetiology of

ASD (Diarmid et al.,2020). Recent studies prove that ASD is caused by multiple genetic mutations that collectively influence the brain network pathways (Liu et al.,2022). Many researchers believe that the majority of ASD-associated genes control the expression of other genes (Rea &Raay,2020), and each gene is responsible for < 1% of autism cases and none of them have a complete contribution to the pathogenesis of ASD (Diarmid et al.,2020). Studies on twins revealed that monozygotic twins have a 90% concurrence rate, whereas in dizygotic twins, it is approximately 10% (Faras et al.,2010). The pleiotropic nature of ASD-connected risk genes (Domadenik et al.,2018) is the major challenge in diagnosing and treating ASDs (Diarmid et al.,2020).

Numerous non-syndromic factors contribute to the development of ASD (Sandhu et al.,2023). Multiple environmental factors accompany the development of ASD, including parental age, pregnancy and birth issues, vitamin D deficiency (Lopez et al.,2020), maternal diabetes (Liu et al.,2022), maternal smoking (Lyll et al.,2014) and prenatal exposure to toxins, teratogenic agents like valproate acid, (Canitano&Bozzi,2017) bacterial, viral pathogens, (Gordon & Geschwind,2017) certain medications and heavy metals (Domadenik et al.,2018). The epigenetic mutations and parental and infant microbiome will also influence the pathogenesis of ASD (Rea &Raay,2020). Chaste and Leboyer demonstrated that ASD-associated children will experience more prenatal and perinatal complications than their siblings, revealing that environmental risk factors easily influence children with ASD. ASD is also correlated to the birth order; the first-born offspring have a higher risk rate of ASD than later individuals (Liu et al.,2022). These factors may induce genetic mutations and cause damage to genetic codes that result in the impairment of brain development (Lyll et al.,2014).

It is evident that there is no unanimously agreed cause of ASD, as multiple genetic and environmental traits contribute to ASD (Lyll et al.,2014); hence, it is referred to as a highly heterogeneous and inheritable disorder (Sandhu et al.,2023). Despite many environmental stimuli, the pleiotropic nature of ASD-associated genes has a more significant contribution to the aetiology of ASD (Ayhan& Konopka,2018). The genetic diversity combined with various environmental factors is a challenging problem for developing potential therapeutics against ASD (Rea &Raay,2020).

- 2. Genetic Iceberg of ASD:** In 2020, the whole genetic information of ASD risk genes was assembled in a promising database known as the Simons Foundation for Autism Research Initiative (SFARI) Gene Database (Lopez et al.,2020). Recent studies have reported that approximately 913 genes have enrolled in the SFARI database (Lu et al.,2020). Depending on the contribution of individual genes in the progression of ASD, a gene scoring system has been established by the SFARI database (Rea &Raay,2020). ASD-associated genes are categorized into three idiopathic categories: Score 1 genes are high confidence ASD risk genes with at least three de novo mutations in ASD individuals, whereas Score 2 genes are strong candidates with two de novo disrupting mutations in ASD patients and score three genes are suggestive evidence with one recorded de novo disrupting mutation associated with ASD (Lopez et al.,2020). Current studies have discovered 255 high-confidence ASD risk genes. Among them, 161 genes regulate the progression of ASD by haploinsufficiency (Tamura et al.,2022).

The present era has gained much success in detecting risk genes associated with Autism Spectrum Disorder (ASD) (Hong & Lakoucheva, 2023). The genetic anomalies of ASD are classified into three categories: monogenic mutations (shank3, MeCP2, Cntnap, Fmr, Ube3a, Chd, Tsc, Otr), polygenic mutations and copy number variations. (Wang et al., 2022). Current GWAS studies revealed that the specific phenotype of autism is contributed by single-nucleotide polymorphisms (Choi et al., 2017), and the function of individual gene and their interactions will be associated with the complex genetic architecture of ASD (Rawsthorne et al., 2020). The rare de novo mutations of ASD-connected genes (Santos et al., 2023) will code for the proteins that regulate transcription and neural and synaptic development (Gabellini et al., 2022), and their mutations will eventually lead to impairment in the brain regions that regulate cognitive functions of the individual (Sandhu et al., 2023). It is observed that the expression of these risk genes is considerably higher during prenatal brain development (Gordon & Geschwind, 2020). Therefore, this plethora of studies will help better understand molecular and cellular mechanisms underlying ASD (Hoffmann et al., 2021).

- **Shank:** SH3 and multiple ankyrin repeat domain proteins are scaffold proteins of the postsynaptic density that help bind neurotransmitter receptors and ion channels to actin cytoskeleton and G-protein-coupled signalling pathways. Genetic mutations in the shank are associated with ASD, characterized by deficits in social interaction and communication, restricted interests, repetitive behavioural phenotype (Wang et al., 2022) and neuronal morphological impairment (Lu et al., 2020). Shank3, a member of the Shank gene family (Kumita et al., 2019), is located in the 22q13.3 chromosome (Liu et al., 2022). Single gene mutations in the SHANK3 gene, which encodes for scaffolding proteins, play a vital role in the development of ASD (Tu et al., 2018). Additionally, deletion of the SHANK3 gene results in the development of Phelan-McDermid syndrome, which is characterized by low muscle tone and intellectual disability that are similar to ASD-associated individuals. It is observed that the disruptions of the SHANK3 gene were identified in 10% of ASD patients (Liu et al., 2022). The principal mechanism involved in the Shank 3 mutation is haploinsufficiency (Tu et al., 2018), which highly contributes to the development of autism and schizophrenia in humans (Kumita et al., 2019). In addition, genetic modifications in Shank 2 gene will enhance the number of synapses and alter the diversity of the dendritic tree (Gordon & Geschwind, 2020), which results in the abnormal functioning of chromatin, ribosomes, mitochondria, GABA (γ -amino butyric acid) neurons, and oligodendrocytes of ASD associated individuals (Wang et al., 2022).

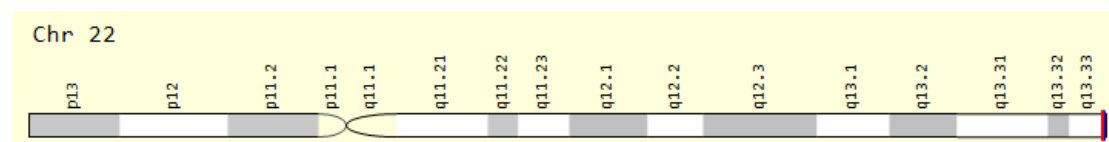


Figure 1: The Genomic Location of SHANK3 Gene in Humans.

- **MECP:** Methyl CpG binding protein-2 (MECP2) is an epigenetic regulatory protein encoded by an X-linked gene MECP2 (Cleber & Trujillo, 2021). Current studies

revealed that MECP2 is a transcriptional regulator that can act as an activator and repressor based on its accompanying partner (Rea & Raay, 2020). It has a broader role in regulating synaptic development (Trujillo et al., 2021), chromatin modification, alternative splicing, and micro RNA synthesis, ultimately stabilizing the whole genome transcription (Xiang et al., 2020). Mutations in the MECP2 gene may impact synaptogenesis and neural circuit organization (wang et al., 2022), which leads to neurodevelopmental impairment (Trujillo et al., 2021). Disruptions in MECP2 in hemizygous males result in encephalopathy in infants, which is lethal. In contrast, in females, it can cause a complex neurodevelopmental syndrome on the wide range of autism spectrum referred to as Rett syndrome (RTT) (Rea & Raay, 2020). The prevalence of Rett syndrome in females is estimated to be approximately 1 in 10,000. Individuals with RTT are characterized by neurological traits such as cognitive deficits, motor anomalies, epilepsy and autistic behaviours (Tsuchiya et al., 2015).

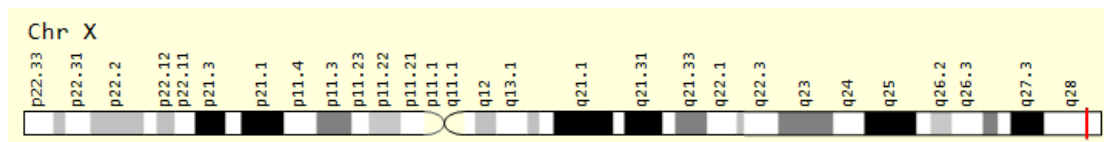


Figure 2: The Genomic Location of MECP2 Gene in Humans

- UBE3A:** Ubiquitin protein ligase E3A (UBE3A) encodes an E3 ubiquitin ligase, which controls the function of various neurodevelopmental-associated genes and regulates target protein degradation by proteasome. Additionally, it can perform transcriptional coactivator activity for steroid receptors (Elamin et al., 2023). Recent human neuron and brain organoids studies reported ubiquitin-mediated degradation of calcium and voltage-dependent amperipotent potassium (BK) channels silence neuronal anxiety (Sun et al., 2019). UBE3A is the only gene that is expressed exclusively from the maternal allele, suggesting that any duplication (Elamin et al., 2023) in this gene will result in the maternally heritable syndrome called Dup15q (Elamin et al., 2023). Globally, the duplication in the maternal isodicentric 15 chromosome 15q11-q13 is observed in 1%-3% of ASD-associated individuals (Xu et al., 2017). It is identified that both deletion and over-expression of UBE3A will change the dendritic organization, revealing that the balanced expression level of *Ube3a* is essential for a stable dendritic network (Wang et al., 2022).

