Towards understanding the genetics of Autism

Harshita Shailesh¹, Ishita Gupta², Said Sif¹, Allal Ouhtit¹

¹Department of Biological and Environmental Sciences, College of Arts & Sciences, Qatar University, Qatar, ²Department of Genetics, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, PC 123, Al Khoud, Sultanate of Oman

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1. ABSTRACT

Autism spectrum disorder (ASD) includes a group of neurodevelopmental disorders that affect communication skills, social interaction and intellectual ability. Despite evidence suggesting a strong genetic link with ASD, the genetic determinant remains unclear. Early studies focusing on candidate genes have shown that several genes associated with neuronal synaptic function are involved in development of ASD. Linkage studies have identified several single nucleotide polymorphisms (SNPs) associated with ASD, and genome-wide association studies have implicated several loci, but failed to recognize a single specific locus with strong significance, indicating heterogeneity in ASD genetic determinants. Detection of de novo copy number variations and single nucleotide variants in several ASD probands has confirmed the genetic heterogeneity of the disease. More interestingly, next generation sequencing approaches have recently identified novel candidate genes and several point mutations in sporadic ASDs, thus increasing our knowledge of ASD etiology. The current review summarizes the findings of recent studies using genetic and genomic approaches to understand the underlying molecular mechanisms of ASD.

2. INTRODUCTION

Autism spectrum disorder is a group of heterogeneous disorders that usually develop during childhood, characterized by a wide range of symptoms, such as difficulties in communication and social interaction, restricted and repetitive behavior. ASDs often exhibit atypical behaviors such as hyperactivity, attention deficiency, aggressiveness, impulsive actions, and tendency to self-injuring and tantrums. Some ASD individuals are hypersensitive to normal level of light, pain, sound, smell and touch; however, some ASD patients develop normal and even advanced skills in specific areas (1). Moreover, ASD is comorbid with other conditions such as epilepsy, gastrointestinal disease and immune deficiency (2).

The word "Autism" was first coined in 1911 by German psychiatrist Eugen Bleuler to describe the severe symptoms of schizophrenia (3). Later in 1943, Leo Kanner, an American professor of childhood psychiatry, redefined autism as a disorder of mental retardation associated with innate inability to establish effective social contact and obsessiveness with specific actions (4). In the same period, another scientist Hans Asperger also observed similar but milder symptoms in several children and referred to the disorder as autistic psychopathy (5), which was later defined as Asperger's syndrome, a subtype of autism spectrum disorder. Infantile autism was included in the Diagnostic and Statistical Manual of mental disorder (DSM-III) in 1980 for the first time (6), and ever since, its periodic revision has increased the number of autism probands. DSM-IV considers autism as a group of similar disorders composed of Autistic disorder, Asperger syndrome, Rett syndrome, Childhood Disintegrative Disorder (CDD) and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS) (7).

Autistic disorder has an early onset, before the age of 3 years, with severe impairment in social interaction,
communication, restricted behavior and repetitiveness. In stark contrast, Asperger syndrome children show relatively milder symptoms with improved language skills, but exhibit lonely behavior (8). Rett syndrome is mostly confined to female children, who show normal postnatal development, however, clinical symptoms such as motor and respiratory problems, social unresponsiveness, frequent seizures and delayed development start to appear at later stages (9). CDD patients show normal development up to age 3, followed by deterioration of language skills, social function and motor skills (10). PDD-NOS children have heterogeneous symptoms of both autistic disorder and Asperger’s syndrome, but improved social interaction skills and thinking capability (11). The latest edition of the Diagnostic and Statistical Manual (DSM-V) has combined all of these subgroups (excluding Rett Syndrome) into a single group referred to as autism spectrum disorder (ASD) (12).

