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REVIEW PAPER

New Genetic Approaches for Early Diagnosis and Treatment of Autism Spectrum Disorders

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Abstract

Autism spectrum disorders (ASD) are common heterogeneous neurodevelopmental disorders, characterized by disruptions in social interactions, communication, and limitations in behavior. Early diagnosis is an important step to prevent progression of ASD. Recent developments in genetic technology provide useful tools to investigate the molecular mechanisms involved in autism. Despite a number of noteworthy studies, there is not yet enough understanding of the genetic etiology of ASD. Research should focus on multidisciplinary approaches to improve early diagnosis and intervention of autism. It is important to study the combinatorial effects of genetic, epigenetic, and environmental factors. This review focuses on current research in ASD, highlighting the importance of identifying new approaches, such as next generation sequencing (NGS) and microRNA (miRNA) technologies, to introduce possible ways for developing new biomarkers and drugs.

Keywords Autism spectrum disorders · Next generation sequencing · Early diagnosis and treatment · New genetic approaches

Autism spectrum disorder (ASD) is a highly heritable neurodevelopmental condition characterized primarily by social interaction, language, and communication abnormalities that can be distinguished in children during the early years. Language disabilities, difficulties in sensory integration, lack of reciprocal interactions and, in some cases, cognitive delays are other phenotypic and clinical features of the disorder that may appear. According to the Centres for Disease Control, ASD occurrence is about 1–2% of the population and is four times more common in boys than girls (Ahn et al. 2013). ASD is heterogeneous and its manifestation occurs owing to genetic, epigenetic and environmental factors. Despite the numerous studies conducted on ASD so far, the certain etiology of ASD still remains unclear (Ansel et al. 2017). It consists of three subgroups based on different clinical features. These are pervasive developmental disorder (PDD), including 'classical' autistic disorder and Asperser's syndrome, and pervasive developmental disorder–not otherwise specified (PDD–NOS, also called atypical autism) (Li et al. 2012). Because autism is identified with a common set of behaviors, in the latest version of the Diagnostic and Statistical Manuel of Mental Disorders, fifth edition (DMS-5), the diagnosis of 'autistic disorder' has been changed to 'autism spectrum disorders' (Chung et al. 2014). Until the 1980s, genetically strong component of autism was not acknowledged, as in several other complex diseases. However, with the increase in genetic and epidemiological studies, our understanding of the genetic contributions to autism has slightly differentiated. Primarily, it is predicted that approximately 10% of children with ASD have a determined co-occurring genetic, neurological and metabolic disorder like fragile X syndrome or tuberous sclerosis (Caglayan 2010).

Secondly, if a new-born infant has an affected sibling, the risk of ASD development increase to 25-fold compared to the normal population risk (Abrahams and Geschwind 2008). Thirdly, twin studies have shown that identical twins have around a 60–90% chance of being diagnosed with autism, while the risk of autism in nonidentical twins declines noticeably to 0–24% (Bailey et al. 1995; Steffenburg et al. 1989). Nevertheless, according to the most comprehensive study, completed with 503 ASD twins in California, the risk levels in the heritability rate have been overestimated. Depending on their study, the concordance rate was 57% in monozygotic male twins and 67% in female twins, whereas the rate was 33% in same sex dizygotic twins. Similar studies have confirmed that an increase of mutations or structural variations

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makes a significant impact on the risk of ASD (Sztainberg and Zoghbi 2016). When the results are considered together, the etiology of ASD seems to vary according to the individual's genome and interactions with environment. Over the past few decades, significant progress has been made to illuminate the genetics of ASD. First, the main function of de novo germline mutation was identified clearly. Later on, these mutation types helped in the discovery of many ASD risk loci and genes (De Rubeis et al. 2014; Dong et al. 2014). One of the studies of this cohort, called The Simons Simplex Collection (SSC), made possible for the discovery of de novo variations among ASD families, and has had a great impact on this progress (Fischbach and Lord 2010). SSC analysis has revealed many rare de novo mutation types, such as copy number variants (CNVs), small insertion/deletions (indels), and single nucleotide variants (SNVs), in unaffected siblings. With the advantage of whole exome and genome analysis of large ASD cohorts, studies have significantly accelerated gene discovery in ASD (Li et al. 2012). In this paper, we overview etiological risk factors and biomarkers of ASD that are discovered to date. Finally, we present current diagnostic and therapeutic features of techniques which are able to adequately address difficulties in treatment of ASD and its symptoms. The studies with relevant updated information are included in this review to discuss current development on ASD. We aim to provide recent detailed analysis on genetic, environmental, and epigenetic factors affecting the occurrence of ASD as well as applied therapeutic means to give insight into designing new approaches that can maximize treatment outcomes and to improve patients' lives.

Etiopathogenesis of ASD

Etiopathology of disease is defined as various causes and consequences of any disorder or disease. Studies indicate that genetic, epigenetic, and environmental factors are the cause of ASD. Epigenetic factors are thought to be affected and altered by genetic and environmental factors during several conditions that result in ASD development (Siu and Weksberg 2017). The following aspects are crucially significant in understanding the disease as well as designing therapeutic or treatment approaches to reduce occurrence rate of the disease.

Genetic Background of ASD

Since discovery of the first genetic findings in the 1970s, illustrating the pivotal role of genetic factors associated with ASD, many new studies have been adopted by different groups to define a molecular pathology that explains the disorder based on genetic alteration. As will be debated below, in order to determine the common characteristics of different genetic variants and to narrow its relation with a particular gene or functional defects associated with ASD, a wide range of studies were taken into account.

