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Gene Therapy as a Promising Approach for the Underlying Causes of Autism Spectrum Disorder

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Abstract

Autism spectrum disorder (ASD) is a range of developmental disorders characterized by impaired traits associated with three distinct domains: communication, social interaction, and stereotypic repetitive behavior. Although the etiology of ASD depends on various components, current research mainly focuses on the genetic factors that contribute to the development of ASD and its effects. A big treatment consideration is gene therapy, which has disease-modifying potential. This article provides insight into the foundation of ASD and the leading gene therapies that aim to address impaired neurological behavior. We will discuss how certain genetic factors can have large contributions to ASD development and how scientists can go about targeting these factors for potential remedies.

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Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition that affects communication and social interaction. It is a complex condition with a range of symptoms and severity levels, and the exact causes are still not fully understood.¹ However, genetic factors are believed to play a significant role in the development of ASD.²

Symptoms of ASD, which typically appear in early childhood, include impaired social interaction and communication, repetitive behaviors and interests, and sensory processing issues. These symptoms range from mild to severe and may affect an individual's ability to function in daily life.^{1,3}

At a brain developmental level, ASD is thought to result from abnormal development, particularly in regions of the brain involved in social interaction and communication. Some studies have suggested that individuals with ASD may have differences in brain structure and function, such as altered connectivity between different regions.⁴

At a genetic level, ASD is believed to be caused by a combination of genetic and environmental factors. While the exact causes of ASD are still not fully understood, research has identified a number of genetic variations that are associated with an increased risk of developing the condition. These genetic factors may interact with environmental factors to influence brain development, ultimately resulting in ASD.¹

Autism is one of the most heritable neurodevelopmental disorders, affecting 78 million people or 1.5 % of the world's population.⁵ Despite its ubiquitous existence, there are aspects of the disorder that science has yet to unveil. With the etiology of autism still in question, scientists have been able to conclude that at least 40% of neurobehavioral disorders within the autism spectrum are the result of genetic abnormalities.⁶

The current treatment for ASD falls into two categories: those that target core symptoms which include impaired communication, social interaction, and repetitive behaviors; and those that target secondary or consequential symptoms such as ADHD and irritability. Gene therapy, a treatment that targets core symptoms, focuses on mending genetic building blocks. It aims

to alter abnormalities in hopes of preventing domino-effects in the body that result in the neurobehavioral deficits which characterize ASD. Using viral vectors such as recombinant adeno-associated viruses, gene therapy is able to introduce new genetic material to counteract the establishment of ASD symptoms.⁷ This paper will be discussing studies and their adaptation of gene therapy to varying pathophysiologies of ASD in order to offer effective treatment methods. This paper will cover the underdevelopment of neurons, impaired neural migration, impaired synaptogenesis, and dendritic morphogenesis.⁸

Etiology of Autism Spectrum Disorders

Although the details of the origin of ASD have not yet been discovered, scientists have come up with multiple theories answering the question of “how” ASD comes to be in individuals. One such theory on the pathophysiology of ASD is linked to neural connectivity. In a typical human body, an individual develops a surplus of neurons where over time, non-functional and unnecessary neurons are removed through various mechanisms. In patients who have ASD, the mechanism that targets the elimination of underdeveloped neurons is damaged. As a result, the excess neurons impair the shaping and fine-tuning of neural circuits.⁹

Another theory focuses on neural migration. Similar to neural connectivity, neural migration also contributes to well-formed neural circuits which aid in proper communication and other neural behaviors. In ASD patients, the misplacement of neurons as a result of faulty migration during development increases the thickness of the cortex and “smudges” the boundaries of white matter. This mostly affects the frontal and temporal lobes: key elements in processing language and emotion—functions commonly found to be impaired in ASD patients.⁹

