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Treatment of autism spectrum disorder by regulating neurotransmitters and modifying genes

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Abstract. Autism spectrum disorder (ASD) is a neurological and developmental disorder, which has many underlying causes, making it much more challenging to find an appropriate treatment to cure. It has been found that ASD is related to a wide variety of neurochemical changes, including GABA, oxytocin, and several monoamine neurotransmitters such as dopamine, norepinephrine, and serotonin. Since ASD is heterogeneous, precise technology such as PET, LP, iEEG, rs-fMRIs, and MRS had been emerged to discover the site of illness. It requires a persistent effort since the ASD phenotype is invertible instead of unchanged, which will be explained soon. Until now, only genetic modified virus (rAAV) have so far successfully incorporated with transduction and gene expression in vivo. However, whether the presence of neutralizing circulating antibodies will preclude intravenous administration is unsure. Instead of systemic delivery, intrathecal administration is discovered as a substitute, which can solve the problem and have advanced to a clinical trial. More gene therapies are hypothesized, for instance, using rAAV for gene replacement, ASOs for RNA knockdown, and CRISPR/Cas9 for gene editing. Unfortunately, these gene therapies still have a long way to go to overcome the restrictions. As a result, this research will analyze the different treatments for the ASD.

Keywords: monoamine neurotransmitters, heterogeneous, gene editing.

1. Introduction

Autism spectrum disorder (ASD), better known as autism, is a mental disorder. It affects individuals' ability to speech, think, or interact with others. It is noticeable that the diseased are found to start from a patients' early age of their life, usually the first two year, so it is described as a neurodevelopmental disease. The Diagnostic and Statistical Manual of Mental Illness (DSM-5) states that people who suffer from ASD are always struggling with daily communication and social interactions, have limited interests and reduplicative behaviors, or experience tribulations that impair their ability to act as the normal population in social occasions such as work or school. The kind and severity of symptoms that people encounter vary greatly. Certain treatments can assist with daily well-being, even though ASD can potentially be a chronic condition.

Social communication issues, including seem never gazing at or responding to those who are chatting, making very little visual contact, or irregularly expressing interest, emotion, or enjoyment in items or activities, are possible signs and symptoms of autism. Additionally, individuals with ASD exhibit restrictive or repetitive behaviors. These abnormalities include repetition of certain unusual behaviors, a persistently intense interest in a particular subject, such as figures, specifics, or facts, or

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behaving vigorously or unperturbed than other individuals to sensitive output, such as pressure, light intensity, or even temperature change. Additionally, people with ASD are seem to be extremely talented in a variety of areas, such as math, art, painting, or music.

ASD is actively monitored by the association named Autism and Developmental Disorders Monitoring (ADDM) Network. This study focuses on 8-year-old youth in 2018 whose guardians resided around 11 ADDM Network sites in the U.S. It also examines and features of ASD in those tested samples towards those children. The likelihood of ASD in children of 8 years old was 23.0 per 1,000 (one in 44), and it was 4.2 times more common in boys than in girls. 5.4% of the 5,058 kids who fit the criteria for ASD had an ASD ICD code alone, 18.8% had the phenotypes for ASD whilst so far, no psychiatric diagnosis for it, and 75.8% had been diagnosed to have ASD. Based only on recorded ASD diagnostic claims, the prevalence of ASD per 1000 children aged 8 years is 17.4. The average age at which the first recorded ASD diagnosis was made from 144 weeks in California to 250 weeks is Minnesota [1].

Researchers didn't figure out the primary reasons for ASD, while researches gave information about an individual's DNA is impacted by nurture in manners that trigger the early development of ASD, while part of the environmental elements are perceived to link to the formation of ASD. The study initially only had a tiny sample size, but it was the first indication that autism might have a genetic basis. More than a dozen other twin studies conducted since this ground-breaking study has supported the initial finding. In 1977, 11 identical and 10 fraternal twins from different parts of Great Britain participated in the first twin study on autism, and at least one of the twins was autistic. Identical twins have a percentage of 36% that both are autistic, while fraternal twins are never found to be the case. The best estimate at this time is that identical twins have a concordance of 50–80% and fraternal twins have a concordance of 5-20%. This suggests that the illness has a significant hereditary component. The percentage for fraternal twins, which is 5-20%, also illustrates the likelihood that a couple who already has an autistic kid will produce another autistic child. The exact reason for ASD is not yet been clearly identified. This is due to two factors. The first is that the relevant genomic areas are probably exceedingly complicated. To study them, scientists have had to create new methodologies [2]. The second is that it's likely that the genetic alterations are both complex and extremely rare.