Beginning in the 1950s, due to the advantage of karyotyping techniques, approximately 7–8% of patients were identified with chromosomal abnormalities associated with ASD. Although if there are numerical and structural aberrations in nearly every chromosome, the most striking effects have been found in fragile X and maternal 15q11–13 duplication (Xu et al. 2004) 2q37 and 22q13.3 deletion (Jacquemont et al. 2006). A molecular cytogenetic technique known as Chromosome microarray (CMA) gives scientists the ability to overcome the limited resolution of conventional cytogenetics and provides genome-wide scanning for both microscopic and submicroscopic chromosomal aberrations (Zahir and Friedman 2007). This technique has been very convenient in finding ASD candidate genes and has become the best-known example among CNV studies for pediatric diseases (Connolly and Hakonarson 2012). Sebat et al. carried out the first family-based CNV research with 264 ASD families in 2007, confirming 17 loci on 11 chromosomes that had also been validated by higher-resolution microarray scans, G-banded karyotype, FISH, and microsatellite genotyping. Since then, CNVs have arisen as a potential genetic contribution to the development of autism (Sebat et al. 2007). The next report on deletion CNVs at 8p23.1 and 17p11.2 was done by a Korean group, using the aCGH technique (Cho et al. 2009). Qiao et al. applied the same technique using 100 samples from autistic patients and determined that out of nine CNVs, three of them belonged only to their cohort (Qiao et al. 2009). Additionally, Spanish group also characterized 13 CNVs, including 24 various genes using samples from 96 ASD patients (Cuscó et al. 2009). Another group, Girirajan et al., identified CNV genes consisting of DPP10, PLCB1, TRPM1, NRXN1, FHIT and HYDIN that tend to cause gene disruption events. Depending on the results, changes in the CNV levels or the length of DNA have an impact on the phenotypic characteristics of autistic individuals (Girirajan et al. 2013). There are multiple genes with similar functions in the brain that are considered to be possible ASD sensitivity genes, like GABA receptors, GABRA5 and GBARB3, components of the proteosome complex, UBE3A and HERC2, ribonucleoprotein complex unit, SNRPN, and the FMRP interacting protein CYFIP1 (Yeh and Weiss 2016). Although the region was identified almost two decades earlier, there is no consensus that duplication of only one gene is strongly associated with the behavioral phenotype.

Monogenic factors alongside cytogenetic abnormalities of ASD supply primary insight into genetic composition of ASD (Folstein and Rosen-Sheidley 2001). By following these clues, a great number of studies have been carried out to introduce tools that are capable of identifying genetic as well as

biological mechanisms of ASD. Nearly 10% of ASD patients have single gene disorders, such as Rett syndrome, Tuberous sclerosis (TSC), Fragile X syndrome (FXS), and others (Lokody 2014). Mutations appear at TSC1 and TSC2 genes cause TSC (Van Slegtenhorst et al. 1997; E.C.T.S. Consortium 1993). TSC1 and TSC2 genes take part in the local translation at the synapse in order to control mechanisms in the mammalian rapamycin pathway (mTOR) as an inhibitor. Mutation in the MeCP2 gene causing Rett syndrome was found in nearly 1% of ASD patients (Amir et al. 1999). As a multifunctional protein, MeCP2 has functions in transcription regulation by activating and repressing neurons. Mutations in the PTEN gene are responsible for a few diseases, such as ASD, cancer, and seizures. PTEN has a key role in the regulation of the PI3K/AKT mechanism and it also suppresses the mTOR mechanism indirectly. Therefore, these data suggest that functional abnormalities in the transcription or translation factors in neurons or the PI3K/AKT/mTOR mechanisms lead to ASD (Endersby and Baker 2008).

Many scientists have focused on linkage mapping in order to examine the chromosomal regions which are co-segregated among affected Individuals (Ivanov et al. 2015). Linkage studies are successful in determining Mendelian diseases, specially the ones with adjacent alleles are close together on the same chromosome. Using this technique, a full genome screening with intense genetic markers covering all chromosomes or a certain specific area of interest can be examined (Freitag et al. 2010). So far, only the 17q11–17q21 and 7q22–7q32 regions were replicated at the important level that contain CNTNAP2, RELN, and MET; the most comprehensively analyzed genes that are linked with autism spectrum disorder (Ivanov et al. 2015).

Linkage data analysis supports the fact that many loci on different choromosomes, along with many genes, play a significant role in the emergence of autism (Ivanov et al. 2015; Freitag 2010). Many studies have mainly focused on increasing homogeneity by concentrating on known features of autism to identify risk alleles related to ASD (Volkmar et al. 2009), enhance therapies efficiently, and help to determine differences in homogenous subgroups for mental behaviors (Schreibman 2000). Language ability (e.g., age of baby's first words) has been found to be correlated with cognitive ability as well as interactive skills of children which were measured in later years of childhood (Mayo et al. 2013; Matson and Neal 2010).

These endophenotypes that may exist in the general population or in normal individuals can assist to trace the quantitative trait loci (QTL) that contribute to autism phenotype (Kumar and Christian 2009). The 7q34-7q36 locus, which was specified by Yonan and colleagues, was found to be an autism locus (Yonan et al. 2003) and was represented in other studies (Schellenberg et al. 2006). Moreover, age-related language ability delay was linked with the 9q34 locus using similar measurement of the age of baby's first word (Schellenberg et al. 2006). It is thought that QTL studies may be useful in the classification of susceptibility-sensitive genes in the subtypes of autistic patients. In fact, linkage studies have not fully elucidated autism genes, which is not related to faulty method but rather the complexity of the disease, leading future studies in need of more homogeneous specimens (Ivanov et al. 2015).