An additional theory that defines the mechanism by which ASD is established focuses on impaired synaptogenesis and dendritic morphogenesis. The normal development of synapses and dendrites entails excess formation followed by the purging of faulty expression. In patients with ASD, the suppression mechanism responsible for the expulsion of

faulty signaling factors is impaired. The overwhelming number of defective synapses and dendrites damages the pathway of signals within the body, resulting in the core symptoms associated with ASD.⁹

Key Genetic Factors

The build-up of faulty factors as a result of impaired mechanisms stems from mutations present in the genetic factors that regulate such processes within the human body. The existence of these mutations is what has led scientists to link ASD to genetic abnormalities. In typical human physiology, genetic factors work in a factory-line fashion to facilitate proper operations in the formation of neurons, synapses, and dendrites as well as key neural factors. The malfunction of any genetic factor in the factory line creates a “domino effect” that leads to forms of neurobehavioral impairment associated with ASD. Although there are many different factors that contribute to proper neurobehavior, this paper focuses on the mutation of three in particular: TCF4, RELN and MECP2.^{10, 11, 12, 13, 14}

Studies: Linking Etiology of ASD and Mutations of ‘Key Genetic Factors’

4.1 TCF4 Gene Study

Transcription Factor 4 (TCF4) is responsible for encoding a helix-loop-helix transcription factor expressed most often during brain development. An alteration in TCF4 causes Pitt-Hopkins Syndrome (PTHS): a disorder characterized by profound cognitive and motor disabilities and defined under the umbrella definition of autism disorders. Patients with PTHS have mutations that either knock out the functional TCF4 gene, eliminate its essential DNA-binding domain, or impact one of its transcriptional activation domains.¹⁰

The University of California San Diego Medical School sought to understand how a mutated TCF4 gene results in this form of autism.^{11, 12} To

explore this idea, scientists used pluripotent stem cells (iPSC), adult somatic cells that have been reprogrammed to be in an embryonic state, of 5 patients with PTHS.¹² To guarantee a controlled experiment, mutated TCF4 was the only chromosomal abnormality present in the experimental PTHS iPSC lines. These iPSC lines were used to generate neuron progenitor cells (NPCs), the precursors of most of the neurons that make up the central nervous system. The PTHS NPCs, produced by the PTHS iPSC lines, presented a reduction in TCF4 expression. This resulted in neurons with altered expressions of CNTNAP2 and KCNQ1, which are usually regulated by the TCF4 gene.^{10,11} For further analysis, scientists used the control and PTHS iPSCs line to create brain cortical (CtO) organoids, an artificially manufactured tissue resembling the functionality and structure of the human brain.¹¹

In comparison to the control organoid's normal spheroid form, the PTHS CtO organoid presented an abnormal structure and size. Through single cell RNA sequencing, it was concluded that the PTHS cortical organoid's diminished size and characteristic factors were the result of a loss of cellular diversity. To make certain that this loss was not a result of mispatterning, the UC San Diego team performed single cell transcriptomic testing on the organoids. The telencephalic marker, FOXG1, expression analysis in combination with the single cell RNA sequencing concluded that the loss of cell diversity in the PTHS cortical organoid was due to a higher percentage of underdeveloped neurons, known as neural progenitor cells (NPC). The effects of the mutated genetic factor TCF4 on the brain correlates to the neural connectivity theory. This theory posits that a surplus of deficient development of neurons due to impaired progenitor proliferation, alters the function and structure of the brain and its neural circuits responsible for functions such as communication and behavior. This excess of underdeveloped neurons prevents the idealized function of neural circuits.¹¹

With an understanding of the first domino in the developmental pathway of PTHS, the UC San Diego team focused on abolishing its molecular and cellular characteristics by correcting the expression of the TCF4 gene. The first method used two viral vectors and a CRISPR- based transepigenetic

strategy. All three cassettes worked together to enhance the transcription of the TCF4 gene, ultimately correcting the downstream targets.¹¹