There are more than 3 billion base pairings in the double stranded DNA chain that makes up the human genome. Researchers must examine a huge number of autistic persons to find little bits of DNA amid so many base pairs that may be related to the onset of autism. Yet, with the rapid advancement of genetic technologies and international scientific collaboration that will enable the study of a huge number of people, important strides in our understanding of the origins of autism are highly likely to be made very soon. A large number of cases of autism may be linked to so-called common genetic variation. This is a reference to genetic variations that are common in people without autism but which do not, by themselves, cause autism. The development of the brain is significantly impacted when many genetic risk factors are present in the same individual. There is no known cause of autism and no single treatment that works for all. The purpose of the treatments is to minimize the symptoms and increase the patient's ability to function normally. Several therapies are available as therapy choices. Some institutions and organizations offer behavior and communication therapy intending to improve language, social, and behavioral challenges related to autism. Applied behaviour analysis could help kids acquire new skills and transfer those talents to a variety of circumstances by using a reward incentive system [3-4]. Children with autism are very appreciative of the highly structured educational programs included in educational therapy. Home psychotherapy educates adults in the family ways to interact with and connect with their children in approaches that promote capacities for interpersonal activities, regulate behavioral issues, and carry on communicating and everyday life abilities [5]. Medication is also a type of therapy. Certain drugs can assist regulate symptoms, but no treatment can relieve the primary symptoms of ASD. For instance, some pharmaceuticals may be prescribed if the child is hyperactive, serious issues in terms of abnormal behaviors can be treated with anti-psychotic medications, along with anxiety, which could be controlled by applying amitriptyline. Any pharmacies or nutritional supplements that the autistic kid is taking should be disclosed by professional medical

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practitioners, because certain drugs can bring severe side effect and easily damage to the body. The American Academy of Paediatrics recommends that all parents are responsible to take their children to be scanned for ASD in a young age and parents should discuss the results with doctors. Therefore, this research will summarize current reasons found to relate to the formation of ASD, and analyze the treatment of autism by targeting the different causes [6].

2. Neurotransmitter regulation

Neurotransmitters are crucial for the daily function and development of the nervous system as it contributed as a ligand during the transmission of eclectic impulses. Many psychological diseases are highly related to changes of sensitivities, or the number of neurotransmitters secreted in the CNS.

Since the diagnosis of ASD is according to behavioral appearance without a clear neurological basis at this time, even though many different systems have been proved to relate to autistic actions, ASD is known as a heterogeneous neurological disorder. It has been suggested there are a variety of underlying causes, making it challenging to find the best course of treatment for ASD without a time-consuming trial-and-error procedure. ASD has been linked to a wide variety of neurochemical changes. The neurotransmitter which excites the brain cortex is mainly glutamate and the inhibitory neurotransmitter is mostly consist of gamma aminobutyric acid (GABA). Both of them are thought to exhibit not just an excitatory-inhibitory imbalance, but also defects in GABAergic transmission between neurons. ASD has also been linked to oxytocin, a crucial neurotransmitter thought to play a role in social motivation and bonding, and it is currently the subject of extensive research. Moreover, ASD are thought to be highly related to abnormalities in dopamine, acetylcholine, and serotonin. These abnormalities can sometimes be brought on by flaws in biosynthesis brought on by a lack of the cofactors' pyridoxal-5-phosphate, tetrahydrobiopterin, and/or folate [7].