Epigenetics of ASD

Epigenetics is the study of phenotypic changes that influence gene expression without any change in the primary DNA sequence. Multiple studies show that epigenetics has a crucial role in many mechanisms, such as regulation of neurogenesis, neuronal plasticity, learning, and memory, which are partially responsible for development of autism. Thus, it is widely accepted that changes in the epigenetic state of DNA may significantly affect autism risks (Sivanesan et al. 2017). Some genes, which are observed to have undergone alteration in ASD, play an important role in hormonal pathways. The *RORA* gene, involved in both immune and neurodevelopmental processes, has been shown to be associated with many genes at the transcription level, such as *NLGN1* and *NTRK2*, which encode catalytic receptors for neurotrophins; *RBFOX1*, which encodes insertions to provide synaptic conduction and membrane stimulation; and *ITPR1*, which plays a role in the regulation of calcium channels in neurodegenerative disease. Thus, any changes leading to alteration in normal volume of RORA protein may affect the transcription levels of all these genes, resulting in the impairment of multiple neural pathways in autism (Sarachana and Hu 2013). Although previous studies show relevant correlation between *RORA* gene and ASD, recent study on blood lymphocytes of autistic and healthy children has shown no significant difference in its level, which indicates different DNAm in different cell types (Salehi et al. 2017).

Some studies have focused on DNA methylation analysis in autism cases to find correlation of such event as a risk factor for development of autism. Genes involved in the regulation of gene expression levels by the methylation of DNA have also been associated with ASD. For example, hypermethylated CpG fragments in the promoter region of the BCL-2 gene have been shown to cause the dysregulation of downstream genes (Nguyen et al. 2010). Similarly, the methylation of the oxytocin receptor gene (OXTR), Engrailed 2 (EN2), and methyl CpG binding protein 2 (MECP2) has been shown to be effective in autism as well as social sensations in the brain (Kumsta et al. 2013; Loke et al. 2015). At the same time, hypermethylation of the promoter regions has been found in the SHANK3 and FMR1 genes in ASD patients (Li et al. 2015).

In addition to DNAm alteration in autism, scientists found placenta to be an easily accessible and efficient source for analysis of methylation during the whole period of pregnancy

by studying partially methylated domains (PMDs) and highly methylated domains (HMDs) (Schroeder et al. 2013) on pregnant women and candidates intending to conceive considering their current offspring is suffering from ASD, as they have 13 times higher chance to have another child with ASD (Ozonoff et al. 2011). The result indicated higher methylation rate of PMDs compared to HMDs. However, a specific methylated region was detected in fetal brain enhancer near DLL1 locus on HMDs (Schroeder et al. 2016) that is thought to be responsible for neural differentiation as well as neurons' proliferation, which is crucial in development of ASD during pregnancy (Shimojo et al. 2016). Such methylation was observed to cause dysfunction of DLL1, which further inhibits the delta-like 1 receptor responsible for the neural developmental process in embryonic stage (Schroeder et al. 2016).

Other DNAm alteration studies have expanded their analysis arrays to more than genes and loci and presented rather interesting findings on H3K27me3 and H3K9me3 histones methylation leading to lack of accessibility of adjacent DNA area during transcriptional stage caused by CpH hypermethylation of H3K27me3 and H3K9me3 histones in ASD individuals (Ellis et al. 2017; Wiles and Selker 2017).

Other studies have detected a connection between X-linked genes' mutation and ASD patients. The *KDM5C* gene, known as *SMCX* or *JARID1C* located on Xp11.22-p11.21, encodes H3K4me2/3 (di- and tri-methylated histone H3 lysine 4), and its mutation causes disruption of crucial genes' transcriptional phase, such as *SCN2A* (sodium voltage-gated channel), *CACNA1H* (calcium voltage-gated channel), *BDNF* (brain derived neurotrophic factor), and *SLC18A1* (solute carrier; vesicular monoamine transporter) that are associated with neural functional development as well cognitive and psychiatric dysfunction (Siniscalco et al. 2013; Adegbola et al. 2008). Moreover, the R1115H variant, which is located in the C-terminal segment of *KDM5C*, undergoes missense and truncation mutation causing MRXSCJ (mental retardation, X-linked, syndromic, Claes-Jensen type) (Vallianatos et al. 2018) that has been frequently reported in ASD patients (Santos-Rebouças et al. 2011; Ounap et al. 2012; Abidi et al. 2008; Brookes et al. 2015).

Study on the *Ankrd11* gene reveals the fact that the change caused by acetylation from histone modifications may also be effective in ASD. Since *Ankrd11* is a potential chromatin regulator, there is no other known function in the brain. However, knockdown of *Ankrd11* has been found to inhibit histone acetyltransferase activity. Thus, as a chromatin regulator, it regulates histone acetylation and gene expression levels during neural development that can lead to ASD (Gallagher et al. 2015).

Environmental Factors on ASD

Genetic and epigenetic factors have been evidently presented to support autism etiology. Nevertheless, thorough examination of identical twins with autism corroborates the significance of environmental factors on etiology of related genes (Ronald and Hoekstra 2011; Hallmayer et al. 2011). Environmental factors' impact on quality and quantity of genes and their expression result from the occurring changes on DNA sequence epigenetically, such as DNAm alteration and histone modification, which can be passed on to the next generation (Perera and Herbstman 2011). Subsequently, such environmental factors, if detrimental, can cause genetic dysfunction during embryonic development, leading to genomic diseases such as ASD (Foley et al. 2009). However, a single environmental factor is never sufficient enough to develop autism. Therefore, a combination of these factors during various stages of offspring development, including prenatal, natal, and postnatal stage, yields autism.