ASD is associated with altered brain function leading to abnormal behavioral phenotype. Several studies have reported defective early brain development in ASD probands due to abnormal neural growth corresponding to age, such as increased volume of white matter and grey matter (13), increased size and number of neuronal cells in prefrontal cortex (14), small cerebellar vermis area (15), structural differences in the grey matter (16) and reduced cortical thickness (17). A recent study using the functional connectivity MRI technique has identified local functional over connections in the posterior brain of diseased individuals, indicating a role of altered neuronal communication in ASD (18). Collectively, these reports indicate that several anatomical and physiological alterations of the brain play a crucial role in the onset of ASD.

Genetics, environment and gene-environmental interactions play a vital role in the onset of abnormal physiology and anatomical defects in the brain. Extensive evidence indicates that the brain of autistic patients is subjected to several genetic and environmental influences, which in turn lead to abnormal development and functioning of the brain (19, 20). Early conventional genetic analyses such as candidate gene studies (21), linkage studies (22), cytogenetic approaches (23), and recent advanced genomic approaches such as genomewide association studies (24), exome sequencing (25), whole genome sequencing (26) and targeted sequencing by next-generation sequencing (27) have attempted to identify the genetic alterations in ASD. The current review will summarize our present understanding of the genetic infrastructures that might play a crucial role in the onset and development of ASD.

3. GENETICS OF AUTISM

The genetic link of ASD was discovered in the studies of twins, which indicated that monozygotic twins have 60–70% chance of disease incidence (28) and that of dizygotic twins is 0–30% (28, 29). The role of the genetic makeup in ASD was further supported by another study, which indicated that siblings of affected children have an 18% risk of developing ASD compared to the normal population (30). Despite these reports showing strong genetic link, ASDs show heterogeneity in clinical symptoms and genetic architecture. Currently, researchers are focusing on deciphering the various genetic variations, which might play role in the onset of ASD.

ASD has higher prevalence in male children compared to female (31). Although several hypotheses have been proposed to explain the cause of sex difference in ASD incidence, none of them were able to provide significant evidence. One of the best fitting hypotheses, supported by clinical studies, suggests that in males, exposure of high levels of secreted testosterone during early gestation results in cognitive hyper-masculinization of the brain during early development, which triggers onset of the autistic syndrome (32-34).

3.1. Candidate gene studies

Candidate gene studies aim to investigate the gene variant in affected individuals, which could play a vital role in disease onset (35). Researchers have focused on identifying potential autism-causing candidate genes that are crucial for brain development, synapsis formation, and neurotransmission. Mutation in brain developmental pathway genes such as WNT-2, which is involved in neuronal proliferative signaling, has been observed in several ASD individuals, indicating a potential role of discrete genes in disease onset (36). Genes involved in establishing neuronal connectivity during brain development such as RELN, which has shown polymorphism in some ASD patients, has been identified as a potential candidate gene (37). Moreover, genes involved in neurotransmission such as serotonin 2A receptor (HTR2A) (38) and serotonin transporter gene (SLC6A4) have also been identified as ASD candidate genes (39). Variation in genes coding for the hypothalamic hormonal pathway such as OXTR, which is considered an ASD candidate gene, has been found in ASD probands (40, 41). In addition, a recent study involving Chinese ASD patients showed that the schizophrenia candidate gene, CACNAIC (calcium channel, voltage-dependent, L type, alpha 1C subunit), could be a potential ASD candidate gene (42). Taken together, these candidate gene studies indicate that polygenic inheritance is prevalent in ASD.

3.2. Association studies and linkage studies

Association studies involving case-control groups have led to the discovery of several novel genetic loci involved in ASD development. Massive parallel sequencing of several ASD candidate genes in a large case-control cohort revealed accumulation of novel
Genome-wide linkage studies have identified many novel loci present in coding and non-coding regions of affected ASD individuals. Early studies of whole genome scanning using microsatellite markers have detected several loci linked to ASD. International Molecular Genetic Study of Autism Consortium (IMGSAC) identified linkage of novel loci on chromosome 7 and 16 in ASD individuals (47). Furthermore, the Paris Autism Research Sibpair Study identified novel potential markers on different chromosomes including 2, 4, 5, 6, 7, 10, 15, 16, 18, 19, and X in ASD individuals from 51 multiplex families (48). An autism sib-pair study of 110 multiplex families using a high-throughput array of 335 microsatellite markers indicated significant linkage of loci located on chromosomes 5 and 8 (49). In light of the lack of consistency among these reports, it is reasonable to conclude that there is a great genetic heterogeneity in ASD. However, it should be noted that these studies had major drawbacks such as small sample size, lack of replication, and population heterogeneity, which might have contributed to variation.