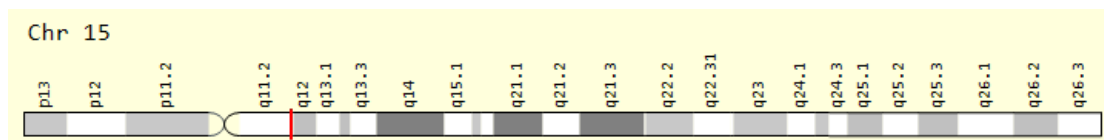


Figure 3: The Genomic Location of UBE3A Gene in Humans

- Chromatin Helicase DNA-Binding Protein (CHD):** The members of the Chromatin helicase DNA-binding protein family have a wider role in axon progression and synaptic adherence that are essential for brain development. CHD8, CHD9 AND CHD11 are exclusively involved in the aetiology of ASD (wang et al., 2022). CHD8 is one of the most commonly mutated genes observed in ASD patients (Hoffmann &

Spengler,2021). That encodes a transcriptional repressor protein called CHD8 that binds with the β -catenin to control the WNT signalling pathway(Rea & Raay,2020). Mutations in CHD8 will create a fluctuation between excitatory and inhibitory signals that are associated with ASD (Wang et al.,2022) and are characterized by facial dysmorphisms commonly observed in the forehead and eyes and dorsally rotated ears (Hoffmann & Spengler,2021) impairment in social interaction followed by macrocephaly. Slow myelination is observed in one-third of the individuals carrying CHD8 mutations. The Past research studies on deletions in the CHD8 gene reported that up-regulated genes enhance the functions of the cell cycle, RNA splicing and transcription down-regulated genes promote neuron differentiation and cognitive development (Hoffmann et al.,2021). The heterozygote disruptive de novo mutations in *CHD8* have a high contribution to the pathogenesis of ASD. The most common symptom CHD8 de novo mutated patients exhibit is atypical wide head circumference (Hoffmann & Spengler,2021).Moreover, most of the ASD associated genes such as ARID1B, ADNP, ASH1L, CUL3, DYRK1A, PTEN, RELN, SHANK3, SCN2A, SETD5, and SYNGAPP1 are well known targets for CHD8 (Rea and Raay,2020).

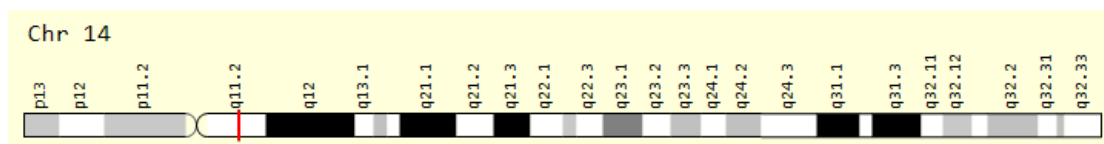


Figure 4: The Genomic Location of CHD8 Gene in Humans

- Patched Domain Containing 1 (PTCHD1):** Patched domain containing 1 (PTCHD1) and its antisense lnc RNA (PTCHD-AS) are located on Xlinked chromosome (Xp22.11). PTCHD1 consists of 12 known transmembrane domains and their expression was observed within plasma membrane of dendritic spines in neural network. It is prominently expressed in pituitary gland and cerebellum regions of human brain. The C-tail PD2 binding domain of PTCHD1 interlink the components within retromer complex and post synaptic density providing a potential evidence of endosomal protein sorting in dendritic spines. Whole genomic analysis of PTCHD1 revealed that it plays an important role in the aetiology of ASD. A study performed in 2008 reported, the copy number variants (CNVs) containing micro deletion of first exon around 167kb in Xp22.11 chromosome were transmitted from a carrier mother to an ASD associated male and his dizygotic cognitive disabled twin brother leads to the production of null allele. (Pastore et al.,2022) The genetic disruption of PTCHD1-AS enhances the number of synapses (Gordon and Geschwind,2020) results in the impairment of excitatory neurotransmission in males (Ross et al.,2023). A plethora of studies on ASD male patients have found, heritable and de novo disruptions in Xp22.11 influence both PTCHD1, PTCHD1-AS and produce point mutations in the coding sequence of PTCHD1 which strongly contribute in the production of missense, nonsense and truncating variants (Pastore et al.,2022)

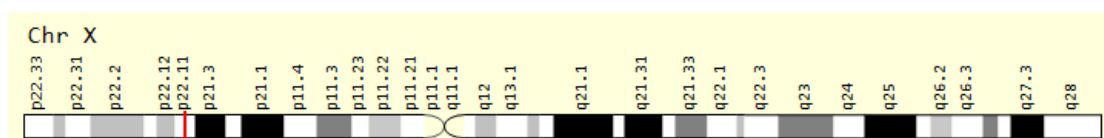
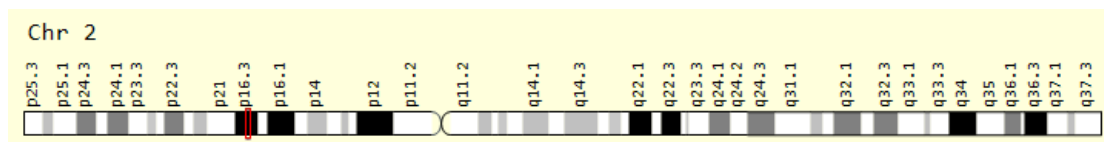
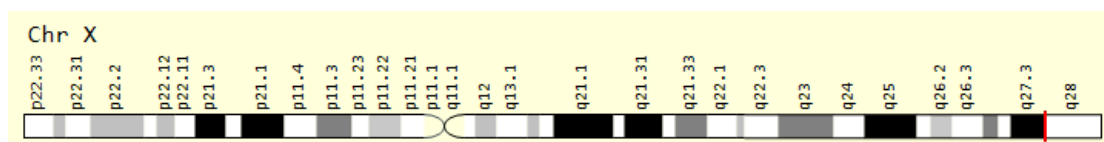


Figure 5: The Genomic Location of PTCHD1 Gene in Humans

- **Neurexins 1 (NRXN1):** Neurexins are pre-synaptic cell adhesion proteins (Wang et al.,2022) that regulate synaptic function and its adhesion in the central nervous system of the vertebrates. NRXN1 is a synaptic adhesion molecule (Rea &Raay,2020) located on chromosome 2p16.3 (Kim et al.,2008). It produces a calcium-associated neurexin network in the synaptic region that contributes to the progression of excitatory neurons (Rea &Raay,2020). A study regarding multiple clinical phenotypes of two subjects with disruptions in the 2p16.3 chromosome has reported NRXN1 as a novel autism risk gene (Kim et al.,2008). Heterozygous microdeletion in NRXN1 is the primary mechanism involved in the pathogenesis of autism (Rea &Raay,2020). Like NRXN1, NRXN2 is accompanied by autism spectrum disorder (ASD), mediating synaptogenesis and maintaining synaptic equilibrium (Lu et al.,2020). The complexity in the expression of NRXN1 is a challenging problem in diagnosing ASD (Rea &Raay,2020).

**Figure 6:** The Genomic Location of NRXN1 Gene in Humans

- **Fragile X Messenger Ribonucleoprotein (FMR1):** The FMR1 gene is located on X- a linked chromosome that encodes fragile X mental retardation protein (FMRP), a polyribosome-dependent molecule that regulates protein translation in neurons (Wang et al., 2022) synaptic plasticity (Giri et al., 2019) and modifications in dendritic spines. The most common mutation of the FMR1 gene is identified in the five ' untranslated regions formed due to the repeated expansion of the CGG triplicate followed by hypermethylation of the X chromosome in males and active X chromosome in females. The polyribosome-dependent protein (FMRP) represses the translation process to regulate the synthesis of other neural proteins in the synaptic region. The disruptive FMR 1 gene is estimated to contribute to 5% of ASD cases worldwide. (Rea and Raay,2020) Mutations in the FMR1 gene associated with Fragile X Syndrome (FXS) are characterized by delayed brain development and autism behavioural phenotypes (Giri et al.,2019).

**Figure 7:** The Genomic Location of FMR1 Gene in Humans

- **Sodium Voltage-Gated Channel Alpha Subunit 2 (SCN2A):** Sodium channel, voltage-gated, type II alpha subunit (SCN2A) encodes a voltage-gated sodium channel (VGSC) expressed in the initial segment (Rea &Raay,2020). It mediates the

synthesis of action potentials in excitatory cortical neurons followed by benign familial neonatal (infantile) seizures and developmental and epileptic encephalopathy-11 (Ghosh et al.,2023). Haploinsufficiency of *SCN2A* is the major contributing factor for ASD (Sanders et al.,2018). Loss of function (LOF) mutations in the *SCN2A* gene are characterized by autism-like behaviour, while gain of function (GOF) mutations are accompanied by neonatal epilepsy (Ghosh et al.,2023). In 2012, a study found that approximately 200 de novo missense mutations are responsible for 1% of epilepsy cases (Wong et al.,2021). Any deletions and mutations in *SCN1A* are associated with Dravet Syndrome, characterized by infantile epilepsy and tonic-clonic seizures. Therefore, *SCN2A* is a potential therapeutic target for treating neurodevelopmental disorders like autism (Ghosh et al.,2023).