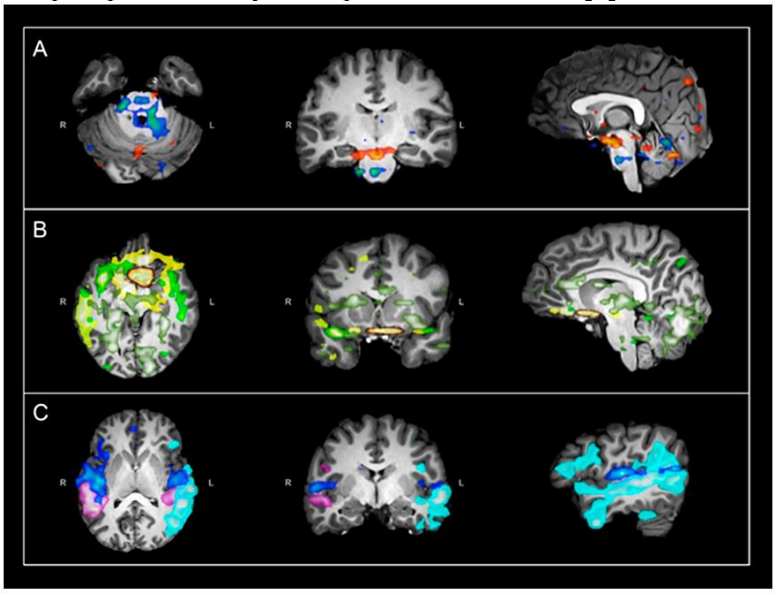

Figure 1. Selective fMRI network for ASD patients [7].

Finding anomalies in monoamine neurotransmitters is crucial since ASD is being actively researched and treated with drugs that regulate dopamine, norepinephrine, and serotonin. ASD, like many psychological illnesses, is quite heterogeneous, as was before established. Because therapeutic procedures attempt to target anomaly of the particular individual with a unique phenotype, who is probably belonging to a group of patients with the similar symptom, much research does not necessarily transfer effectively into medical treatments. This is due to the academic contribution usually reflect a group of autistic individuals. For instance, even though serotonin system abnormalities link to ASD, curing all patients with over-the-counter medications to enhance serotonergic neurotransmission does not seem to be useful and may have the opposite effect in certain

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cases. As a result, precision medicine has emerged to aid in the discovery of biomarkers that can further direct a more individualized treatment strategy.

Numerous neurological and neurodevelopmental problems can benefit from effective treatments when neurotransmitter deficiencies are quickly and accurately identified. The spatial configuration to relevant neurotransmitters using current neurotransmitter detection techniques, such as positron emission tomography (PET) and magnetic resonance spectroscopy (MRS), are constrained. Lumbar puncture (LP) neurotransmitter metabolite analysis is the recent discovered screening technique for suspected neurotransmitter deficiencies, but this procedure carries some invasive procedural hazards. Therefore, in the absence of confirmation testing, pharmacological treatments for potential neurotransmitter deficiencies might be administered. To ascertain who might profit from confirmatory LP, a less risky biomarker is required.

It is widely known how neurotransmitter-associated brain networks are distributed spatially. Resting-state functional magnetic resonance imaging (rs-fMRI) can be used in identifying these networks and the cortical and subcortical spatiotemporal changes they exhibit. Clinical uses for rs-fMRI include the assessment of epilepsy as well as other illnesses. Additionally, functional MRI for kids and intracranial electroencephalography (iEEG) have both been used to validate rs-fMRI versus competing measures. These systems can quickly change the activity of cortical network because of their extensive and focused target to geographically far cortical areas in the brain by secreting neurotransmitters. For instance, pharmacological dopamine coordination systems depletion increases node-specific hemodynamic signal variability and diminishes functional connection, indicating the significance of this system for the reliability of particular brain areas in a larger perspective. Therefore, dis-functioning of these neurotransmitter systems may cause an impact on higher cognitive functions at the network level. In the current example, abnormal activation of brain areas contains a monoamine production which can be observed by rs-fMRI network patterns and later verified by measurement of CSF neurotransmitter metabolites, as shown in Figure 1 [7].

3. Gene therapies

Since research in monogenic animal models indicates that the syndrome of autism is changeable instead of fixed, the continual correction for gene mutation would benefit a long-lasting treatment. Although some ASD disorders may be treatable with nucleic acid treatments, as will be mentioned later, only viral packaging techniques have thus far been able to reliably combine effective transduction with sustained gene expression in vivo.