Genome-wide association studies using millions of single nucleotide polymorphism (SNP) markers have gained great success in identifying several new polymorphisms in ASD. The Autism genome project used genome-wide analysis of 10K SNP microarrays in 1,400 families and identified linkage of a novel region on chromosome 11p12-p13 with ASD (50). Another study by John Hopkins University using 50K SNP microarray identified two novel genome-wide association loci on chromosomes 6q27 and 20p13, but with low statistical significance. However, genotyping of the top results of the study in additional families indicated a SNP on chromosome 5p15 with significant association, which was mapped to the region spanning two neuron functional genes SEMA5A and TAS2R. The same study also demonstrated that SEMA5A is downregulated in autism patients. This evidence indicates that the locus might be the principal regulator site of gene expression (51). Moreover, SEMA5A can be a potential ASD candidate gene since it is an axonal guidance molecule, which regulates neuron growth (52). A study genotyped large cohort of ASD families identified six SNPs in a region spanning cadherin 9 (CAD9) and cadherin 10 (CAD10), indicating a role of neuronal adhesion molecules in ASD pathogenesis (53). Genomic approaches to study the parental origin of linked SNPs indicated a strong linkage of SNPs in chromosomes 4, 15 and 20, inherited from paternal side. Although few maternal inherited linked regions were identified, they had low statistical significance (54), thus suggesting the pattern of inheritance from the paternal side. Taken together, genome-wide linkage studies have identified novel loci with high statistical significance and with most of the results confirmed in more than one cohort.

3.4. Copy number variants (CNVs) and de novo single nucleotide variants (SNVs)

Copy Number Variants (CNV) are variation in copy numbers of DNA segments larger than 1kb due to chromosomal aberrations such as deletions, duplications, and insertions, accounting for heterogeneity among the normal individuals (55, 56). CNVs are extensively studied in mental disorders as they are found to be associated with many neuropsychiatric disorders and learning disabilities (57). Evidence indicates that de novo CNVs as well as inherited CNVs play vital role in onset of neurological disorders such as obsessive-compulsive disorder, schizophrenia and bipolar disorder (58-60).

Identification of rare de novo CNVs in ASD individuals by Sebat et al (2007) using comparative gene array hybridization (CGH) technique was a major
breakthrough, which answered most of the “missing heritability” in ASD (61). Rare CNVs, both de novo and inherited have been identified in many simplex and multiplex ASD families. Deletion and or duplication of chromosomal loci such as 16p11.2, 15q24 (62), 17q12 (63), 7q11.23, 16p13.2 (64), and also some genes including SHANK3, NEUREXIN1, NEUROLigin4 (62, 65), CONTACTIN4, UBE3A, PARK2, RFWD, FBXO40 (65), CADHERIN13 (64) and CONTACTIN6 (66) have been reported in many ASD cases. A recent study has shown CNVs in GABAergic signaling pathway genes coding for GABA receptor DBI, GABA receptor-associated protein GABARAPL1, and post-synaptic GABA transporter protein SLC6A11 (67). Whole exome sequencing of ASDs has identified multiple de novo indels, which result in loss of the epigenetic regulator gene, Lysine (K)-specific methyltransferase 2E (KMT2E) and RIMS1 gene, which regulates synaptic vesicle release (68). A recent study in Finnish case-control data sets identified de novo CNVs in genes, which are involved in neuroactive ligand-receptor interaction pathways, calcium signaling pathways, and metabolic pathways such as BDJKR1, BDKRB2, AP2M1, SPTA1, PTH1R, CYP2E1, PLCD3, F2RL1, UQCRCC2, LILRB3, RPS9, and COL11A2 (69). Although, several de novo and inherited CNVs have been detected in various ASD cases, their causal role has yet to be established. Functional study of recurrent CNVs in animal models will help to identify their actual role in ASD onset.