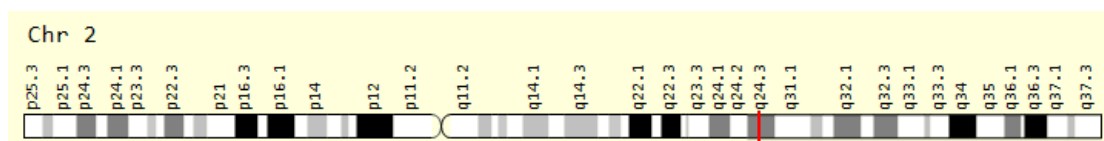


Figure 8: The Genomic Location of *SCN2A* Gene in Humans

- Neuroligins (NLGNs):** Neuroligins (NLGNs) are post-synaptic cell adhesion proteins (Rawsthorne et al.,2020) with different domains, such as extracellular cholinesterase-like globular domain and PDZ binding intracellular domain located on multiple genes in humans. NLGN1 and NLGN2 are located on autosomal chromosome (3q26) and (17p13), respectively, while NLGN 3 (Xq13), 4X (Xp22.3) are located on X- linked chromosome and 5 (Yq11.2) located on Y- linked chromosome(Zhen Liu1 et al.,2022). The X-linked genes play a broader role in neural synaptic function (Choi et al., 2017).NLGNs are transmembrane proteins that interact with pre and post-synaptic receptors to promote signal transmission between neurons. R451C mutation is the first recorded mutation on NLGN3, identified in two siblings associated with ASD that are characterized by membrane trafficking of NLGN3, unfolded protein response (UPR), catalytic degradation of NLGN3 by the proteasome (Liu et al.,2022), misfolding, impaired synaptic function and plasticity (Rawsthorne et al.,2020).

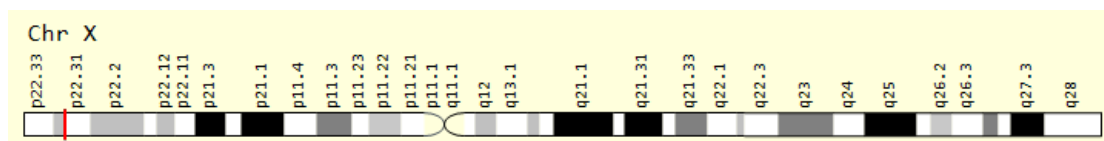


Figure 9: The Genomic Location of *NLGN 4X* Gene in Humans

- Contactin-Associated Protein-Like 2 (CNTNAP2):** Contactin-associated protein-like 2 (CNTNAP2) is considered one of the most significant genes that encode an axon-linked cell-anchored protein, contactins with 1,331 amino acid residues. CNTNAP2 is a trans-membrane molecule that belongs to the NLGNs family of post-synaptic cell adhesion proteins that promote synaptogenesis in the neural network. Genetic modifications in CNTNAP2 include copy number variants, deletions and missense variants, which are associated with delayed growth of cortical nerves and impaired excitatory and inhibitory neural transmissions, resulting in the development

of neurological syndromes such as Tourette syndrome and ASD (Rea & Raay, 2020). Studies on the contactin-associated protein-like (CNTNAP) family showed that they play an essential role in synaptic progression and social skills. For example, CNTN4 regulate the changes in neural morphology. Similarly, CNTNAP3 forms complexes with scaffold proteins and synaptic adhesion molecules (Wang et al., 2022).

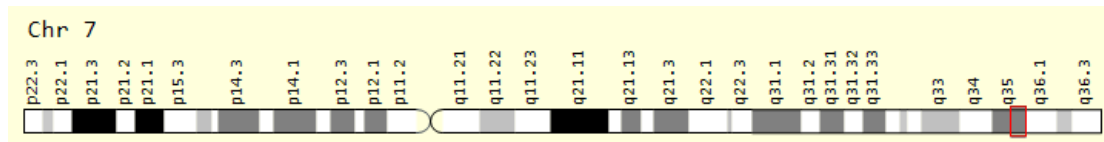


Figure 10: The Genomic Location of CNTNAP2 Gene in Humans

Transient receptor potential canonical (TRPC) channels are encoded by the TRPC6 gene that belongs to a family of Calcium cation permeable channels. De novo disruptions in TRPC6 induce hyperexcitability associated with impairment in socialization, suggesting TRPC6 have a solid contribution to the aetiology of ASD (Shin et al., 2023). (Kaiser et al., 2022) addressed PAX5 as a potential risk factor for ASD as its breakdown may produce a monogenic form of ASD. Phosphatase and tensin homolog (*PTEN*) has been observed as a candidate risk gene of ASD associated with impaired social interaction (Rea & Raay, 2020) and protein instability caused by missense mutation of the gene (Wong et al., 2020). Another gene, *DYRK1A*, contributes to the onset of ASD and is described as a significant driver of 21q22.13 truncation, leading to the inherited cause of cognitive disability microcephaly and autism spectrum disorders (Kim et al., 2017). *MBD5*, a candidate risk gene for neurodevelopmental disorders like ASD, encodes methyl-CpG-binding domain five protein. In addition to the haploinsufficiency of *MBD5*, de novo mutations and significant copy number variants (CNVs) collectively contribute to the pathogenesis of ASD (Seabra et al., 2020). *POGZ* encodes Pogo transposable element derived with ZNF domain (*POGZ*), a transcriptional repressor protein (Papadimitriou et al., 2021). Individuals with a missense mutation of the *POGZ* gene have an intellectual disability, a characteristic feature of ASD (Matsumura et al., 2020). *ITGB3* is an ideal risk gene for ASD that encodes the extracellular matrix β 3 integrin receptor. Haploinsufficiency of *ITGB3* induces hyperexcitability of cortical neurons that causes fragile X syndrome, a monogenic form of ASD (Jaudon et al., 2022).

- 3. ASD Associated Syndromes:** The term autism spectrum disorders (ASD) defines the complex aetiology of ASD (Faras et al., 2010). Especially syndromic ASD is caused by various pleiotropic genes, contributing to a wide range of ASD-connected syndromes (Domadenik et al., 2018). A recent study on genomic analysis reported that over 10% of individuals with genetic deficits express ~100 genetic syndromes characterized by autism-like behaviour (E Deneault et al., 2018). Moreover, ASD cohorts exhibit multiple symptoms, including hyperexcitability, impaired socialization, depression and cognitive deficits that are associated with various genetic syndromes like Rett syndrome, Angelman syndrome, Timothy epilepsy, Fragile-X syndrome (Sandhu et al., 2023), duplication syndrome. Globally, over 4–5% of ASD individuals are associated with Fragile X and Rett syndrome, while 1–3% of them exhibit Angelman syndrome (Rea & Raay, 2020).

Even though the exact cause of ASD is unclear, (6) the pathogenesis of ASD is highly diverse and notably heterogeneous (Hong & Lakoucheva, 2023).

- **Fragile X Syndrome:** Fragile X Syndrome (FXS) is a neurodevelopmental disorder caused by a monogenic mutation of X- the linked FMR gene. The healthy FMR1 gene consists of 6–45 CGG trinucleotide repeats, encoding fragile X mental retardation protein (FMRP) protein essential for normal cognitive development. In permutation, the expansion of CGG repeats vary from 55 to 200; if the range exceeds 200, it is referred to as a complete mutation characterized by severe deficits, including delayed brain development, abnormal behaviour (S Clifford et al., 2007), hyperactivity, anxiety and intellectual impairments are prominently observed in males due to X-linked mode of inheritance (Rea & Raay, 2020). A recent study used the Autism Diagnostic Observation Scale-Generic (ADOS-G) and the Autism Diagnostic Interview (ADI-R) to assess the autism behaviour in FXS patients and reported that 90% of male FXS patients exhibit autism features. In comparison, 3% of females met this criteria. Moreover, FXS carrier mothers play an essential role in their children's early development. It is identified that intellectual disability is the ultimate cause of co-morbidity between FXS and Autism (S Clifford et al., 2007).
- **Rett Syndrome:** Rett syndrome (RTT) is a critical X-linked genetic disorder caused by de novo mutations in methyl-CpG binding protein 2 (MeCP2), a candidate risk gene of ASD (Xiang et al., 2020). The characteristic features of Rett syndrome are early onset of cognitive regression (Rea & Raay, 2020) characterized by delayed head growth, regression of acquired abilities, and stereotypies (Trujillo et al., 2021) followed by impaired speech and motor function deficits. Other symptoms include gait anomalies, primary microcephaly and seizures (Rea & Raay, 2020) and synaptic deficits (Trujillo et al., 2021). Recently (Xiang et al., 2020) found that MeCP2 has a significant role in neural development; its loss of function mutation leads to dysregulation of neural development genes, neuronal function regression and plasticity. Although mutations in MECP2 solely account for Rett syndrome (RTT), the severity of symptoms depends on the cellular distribution of MECP2 (Trujillo et al., 2021). RTT individuals exhibit autism-like symptoms such as intellectual disability, impaired motor skills, and declined cognitive development (Wang et al., 2022).
- **Angelman Syndrome:** Angelman syndrome (AS) is a monogenic (Kennedy & Henderson, 2017) neurodevelopmental disorder caused by the breakdown of the maternally inherited ubiquitin-protein ligase E3A (*UBE3A*) gene (Sandu et al., 2023). *Most AS individuals are associated with suppression of the UBE3A gene on (Sun et al., 2019) maternal 15q11.2-q13 chromosome.* This loss of function is likely a significant drive for Uniparental disomy (UPD) observed in 5-10% of AS patients characterized by irregular ubiquitin-proteasome signalling resulting in the dysregulation of brain neurons. In humans, AS cohorts clinically exhibit physical anomalies, including obesity, muscle stress, delayed growth, speech deficits, uncontrolled laughing and smiling. Most of the defining features of AS are similar to autism phenotype, which impacts brain development, speech deficits and social interactions (Kennedy & Henderson, 2017).