Prenatal factors include physical, mental, and psychological factors of parents during the whole pregnancy. Parental age plays a rather substantial part in development of autism. Studies on parents' age in different countries indicate a higher chance of autism development with higher maternal age (Lee and McGrath 2015; Parner et al. 2012; Alter et al. 2011; Hultman et al. 2011; Kong et al. 2012; van Balkom et al. 2012; Sandin et al. 2012). The effect is explained owing to the occurrence of de novo mutation in germline cells and DNAm alternation leading to neural damage and eventually ASD development (Parner et al. 2012; Alter et al. 2011; Hultman et al. 2011; Kong et al. 2012). High maternal age is correlated with chromosomal abnormalities in ovules resulting in neural development dysfunction (Kolevzon et al. 2007).

Maternal physical health is highly significant in a child's autism. Maternal bleeding during pregnancy results in placental and fetal complications and increases the risk of autism development to 81% (Gardener et al. 2009). Additionally, maternal diabetes are found to be greatly linked to offspring with ASD (Xu et al. 2014), which is due to hypoxia in utero caused by hyperglycaemia that impairs the neurodevelopmental stage of the fetus, eventually leading to a higher chance of ASD (Burstyn et al. 2011). Maternal infection, specifically during the first trimester of pregnancy, shows a higher chance of a child with ASD (Zerbo et al. 2015; Hsiao et al. 2012). Bacterial infection, in particular urinary tract infection, was observed with children who were diagnosed with ASD (Zerbo et al. 2015). The mechanism of such event is unknown. However, some studies on animals and humans' monitoring suggest the abnormal brain formation and other neurodevelopmental disorder is due to activation of the maternal immune system, which generally occurs in the presence of bacterial or any other infection that can penetrate and reach the fetus through the placenta (Hsiao et al. 2012; Malkova et al. 2012; Patterson 2011). A high level of cytokines interferon γ (IFN-γ), IL-4 and IL-5 in serum during mid-pregnancy (Goines et al. 2011) and IL-4, IL-10, TNF-α, and TNF-β and chemokines (MCP-1) in amniotic fluid (Abdallah et al. 2013), was associated with autism. Nausea and vomiting

during pregnancy (NVP) is experienced by 70–80% of pregnant women (Pepper and Craig Roberts 2006). However, the severity of the condition varies in every individual affecting its impact on fetal abnormality. Dysregulation of maternal human chorionic gonadotropin (hCG), thyroid hormones, and estrogens by sever NVP (Bustos et al. 2017; Niemeijer et al. 2014) are found to be associated with offspring diagnosed with ASD (Windham et al. 2016).

Maternal mental health also appears to be a crucial impact factor on autism. Analysis of pregnant women who suffered from a mental health disorder, such as schizophrenia, shows three times higher chance of having a child with autism than control cases (Holtmann et al. 2007; Daniels et al. 2008). Additionally, depression and anxiety, specifically during 21–32 weeks of pregnancy, are also related with ASD, which epigenetically affects the fetus stress response gene during fetal brain and neural development (Weinstock 2008).

Natal risk factors have also been associated with autism. Assisted reproductive technologies (ART), such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), are observed in cases with autism. This correlation is thought to be derived from abnormal DNAm during genome imprinting (Melnyk et al. 2012). Recent analysis shows a higher rate of autism with ICSI rather than IVF (Liu et al. 2017) possibly due to higher risk of abnormal genome imprinting by using surgically extracted sperm that may cause deregulation in DNAm.

Some dietary factors are considered to be the probable cause of ASD. For instance, unsaturated fatty acids, such as omega-3 and omega-6, significantly affect retinal and brain development, signal transduction, and gene expression during the first 2 months of pregnancy (Deckelbaum et al. 2006; Lyall et al. 2013), and increased maternal intake of omega-6 has shown to reduce the possibility of ASD in offspring by 34% and low intake was associated with high risk of ASD development (Lyall et al. 2013; Parletta et al. 2016). Studies on omega-3 intake level during pregnancy presented the same result; however, they failed to show any correlation between higher intake of omega-3 and lower chance of ASD (Lyall et al. 2013). In addition to fatty acid, folic acid (FA) consumption 1 month before pregnancy and 2 months after pregnancy lowers the risk of autistic disorder development (Schmidt et al. 2012; Surén et al. 2013; Wang et al. 2017), such as language delay at age 3 (Roth et al. 2011), compared to the individuals who did not receive any FA supplement (Wang et al. 2017).

Micro RNAs and ASD

Despite the fact that genomic evidence only established a relationship between miRNAs and ASD, miRNA expression studies reveal the interaction more directly. Various studies have focused on miRNAs dysregulation and deregulation in different tissues, such as brain tissue, olfactory mucosal root cells and primary skin fibroblasts (Fregeac et al. 2016).

The connection between ASD and miRNAs is based on the ability of ASD-related genes to function as regulators in the biogenesis of miRNAs, and to function on the RNA pathway. This is the case for the mutated FMR1 and MECP2 genes causing, respectively, Fragile X and Rett syndrome, which are often associated with ASD (Nguyen et al. 2016). Many studies have shown that the FMPR protein, the product of FMR1 gene, has both biochemical and genetic interactions with many components of the miRNA pathway. For example, in mammalian cells, Jin et al. illustrated FMPR interaction with endogenous miRNAs and EIF2C2, an argonaute family protein (Jin et al. 2004). Recombinant protein studies later revealed that FMPR also serves as a miRNA receptor protein for ribonulease DICER. The regulatory role of MECP2 in miRNA expression was first demonstrated by the finding that miR-137 expression in adult stem cells is subject to an MECP-mediated epigenetic mechanism. MECP2 interacts with DGCR8 to support the release of DROSHA from DGCR8, which inactivates miRNA function. The same study has shown that phosphorylation of MECP2 at Ser80 facilitates the binding of MECP2 with DGCR8. Thus, direct interaction with MECP2 DGCR8 plays a role in the regulation of gene expression by suppressing nuclear miRNA processing (Cheng and Qiu 2014). In general, all of these studies show that there is a link between miRNA mechanisms and ASD.

