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Review Article

Role of Genetic Mutations in Development of Autism Spectrum Disorder

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ABSTRACT

Autism Spectrum Disorder (ASD) is a highly heritable neurodevelopmental disorder characterized clinically by repetitive stereotyped behaviors, restricted interests, and impaired social and communication skills and it is defined as a developmental disorder. In the past years, autism was confused with other monogenic disorders because the findings were similar. However, with the development of technology over the years, there have been many studies in the field of autism. Recent studies show that there are rare variants of autism as well as small-effect gene variants. Rare variants have more impact than known variants. The discovery of rare variants challenges traditional diagnostic boundaries and demonstrates the heterogeneity of autism. Studies on twins have proven that autism is one of the most common inherited disorders. In addition, it has been observed that the heritability rate of autism is lower in dizygotic twins compared to monozygotic twins. Based on the microarray, deletion, and duplication analysis, single-molecule fluorescence hybridization methods, various mosaic mutations, microdeletions, microduplications, and mutations in Cytoplasmic FMR1-Interacting Protein 1 (*CYFIP1*), NIPA Magnesium Transporter 2 (*NIPA2*), and Ubiquitin protein ligase E3A (*UBE3A*) genes were found in autism. As a result of research not conducted at the molecular level, a broad spectrum of autism with mild symptoms has been found in families of individuals with autism. The likelihood of autism recurring due to various mutations is not known. Therefore, a genetic counselor should be consulted when planning a family. In summary, this review explains in detail how autism has been shaped by recent studies.

Keywords: Autism Spectrum Disorder (ASD), Genetic factor, Neurodevelopmental disorder, Mutations

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INTRODUCTION

Autism Spectrum Disorder (ASD) is one of the most common developmental disorders today. ASD is seen in all racial and ethnic groups in the world, but there is no precise information about its prevalence, although it is stated that it has increased over the years. Today, the reason for the increase in the incidence of autism is estimated to be the increase in autism awareness, the broad determination of diagnostic criteria, and the early diagnosis methods becoming known.

LITERATURE REVIEW

Inheritance of autism in twin studies

The concept of autism is more clearly defined by the clinical studies of Leo Kanner in 1943. Leo Kanner observed 11 children during this period and observed that each of the children showed language impairment and resistance to variability. He even called this condition early infancy autism, meaning "extreme loneliness", in an article published in those years. While the prevalence of autism was low in those years, today it is thought to affect 1% of the world population. While the prevalence of autism in boys is 5 times higher than in girls, considering this knowledge, it is estimated that 1 in 68 children have autism. Autism is referred to as ASD in Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) and International Classification of Diseases 11th Revision (ICD-11). Since the causes of autism are quite different and are caused by mutations in various loci (gene regions), the range is quite wide. It is even confused with other monogenic disorders. Before detailed research, Rett syndrome, a monogenic disorder, was thought to be typical autism seen only in females. However, genetic tests show that a mutation in the Methyl-CpG Binding Protein 2 (*MECP2*) gene causes Rett syndrome. In addition, unlike autism, individuals with Rett syndrome can make eye contact and cope with more severe autonomic nervous system disorders. In addition, monogenic disorders such as Tuberous Sclerosis and Fragile X syndrome can also present with autism symptoms and are often confused with autism. Some researchers describe these disorders as syndromal autism or high penetrance of autism. On the other hand, new genetic and biological findings emphasize that there is no clear-cut distinction between these rare disorders (Thapar A and Rutter M, 2021; Şener EF and Özkul Y, 2013).

Kanner emphasizes that autism is congenital. In addition, according to a psychoanalytic understanding that emerged later, the cause of autism is seen as unloving mothers or "refrigerator mothers". To see the causes and effects of autism, research is being conducted on twin individuals. In these studies, twins were selected as monozygotic and dizygotic. The first twin studies in history were conducted by Folstein and Rutter in 1977. In a study of 21 same-sex twins, Folstein and Rutter found the heritability rate for autism to be 0.0% in dizygotic twins and 3.0% in monozygotic twins. When they evaluated speech impairment, mental retardation, and mild abnormalities, the heritability rate was 10% in dizygotic twins and 82% in monozygotic twins. As a result, the researchers observed that autism is a condition that can be inherited. Several subsequent twin studies also found higher rates of heritable autism in monozygotic than in dizygotic. In a 2016 twin study by Tick, a meta-analysis of all twin studies gave a heritability estimate of 64%-91%. As a result, it was observed that environmental contribution was less than is thought in autism (Thapar A and Rutter M, 2021). In addition to this information, in twin studies, it was observed the higher the estimated heritability, the lower the likelihood of environmental factors. The lower the heritability, the greater the likelihood of the influence of environmental factors.