- **Duplication Syndrome:** Duplication syndrome (Dup15q) is a maternally inherited neurodevelopmental disorder caused by the mutation on the long arm of the 15th chromosome in the 11.2-13.1 position. E3 ubiquitin ligase, encoded by the UBE3A gene, has a solid contribution to the aetiology of duplication syndrome as it is the only gene that is inherited from the maternal allele, revealing that maternal duplications are the primary drive of Dup15q syndrome. High mutations in UBE3A exhibit Dup15q phenotype such as hypersensitivity, which can be treated by regulating UBE3A levels. The only difference in the array of neurodevelopmental disorders caused by overexpression of UBE3A and Dup15q is the synaptic behaviour of Dup15q-associated neurons. This suggests that overexpression of UBE3A is the major contributor to disease phenotype, but a small portion of it is also associated with other alleles. The two defining characteristics of this syndrome are Autism and seizures, which are also associated with intellectual deficits and motor function regression. It is identified that ~77% to 100% of Dup15q individuals exhibit autism behaviour phenotype (Elamin et al.,2023).
 - **Timothy Syndrome:** A mutation in a calcium channel-regulating allele, CACNA1C, causes Timothy syndrome (TS). Various autism symptoms characterize this sporadic autosomal dominant disorder. CACNA1C encodes the alpha 1 C subunit of voltage-gated calcium channels (Cav1.2). It was independently discovered from 3 studies performed in 1992, 1995 and 2004. In the majority of the TS-associated children, de novo mutations are observed rather than inherited mode of modifications. De novo disruptions are produced during gametogenesis and expressed after fertilization. The behaviour anomalies associated with TS were first reported in 13 individuals displaying syndactyly of fingers and toes, neuro-atypical features resembling ASD (Bauer et al.,2021).
4. **CRISPR /Cas9:** In 1970, the evolution of rDNA technology unlocked the door for many advancements in gene editing technology (Hsu et al.,2014). Gene editing system contains highly efficient gene tracing tools to alter the disease-causing gene that is used for a better understanding of gene interactions and the aetiology contributing to the disorder(Wang et al.,2022). Gene modification involves the correction of DNA fragments from a single base pair to large segments, followed by the modification of desired cells to an individual organism.(Roderguez et al,2023) The double-stranded breaks (DSBs) generated during gene editing will be removed by two prominent mechanisms, including homology direct repair and non-homologous end joining; the prior is resistant to errors, while the latter is susceptible to errors. They correct the errors either by insertions, deletions or substitutions in the gene of interest (Rodriguez et al.,2019).

In genetic engineering technology, Meganucleases, zinc-finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN) are the most frequently used programmable nucleases. Despite possessing numerous applications, they are highly laborious and show low accuracy (Shao et al.,2016). In 2013, a DNA-RNA recognition-dependent technique called CRISPR was overcome by these limitations (Jiang &Doudna,2017). It is an excellent gene tracing method with a highly efficient

delivery system(52). In contrast to former methods, the CRISPR/Cas9 is cost-effective, requires a short experimental period and can be performed easily. (Rodriguez et al,2023)

- **Mechanism:** In the present era, many researchers are aiming to study the mechanism involved in CRISPR/CAS9 (Hsu et al.,2014). The mechanism of RNA-guided DNA degradation mediated by Cas9 protein involves stages of recognition, cleavage followed by repair (Sandhu et al.,2023)
 - **Recognition:** The attachment of sgRNA to its complementary sequence on the gene of interest initiate the recognition process. In the initial stage, to connect sgRNA and cas9, the REC domain plays a crucial role in developing the sgRNA -cas9 complex. The cas9 nuclease identifies the target DNA. PAM enormously contributes to the accurate binding of the sgRNA-cas9 complex to the target sequence. The DNA recognition and unwinding is carried by PAM-dependent cas9 nuclease. The cas9 identifies PAM followed by the pairing of sgRNA-cas9 complex with its complementary sequence to capture the gene of interest and regulate target site binding while preventing spontaneous self-mutilation. Accurate recognition is essential for precise cleavage (Sandhu et al.,2023).
 - **Cleavage:** The RNA-DNA heteroduplex mediates the unwinding of dsDNA at specific PAM regions. The unwinding enhances the catalytic activation of RuvC and cas9's HNH. The PAM molecule highly regulates the endonuclease activity of cas9. In the cleavage process, the cas9 mimics the role of molecular scissors. The HNH nuclease domain of cas9 breaks the target DNA, complementary to sgRNA, while the RuvC domain cuts the displaced strand, promoting the site-specific cleavage (Shao et al.,2016).
 - **Repair:** The two fundamental cellular pathways involved in the repair mechanism include non-homologous end joining (NHEJ) and homology-directed repair process. The generation of double-stranded breaks (DSBs) by the nuclease activity initiates the repair process. NHE mediates the error-susceptible DNA repair. In NHE-mediated repair, the endogenous DNA repair machinery produces arbitrary insertions, deletions and modifications by breaking and ligating the ends of double-stranded breaks at the gene coding sequence, resulting in the disintegration of targeted gene followed by frameshift mutations and the formation of early stop codons. While HDR mediates error-resistant DNA repair. Double-stranded breaks start the HDR-mediated repair in this mechanism, providing accurate modification (Shao et al.,2016).
- 5. CRISPR/Cas9 Engineered Cellular Models:** ASD, a strongly heterogeneous genetic neuroatypical disorder, requires a highly realistic and reliable technique to detect the modifications in the ASD-associated genes underlying the disease pathology (Hayek et al., 2020). Although in vitro models are heterogeneous, they have notable advantages in discovering candidate ASD risk genes, mechanisms involved and associated symptoms underlying the aetiology of ASD. CRISPR/cas9 overcomes the heterogeneity of in vitro variants and helps generate efficient homogenous cells (Sandhu et al., 2023). In the present era, CRISPR/Cas9 has opened the door to discovering numerous in vitro models with abundant clinical applications. Gene therapy has been extensively used to treat

human neuropsychiatric disorders (Choi et al.,2017). Whole genome sequence analysis identified CRISPR/Cas9 as a potent tool in cell therapies with high specificity (Geen et al.,2015). CRISPR cellular models have numerous advantages over traditional methods, including that they can be easily maintained in laboratory conditions, are inexpensive, have a short proliferation period and have no ethical concerns (Sandhu et al.,2023). From individuals while presenting the genetic notations underlying the ASD (Lopez et al.,2020). The most commonly used cellular models include pluripotent stem cells, human embryonic cells (hESCs), and induced pluripotent cells(iPSCs) (Choi et al.,2017).

(Sun et al.,2019) produced a UBE3A deficient human embryonic stem cell lines (hESC) by using the CRISPR/cas9 technique and found UBE3A knockout hESCs were (karyotypically typical with stable cell division but exhibit hypersensitivity to neuronal networks associated with ASD-like behaviour.Pluripotent stem cells are efficient models for understanding human neurocytological disorders (Martinez et al.,2015). (Trujillo et al.,2023) They validated the significant role of Mecp2 in neuropsychiatric disorders like ASD by using pluripotent stem cells. Loss of Mecp2 in PSCs displayed abnormalities in the synaptic network characterized by cognitive impairments.(Lu et al.,2020) described a CRISPR-mediated miR-873 mutation in neuroblastoma cells that regulates the expression of ASD candidate's risk genes. The loss of function mutated miR-873 cells enhances the levels of targeted genes such as ARIRIB, shank3, and CH8. In knockout neuroblastoma cells, the expression of miR-873 is reduced while the targeted gene expression increases gradually, resulting in the regression of neuronal differentiation.(Elamin et al.,2023) identified, UBE3A solely accounts for duplication syndrome (Dup15q), overexpression of UBE3A associated with the cellular phenotypes. (Elamin et al.,2023) silenced UBE3A allele by using antisense oligonucleotides(ASOs) and introducing it in induced pluripotent stem cells do not exhibit cellular dysregulation. At the same time, the over-expressed UBE3A iPSCs possess irregular neuronal firing and delayed inhibitory synaptic activity, suggesting the role of UBE3A in Dup15q.(Matasmura et al.,2020) They performed a study to reveal the pathogenesis of the POG2 gene associated with ASD. The de novo mutant POG2 gen was derived from ASD patients and was introduced into pluripotent stem cells (iPSC) (Matsmura et al.,2020). POG2 deficient cells exhibit irregular localization of POGZ protein and slow cognitive development.A study described by (Shin et al.,2023) showed the significant role of TRPC6, a candidate gene associated with ASD. De novo mutations are introduced in TRPC6 through CRISPR/Cas9. The loss of TRPC6 in human pluripotent stem cells is characterized by decreased intake of Ca⁺² in hPSCs.

- 6. CRISPR/Cas9 Engineered Organoid Models:** The diverse origin of ASD is one of the significant obstacles in generating target therapeutics for ASD. In this aspect, 3D organoids play a prominent role in understanding the structural and functional properties of the human brain. Researchers aimed to mimic the human brain's neural network, allowing them to discover 3D organoids that have broader applications in the study of autism spectrum disorder. The human stem cells mimic the essential organs, resulting in the formation of organoids. 3D organoids have additional strengths than 2D in vitro models; they maintain homeostasis and can be preserved long (Choi et al.,2017). The formation of 3D organoids has a high resemblance to the developmental stage of the human brain (Ayhan& Konopka,2018). These clinical applications assured the 3D organoids a better model for studying the architecture of the cellular network of the human brain associated with ASD (Gordon & Geschwind,2020). The FOXG1 deficient

organoid model has been generated using CRISPR/Cas9 technology. The genome transcriptional study of these organoid models represents autism-like behaviour corresponding to dysregulation in the size and function of the human brain (Choi et al.,2017). The *Mecp2* knockout model associated with Rett syndrome is characterized by defects in neuronal formation (Gordon & Geschwind,2020). While the loss of function of the *UBE3A* model displays anxiety, cognitive impairment representing ASD (Sun et al.,2019). A healthy organoid is induced with *RECN* disruption mediated by the CRISPR/Cas9 system. The breakdown of the *RECN* gene is characterized by intellectual disability. The *RECN*-associated organoid is notably used for the discovery of target therapeutics against ASD (Choi et al.,2017).