When it comes to viruses that may infect cells in there G2 phase, AAVs is the best option for delivery into the central nervous system. Using AAV vector technology, which results in the stable expression of gene in cells that do not divide, the CNS may be targeted or extensively addressed. Wild type serotypes give wide variety of usable vectors, in contrast to next-generation synthetic vectors, which is much more effective and focused delivery of the trans-gene in therapies and may be customized to particular disorder approaches. Transgene expression can be universal or restricted to certain cell types depending on the vector, delivery technique, and/or promoter control. This is the case because they have a lower possibility to cause immune response (in comparison to adenovirus and herpes simplex virus), the capacity to persist in the form of episomal, a lower oncogenic probability (than retroviruses), high production titers, and the capacity to persist in episomal form. Some early clinical investigations in CNS gene therapy, for diseases from SMA to idiopathic Alzheimer's disease, have already employed RAAV vectors without incident [8].

Moreover, the mode of delivery is modifiable. AAV vectors have previously been stereotactically injected into the parenchyma, results in high concentrations in particular regions and little vector dissemination. The localizing solution of delivering vectors inside the body may be helpful in correcting particular dysregulated ASD circuits linked to particular clinical phenotype, which is similar to the development so far in motor scores observed after lentiviral vector delivering method of gene therapy towards gene responding for producing dopamine to the nigrostriatal pathway in Parkinson’s. A craniotomy is required for each injection. Global CNS gene repair will be required in order to fully

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reverse the cognitive symptoms of ASD, which appear to include global synaptic dysregulation. The finding that rAAV9 shifts CNS neurons and spongiocyte cells worldwide and so crosses the blood-brain barrier (BBB) as well as the subsequent creation of more efficient entrance through BBB, rAAVs has opened the potential of employing an intravenous injection in the genetic treatment of autism.

The use of intravenous injection in various ASDs may be prevented by adverse effects related to peripheral tissue transduction and the existence of neutralizing circulating antibodies (47% of persons have anti-AAV9 antibodies). Altering the viral vector's nucleic acid sequence apart from the transgene, like the addition of sequences that make it unable to target off-target cells recognized by microRNA, may be able to solve the first problem, but they take up a lot of space (the length of DNA carried by rAAV is restricted to 5000 bps maximum). By using a modified AAV coat, where there is lower neutralizing antibody seroprevalences, the latter problem may be resolved [9].

As a substitution to the delivery mentioned, intrathecal administration has the potential to combine safer comparison administration with widespread transduction in the central nervous system, less problem in the peripheral system, and improved resolution, hence lowering dosage needs. Contradictory information is available on the efficiency of intrathecally injected rAAV in transducing tissues outside the spinal cord, at the same time it considers the peripheral leakage and the viability of neutralizing immune responses. Interestingly, the AAV9 technology injection shows efficacy when facing large axon diseases, which has been moved to clinical therapies.

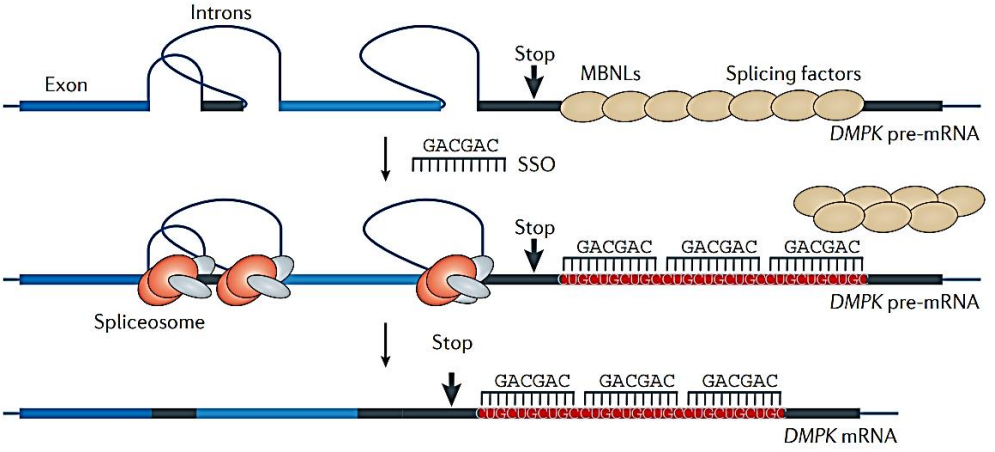

Figure 2. Oligonucleotide-induced regulatory mechanisms for gene expression [10].