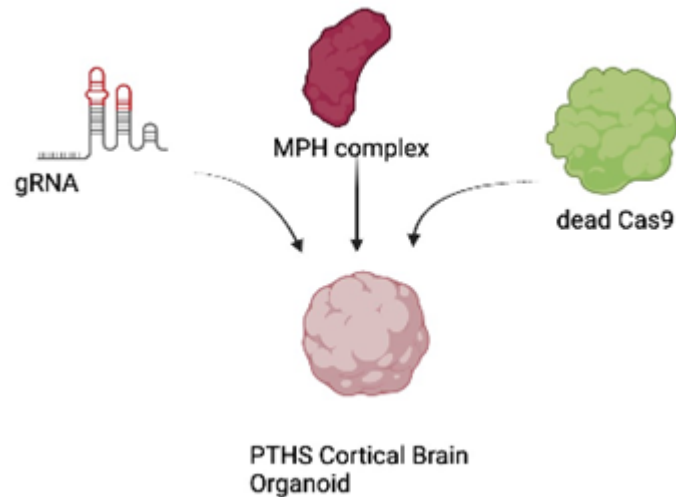


Figure 1: CRISPR-based transgenetic strategy for correction of abnormally low TCF4 expression; a complex comprising of gRNA targeting promoters of TCF4, a transcriptional activation complex (MPH) and dead Cas9

However, the use of two viral vectors created another problem: a decrease in cellular aggregation in the brain organoids. The scientists attempted another method called virus-mediated overexpression in which a wild-type copy of TCF4 was overexpressed in hopes of overriding ectopic expression of the mutation. PTSH organoids exposed to overexpression-type gene therapy showed improvements in two key regions that marked the downstream corrected function of the TCF4 gene: increased firing rates and number of network electrical bursts. The combination of these two methods, provided that the impaired proliferation of neurons from a mutated TCF4 can be genetically corrected, could prove to lessen the symptoms of other genetic autism disorders.¹¹

4.2 RELN Study

RELN is a gene that encodes for a RELN glycoprotein in the extracellular matrix of GABAergic (Gamma-Aminobutyric Acid) neurons. These cells perform important functions in neural migration and cortical lamination.¹³ Neural migration is an important process that occurs in mammalian nervous system development.¹⁴ Cortical lamination is the layering of cells of

the outer regions of the brain's cerebrum, helping to maintain neuronal cytoskeletal stability. Reduced RELN levels have been shown to be associated with different psychological disorders including bipolar disorder, Alzheimer's disease, and autism spectrum disorder. Studies have shown significantly lower levels of RELN in ASD patient's superior frontal cortex, parietal cortex, cerebella and plasma. This could potentially be because of RELN promoter hypermethylation that occurs in GABAergic neurons, as seen in seizure disorder patients.¹³

In addition, sex hormones can also play a role in methylation of the RELN promoter, which has shown to increase ASD-associated behaviors. After postmortem cerebellar studies of ASD patients, it was found that lower RELN mRNA levels are associated with higher MECP2 (another essential protein for nerve cells) binding, increasing gene regulator 5-hmC at the RELN promoter and in turn decreasing transcription and protein levels. Such RELN mutations that cause disruptions in signaling pathways are connected to ASD disorders. One example is the loss of Purkinje cells, regulated by RELN, which increases the risk of cognitive delay and epilepsy. This is a phenomenon also seen in ASD patients.¹⁵

Investigating the role of RELN on neuronal signaling and ASD development can produce promising leads for potential treatments of ASD. Increasing levels of RELN protein can alleviate behavioral symptoms of RELN that ASD patients also experience. However, although there is much evidence for RELN's influence on ASD, it is not the sole factor of ASD development. For the diagnosis of ASD, there are usually secondary genetic or environmental factors that contribute to the disorder's development.¹⁶

4.3 MeCP2 Gene Study

Many studies have shown MeCP2 protein's role in brain development and regulation, such as expression of the brain-derived neurotrophic factor (BDNF) gene and regulation of synaptic homeostatic plasticity. MeCP2 is a part of the methyl-CpG-binding protein family which regulates gene expression by modifying chromatin, a protein complex of DNA and histone proteins that package DNA in chromosomes. MeCP2 performs regulation of DNA methylation via recruitment of histone deacetylases.^{17,18}