7. CRISPR-Cas9 Engineered Animal Models of ASD: Animal models are versatile tools for studying the pathogenesis of autism spectrum disorder (ASD) and discovering potential therapeutics (Shao et al.,2016) by conducting continuous preclinical trials to enhance the efficiency of novel drugs (Kareklas et al.,2023). Despite numerous advantages, cell culture models may not interpret the complete aetiology of ASD (Lopez et al.,2020).In the present era, CRISPR/CAS9 has vastly increased the efficacy of producing gene-targeting animal models (Zhao et al.,2016). Since the CRISPR/Cas system is the only genome engineering technique that depends on Watson-Crick base pairing rather than the possibly less specific protein-DNA interaction (Hruscha et al.,2013), Such advancements are economical and promote large-scale production of autism-associated models (Hsu et al.,2014).(Lopez et al.,2020) demonstrated the fundamental characteristics of an efficient model, including a high resemblance to human phenotype. This similar mutation contributes to human disorders and is homologous to target therapeutics against human disease. They are the two most commonly used techniques for generating transgenic ASD animal models. In forward genetics, mutations are observed in the preferred animal model that exhibits autism behaviours. In contrast, the selected mutations are introduced into the animal model in backward genetics to observe the disease phenotype(Lopez et al.,2020). Although creating an ASD animal model is laborious and includes ethical issues, it is helpful for a better understanding of autism spectrum disorder and to examine the function of candidate genes in the aetiology of ASD (Sandhu et al.,2023). The most accepted species for the monogenic mutation studies of ASD include mice, rats, monkeys, *Drosophila* and zebrafish, based on the necessity and objective of the study(Wang et al.,2022).

- **Drosophila:** *Drosophila melanogaster*, the fruit fly, is a potent animal model for genetic analysis of ASD as it has numerous advantages, including inexpensive and genetically very accessible (Tane et al.,2021), less breeding period, and massive production of offspring for accurate and comprehensive analysis. A study found that 75% of *drosophila* genes are analogous to human disease-causative genes and have identical neural functions as humans (Wang et al.,2022). These characteristics suggest that *Drosophila* is an ideal model for ASD studies with considerable potential for both high-throughput and in-depth research (Tane et al.,2021).A recent study used *Drosophila* as a research tool and developed a *Drosophila* fragile X mental retardation model (*dFmr1*). The disruptions in the *Fmr1* gene cause fragile X syndrome in *Drosophila*. The mutant flies are characterized by delayed synaptogenesis, perturbation of neurotransmission and synaptic type-specific mutations in the central

and peripheral nervous system. The results revealed dFmr1 flies exhibit anomalies in *Drosophila* stress odorant (dSO) response (Wang et al.,2022).(Cao et al.,2022) experimented on *Drosophila* to study the impact of serotonin levels on autism-like phenotype and revealed that the 5-HT2B receptor mediates the effect of serotonin levels on *Drosophila*'s social behaviour and repetitive stereotype. (Cao et al.,2022) The CRISPR/Cas9 technology deleted the gene encoding the 5-HT2B receptor and observed impaired social communication in mutant flies. When these flies are fed with 5-HTP, social deficits are regulated by serotonin through 5-HT2B receptors in dFB neurons, proving the functions of 5-HT2B receptors in social interaction are independent of circadian rhythm or olfactory neurons.

- **Caenorhabditis Elegan:** *Caenorhabditis elegans* is a model organism that provides an excellent experimental framework for examining the impact of genetic variations linked to ASD. The genetic flexibility, basic nervous system and gene homology of humans promote synaptogenesis and systems-level investigation in *C. elegans* (Rawsthorne et al.,2020).(Rawsthorne et al.,2020) performed an analysis to know the impact of genetic modifications on autism-like phenotype(Elamin et al.,2023) have discovered the gene *neurologin* has a broader role in the regulation of crucial social interaction and (Elamin et al.,2023) used CRISPR/Cas9 to modify R-C mutation that closely resembles a fully penetrant human variant linked to Autism. *C. elegans* possessing this mutation will inherit the behavioural abnormalities of a *C. elegans* *neurologin* null mutant associated with impaired synchronous activity.
- **Zebrafish:** The unique characteristics of zebrafish have gained the attention of numerous researchers working on neurological disorders like ASD (Kareklas et al.,2023). They are well-established models for drug discovery due to their short life cycle and large number of offspring each reproductive session, the tiny size of the body, minimal maintenance requirements, and ease of housing a large number of them in a small aquarium. Regarding the structure of the brain, nervous system, and neuronal signalling pathways, zebrafish exhibit strong similarities to humans. Additionally, zebrafish exhibit outstanding ability for the development of particular social behaviours that are crucial for replicating the symptoms of ASD (Beik et al.,2022). Zebrafish are notable for having vibrant colour vision, which gives them a clear edge over rodent models (Park et al.,2016). Genetic studies suggest that zebrafish and humans share approximately 62% of the 858 genes involved in ASD pathogenesis, which is extremely useful for preclinical research. (Beik et al.,2022) With techniques like CRISPR, it is relatively simple to alter the zebrafish genome, which enables the use of large samples that are usually impossible in mammalian models. (Dallman et al.,2020). Zebrafish with *shank3* mutations exhibit reduced locomotion activity and lower amounts of synaptic proteins produced by CRISPR/Cas9, leading to ASD-like symptoms. Additionally, gastrointestinal dysmotility, which is similar to gastrointestinal symptoms frequently observed in people with ASD, is caused by diminished *shank3* expression in the zebrafish model system, whereas zebra fish with *Cntnap2* mutations exhibited vulnerability to drug-induced seizures, were hyperactive at night, and had GABAergic deficits, particularly in the forebrain (Wang et al.,2022). (Kareklas et al.,2023) It generated a zebrafish *shank3a* mutant to identify the contribution of the *shank3* protein in the expression of

emotion and empathy that are associated with the aetiology of human ASD. (Kareklas et al.,2023) Delayed cognitive development, coordination abilities, and communication defects are joint in humans with ASD and SHANK3 mutant zebrafish. All of the evidence points to the possibility that shank3 mutations in zebrafish cause impairments in social skills.(Rea &Raay,2020) They addressed a knockdown model of the ARID1B gene in zebrafish by utilizing morpholino oligonucleotide injection to study delayed embryonic growth, a significant clinical symptom of ARID1B mutations in humans. In addition, (Rea &Raay,2020) verified two models used to examine the social interaction of fmrl knockout zebrafish, showing specific characteristics like those of human FXS, such as hyperexcitability and memory loss. FMR1 disruptions in zebrafish cause aberrant phenotypes and impairments in craniofacial growth. The knockout shows specific symptoms associated with anxiety but no common morphological abnormalities. (Rea &Raay, 2020) Demonstrated in a study conducted in the Harold Burgess lab, an altered zebra line (chd8y608/+) was generated through a CRISPR-mediated 5-base pair removal in the fourth exon, which led to a frameshift mutation in the resultant transcript. Zebrafish chd8 was previously knocked down by morpholino injection in two separate experiments validated by (Rea &Raay, 2020). In line with CHD8 loss of function in human ASD patients, morpholino-mediated knockdown of CHD8 causes macrocephaly (extension of the forebrain/midbrain). Breakdown of CHD8 in zebrafish is compatible with macrocephaly, and a decrease in post-mitotic enteric neurons that results in gastrointestinal motor deficits contributes to gastrointestinal concerns in ASD patients (Rea &Raay, 2020).With the help of the CRISPR/Cas9 system, (Zhang et al.,2022) created NDE1 knockout zebrafish to study this gene's expression pattern during development. In exon 2, the deletion causes a frameshift mutation and an early stop codon. Additional research was done on shoaling phenotype, social interest, and kin preferences. The findings show that the group tendency is minimal in NDE1 knockout zebra fish during adolescence as well as in adulthood. Analyses of morphology, anatomy, and behavioural phenotype indicate that NDE1 deletion mutations lead to neuron morphological and functional anomalies.(Gabellini et al.,2022) Created zebra fish setd5 mutants using the CRISPR/Cas9 technology and studied their morphological, behavioural, and molecular phenotypes to model human SETD5 haploinsufficiency and observed that setd5 heterozygous mutants exhibit impaired socialization and motor function required for shoaling activity as well as they are uninterested in sociality.

Using CRISPR/CAS9, the functional significance of genes including CHD8, FMR1, nuclear receptor subfamily 3 group c member 2, and SHANK3 in the zebrafish model of autism spectrum disorder has been investigated. These zebrafish models exhibit defined features of ASD, such as macrocephaly, hyperactivity, anxiety, decreased social behaviour, sleep difficulties, and abnormal neural development (Sandhu et al.,2023).

- **Rodents:** Rodents have been considered a standard preclinical tool for better understanding the complicated pathogenesis of ASD and to validate new therapeutics before clinical trials. One of the factors contributing to their success is their ability to replicate the primary symptoms of ASD. They have a brief generation period and can

be maintained easily in the laboratory because of their small size and gregarious nature. Their DNA has also been sequenced, demonstrating a high resemblance to humans. Additionally, techniques to alter both species genomes are established to identify the ASD-associated mutations. (Lopez et al.,2020). In a mouse model, early mutation induces the expression of FMRP protein, cognitive bioenergetics, Zinc-dependent synaptic protein, SH3 and SHANK3, which are associated with fragile X syndrome. The mutant mice exhibit irregular cognitive bioenergetics, impaired SHANK3 expression and abnormal zinc levels, contributing to neurodevelopmental disorders (Canitano and Bozzi et al.,2017).In the MECP2 knockout mouse model, the pyramidal cells within the primary somatosensory cortex are associated with Rett syndrome, characterized by delayed temporal summation and irregular neuronal firing and synaptic network of the neurons. The RTT-linked behaviour phenotypes are reversed by using CRISPR CAS to treat the mutant allele of MECP2, suggesting a novel therapeutic technique for RTT individuals (Wang et al.,2022).