Recent study detected deregulation of other miRNAs, such as miR-146a, miR-221, miR-654-5p, and miR-656, which are found abundant in primary skin fibroblasts, associated with ASD. The same study presents the significance of miR-146a and miR-221 for neural development and cognitive functions in mouse brain. In addition to hsa-miR-146a-5p, miR-146a up-regulation in ASD neurons is thought to cause alteration in the biology of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor (Chen and Shen 2013) through both impaired MAP1B-mediated endocytosis and a reduced amount of GRIA3, thus altering neuronal dendritic complexity and astrocyte glutamate uptake capacities in ASD (Nguyen et al. 2016). hsa-miR-146a-5p has been found to represent a reliable biomarker for ASD pathology. This miRNA has been shown to be actively up-regulated in four different cell types from four different cohorts of ASD patients. Deregulation of this miRNA may negatively impact deregulation of target genes, such as *NOTCH1*, *GRIA3*, *SYT1*, and *NLGN1*, that are known to be highly crucial for the functional and developmental stage of brain. In addition, the same miRNA has a deleterious effect on differentiation of neuro-progenitor cells into neurons and astocytes due to deregulation (Jovičić et al. 2013; Mei et al. 2011). Moreover, levels of hsa-miR-146a-5p were observed in temporal lobe epilepsy (Aronica et al. 2010) and frontal cortical dysplasia (Iyer et al. 2012), which suggests similar pathophysiological mechanisms of these disorders with ASD.

Analysis of brain tissues from post-mortem ASD cases detected up-regulation of hsa-miR-21-3p in hNPCs (human neural progenitor cells) causing down-regulation of M16 mRNA, which is responsible for M16 gene transcription. Such dysregulation decreases expression of *PAFAH1B1/LIS1*, *DLGAP1*, and *ATP2B1/PMCA1* genes that are highly important in development of neurons, synopsis, and calcium regulation in brain cells and are observed in ASD patients. Another interesting miRNA to be considered as ASD biomarker was hsa_can_1002-m, which is shown to be highly down-regulated in the cerebral cortex of ASD patients and over-activates epidermal growth factor receptor (EGFR) and the fibroblast growth factor receptor (FGFR) that disrupt the natural development of glia cells (Wu et al. 2016). A study of ASD based on sex indicates more sncRNA and miRNAs dysregulation in ASD females compared to ASD males (Schumann et al. 2017). This could indicate sexual dimorphism of miRNAs and ASD related genes. For instance miR-219 and miR-338 down-regulation that leads to oligodendrocyte differentiation had greater volume in female superior temporal sulcus (STS) with ASD (Schumann et al. 2017). Serum analysis of ASD patients by stem-loop qRT-PCR assay suggested up-regulation of miR-365a-3p, miR-619-5p, miR-664a-3p and down-regulation of miR-3135a, miR-328-3p, miR-197-5p, miR-424-5p, miR-500a-5p, which could be an excellent biomarker in ASD diagnosis (Kichukova et al. 2017).

Early Diagnosis of ASD Using Advanced Genetic Approaches

The emergence of thousands of low-cost genotyping techniques in the 2000s allowed genome-wide association of large-scale experimental designs of multi-factor complex diseases (Beckmann and Antonarakis 2010). The basis of genome-wide association studies (GWAS) is the search for common variants such as single nucleotide polymorphisms (SNPs) in the genome that cause common diseases. Weiss and her colleague first examined SNPs of more than 1000 families, but could not reach any statistical conclusions associated with disease. Subsequent studies revealed the genotyping of the most noticeable SNPs in autistic families and the fact that the mutation in the 5p15 locus is significant. This SNP is located between the gene encoding the SEMA5A protein, the family member of the semaphorin, and TAS2R1, the bitter taste receptor. Another independent study can be shown as evidence that the level of expression of SEMA5A falls in the lymphoid cell lines of autistic individuals, based on all blood and brain samples from control subjects (Weiss et al. 2009). In addition, a few suggestive association signals were detected at loci containing JARID2 and JMJD2C that are thought to be ASD candidate genes (Liu and Takumi 2014). Two different populations were used in another GWAS conducted by Wang et al. One of them overlapped with the sample used in the work of Weiss and her colleagues, while the other contained more than 1200 cases and more than 6500 controls. In this study, the 5p14 region is defined as a significant region. The signal is generated by the genes CDH9 and CDH10, which encode neuronal cell adhesion molecules (Wang et al. 2009). In 2010, the consortium of the Autism Genome Project scanned 1558 ASD families and nearly one million single-nucleotide polymorphisms, resulting in the MACROD2 gene being identified as taking part in genome-wide association (Anney et al. 2010).

It has been accepted that ASD has broad phenotypic and genotypic heterogeneity as a result of rich data and different results obtained independently from each GWAS. Thus, many of the following studies have sought to find variants associated with autism subtypes in intense QTL searches. GWAS studies can help in this regard to allow for deeper screening of relevant targets in determining the etiology of ASD (Surén et al. 2013). GWAS was used for identification of biomarkers during pregnancy that are thought to be correlated with autism. In some studies, carried out in Finland and the Netherlands, CRP was increased in the serum of mothers with an ASD child (Brown et al. 2014; Koks et al. 2016), while another study reported a lower level of CRP during mid-pregnancy (15–19 weeks), which was analyzed on a more heterogeneous group where cases were selected from different ethnic groups (Zerbo et al. 2016). These observations suggest ethnicity can be an influential factor on CRP level during pregnancy. However, all studies could not find any relationship between autism and CRP level (Zerbo et al. 2016; Brown et al. 2014; Koks et al. 2016).