Single nucleotide variance (SNVs), due to de novo point mutation, refers to a single nucleotide change, especially within the coding region of a gene, resulting in silent mutations, missense mutations, or nonsense mutations, and are frequently reported in ASD (70,71). Whole exome sequencing of ASD individuals identified several disruptive mutations in brain-expressed genes (72). SNVs leading to de novo loss of function of genes involved in synaptic functions, histone-modification and chromatin remodeling have been detected in ASD (73). Sequencing the exons and exon –intron boundaries of Glycine receptor alpha 2 (GLRA2) in 400 males from the Paris Autism Research International study (PARIS) has revealed de novo missense mutations in the gene leading to loss of function. Furthermore, functional analysis indicated that GLRA2 plays a role in axonal branching, synaptic plasticity, cognition and memory, which are deregulated when the gene is mutated (74).

3.5. Autism and epigenetics

Epigenetic modifications such as promoter DNA methylation at CpG sequences and post-translational modification of histones regulate gene expression. Differential methylation of promoter DNA of neurodevelopmental genes has already been reported, indicating the role of epigenetic regulation in the development of neurological disorders (75). Prevalence of significant differences in clinical manifestations of monozygotic twins, despite similarity of copy number variations among these ASD twins, indicate the role of epigenetics in ASD development (76). Several ASD candidate genes are involved in chromatin structure regulation, indicating the role of epigenetic alterations in disease development. Mutations in SWI/SNF-based chromatin remodeling complexes such as ADNP and ARID1B, and genes coding for histone demethylases such as JARID1C/SMCX, have been reported in ASD (77-79). Multiple de novo indels in the exons of the chromatin regulator gene, Lysine (K)-specific methyltransferase 2E (KMT2E), are reported in many ASD cases (80). Elevated levels of microRNAs including miR-142-5p, miR-142-3p, miR-144-3p, miR-21 and miR-451a have been reported in the brains of autism individuals, of which miR-21 and miR-451a have binding region at 3'UTR of oxytocin receptor gene transcript. Gene expression analysis and western blotting studies indicated that mRNA level of OXTR is increased in these individuals, however OXTR protein level is kept low, due to increased miR-21 level, (81). Oxytocin receptor is involved in an important pathway that regulates social behavior and found to be deregulated in ASD (82).

Environmental toxins have an adverse impact on DNA methylation and other epigenetic modifications, altering many developmental processes, including neurodevelopment (83). Prenatal exposure to various environmental conditions has been found to increase ASD risk in children (84). Increased prevalence of ASD has been observed in children residing in close proximity to industries emitting air-pollutants such as arsenic, which changes global DNA methylation, histone acetylation and methylation (85, 86). High levels of heavy metals as well as minerals including sulfur, sodium, magnesium, potassium, zinc, and iron, and low levels of calcium and copper are present in the hair of children with autism (87). Autism is also associated with reduced levels of both antioxidant resources and methylation capacity along with high levels of mercury in hair (88). Maternal consumption of folic acid, a potential methyl donor to DNA methylation enzymes, and S-adenosylmethionine (SAM) during pregnancy is a known protective mechanism against ASD (89).