In the cortical hippocampus of CHD8 deficient mice, ASD-associated symptoms include hyperactivity to chemical stimuli, decreased motor function, impaired interactions with peers, and enhanced synchronization. In the female carrier of *Chd8*+/*N2373K* mice, declined coordination activity, inhibitory drive regression and high spontaneous firing are strongly expressed (Wang et al.,2022). (Hayek et al.,2020) developed a *Kdm5A* deficient mice model (*kdm5a*+) using Forward genetics .The loss of function of *kdm5A* knockout mice exhibits repetitive stereotypic patterns, indifference to social responses, impaired brain bioenergetics, and anomalous dendrite production. The defining features of disrupted *kdm5A* variants are the autism-like phenotype and impaired speech. (Hayek et al.,2020) revealed the role of forward genetics in examining the pathogenesis of ASD-associated genes.(Rea &Rayy,2020) addressed multiple studies on heterozygous *Arid1b* mouse model, expressing irregular GABAergic neuron network resulting in high cell death rate and low cell division during juvenile development. The carrier *Arid1b* possesses hydrocephalus that is associated with ASD. Mutations in the cerebellum strongly contribute to neuroatypical ASD cognitive impairment.

In a study described by (Benger et al.,2022), the chemical stimulation of the Right crus I region of the TSC mouse cerebellum is repetitively manifested with behavioural impairments. A knockout mouse with *ARID16* disruptions expressed ASD behaviour, including decreased dyskinesia, hypersensitivity, decreased synchronized activity and weight loss. (Wang et al.,2022). A CRISPR CAS-mediated MECP 2 deficient mouse is responsible for developing Rett syndrome. It is associated with psychiatric characteristics such as epilepsy, intellectual impairments, and motor skill defects (Tsuchiya et al.,2015). Overexpression of *Ube3A* in a mouse model exhibits fundamental autism phenotypes. While phosphorylation of *Ube 3A* by disrupting protein kinase A leads to hyperexcitability of *Ube3A* that manifests with impaired synaptogenesis (Xu et al.,2017). (Xu et al.,2023) *Ctnd2* knockout mice model, characterized by sleep disturbances, is a defining phenotype of Autism. The deletions of *Ctnd2* in ASD cohorts enhance the production of copy number variants.

- Non-Human Primate Models:** Non-human primates are more outstanding models than other species due to their high resemblance of cognitive physiology (Wang et al.,2022). These models are simple to understand (Kumita et al.,2019). Therefore, they are widely used in studies of neurodevelopmental disorders such as Autism and the complex pathogenesis and diverse genes associated with Autism (Wang et al.,2022). (Sandhu et al.,2023) Shank 3 mutated the ASD monkey model by altering exon 21 using CRISPR technology. The F1 progeny manifested with autism-linked symptoms, including intellectual disability and stereotypic behaviour. Overexpression of Mecp 2 in CRISPR-mediated transgenic cynomolgus monkey displayed a harmful number of rotatory motions, anxiety, and low synchronization similar to ASD behaviour. While the loss of function of Mecp 2 in rhesus monkey exhibit core autism phenotypes, including sleep-wake behaviour, hyperactivity, and repetitive hand movements (Wang et al.,2022). They also explored a study on cynomolgus macaques possessing Shank 3 heritable mutations. The F1 progeny of the mutants express stereotypic behaviour, irregular sleep patterns, impaired coordination functions, and defects in cognitive activity resembling ASD phenotype. (Kumita et al.,2019) developed a transgenic marmoset model by introducing a mutation in Shank 3, C- kit through the CRISPER CAS technique. (Kumita et al.,2019) observed the ASD-like features, including intellectual disability, in the knockout marmoset model.

Table 1: Representative Clinical Studies of Neuro Developmental Disorders in CRISPR/CAS9 Mediated Models. This Table Provides an Extensive Overview of Genetic Models Used to Study Neuro Developmental Disorders, Including Associated Genes, Syndromes and Phenotypes.

Model	Gene	Syndrome	Phenotype	Reference
Human embryonic stem cells	Ube3A	ASD	Hypersensitivity	Sun et al.,2019
Pluripotent stem cells	Mecp2	ASD	Cognitive impairment	Trujillo et al.,2023
Neuroblastoma cells	miR-873	ASD	Regression of neuronal differentiation	Lu et al.,2020
Induced pluripotent stem cells	Ube3A	Dup15q	Irregular neuronal firing	Elamin et al.,2023
Induced pluripotent stem cells	POGZ	ASD	Slow cognitive development	Matasmura et al.,2020
Human pluripotent stem cells	TRPC6	ASD	Decrease intake of Ca ⁺²	Shin et al.,2023
Organoid	FOYG1	ASD	Dysregulation in size and function of human brain	Choi et al.,2017
Organoid	Mecp2	Rett	Defects in neuronal formation	Gordon and Geschwind, 2020

Organoid	Ube3A	ASD	anxiety	Sun et al.,2019
Organoid	RECN	ASD	Intellectual disability	Choi et al.,2017
Drosophila	5-HT2B	ASD	Impaired social communication	Cao et al.,2022
C. elegans	Neurologin	ASD	Impaired synchronous activity	Rawsthorne et al.,2020
Zebra fish	Shank 3	ASD	Delayed cognitive developmnt	Kareklas et al.,2023
Zebra fish	Shank 3	ASD	Irregular motility	Wang et al.,2022
Zebra fish	Cntnap2	ASD	GABAergic deficits	Wang et al.,2022
Zebra fish	Arid1b	ASD	Delayed embryonic growth	Rea and Raay,2020
Zebra fish	Fmr1	Fragile X syndrome	Impairments in craniofacial growth	Rea and Raay,2020
Zebra fish	Chd8	ASD	Motor defects	Rea and Raay,2020
Zebra fish	NDE1	ASD	Neuron morphological and functional anomalies	Zhang et al.,2022
Zebra fish	Sedt5	ASD	Impaired socialization	Gabellini et al.,2022
Mouse	Shank 3	Fragile X Mouse syndrome	Cognitive bioenergetics	Canitano and Bozzi,2017
Mouse	Mecp2	Rett	Irregular neuronal firing	Wang et al.,2022
Mouse	Chd8	ASD	Impaired interactions with peers	Wang et al.,2022
Mouse	Kdm5A	ASD	Repetitive stereotypic patterns	Hayek et al.,2020
Mouse	Arid1b	ASD	Irregular GABAergic neuron network	Rea and Raay,2020
Mouse	TSC	ASD	Behavioural impairments	Benger et al.,2018

Mouse	Arid1b	ASD	Weight loss	Wang et al.,2022
Mouse	Mecp2	Rett	Epilepsy	Tsuchiya et al.,2015
Mouse	Ube3A	ASD	Hyper excitability	Xu et al.,2017
Mouse	Ctnnd2	ASD	Sleep disturbances	Xu et al.,2023
Monkey	Shank 3	ASD	Intellectual disability	Sandhu et al.,2023
	Mecp2	ASD	High rotatory motion	Wang et al.,2022
Rhesus monkey	Mecp2	ASD	Hyperactivity	Wang et al.,2022
Macaques	Shank 3	ASD	Irregular sleep patterns	Wang et al.,2022
Marmoset	Shank 3	ASD	Intellectual disability	Kumita et al.,2019

V. LIMITATIONS

Despite numerous notable advancements in CRISPR/CAS9 technology, multiple obstacles remain (Hsu et al.,2014). The high off-target rate of Cas9 is the significant challenging aspect of the CRISPR/CAS9 system that enhances the frequency of mutations in undesired regions with unknown characteristics (Sandhu et al.,2023) associated with various neurological disorders(Lopez et al.,2020). The DSBs produced by CRISPR frequently cause apoptosis, which is also a safety concern. Another significant issue is the immunogenicity employed by viral carriers in Cas9 delivery systems. Cas9 is produced from streptococcus pyogenes, a pathogen that contributes to multiple human diseases, and most patients have developed anti-cas9 antibodies within them. In a therapeutic approach, if Cas9 is introduced into an ASD individual to treat a mutated gene, it will be recognized as a foreign antigen, and immediately, an immune cascade will develop to disintegrate the Cas9 molecule, which would ultimately stop the gene editing process (Sandhu et al.,2023).

VI. CONCLUSION

The complexity and pleiotropic trait of ASD genes expanded the spectrum of autism resulting in the development of multiple neurodevelopmental syndromes mimicking the behavioural phenotype of ASD. Therefore, it is a highly challenging task to identify and classify them based on the phenotype of individuals. Despite of many gene editing technologies, CRISPR/CAS9 have been proven to be the most specific, efficient gene editing tool with promising results. Currently, CRISPR/CAS9 is at the foreground of biotechnological innovation in behavioural and neuroscience domain. The CRISPR/CAS9 mediated in vitro and in vivo models have extended the study of ASD pathology for the discovery of highly efficient target therapies.