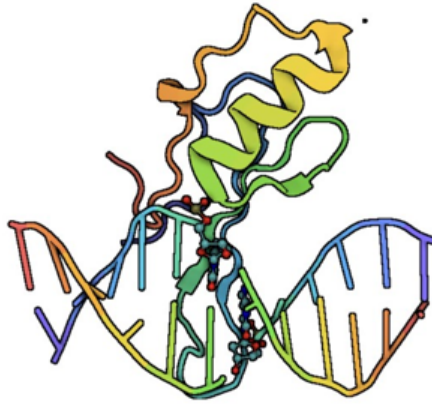


Figure 2: MeCP2 protein structure

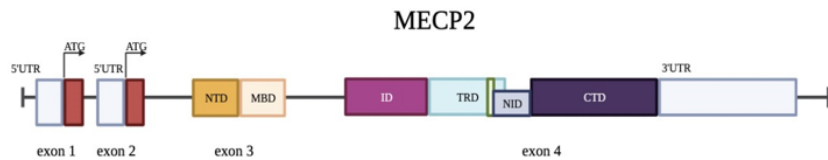


Figure 3: The MECP2 gene

MeCP2 is found in high concentrations in neurons. The gene locus is located on the long (q) arm of the X chromosome in band 28 (“Xq28”). MeCP2’s main role is “repressing” or “silencing” other genes, preventing transcription and translation when they are not needed. Recent studies have suggested that MeCP2 can act as an activator, but this is still a new and controversial theory. MeCP2 represses gene expression by recognizing and binding to methylated cytosine residues in DNA called 5MeCyt; regions enriched with A/T neighbor bases. MeCP2 is also able to bind to hydroxymethylated DNA called 5-hydroxy methylated cytosine.¹⁸

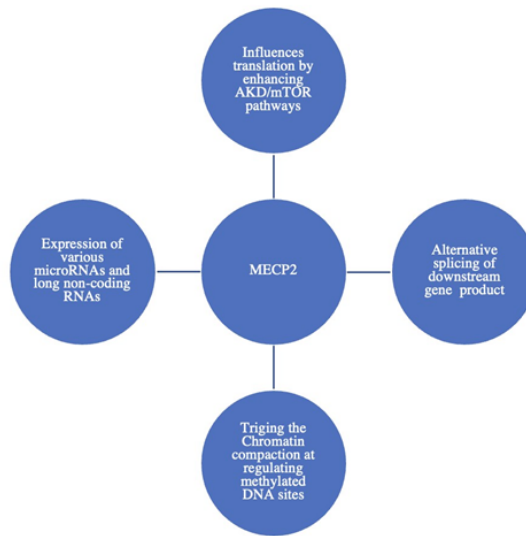


Figure 4: The varied molecular functions of MECP2 protein

The Relationship Between Autism and MECP2

Mutations that alter typical MeCP2 function will lead to severe neurodevelopmental disorders such as Rett syndrome (RTT) and ASD. More than 95% RTT patients carry Mutant MeCP2, and some MeCP2 mutations have been reported in ASD patients. ASD and RTT overlap in certain phenotypes, including stereotypical body movements, social avoidance, and anxiety.^{17, 18}

In a study conducted by Zhu Wen at School of Life Sciences in Peking University, Whole-Exome Sequencing (WES) was performed on 120 ASD cases. This experiment was able to identify three mutations in the coding regions of the MeCP2 gene. They found that the MECP2 gene was linked with a host of neuropsychiatric disorders and neurological phenotypes.¹⁸

5.1 Methods:

120 Han Chinese families were selected with probands diagnosed with ASD from 2013-2015 in the Department of Child and Adolescent Psychiatry in Shanghai Mental Health Center. The range of age of the patients was between 2 to 18 years old and included 18 females and 102 males. The study excluded patients with severe somatic disorders RRT.¹⁸