Broad Autism Phenotype (BAP)

Broad Autism Phenotype (BAP) is a condition that presents with mild symptoms of autism. It is often found in relatives of individ-

uals with autism. In a study by Bai the inclusion of the offspring of sisters contributes to the likelihood of BAP. Bolton P examined 137 individuals who were diagnosed with autism in their families and reported that 5.8% of the siblings were diagnosed with autism or Asperger syndrome, while no ASD diagnosis was reported in individuals whose siblings had Down syndrome. In a study by Ghaziuddin, 4.3% of the siblings of 114 children with autism were diagnosed with autistic disorder. As a result, the phenotype of milder cognitive and language symptoms in family members of individuals with autism has begun to be defined as BAP. Since there are no accepted methods for evaluating BAP, the measurement tools used in the studies may differ in some aspects. One of the first and most widely used instruments is the Autism Family History Interview (AFHI) developed by Folstein and Rutter. The AFHI asks detailed questions about childhood and adolescence in parallel with the symptoms that define ASD. The scores obtained based on the review are summed and used to determine whether the individual exhibits BAP or not. In a study of 99 probands with autism and 36 probands with Down syndrome (the first representative of a hereditary disease in the family), it was found that there were more BAP (+) individuals in the families of autistic individuals than in the control group (Küçük Ö, *et al*., 2018; Trevis KJ, *et al*., 2020). Individuals with BAP have deficits in social functioning. In early adolescence, these deficits are associated with avoidance of social reciprocity, demanding, social smiling, and lack of eye contact. In adulthood, these problems manifest themselves in a reduced need for close relationships, low quality, and a small number of friendships. BAP is also associated with hidden language problems. In early childhood, these problems are seen as a delay in language development.

In children and adults, the problem appears to be primarily in pragmatic language. There is evidence of some hidden deficits in the cognitive abilities of patients with BAP. It has been observed that individuals with BAP have less reflection of emotions in facial expressions and have difficulty conveying their mental states to others. As a result of many studies, BAP traits are more common in families with 2 or more children with ASD than in families with a single child with ASD. These results suggest that the genetic transmission of autistic traits may depend on different models (Küçük Ö, *et al*., 2018).

Genetics of autism

As a result of genome scans, chromosome regions and genes associated with autism have been found (Philippi A, *et al*., 2007). The associated chromosome regions are 2q, 7q, and 17q (Szatmari P, *et al*., 2007). Today, 7 chromosome regions are prominent. These regions are associated with chromosomes 2, 3, 7, 11, 15, 17, and X (Yüksel A, 2005). Mutations in Neurexin 1 (*NRXN1*), Neuroligin 3/Neuroligin 4 (*NLGN3/NLGN4*), and SH3 and Multiple Ankyrin Repeat Domains 3 (*SHANK3*) genes and copy number variants at 15q11-q13 and 16p11.2 are responsible for approximately 10% of autism disorders (Miles JH, 2011; Weiss LA, *et al*., 2009). Szatmari P, *et al*. found a significant linkage in the 11p12-p13 and 15q23-q25 region in the whole genome screening study with 10000 markers on 1181 families in 2007. However, they could not identify a single locus region carrying variation (Szatmari P, *et al*., 2007). Engrailed-2 (*EN2*), Mesenchymal Epithelial Transition (*MET*), and Contactin Associated Protein 2 (*CNTNAP2*) have been identified as important candidate genes in recent locus association studies. *EN2* is a transcription factor located at 7q that is involved in midbrain and cerebellum development (El-Fishawy P and State MW, 2010). The *MET* gene is located in the 7q31 region. Accordingly, Campell DB, *et al*., 2010 reported the risk of *MET* and autism as 2.27 in the family-based study.