REFERENCES

- [1] Aishworiya, R., Valica, T., Hagerman, R., & Restrepo, B. (2022). An update on psychopharmacological treatment of autism spectrum disorder. *Neurotherapeutics*, 19(1), 248-262.
- [2] Ayhan, F., & Konopka, G. (2018). Genomics of autism spectrum disorder: approach to therapy. *F1000Research*, 7.
- [3] Bailey, A., Palferman, S., Heavey, L., & Le Couteur, A. (1998). Autism: The phenotype in relatives. *Journal of autism and developmental disorders*, 28, 369-392.
- [4] Bauer, R., Timothy, K. W., & Golden, A. (2021). Update on the molecular genetics of Timothy syndrome. *Frontiers in pediatrics*, 9, 668546.
- [5] Bengler, M., Kinali, M., & Mazarakis, N. D. (2018). Autism spectrum disorder: prospects for treatment using gene therapy. *Molecular autism*, 9(1), 1-10.
- [6] Canitano, R., & Bozzi, Y. (2017). autism Spectrum disorders: developmental trajectories, Neurobiological Basis, treatment Update. *Frontiers in Psychiatry*, 8, 125.
- [7] Cao, H., Tang, J., Liu, Q., Huang, J., & Xu, R. (2022). Autism-like behaviors regulated by the serotonin receptor 5-HT2B in the dorsal fan-shaped body neurons of *Drosophila melanogaster*. *European Journal of Medical Research*, 27(1), 203.
- [8] Choi, H., Song, J., Park, G., & Kim, J. (2017). Modeling of autism using organoid technology. *Molecular Neurobiology*, 54, 7789-7795.
- [9] Clifford, S., Dissanayake, C., Bui, Q. M., Huggins, R., Taylor, A. K., & Loesch, D. Z. (2007). Autism spectrum phenotype in males and females with fragile X full mutation and premutation. *Journal of autism and developmental disorders*, 37, 738-747.
- [10] Coll-Tané, M., Krebbers, A., Castells-Nobau, A., Zweier, C., & Schenck, A. (2019). Intellectual disability and autism spectrum disorders 'on the fly': insights from *Drosophila*. *Disease Models & Mechanisms*, 12(5), dmm039180.
- [11] Deneault, E., White, S. H., Rodrigues, D. C., Ross, P. J., Faheem, M., Zaslavsky, K., ... & Scherer, S. W. (2018). Complete disruption of autism-susceptibility genes by gene editing predominantly reduces functional connectivity of isogenic human neurons. *Stem cell reports*, 11(5), 1211-1225.
- [12] DOMADENIK, A. (2018). Overview of current mouse models of autism and strategies for their development using CRISPR/Cas9 technology. *Acta agriculturae Slovenica*, 112(1), 19-30.
- [13] Elamin, M., Dumarchey, A., Stoddard, C., Robinson, T. M., Cowie, C., Gorka, D., ... & Levine, E. S. (2023). The role of UBE3A in the autism and epilepsy-related Dup15q syndrome using patient-derived, CRISPR-corrected neurons. *Stem Cell Reports*, 18(4), 884-898.
- [14] El Hayek, L., Tuncay, I. O., Nijem, N., Russell, J., Ludwig, S., Kaur, K., ... & Chahrour, M. H. (2020). KDM5A mutations identified in autism spectrum disorder using forward genetics. *Elife*, 9, e56883.
- [15] Faras, H., Al Ateeqi, N., & Tidmarsh, L. (2010). Autism spectrum disorders. *Annals of Saudi medicine*, 30(4), 295-300.
- [16] Gabellini, C., Pucci, C., De Cesari, C., Martini, D., Di Lauro, C., Digregorio, M., ... & Andreazzoli, M. (2022). CRISPR/Cas9-Induced Inactivation of the Autism-Risk Gene *setd5* Leads to Social Impairments in Zebrafish. *International Journal of Molecular Sciences*, 24(1), 167.
- [17] Genovese, A., & Butler, M. G. (2020). Clinical assessment, genetics, and treatment approaches in autism spectrum disorder (ASD). *International journal of molecular sciences*, 21(13), 4726.
- [18] Gillberg, C. (1998). Chromosomal disorders and autism. *Journal of autism and developmental disorders*, 28, 415-425.
- [19] Gill, P. S., Clothier, J. L., Veerapandian, A., Dweep, H., Porter-Gill, P. A., & Schaefer, G. B. (2021). Molecular dysregulation in autism spectrum disorder. *Journal of Personalized Medicine*, 11(9), 848.
- [20] Giri, S., Purushottam, M., Viswanath, B., & Muddashetty, R. S. (2019). Generation of a FMR1 homozygous knockout human embryonic stem cell line (WAe009-A-16) by CRISPR/Cas9 editing. *Stem Cell Research*, 39, 101494.
- [21] Ghosh, A., Nadella, N., Monaghan-Nichols, A. P., & Chu, X. P. (2023). Gene therapy as an emerging treatment for *Scn2a* mutation-induced autism spectrum disorders. *Fundamental Research*.
- [22] Gordon, A., & Geschwind, D. H. (2020). Human in vitro models for understanding mechanisms of autism spectrum disorder. *Molecular autism*, 11, 1-18.
- [23] Gutiérrez-Rodríguez, A., Cruz-Fuentes, C. S., Genis-Mendoza, A. D., & Nicolini, H. (2023). CRISPR/Cas9 genome editing approaches for psychiatric research. *Brazilian Journal of Psychiatry*, 45, 137-145.
- [24] Hoffmann, A., & Spengler, D. (2021). Chromatin remodeler CHD8 in autism and brain development. *Journal of Clinical Medicine*, 10(2), 366.

- [25] Hoffmann, A., & Spengler, D. (2021). Single-cell transcriptomics supports a role of CHD8 in autism. *International Journal of Molecular Sciences*, 22(6), 3261.
- [26] Hong, D., & Iakoucheva, L. M. (2023). Therapeutic strategies for autism: targeting three levels of the central dogma of molecular biology. *Translational Psychiatry*, 13(1), 58.
- [27] Hruscha, A., Krawitz, P., Rechenberg, A., Heinrich, V., Hecht, J., Haass, C., & Schmid, B. (2013). Efficient CRISPR/Cas9 genome editing with low off-target effects in zebrafish. *Development*, 140(24), 4982-4987.
- [28] Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell*, 157(6), 1262-1278.
- [29] <https://gene.sfari.org/> (assessed on 29 august 2023)
- [30] Hulbert, S. W., & Jiang, Y. H. (2017). Cellular and circuitry bases of autism: lessons learned from the temporospatial manipulation of autism genes in the brain. *Neuroscience bulletin*, 33, 205-218.
- [31] Jaudon, F., Thalhammer, A., Zentilin, L., & Cingolani, L. A. (2022). CRISPR-mediated activation of autism gene Itgb3 restores cortical network excitability via mGluR5 signaling. *Molecular Therapy-Nucleic Acids*, 29, 462-480.
- [32] Jiang, F., & Doudna, J. A. (2017). CRISPR-Cas9 structures and mechanisms. *Annual review of biophysics*, 46, 505-529.
- [33] Kaiser, F. M., Gruenbacher, S., Oyaga, M. R., Nio, E., Jaritz, M., Sun, Q., ... & Busslinger, M. (2022). Correction: Biallelic PAX5 mutations cause hypogammaglobulinemia, sensorimotor deficits, and autism spectrum disorder. *Journal of Experimental Medicine*, 220(1), e2022049812012022c.
- [34] Kareklas, K., Teles, M. C., Dreosti, E., & Oliveira, R. F. (2023). Autism-associated gene shank3 is necessary for social contagion in zebrafish. *Molecular Autism*, 14(1), 23.
- [35] Kennedy, A. J., & Henderson, J. O. (2017). Angelman Syndrome: An Autism Spectrum Disorder. *Journal of Student Research*, 6(2), 56-60.s
- [36] Kim, H. G., Kishikawa, S., Higgins, A. W., Seong, I. S., Donovan, D. J., Shen, Y., ... & Gusella, J. F. (2008). Disruption of neurexin 1 associated with autism spectrum disorder. *The American Journal of Human Genetics*, 82(1), 199-207.
- [37] Kim, O. H., Cho, H. J., Han, E., Hong, T. I., Ariyasiri, K., Choi, J. H., ... & Kim, C. H. (2017). Zebrafish knockout of Down syndrome gene, DYRK1A, shows social impairments relevant to autism. *Molecular Autism*, 8, 1-14.
- [38] Kumita, W., Sato, K., Suzuki, Y., Kurotaki, Y., Harada, T., Zhou, Y., ... & Sasaki, E. (2019). Efficient generation of Knock-in/Knock-out marmoset embryo via CRISPR/Cas9 gene editing. *Scientific reports*, 9(1), 12719.
- [39] Liu, Z., Dong, H., Cui, Y., Cong, L., & Zhang, D. (2020). Application of different types of CRISPR/Cas-based systems in bacteria. *Microbial cell factories*, 19(1), 1-14.
- [40] Liu, Z., Xiao, J., & Xue, H. Practical use of CRISPR-Cas9 on SHANK3 and Neuroligin related to Autism Spectrum Disorder.
- [41] Lu, J., Zhu, Y., Williams, S., Watts, M., Tonta, M. A., Coleman, H. A., ... & Claudianos, C. (2020). Autism-associated miR-873 regulates ARID1B, SHANK3 and NRXN2 involved in neurodevelopment. *Translational Psychiatry*, 10(1), 418.
- [42] Lyall, K., Schmidt, R. J., & Hertz-Picciotto, I. (2014). Maternal lifestyle and environmental risk factors for autism spectrum disorders. *International journal of epidemiology*, 43(2), 443-464.
- [43] Markenscoff-Papadimitriou, E., Binyameen, F., Whalen, S., Price, J., Lim, K., Ypsilanti, A. R., ... & Rubenstein, J. L. (2021). Autism risk gene POGZ promotes chromatin accessibility and expression of clustered synaptic genes. *Cell reports*, 37(10).
- [44] Martinez, R. A., Stein, J. L., Krostag, A. R. F., Nelson, A. M., Marken, J. S., Menon, V., ... & Grimley, J. S. (2015). Genome engineering of isogenic human ES cells to model autism disorders. *Nucleic acids research*, 43(10), e65-e65.
- [45] Matsumura, K., Seiriki, K., Okada, S., Nagase, M., Ayabe, S., Yamada, I., ... & Nakazawa, T. (2020). Pathogenic POGZ mutation causes impaired cortical development and reversible autism-like phenotypes. *Nature communications*, 11(1), 859.
- [46] McDiarmid, T. A., Belmadani, M., Liang, J., Meili, F., Mathews, E. A., Mullen, G. P., ... & Rankin, C. H. (2020). Systematic phenomics analysis of autism-associated genes reveals parallel networks underlying reversible impairments in habituation. *Proceedings of the National Academy of Sciences*, 117(1), 656-667.
- [47] Ming, S. H. A. O., Tian-Rui, X. U., & Ce-Shi, C. H. E. N. (2016). The big bang of genome editing technology: development and application of the CRISPR/Cas9 system in disease animal models. *Zoological Research*, 37(4), 191.