In terms of clinical assessment, comprehensive profiles of each patient were collected in the case report form. Researchers classified the symptoms into 4 categories: social interaction, language, repetitive behaviors, and functional impairment. 2–3 µg of genomic DNA was extracted from each patient and libraries in order to prepare cluster generation and sequencing. Based on the patients' WES experiment, families within probands carrying MECP2 variations were selected for Sanger sequencing to determine if the variations were de novo or inherited. The WT- MeCP2 gene (the E2 isoform of rat *Mecp2* cDNA) and other mutated plasmids such as MeCP2-P152L, MeCP2-R294X, and MeCP2-P376S were used to detect ASD probands.¹⁸

On days 15–16 of mouse embryo development, cortical neurons and HEK-293 cells were individually cultured and electrophoretically transfected into each group at 0 days in vitro individual. HEK-293 cells and mouse cortical neurons were collected after 3 days in vitro for Western blot and immunofluorescence analysis, respectively.¹⁸

5.2 Results:

Three mutations of MeCP2 were detected among 120 ASD patients via WES. p.P152L (c.455C>T) and p.P376S (c.1162C>T) were missense mutations and p.R294X (c.880C>T) was a truncating mutation. Sanger sequencing showed that p.P152L and p.R294X were de novo mutations, but p.P376S was inherited maternally. They did not find any of these mutations in GnomAD, indicating that they were rare mutations.¹⁸

In terms of clinical features, poor social interaction and functional impairment were major symptoms for all three mutations, but language use and repetitive behavior differed greatly between them. Also noted were abnormalities of dendritic and axonal growth found after autism-related MeCP2 mutants were expressed in mouse cortical neurons, suggesting that autism-related MECP2 mutations impair proper development of neurons.¹⁸

The results strongly suggest that MeCP2-P152L, MeCP2-R294X, and MeCP2-P376S affect the proper physiological function of the MeCP2 protein and may contribute to the pathogenesis of autism.¹⁸

MECP2 Gene Therapy Methods

In addition to these studies, there have been a number of experiments utilizing gene therapy for the treatment of ASD. The majority of these trials have utilized a viral vector to deliver a modified version of the MECP2 gene to patients. The results of these trials have been promising, with patients exhibiting improved social interaction, better communication skills, and an improved quality of life.^{6,19}

Recombinant adeno-associated virus (rAAV)-delivered gene substitution has been shown to improve behavior in several investigations employing monogenic animal models. For example, a recent study has shown that systematic administration of a rAAV9-MECP2 vector sufficient for 10% CNS transduction (of primarily neuronal cells) in an RS animal model resulted in modest behavioral improvements.⁶ Comparatively, 25% CNS transduction at a 6-fold higher vector dose led to noticeable behavioral and phenotypic benefits.¹⁹

A promising MECP2 gene therapy strategy is using antisense oligonucleotides. Antisense Oligonucleotides are small, modified nucleic acids that can selectively hybridize with m-RNA from a target gene and silence it. MECP2 duplication syndrome has been associated with autism, intellectual disability, motor dysfunction, and anxiety, and is one of the most common genomic rearrangements seen in male patients. The cause of these deficits is the over-expression of methyl-CpG-binding protein 2 (MeCP2). This syndrome poses a challenge to traditional therapeutic approaches.¹⁹

Some symptomatic mice models of monogenic loss of function neurological disorders, including the loss of MECP2 in Rett syndrome, have demonstrated reversal of phenotypes, indicating that the molecular correction of the underlying dysfunction could potentially restore typical physiology. The study focused on the restoration of normal MeCP2 levels in MECP2 duplication syndrome using mice models.^{17,19}



Figure 5: methods used in Sztainberg et al. trials testing use of human-specific antisense oligonucleotides (ASOs) to normalize MeCP2 levels in mouse models