4. CURRENT TREATMENT STRATEGIES OF ASD

The current treatment strategies for ASD aim to reduce the abnormal behavioral symptoms. Antipsychotics and antianxiety drugs along with adjuvant treatment strategies such as behavioral therapy, special educational approaches, speech therapy and cognitive therapy are generally used to improve the symptoms associated with ASD (90, 91). Moreover, strong evidence suggests that incorporation of an adequate diet plan helps to overcome several co-morbidities associated with ASD (92). Dietary elimination of casein and gluten shows
improved disease status than normal diet consuming ASD individuals (93). Dietary supplementation of deficit nutrients in ASDs such as omega-3 fatty acids, probiotics, vitamins, folic acid, and several minerals have been shown to be beneficial in reducing some autism symptoms (94). Also, in relation to diet, there is a necessity for awareness to ensure sufficient intake of nutrients including vitamin B12, folate and foodstuff containing DHA, to further reduce or reverse any adverse effects of nutrient deficiency (95).

5. CONCLUSIONS AND FUTURE PROSPECTIVE

Identifying the genetic etiology of ASD was challenging 50 years ago. But, recent advances in genetic and genomic techniques including microarray, CGH array, whole exome sequencing and next generation sequencing have lead to the discovery of many novel de novo and inherited chromosomal aberrations, SNPs, CNVs and epigenetic modifications associated with ASD. Genome-wide association studies have indicated that commonly inherited variations have higher potential to increase the disease risk (96); however, the individual effect of each variant is very low (97). Although, several rare inherited mutations have been reported in some ASD probands, their effect in imposing ASD risk still lies nascent, except bi-allelic alterations in neuronal protein coding gene, which contributes significantly in disease onset (98). Taken together, recent investigations redefine ASD as a heterogeneous disorder originating from various genetic patterns such as inherited genetic loci from parents to the affected child, de novo variations due to germ line mutations, and epigenetic modifications.

Identification of novel genetic markers has increased knowledge regarding genetic architecture of ASD; however, wide heterogeneity of these variations has limited their application as diagnostic markers. Combining genetic studies with functional analyses serves as a powerful tool to identify genetic architecture of ASD, while explaining the molecular mechanism of ASD onset. Furthermore, these identified genetic markers may represent potential diagnostic criteria for prenatal diagnosis as well as in newborn babies. Identifying novel markers may pave the way towards targeted treatment strategies, prognosis, and better monitoring of disease recurrence post-treatment. Identification of novel predisposition markers may also aid in delay the onset of ASD. Finding common variations among autism probands helps to subtype the disease population and allows prescribing specific treatment strategies. Early detection of ASD helps therapists to guide and inform families about the chances of pervasiveness of the disease in next siblings (99). Increased genetic understanding of ASD has tried to answer the cause of ASD associated comorbidities. The search for de novo copy number variations has revealed CNV in genes involved in metabolic pathways, indicating the cause of metabolic alterations in ASD patients (76). Researchers continue to identify common and inherited mutations, which may play a potential role in onset and development of autism.

Gaining the immense knowledge about autism genetics has opened the path to search for novel drug treatments. For instance, discovery of oxytocin dysfunction in ASD has led to development of oxytocin as a drug treatment in ASD patients. Oxytocin therapy is considered a promising treatment in betterment of ASD symptoms such as enhanced social interactions; however, this treatment has been found to have no effect in some ASD individuals. The wide spectrum of ASD genetic heterogeneity may be one of the underlying causes for this failure (100), further emphasizing the need of personalized therapy in ASD individuals. To this end, researchers have tried to reverse the malfunctioning of several proteins in autism, and restoration of mutated synaptic protein nurexin (NRXN1-β) has been successful in mice. This reversal reduced the autistic behavior such as repetitive behavior, reduced social interaction in mice (101). Thus, there is great hope that through these molecular and experimental therapeutic trials, more and more corrective approaches will be unraveled which will help guide clinicians better design treatment strategies for eventual clinical trials.

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Send correspondence to: Allal Ouhtit, Department of Biological and Environmental Sciences, College of Art and Science, Qatar University, P.O. Box 2713 Qatar, Tel: 974 44037572, Fax: 974-4403-4531, E-mail: aouhtit@qu.edu.qa