- [48] Muhle, R., Trentacoste, S. V., & Rapin, I. (2004). The genetics of autism. *Pediatrics*, *113*(5), e472-e486.
- [49] Nakagawa, T., Hattori, S., Nobuta, R., Kimura, R., Nakagawa, M., Matsumoto, M., ... & Nakayama, K. (2020). The autism-related protein SETD5 controls neural cell proliferation through epigenetic regulation of rDNA expression. *iScience*, *23*(4).
- [50] O'Geen, H., Abigail, S. Y., & Segal, D. J. (2015). How specific is CRISPR/Cas9 really?. *Current opinion in chemical biology*, *29*, 72-78.
- [51] Park, J. S., Ryu, J. H., Choi, T. I., Bae, Y. K., Lee, S., Kang, H. J., & Kim, C. H. (2016). Innate color preference of zebrafish and its use in behavioral analyses. *Molecules and cells*, *39*(10), 750.
- [52] Pastore, S. F., Ko, S. Y., Frankland, P. W., Hamel, P. A., & Vincent, J. B. (2022). PTCHD1: Identification and neurodevelopmental contributions of an autism spectrum disorder and intellectual disability susceptibility gene. *Genes*, *13*(3), 527.
- [53] Pensado-López, A., Veiga-Rúa, S., Carracedo, Á., Allegue, C., & Sánchez, L. (2020). Experimental models to study autism spectrum disorders: hiPSCs, rodents and zebrafish. *Genes*, *11*(11), 1376.
- [54] Rawsthorne, H., Calahorra, F., Feist, E., Holden-Dye, L., O'Connor, V., & Dillon, J. (2020). Neuroigin dependence of social behaviour in *Caenorhabditis elegans* provides a model to investigate an autism-associated gene. *Human Molecular Genetics*, *29*(21), 3546-3553.
- [55] Rea, V., & Van Raay, T. J. (2020). Using zebrafish to model autism spectrum disorder: a comparison of ASD risk genes between zebrafish and their mammalian counterparts. *Frontiers in molecular neuroscience*, *13*, 575575.
- [56] Redman, M., King, A., Watson, C., & King, D. (2016). What is CRISPR/Cas9?. *Archives of Disease in Childhood-Education and Practice*, *101*(4), 213-215.
- [57] Rodríguez-Rodríguez, D. R., Ramírez-Solís, R., Garza-Elizondo, M. A., Garza-Rodríguez, M. D. L., & Barrera-Saldaña, H. A. (2019). Genome editing: A perspective on the application of CRISPR/Cas9 to study human diseases. *International journal of molecular medicine*, *43*(4), 1559-1574.
- [58] Ross, P. J., Zhang, W. B., Mok, R. S., Zaslavsky, K., Deneault, E., D'Abate, L., ... & Ellis, J. (2020). Synaptic dysfunction in human neurons with autism-associated deletions in PTCHD1-AS. *Biological psychiatry*, *87*(2), 139-149.
- [59] Seabra, C. M., Aneichyk, T., Erdin, S., Tai, D. J., De Esch, C. E., Razaz, P., ... & Gusella, J. F. (2020). Transcriptional consequences of MBD5 disruption in mouse brain and CRISPR-derived neurons. *Molecular autism*, *11*, 1-15.
- [60] Sanders, S. J., Campbell, A. J., Cottrell, J. R., Moller, R. S., Wagner, F. F., Auldridge, A. L., ... & Bender, K. J. (2018). Progress in understanding and treating SCN2A-mediated disorders. *Trends in neurosciences*, *41*(7), 442-456.
- [61] Sandhu, A., Kumar, A., Rawat, K., Gautam, V., Sharma, A., & Saha, L. (2023). Modernising autism spectrum disorder model engineering and treatment via CRISPR-Cas9: A gene reprogramming approach. *World Journal of Clinical Cases*, *11*(14), 3114.
- [62] Santos, J. L. D. S., Araújo, C. D. A., Rocha, C. A. G., Costa-Ferro, Z. S. M., & Souza, B. S. D. F. (2023). Modeling Autism Spectrum Disorders with Induced Pluripotent Stem Cell-Derived Brain Organoids. *Biomolecules*, *13*(2), 260.
- [63] Sharma, G., Sharma, A. R., Bhattacharya, M., Lee, S. S., & Chakraborty, C. (2021). CRISPR-Cas9: a preclinical and clinical perspective for the treatment of human diseases. *Molecular Therapy*, *29*(2), 571-586.
- [64] Shin, K. C., Ali, G., Ali Moussa, H. Y., Gupta, V., de la Fuente, A., Kim, H. G., ... & Park, Y. (2023). Deletion of TRPC6, an autism risk gene, induces hyperexcitability in cortical neurons derived from human pluripotent stem cells. *Molecular Neurobiology*, 1-12.
- [65] Sun, A. X., Yuan, Q., Fukuda, M., Yu, W., Yan, H., Lim, G. G. Y., ... & Je, H. S. (2019). Potassium channel dysfunction in human neuronal models of Angelman syndrome. *Science*, *366*(6472), 1486-1492.
- [66] Tamura, S., Nelson, A. D., Spratt, P. W., Kyoung, H., Zhou, X., Li, Z., ... & Bender, K. J. (2022). CRISPR activation rescues abnormalities in SCN2A haploinsufficiency-associated autism spectrum disorder. *BioRxiv*, 2022-03.
- [67] Tayanloo-Beik, A., Hamidpour, S. K., Abedi, M., Shojaei, H., Tavirani, M. R., Namazi, N., ... & Arjmand, B. (2022). Zebrafish modeling of autism spectrum disorders, current status and future prospective. *Frontiers in Psychiatry*, *13*, 911770.
- [68] Tsuchiya, Y., Minami, Y., Umemura, Y., Watanabe, H., Ono, D., Nakamura, W., ... & Yagita, K. (2015). Disruption of MeCP2 attenuates circadian rhythm in CRISPR/Cas9-based Rett syndrome model mouse. *Genes to Cells*, *20*(12), 992-1005.

- [69] Trujillo, C. A., Adams, J. W., Negraes, P. D., Carromeu, C., Tejwani, L., Acab, A., ... & Muotri, A. R. (2021). Pharmacological reversal of synaptic and network pathology in human MECP2-KO neurons and cortical organoids. *EMBO Molecular Medicine*, 13(1), e12523.
- [70] Tu, Z., Zhao, H., Li, B., Yan, S., Wang, L., Tang, Y., ... & Li, X. J. (2019). CRISPR/Cas9-mediated disruption of SHANK3 in monkey leads to drug-treatable autism-like symptoms. *Human molecular genetics*, 28(4), 561-571.
- [71] Tycko, J., Myer, V. E., & Hsu, P. D. (2016). Methods for optimizing CRISPR-Cas9 genome editing specificity. *Molecular cell*, 63(3), 355-370.
- [72] Wang, H. G., Bavley, C. C., Li, A., Jones, R. M., Hackett, J., Bayleyen, Y., ... & Pitt, G. S. (2021). Scn2a severe hypomorphic mutation decreases excitatory synaptic input and causes autism-associated behaviors. *JCI insight*, 6(15).
- [73] Wang, N., Lv, L., Huang, X., Shi, M., Dai, Y., Wei, Y., ... & Qin, D. (2022). Gene editing in monogenic autism spectrum disorder: animal models and gene therapies. *Frontiers in Molecular Neuroscience*, 15, 1043018.
- [74] Wong, C. W., Wang, Y., Liu, T., Li, L., Cheung, S. K. K., Or, P. M. Y., ... & Chan, A. M. (2020). Autism-associated PTEN missense mutation leads to enhanced nuclear localization and neurite outgrowth in an induced pluripotent stem cell line. *The FEBS journal*, 287(22), 4848-4861.
- [75] Wong, J. C., Grieco, S. F., Dutt, K., Chen, L., Thelin, J. T., Inglis, G. A. S., ... & Escayg, A. (2021). Autistic-like behavior, spontaneous seizures, and increased neuronal excitability in a Scn8a mouse model. *Neuropsychopharmacology*, 46(11), 2011-2020.
- [76] Xiang, Y., Tanaka, Y., Patterson, B., Hwang, S. M., Hysolli, E., Cakir, B., ... & Park, I. H. (2020). Dysregulation of BRD4 function underlies the functional abnormalities of MeCP2 mutant neurons. *Molecular cell*, 79(1), 84-98.
- [77] Xu, M., Wang, L., Wang, Y., Deng, J., Wang, X., Wang, F., ... & Wang, S. (2023). Melatonin ameliorates sleep-wake disturbances and autism-like behaviors in the Ctnnd2 knock out mouse model of autism spectrum disorders. *Genes, Brain and Behavior*, e12852.
- [78] Xu, X., Li, C., Gao, X., Xia, K., Guo, H., Li, Y., ... & Hu, R. (2018). Excessive UBE3A dosage impairs retinoic acid signaling and synaptic plasticity in autism spectrum disorders. *Cell Research*, 28(1), 48-68.
- [79] Zhang, Q., Li, T., Lin, J., Zhang, Y., Li, F., Chen, X., ... & Li, Q. (2022). Deficiency of ndel in zebrafish induces brain inflammatory responses and autism-like behavior. *IScience*, 25(3).