The contributors generated a conditional MeCP2 - overexpression mouse model and showed that the correction of MECP2 levels effectively reversed the molecular, electrophysiological, and phenotypic deficits using antisense oligonucleotides (ASO). It was found that antisense oligonucleotide treatment resulted in broad phenotypic rescue in symptomatic transgenic MECP2 duplication mice and corrected MeCP2 levels in lymphoblastoid cells in a dose dependent manner.¹⁹

Experimental results suggest that delivery of ASOs to CNS could prove to be promising for treating MeCP2 duplication syndrome, with potential applications in ASD and Rett Syndrome. Future directions should focus on testing different ASO dosages, determining safety margins of MeCP2 levels, and screening MeCP2 ASOs for off-target effects.¹⁹

Antisense oligonucleotides (ASOs) and short interfering RNAs (siRNAs) are two methods for sequence-specific suppression of mRNA transcripts which can then be used to mute gene expression. In a conditional MECP2-overexpressing mouse model of MECP2 duplication syndrome, it was demonstrated that halving MECP2 expression restored cellular function and phenotype postnatally. The same study found that intraventricular delivery of ASOs specifically directed against MECP2 caused widespread ASO dispersion throughout the CNS. This resulted in efficient knockdown of MECP2 to levels close to wildtype and a long-lasting phenotypic reversal. Both studies suggest gene replacement and RNA knockdown of MeCP2 gene may be a method worth exploring as a possible remedy for ASD.¹⁹

Future Directions

In addition to the aforementioned genetic influences on ASD cases, there are other factors that can impact the pathophysiology of ASD. For example, many autism patients have reported gastrointestinal disorders (GI). This has prompted interest in possible relationships between the gut microbiome and autism.⁷

Up to 70% of autism patients have reported dealing with GI disorders. These GI disorders can be a result of dysbiosis, an imbalanced gut microbiome, characterized by reduced microbial diversity and increased microflora in gut microbiota. The gut microbiome generates a large portion of metabolites, and is responsible for energy conversion, signaling, and, most importantly, epigenetic signaling. An imbalanced gut microbiome can create an excess formation of S-adenosyl-methionine, the main methyl group in a microbiome, which results in increased methylation. The activation and deactivation of DNA strands through DNA methylation could potentially lead to the gene impairment/variation that results in ASD. Further studies into this phenomenon may have implications for treatment development if methylation events influencing ASD development can be targeted and altered. Future research studying the gut microbiome's ability to interfere with methylation patterns can be used to inform development of gene editing in treating ASD pathogenesis.¹

Conclusion

While there is currently no cure for ASD, there are various approaches to managing its symptoms and improving quality of life. These include behavioral therapy, medication, and support services. Research into the causes and potential treatments of ASD is ongoing, and new developments are constantly emerging.²

Gene therapy is one promising approach that holds significant potential for treating the underlying causes of ASD, rather than just managing its symptoms. However, more research and clinical trials are needed to fully understand the safety and effectiveness of gene therapy for ASD. In the last

five years, clinical research has shown that TCF4, RELN, and MeCP2 are linked to ASD, providing scientists with a basis to explore the possibilities of gene therapy. The CRISPR strategy and viral overexpression in relation to the TCF4 gene, MECP2 levels in relation to the RELN gene, and rAAV-delivered transgenes to alter gene expression all show promise in altering the behavioral phenotypes associated with autism spectrum disorder.²⁰

However, there are also challenges and limitations to the use of gene therapy for ASD. Gene therapy is a complex and expensive approach that may not be accessible to all individuals with ASD. In addition, there are ethical considerations surrounding the use of gene therapy, such as the potential for unintended effects on other genes.²

Overall, gene therapy holds significant potential for the treatment of ASD, but further research and clinical trials are needed to fully understand its safety and effectiveness. If successful, gene therapy could offer a promising new approach for treating the underlying causes of ASD and improving the lives of individuals with the condition.

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