

Implications of the Neurexin Gene Family in Autism Spectrum Disorder

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ABSTRACT

The neurexin gene family, consisting of NRXN1, NRXN2 and NRXN3 are presynaptic cell adhesion molecules and receptors that are needed in the development and differentiation of synaptic function and neural development. When microdeletions and loss of function variations are expressed, they can encode proteins that result in synaptic disruptions and disrupted neurotransmission, leading to a higher risk for developmental disorders and neuropsychiatric conditions. NRXN genes are strong candidate genes linked to ASD risk susceptibility, with all three noted as high confidence risk factors according to the SFARI Gene Database. In this review, we will go over case studies of NRXN mutations in ASD individuals, explain the results and provide insight into incomplete penetrance and further areas of support.

Introduction

Cell adhesion molecules and receptors play significant roles in intercellular communication for a variety of organismal cells. These families of adhesion molecules are involved in neural development and are shown to be highly implicated during neural network formation through processes such as synaptic formation and function, neuronal cell migration and glial network formation to therefore contribute to brain morphology and coordinated brain functionalities including learning, memory and somatosensation.¹

Over the past few decades, multiple groundbreaking studies have identified over 102 risk genes², epigenetic and environmental factors that have each contributed to autism spectrum disorder, commonly referred to as autism or ASD. ASD refers to a spectrum or a group of one developmental disorder with varying severity that affects an individual's social, emotional, and physical behavior. The disorder is frequently characterized by impairments in the form of limited and repetitive behaviors, social interaction, and communication; people with ASD can demonstrate a range of abilities from outstanding disability to above-average intellect.³ Many of these over 102 discovered risk genes encode proteins for synaptic function, transcription regulation, and chromatin-remodelling.⁴ These three critical pathways are most impacted by risk variation; because so many synaptic genes are disrupted in idiopathic ASD, the altering of relevant genes involved in transcription and chromatin-remodeling also points to possible synaptic function impairments as well.⁴

The purpose of this review is to analyze the neurexin protein family involved in presynaptic cell adhesion and reception in the vertebrate system; in particular, this article will go over neurexin 1 (NRXN1), neurexin 2 (NRXN2) and neurexin 3 (NRXN3), out of which NRXN1 and NRXN3 are some of the largest genes in humans. Neurexins are cell-surface receptors that bind to neuroligins (NGLNs) in order to form a Ca²⁺-dependent neurexin/neuroligin complex at synapses necessary for neurotransmission and synaptic function.¹⁴ Because neurexins affect synaptic function and mediate the conduction of nerve signals, all three are high confidence genes for ASD and play an essential role in neural development.⁵ In fact, multiple studies have recorded mutations in this protein-coding gene family as they are linked to ASD, from recurrent to rare variations.

Methods

The paper will first go into the respective neurexin mutations before analyzing individual phenotypes and finally their manifestations in the development of ASD. While neurexins can and do produce alpha-neurexin and beta-neurexin isoforms, this review will focus primarily on its genetic variation and subsequent effects in neural development. Likewise, although neurexins commonly form trans-synaptic complexes with postsynaptic proteins such as NLGNs, LRRTM proteins or cerebellin in the process of synaptic differentiation and neurotransmission⁶, the article will generally focus on the neurexin family and its major genes as a whole with a few studies introduced and their findings explained. To this end, various original research and review papers found on NCBI will be used, as well as the consultation of the SFARI Gene database for ASD research.

NRXN1

The NRXN1 gene, on chromosome 2p16.3, has recorded approximately 214 and 4 rare and common variants respectively. One particular study specifically focused on characterizing rare missense NRXN1 variants in individuals with ASD and schizophrenia (SCZ). After mutation screening of three rare missense variants (T737M, D772G, and R856W) affecting the alpha-neurexin isoform (NRXN1 α), researchers most notably observed that people with the D772G and R856W mutations had more serious social disabilities than T737M carriers, and those with mutated T737M and D772G variants showed a decrease in NRXN1 α -NLGN1 interaction, signifying that NRXN1 mutations, especially ultra-rare missense variants, can produce phenotypes beyond the diagnosis of ASD or SCZ alone.⁷ Another study identified an intragenic NRXN1 3p deletion in a female ASD proband inherited from the unaffected NRXN1 deletion-transmitting mother in a trio family. Researchers recorded an increased number of exonic rare variants in the ASD child compared to the unaffected mother.⁸ One other recent study involved investigating the cognitive and behavioral skills of five children with a heterozygous NRXN1 deletion and four transmitting parents. Three of the five children were diagnosed with ASD and comorbidities, while the other two were diagnosed with an isolated intellectual disability and an unclassified neurodevelopmental disorder respectively.⁹

NRXN1 heterozygous deletions can lead to a manifestation of many neurological and developmental disorders including ASD, SCZ, attention deficit hyperactivity disorder (ADHD), intellectual disability (ID), seizures, mood disorders and congenital malformations. In the same study, 67 NRXN1 deletions were studied from 34 families, resulting in a consistent delay in speech and language at the clinical level.¹⁰ The female proband with the inherited intragenic NRXN1 3p deletion exhibited a phenotype compatible with features of similar 3p deletion carriers such as dysthymic disorder (DD) and macrocephaly.⁸ Epilepsy and seizures were also affiliated with heterozygous NRXN1 deletions, and three of the five children were diagnosed with intellectual disability or global developmental delay along with a major impairment in social communication skills in comorbidity with ASD.⁹