Whole exome sequencing (WES) is another genetic approach that has recently been applied to identify rare or new genetic defects related to genetic disorders. Since the development of ASD is under the influence of many loci and has a weak phenotype/genotype relationship, it is used as a model disease in WES applications (Persico and Napolioni 2013). WES studies, unlike GWAS, offer new possibilities for sporadic cases and make it possible to detect de novo mutations and variations with incomplete penetration (Yin and Schaaf 2017). Many disrupted genes are effective in important gene networks, such as synaptic plasticity and chromatin remodeling. De novo mutations have also been found in other neurological developmental disorders and genes associated with intellectual disabilities (e.g., GRIN2B, SCN1A, and SCN2A) (O'Roak et al. 2012).

Another common use of WAS is to identify candidate recessive mutations passing from known parental ancestry in autistic children (Persico and Napolioni 2013). On the basis of analysis of homogeneity in their studies, Chahrour and his colleagues sought to describe the effects of unrelated marriages in children, and as a result they showed potential evidence of recessive transitions in non-consanguineous marriages. They characterized

four new genes called UBE3B, CLTCL1, NCKAP5L, and ZNF18 that play a role in mechanisms, such as proteolysis, GTP-dependent signaling, and cytoskeleton structure (Chahrour et al. 2012). Puffenberger et al. identified a homozygous missense mutation in HERC2 correlated with ASD and developmental retardation (Puffenberger et al. 2012). In a study by Novarino et al., who scanned families affected by autism, epilepsy, and mental retardation, homozygous inactivation mutations in the BCKDK gene were identified. The data obtained from the WES studies have identified a large number of genes and demonstrated their association with ASD (Table 1), but no consensus has formed around an ASD etiology accepted by scientists (Ivanov et al. 2015).

In addition to the above-mentioned techniques, other next generation sequencing (NGS) techniques have been employed in various studies to determine genetic analysis and diagnosis of ASD in various stages of life. Whole genome bisulfide sequencing and pyrosequencing were employed to study partially methylated domain (PMD) and highly methylated domain (HMD) to determine DNA methylation rate of ASD patients at early ages during pregnancy or before next pregnancy on women with ASD child (Schroeder et al. 2016). Reduced representation bisulfide sequencing (RBBS) has been found to efficiently analyze and detect DNAm alteration in CpG and CpH islands as well as deregulation of histone methylation (Schumann et al. 2017). Further studies can focus on exploiting other NGS techniques which can open new window on future ASD studies to furnish early diagnosis leading to prevent or reduce neurological disorders related to ASD.

Current Approach Used for ASD Treatment and Future Approaches

In the field of gene editing for genetic disorders, adeno-associated viruses (AAVs) are identified to be a perfect vector to transfer a specific gene to target cell. Among these vectors, AAV9 was shown to be precisely useful in treatment of Rett syndrome due to its penetration of brain cells (Gray et al. 2011). To enhance the vector's ability in gene delivery, a self-complementary (sc) AAV9 vector along with a codon-optimized version of the major *Mecp2* brain isoform, known as MCO, was injected intravenously to Mecp2 KO mice (mice whose Mecp2 is knocked-out) and showed phonotypical improvement in mice (Matagne et al. 2017). However, off-target effects need to be eliminated, since an elevated level of Mecp2 was observed in liver cells with high dosage of treatment (Matagne et al. 2017), which has been recently reported to disrupt liver metabolism and its function (Kyle et al. 2016). Therefore, avoiding the side effects by using optimum dosage or modifying the vector can be considered for future use in humans. Other studies have focused on recruiting other AVV vectors in various neurodevelopmental disorders. For instance, AVV8 has also demonstrated interesting effects on astocytes in CNS. Scientists developed AAV8 vectors with minimal promoters to transfer enhanced green fluorescent protein (eGFP) to astocytes mouse striatum, which are abundant in CNS. A high performance of the vector in delivery was demonstrated owing to a high capability of the vector to penetrate through the brain barrier and lower off-target effects (Pignataro et al. 2017), and this method can be further used to transfer genes to astocytes in ASD patients.

The CRISPR-Cas9 genome editing system, which is widely used in various genetic disorders, has been used on neural and brain cells to deliver specific genes to target cells. The clustered regularly interspaced palindromic repeats (CRISPR) system associated with the Cas9 protein is an adaptive prokaryotic immune system with a defense mechanism against foreign genomes following three stages known as adaptation, expression, and interference. The system involves *Cas* genes, which encode Cas proteins, and guide RNA (gRNA) to carry out the mechanism.

The experiment tested the effect of Cas9 RNP-induced (ribo-nucleo protein) using the Simian vacuolating virus 40 nuclear localization sequence (SV40-NLS) on an Ai9 tdTomato mouse to check its capability on neural progenitor cells (NPCs) in hippocampus, striatum, and cortex, which showed significant gene editing in vitro and in vivo, indicating successful neuron-specific targeting to be further used in ASD (Staahl et al. 2017). Other experiments used CRISPR-Cas9 for Huntington's disease to suppress mutant HTT (mHTT). They designed a CRISPR-Cas9 system with four gRNAs (guide RNAs) targeting the human *HTT* gene by using the AAV vector that resulted in significant reduction of mHTT expression in HD140Q-KI mice (Yang et al. 2017). This data can lead scientists to design an efficient gene editing tool as a therapeutic means to combat ASD or minimize severity of symptoms in individuals.