Molecular approaches to autism

In the last decade, there has been a tremendous increase in publications with the rise of molecular genetic approaches to autism. Genome-wide studies in medicine and social sciences have enabled the interrogation of genomic variation. Since genomic variation can be characterized by population frequency, it has been looked at whether or how often the variation involves DNA structure.

Genome-Wide Association Studies (GWAS) involve analyzing the whole genomes of patients and controls to identify genetic variations and to investigate associations of variations such as Single Nucleotide Polymorphisms (SNP). Genome-linked data aims to identify biological cycles and find and exploit the underlying causes of complex diseases. Although GWAS has some limitations, it is one of the most important analysis methods for control-based genetic research. On the other hand, a lot of statistical tests are required for so many variants (Thapar A and Rutter M, 2021).

Array analysis

Microarray studies can detect extra or missing chromosomal regions, so-called copy number variations. Studies using compared cDNA (complementary DNA) sequences and subtraction strategies identify expression changes in genes related to ASD. Nishimura showed that gene expression can distinguish between cases of idiopathic autism and ASD with confirmed common pathways and genetic lesions in neural tissues, glutamatergic neurotransmission by comparing cerebellar cortex from cases and controls. This study identified 68 molecules differentially expressed in cells of patients with Fragile X syndrome and 15q duplication. Apart from this study, there have been several studies using gene expression in peripheral blood to identify pathophysiological cycles and neurodevelopmental disorders related to cycles. However, efforts are underway to validate the results of these studies using independent methods and samples. Although less than 1% of the 900 unique genes were identified in the array studies, several genes in the 15q interval (Cytoplasmic FMR1-Interacting Protein 1 (*CYFIP1*), NIPA Magnesium Transporter 2 (*NIPA2*), Ubiquitin protein ligase E3A (*UBE3A*)) were independently identified in separate experiments. In addition, the alternative addition of proteins containing SH3 domains or ubiquitin conjugation, GTPase regulatory activity, α-protocadherin genes, and SH3 domain-containing or ubiquitin-conjugating proteins in the data sets within the ontological category has increased the potential avenues for future mutation imaging studies.

The use of gene expression data will increase as sample sizes increase. Furthermore, expression analyses will better characterize syndromes and correlate them with phenotypic data. Furthermore, the results of expression studies with high-density SNP arrays will show how disease-associated variants affect cellular function (Chahrour M, *et al*., 2016; D'Gama AM, *et al*., 2017).

Deletion and duplication analysis

The Multiplex Ligation-dependent Probe Amplification (MLPA) technique is a rapid and efficient polymerase chain reaction method for the diagnosis of genetic disorders in which deletions and duplications are frequently observed. Polymerase Chain Reactions (PCR) amplicons (amplification source) are fluorescently labeled, separated, and quantified by capillary electrophoresis. In a study in 2020, MLPA was performed on 256 DNA samples from 240 probands (the first representative of an inherited disease in a family) and 16 family members using the SALSA (Sensor Able to detect Lateral Signaling Activity)-MLPA P343 Autism-1 probe mix (MRC-Holland BV). 234 normal results and 22 abnormal results were obtained. The study yielded 15 probands and 7 abnormal results for parents or siblings of the probands. In addition, 116p11 microdeletion syndrome and 116p11 microduplication syndrome were diagnosed. Finally, 9 single probe alterations and 3 deletions, and 1 duplication were detected in the 15q13 region containing 2, 3 genes (Greenman C, *et al*., 2007).

Single molecule Fluorescence In Situ *Hybridization (smFISH)*

Single-molecule Fluorescence *In Situ* Hybridization (smFISH) allows visualization of single mRNA transcripts *in vitro*. In 2018, smFISH was per-

formed in human inducible pluripotent stem cell-derived cortical neurons targeting the *SHANK3* transcript. Both smFISH and conventional immunofluorescence staining showed a developmental increase in *SHANK3* mRNA and protein, respectively, in control human cortical neurons. Analysis of single *SHANK3* mRNA molecules in neurons derived from an autistic heterozygosis for *SHANK3* can be compared with control levels of S*HANK3* mRNA transcripts in cell soma. A 50% reduction in neuronal processes while remaining wild-type. This suggests that targeting local, dendritic *SHANK3* mRNA may be insufficient to produce the wild-type phenotype of a single copy of the wild-type allele in *SHANK3* (Poduri A, *et al*., 2012).