3.1. Gene replacement

In ASD illnesses brought on by mutations that cause loss of function, a single genetic substitution may recover the synapse (e.g., RS, FXS, and TSC). Based on restrictions applied by imprecise gene packaging techniques, is enough target cell types transduction achievable to impart symptom advantage. The fact that multiple research projects utilizing monogenic animal models have found that the behavior of the animals has improved after gene substitution with rAAV is encouraging. Modest behavioral improvements were seen in an RS mouse model after deliver systemically of a rAAV9-MECP2 vector adequate for 10% CNS transduction. In contrast, 25% CNS transduction at a 6-fold higher vector dosage significantly improved phenotypic and behavioral traits.

3.2. RNA knockdown

It is possible to silence gene expression, through ways such as antisense oligonucleotides (ASOs), which use small RNAs to target specific mRNA transcripts for enzyme-dependent degradation, and other techniques known as short interfering RNAs, which are specifically provided by base pairs to target specific mRNA transcripts and stop their translation, as shown in Figure 2 [10]. These nucleic acids can be altered to lessen degradation and inflammation without requiring a viral vector. Clinical studies for Huntington's disease and SMA are already using these therapies.

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These techniques are most beneficial when complete or partial knockouts of specific transcripts restore synaptic function. RNA silencing may also be helpful when a gene that blocks the expression of a target gene is knocked out, a process known as disinhibition. It's important to note that while motor impairments were remedied in the same research, anxiety and repeated behavior were not. These temporal aspects are certainly significant for deciding the date of a successful intervention in humans, even though they haven't been studied despite the monogenic ASD mentioned [10].

3.3. Gene editing

One of the most inspiring recent advances in gene therapy has been the introduction of easily customized, particular DNA editing techniques, such as CRISPR-Cas9, which uses the combined complex to allow for non-homologous recombination (provided the right template is available) or non-homologous end junctions to silence genes, where the guide RNA is responsible for binding the target PAM sequence and the Cas9 protein forms a cleavage to break the target DNA region, as shown in Figure 3 [11]. The transgene-related toxicity problems associated with both gene replacement and RNA knockdown techniques would normally be eliminated by these techniques, which typically permit physiological gene expression in the target cells [11].