In addition to previous studies, the most recent experiment on Thy1-YFP and Ai9 mice with X-fragile syndrome, which is linked to autism, has brought up a great achievement on reducing repetitive symptoms of X-fragile syndrome (XFS) by using CRISPR-Gold (CRISPR designed with gold nanoparticles) to deliver Cas9 or Cpf1 to striatum by local intracranial injection. Compared to viral vector, which caused gene expression of Cas9 in brain cells and neurons, the CRISPR system has shown no sign of immune system activation and the least off-target effect. In this study, the metabotropic glutamate receptor subtype 5 (mGluR5) gene was selected as an editing target (Lee et al. 2018), since its signaling was found to be over-activated in XFS and other ASD syndromes (Tao et al. 2016; Silverman et al. 2012). The following experiment showed significant reduction of side effects, indicating successful Cas9 or Cpf1 delivery and gene editing. The same study presented CRSPR-Gold effect for gene editing of other cell types, such as glia cells whose dysfunction are present in

Table 1 Recent variations identified by whole exome sequencing associated with ASD disease

Gene symbol	Gene name	Mutation type	References
FOXP1	Forkhead Box P1	Frameshift	O'Roak, 2011
TGM3	Transglutaminase 3	Missense	O'Roak, 2011
LAMC3	Laminin, gamma 3	Missense	O'Roak, 2011
MYO1A	Myosin IA	3'UTR	O'Roak, 2011
GPR139	G protein-coupled receptor 139	Missense	O'Roak, 2011
SCN1A	Sodium channel, voltage-gated, type I, alpha subunit	Missense	O'Roak, 2011
PLCD1	Phospholipase C, delta 1	Synonymous	O'Roak, 2011
SYNE1	Spectrin repeat containing, nuclear envelope 1	Missense	O'Roak, 2011
TAF1L	TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor	3'UTR	O'Roak, 2011
DCTN5	Dynactin 5	3'UTR	O'Roak, 2011
SLC30A5	Solute carrier family 30 (zinc transporter), member 5	Missense	O'Roak, 2011
IL1R2	Interleukin 1 receptor, type II	Synonymous	O'Roak, 2011
AFF4	AF4/FMR2 family, member 4	Synonymous	O'Roak, 2011
GRIN2B	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	3′ splice	O'Roak, 2011
EPHB6	EPH receptor B6	Synonymous	O'Roak, 2011
ARHGAP15	Rho GTPase activating protein 15	Synonymous	O'Roak, 2011
XIRP1	Xin actin-binding repeat containing 1	Missense	O'Roak, 2011
CHST5	Carbohydrate(N-acetylglucosamine6-O) sulfotransferase 5	Synonymous	O'Roak, 2011
TTN	Titin	Synonymous	O'Roak, 2011
TLK2	Tousled-like kinase 2	Missense	O'Roak, 2011
RBMS3	RNA binding motif, single stranded interacting protein 3	Missense	O'Roak, 2011
ADAM33	ADAM metallopeptidase domain 33	Nonsense	.Sanders, 2012
CSDE1	Cold shock domain containing E1, RNA-binding	Nonsense	Sanders, 2012
EPHB2	EPH (Ephrin) receptor B2	Nonsense	Sanders, 2012
FAM8A1	Family with sequence similarity 8, member A1	Nonsense	Sanders, 2012
FREM3	FRAS1 related extracellular matrix 3	Nonsense	Sanders, 2012
MPHOSPH8	M-phase phosphoprotein 8	Nonsense	Sanders, 2012
PPM1D	Protein phosphatase 1D magnesium-dependent, delta isoform	Nonsense	Sanders, 2012
RAB2A	RAB2A, member RAS oncogene family	Nonsense	Sanders, 2012
SCN2A	Sodium channel, voltage-gated, type II, alpha subunit	Nonsense	Sanders, 2012
BTN1A1	Butyrophilin, subfamily 1, member A1	Splice site	Sanders, 2012
FCRL6	Fc receptor-like 6	Splice site	Sanders, 2012
KATNAL2	Katanin p60 subunit A-like 2	Splice site	Sanders, 2012
NAPRT1	Nicotinate phosphoribosyltransferase domain containing 1	Splice site	Sanders, 2012
RNF38	Ring finger protein 38	Splice site	Sanders, 2012
SCP2	Sterol carrier protein 2	Frameshift	Sanders, 2012
SHANK2	SH3 and multiple ankyrin repeat domains 2	Frameshift	Sanders, 2012
ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	Missense	Bi, 2012
ANK3	Ankyrin 3	Missense	Bi, 2012
CLCN6	Chloride channel, voltage-sensitive 6	Missense	Bi, 2012
HTR3A	5-hydroxytryptamine (serotonin) receptor 3A	Missense	Bi, 2012
RIPK2	Receptor-interacting serine-threonine kinase 2	Missense	Bi, 2012
SLIT3	Slit homolog 3	Missense	Bi, 2012
UNC13B	Uncl3 homolog B	Missense	Bi, 2012
BRCA2	Breast cancer 2	Missense	Neale, 2012
FAT1	FAT atypical cadherin 1	Missense	Neale, 2012
KCNMA1	Potassium large conductance calcium-activated channel, subfamily M, alpha member 1	Missense	Neale, 2012

Table 1 (continued)