Gene variant study

Hakonarson conducted a genome-wide study including 780 families with 3,101 participants with children with autism and then the second group of 1,204 with ASD. A genome-wide study involves screening genetic markers across the entire DNA (or genomes) of many people to find genetic variations associated with specific diseases. Hakonarson's genome scan revealed only one common region. This was identified as the region of chromosome 5 containing the Cadherin 9 (*CDH9*) and Cadherin 10 (*CDH10*) genes. Hakonarson thought that these genes code for the production of important neuronal cell adhesion molecules that sit on the surface of nerve cells and facilitate cell-to-cell communication. Hakonarson declared that no other common gene variants have been identified and replicated in independent studies with the same scientific robustness. He claimed that with the discovery, identifying children with these variants in utero means that intervention can start early. In contrast to the previously found common gene variants in autism, research on rare genetic variations provides us with more detailed information about the variants of autism. Generally, rare variants have a wider range of effects than common variants (Chahrour M, *et al*., 2016). This rare variant research has focused on copy number variation. Copy variants are defined as regions of DNA containing thousands of base pair variations. Rare variants are passed on to individuals with autism from their parents (Thapar A and Rutter M, 2021).

Mosaic mutations in autism

Mosaic mutations are defined as *de novo* mutations that occur after fertilization in some body cells of different neurological disorders and cancer cycles (D'Gama AM, *et al*., 2017; Greenman C, *et al*., 2007; Poduri A, *et al*., 2012; Rodin RE and Walsh CA, 2018; Shirley MD, *et al*., 2013). These mutations add anatomical changes to the cellular phenotype. Although still inconclusive, several recent studies suggest that autism is influenced by somatic variants. For example, recent somatic mutation studies suggest that 5.5%-7.5% of *de novo* mutations may be postzygotic mutations (Freed D, Pevsner J, 2016; Lim ET, *et al*., 2017).

Genetic counseling in autism

Since the cause of autism is not known with certainty, it is easier to determine the probability of recurrence of the disease. If no genetic cause is found, there is a relative 10%-20% chance of recurrence (Ozonoff S, *et al*., 2011). If the siblings of an individual with autism have symptoms of the genetic autism phenotype, this value increases slightly when the probability of the presence of autism is considered. If there is only one individual with autism in the family, the probability of recurrence increases up to 25-30. In autism with an undetermined cause, the probability of recurrence decreases to 1%-2% (Pickles A, *et al*., 2000). In 50%-70% of families with individuals with autism, autism is thought to be genetic. In these families, it is among the duties of the medical geneticist to inform the family by evaluating the expectations with genetic testing applications. It should be explained to the family in an appropriate and understandable language that genetic test results may not answer all their questions but they could help. A detailed clinical and genetic evaluation is required to determine the appropriate tests for the condition of the individual with autism. Tests to find a genetic cause in all individuals with autism are not medically accurate. It should be known that a genetic cause can be found in less than 25% of individuals with autism even when the necessary clinical studies are conducted (Gurrieri F, 2012; Abrahams BS and Geschwind DH, 2008; Łaczmańska I, *et al*., 2020; Taylor SE, *et al*., 2018).

DISCUSSION AND CONCLUSION

Autism is defined as a spectrum disorder caused by mutations, gene dosage, and copy number variants in some genes linked to chromosome regions 2q, 7q, and 17q, the cause of which is still not fully determined in a variable structure. Autism contains *de novo* mutations that occur after fertilization, called mosaic mutations. These mutations can also damage the cellular phenotype. Autism due to mutations can be easily detected with current molecular approaches. These approaches include microarray, MLPA, and smFISH. Microarray studies have found *CYFIP1*, *NIPA2*, and *UBE3A* genes in the 15q interval. In the MLPA technique, microdeletion syndrome and microduplication syndrome were diagnosed. Single-molecule fluorescence hybridization targeting dendritic *SHANK3* mRNA by visualization of mRNA transcripts *in vitro* showed that a single copy of the wild-type allele in *SHANK3* may be insufficient to produce the wild-type phenotype. Because the mutations of autism are so diverse, the likelihood of recurrence cannot be accurately predicted. Therefore, there is a constant need for support from a genetic counselor.

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