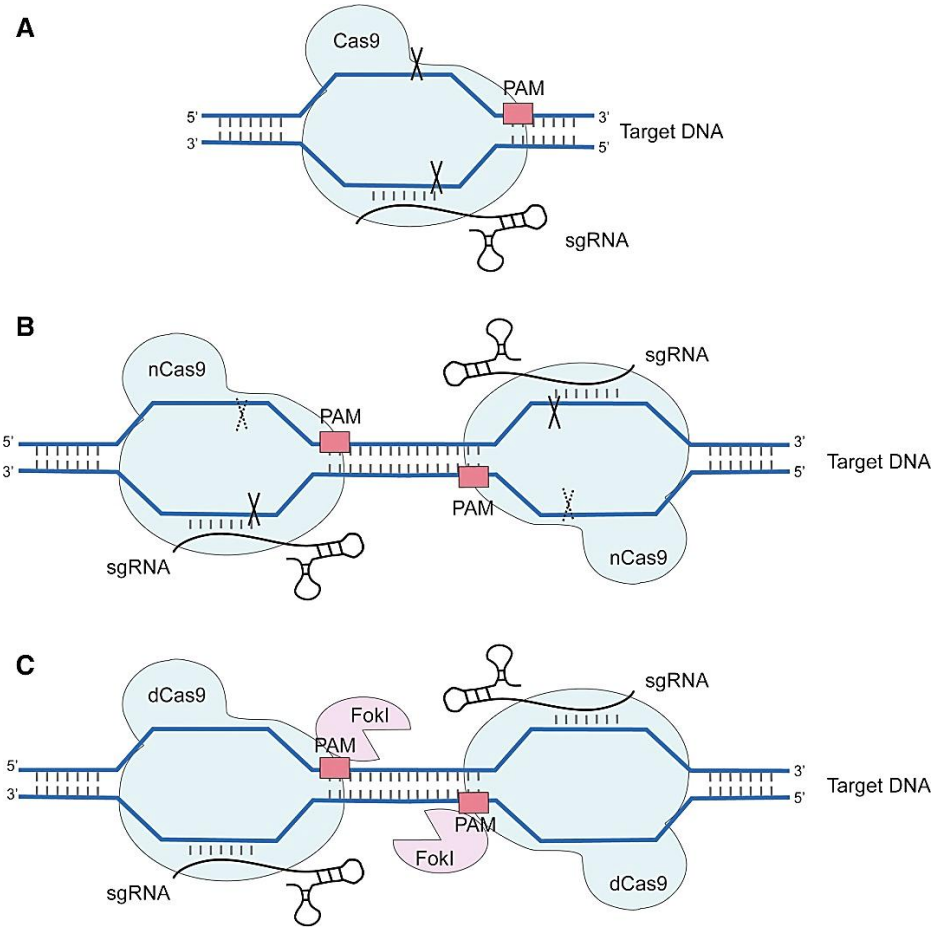

Figure 3. Application of CRISPR/Cas9 for genome editing [11].

However, there is still a long way to go before gene editing techniques can be applied therapeutically, at least in vivo. Delivery techniques, off-target effects, and the harmony of HDR/NHEJ pathways are only a few of the challenges that the current CRISPR-Cas9 technologies still have to overcome. In addition, certain fundamental aspects of the system, such as Cas9's catalytic role, the techniques utilized to locate the target sites underneath them, and the cause of PAM dependence, are yet unclear. It will be easier to develop highly specialized Cas9-mediated tools, expand our target

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site options, and boost the catalytic effectiveness of Cas9. A new study by Doudna and his colleagues offers encouragement in this regard. Postmitotic neurons are edited when the Cas9 ribonucleoprotein (RNP) complex is injected into the cortex, striatum, and hippocampus. Modified Cas9 variants with multiple SV40 nuclear localization sequences increased the efficiency of neuronal editing in vivo by a factor of 10 These advancements demonstrate how genome editing may be used to change or suppress the genetic basis of neurological diseases [12].

4. Conclusion

By now, scientists already discovered the treatment for ASD in two particular ways, which are regulating neurotransmitters and genetic therapies. The iEEG and LP methods can identify the exact location in the CNS where any particular sort of neurotransmitter is not functioning well, while the specific biomarkers with lower risk and higher viabilities are expected to appear to cooperate with these techniques, to perform a precision therapy since there are potentially invasive hazards brought by recent neurotransmitter metabolite analysis. For gene therapies, although plenty of genetic modifying strategies are found, for example, the CRISPR/Cas9, most of them are unfortunately not available, or in another word, not accessible towards to CNS due to the body's protection system constitutes the immune system and BBB. The rAAV is the technique with the most potential to be applied in therapies, while how to package the gene modifying tools inside to a specific target are still be discussed. The essay concluded recent analysis, suggestions along with the problems of therapies for ASD, giving directions for researchers about which specific regions are they aiming to work on to make these therapies function and cure ASD completely. Apart from that, it is noticeable that mental disorders usually appear together instead of alone, which makes it unsuitable to define the reason for ASD as a simple point mutation, or even several point mutations combined. Furthermore, the definition of each mental disorder should be re-discussed, since it is more scientific and convictive to tell the exact reason that causes the disease but not by diagnosing the symptom which we have been always doing. Therefore, the therapies can be done with a clear target so that diseases such as ASD, schizophrenia, or bipolar can be cured. Another problem is, as the statistic is hard to achieve credibility from a general perspective because the educational, ethical, cultural, or economic reasons, therefore it is difficult to do group analysis towards specific types of autism. When collecting data for mental diseases such as ASD, it should be considered that poor regions have less awareness of what actually a mental disorder is, so the statistics should not be considered as fair one. However, as it is defined as a disease, the situation is better in a scientific way.

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