Gene symbol	Gene name	Mutation type	References
CHD8	Chromodomain helicase DNA binding protein 8	Frameshift indel, missense	O'Roak, 2012
NTNG1	Netrin G1	Missense	O'Roak, 2012
GRIN2B	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	Frameshift indel, nonsense, splice site	O'Roak, 2012
LAMC3	Laminin, Gamma 3	Missense	O'Roak, 2012
SCN1A	Sodium channel, voltage-gated, type I, alpha subunit	Missense	O'Roak, 2012
CTTNBP2	Cortactin binding protein 2	Frameshift deletion	Iossifov, 2012
RIMS1	Regulating synaptic membrane exocytosis 1	Frameshift deletion	Iossifov, 2012
DYRK1A	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	Frameshift deletion	Iossifov, 2012
ZFYVE26	Zinc finger, FYVE domain containing 26	Frameshift deletion	Iossifov, 2012
DST	Dystonin	Frameshift deletion	Iossifov, 2012
ANK2	Ankyrin 2	Nonsense	Iossifov, 2012
UBE3B	Ubiquitin protein ligase E3B	Missense	Chahrour, 2012
CLTCL1	Clathrin, heavy chain-like 1	Missense	Chahrour, 2012
ZNF18	Zinc finger protein 18	Missense	Chahrour, 2012
AMT	Aminomethyltransferase	Missense	Yu, 2013
PEX7	Peroxisomal biogenesis factor 7	Missense	Yu, 2013
SYNE1	Spectrin repeat containing, nuclear envelope 1	Missense	Yu, 2013
VPS13B	Vacuolar protein sorting 13 homolog B	Missense, frameshift	Yu, 2013
PAH	Phenylalanine hydroxylase	Frameshift	Yu, 2013
POMGNT1	Protein O-linked mannose N-acetylglucosaminyltransferase 1	Missense	Yu, 2013
ANK3	Ankyrin 3	Missense	Shi, 2013
CIC	Capicua transcriptional repressor	Missense	De Rubeis, 2014
GLUD2	Glutamate dehydrogenase 2	Missense	De Rubeis, 2014
SEZ6	Seizure related 6 homolog	Missense	De Rubeis, 2014
CEP290	Centrosomal protein 290 kDa	Missense	De Rubeis, 2014
CSMD1	CUB and Sushi multiple domains 1	Missense	De Rubeis, 2014
FAT1	FAT atypical cadherin 1	Missense	De Rubeis, 2014
STXBP5	Syntaxin binding protein 5	Missense	De Rubeis, 2014
CHD2	Chromodomain helicase DNA binding protein 2	Frameshift	Dong, 2014
KMT2E	Lysine (K)-specific methyltransferase 2E	Frameshift	Dong, 2014
PHF3	PHD finger protein 3	Frameshift	Dong, 2014
RIMS1	Regulating synaptic membrane exocytosis 1	Frameshift	Dong, 2014
KCND2	Potassium voltage-gated channel, Shal-related subfamily, member 2	Missense	Lee, 2014
BICC1	BicC family RNA binding protein 1	Missense	Lee, 2014
SLC8A2	Solute carrier family 8 (sodium/calcium exchanger), member 2	Missense	Lee, 2014
GPR124	G protein-coupled receptor 124	Missense	Lee, 2014
IL1RAPL1	Interleukin 1 receptor accessory protein-like 1	Missense	Butler, 2014
GPRASP2	GPRASP2	Missense	Butler, 2014
GABRQ	Gamma-aminobutyric acid (GABA) A receptor, theta	Missense	Butler, 2014
SYTL4	Synaptotagmin-like 4	Missense	Butler, 2014
PIR	Pirin (iron-binding nuclear protein)	Missense	Butler, 2014
CHAC1	Cation transport regulator homolog 1	Frameshift	Butler, 2014
RPS24	Ribosomal protein S24	Nonsense	Inoue, 2015
CDKL5	Cyclin-Dependent Kinase-Like 5	Missense	Codina-Solà, 2015
SCN2A	Sodium channel, voltage-gated, type II, alpha subunit	Nonsense	Codina-Solà, 2015
CUL3	Cullin 3	Missense	Codina-Solà, 2015
MED13L	Mediator complex subunit 13-like	Frameshift	Codina-Solà, 2015
KCNV1	Potassium channel, subfamily V, member 1	Frameshift	Codina-Solà, 2015
MAOA	Monoamine oxidase A	Splice site	Codina-Solà, 2015

Table 1 (continued)

Gene symbol	Gene name	Mutation type	References
PTEN	Phosphatase and tensin homolog	Splice site	Codina-Solà, 2015
SLC7A11	Solute carrier family 7 member 11	Missense	Egawa, 2015
ICA1	Islet cell autoantigen 1	Missense	Egawa, 2015
DNAJC1	DnaJ (Hsp40) homolog, subfamily C, member 1	Missense	Egawa, 2015
C1S	Complement component 1, s subcomponent	Missense	Egawa, 2015
TRAPPC12	Trafficking protein particle complex 12	Missense	Egawa, 2015
CLN8	Ceroid-lipofuscinosis, neuronal 8	Missense	Egawa, 2015

numerous neurological and brain disorders (Almad and Maragakis 2012). The revolutionary results of CRISPR-Gold can open a window to future treatment of various disorders on humans with minimal side effects and better efficiency.

Discussion and Future Perspectives

Diagnosis and treatment of various neurological and brain disorders, including ASD, have been revolutionized by development of diagnostic techniques, such as NGS and WES, and therapeutic techniques, such as the CRISPR system. Use of these techniques has enabled scientists to extend diagnosis and further knowledge on the etiology of ASD by combining the techniques with GWAS. Furthermore, current developmental changes and cost reduction of bioinformatics tools has made WGS studies more economical and widely used. In contrast with targeted sequencing, WES can cover analysis of wide ranges of genes and detect biomarkers or risk factors related to ASD. More comparative studies on ASD occurrence must be studied globally, which could lead to elimination of SNP variations and identification of the essential regulatory regions associated with ASD. As ASD's causative factors are not limited to genetic factors, other aspects, including epigenetics and environmental factors, should be further studied and analyzed in various ethnical groups.

Gene editing therapies, such as CRISPR-Gold, have shown great value of achievement in reversing and reducing ASD's related side effects; however, success has been constrained to mice. Therefore, future studies must focus on further modification of gene delivery as well as the induction means to design a genome editing technique that will efficiently knock out causative genes of ASD and result in significant behavioral changes of autistic patients. With all this in mind, such technological advancement in ASD treatment to minimize the most life threatening symptoms can be foreseen.

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