NRXN2

19 rare and 1 common NRXN2 variants have been associated with ASD; the gene itself is located on chromosome 11q13.1. One example of an NRXN2 variant is a truncating mutation that occurs when proteins fail to promote synaptic differentiation in glutamatergic and GABAergic neurons and fail to bind to either of the postsynaptic binding partners LRRTM2 or NLGN2. After resequencing the three neurexin genes in individuals with ASD, SCZ or non-syndromic ID, a NRXN2 truncating mutation was found in an ASD patient inherited from a father with language delay and a history of SCZ, along with a de novo truncating mutation in the NRXN1 gene of a person with ASD.¹¹ As one of the first studies done that linked a mutation on the NRXN2 gene with ASD etiology, future studies have shown stronger correlations of NRXN2 mutations contributing to symptoms and increased risk of ASD. In particular, a Chinese population case-control study done to investigate six genetic variants in the three neurexin genes found that the

NRXN2 rs12273892 polymorphism T allele and AT genotype resulted in a significantly higher risk for ASD diagnosis: patients with the rs12273892 T allele and a TT/AT genotype were more susceptible to ASD than AA carriers.⁵ Microdeletions are in actuality very rare in chromosome 11q13.1. Another study reported a de novo 921 kb microdeletion on a 2-years and 9-months-old boy exhibiting short stature, significant language and developmental delay and other congenital disturbances. However, in comparison to previously conducted cases, the boy did not exhibit autistic symptoms and did not meet the clinical diagnosis threshold for ASD, suggesting that the NRXN2 gene had incomplete penetrance for ASD behaviors.¹²

In comparison to NRXN1 and NRXN3 variants, significant deletions and mutations in NRXN2 are not as common. Most significantly, however, NRXN2 deletions have been reported to disturb the membrane anchor, leading to an absence of the C-terminal trans-membrane and cytoplasmic domains and affecting protein coding.¹¹ Another NRXN2 mutation examined in the same study has shown to remove laminin/neurexin/sex hormone-binding globulin (LNS) domains, which serve as the binding site for NLGNS.¹¹ These mutations are produced from the dysfunction of the neuronal membrane structure and presynaptic cell adhesion that affects synaptic function and signal transmission, which end up as pathological components of neurodevelopmental disorders.⁵

NRXN3

The third gene of the neurexin protein family, located on chromosome band 14q24.3-q31.1, has a reported 21 rare and 5 common variants. The same study of a Chinese population that identified the NRXN2 rs12273892 polymorphism T allele and AT genotype as a marker for an increased risk of ASD also found the NRXN3 rs12879016 polymorphism to play a noteworthy role in ASD susceptibility, with the same pattern depicted in the G allele and GT genotype as well.⁵ Patients with the rs12879016 G allele and a GG/GT genotype had a smaller risk for ASD than TT carriers.⁵ This SNP is within the 3' untranslated region (UTR) and may be involved in gene transcription, but while NRXN3 has been certainly linked to ASD, the mechanistic connection requires further research.⁵ A clinical study of four index cases diagnosed with ASD who possess rare inherited or de novo microdeletions at 14q24.3-q31.1 showed NRXN3 deletions in a father with subclinical ASD and in a carrier set of parents without formal ASD diagnoses.¹³ One other report of a three-generation Chinese family also reported identification of a rare 222 kb heterozygous microdeletion affecting the NRXN3 α isoform; the 7-year-old male proband met the clinical diagnosis threshold for ASD, while also exhibiting motor and language delay, moderate ID, ADHD and facial dysmorphic features.¹⁴ This particular NRXN3 deletion was also found in the mother and the maternal grandfather who were both deletion carriers with variable degrees of communication difficulties and neuropsychiatric conditions.¹⁴

Some NRXN3 variations have been shown to induce synaptic dysfunction and therefore affect neurodevelopment⁵, while other mutations have been associated with comorbidities such as epilepsy¹⁵, low to normal cognitive abilities¹⁶ and ID, ADHD and SCZ.¹⁴ Such evidence continues to point to incomplete penetrance in regards to NRXN3 mutations and a wide range of resulting phenotypes. Particularly, it is interesting to note that the 7-year-old male proband met the clinical diagnostic test for ASD, while the mother and maternal grandfather who also carried the deletion were presented with language and communication difficulties along with comorbidities such as SCZ and temper tantrums but did not meet the ASD diagnostic threshold. The study further supports the role of intra-family variable expression due to this NRXN3 deletion and could point to SCZ and facial dysmorphism as potential features of NRXN3 variation.¹⁴

Discussion

While NRXN has shown a positive trend towards ASD etiology, the mechanistic connections of how the variants work to affect neural development must be studied in greater depth moving forward. It may also be interesting to conduct multi-generational and multi-familial studies of NRXN mutation carriers to research how the same loss of

function variation can lead to a manifestation of different behaviors. The discovery and further exploration of rare NRXN mutations not only serves as a source of reference for future gene studies but may also provide information on creating new therapeutic strategies and means of support to target some variations.

Conclusion

Each of the 102 implicated risk genes contributes its own variation that, when combined with any other ASD risk gene, can influence the severity of the disorder. Much research has been conducted to successfully identify NRXN1 loss of function variants, while fewer such variants have been found in NRXN2 and NRXN3 genes. Incomplete penetrance of ASD etiology has been noted numerous times in multiple studies, further supporting the idea that these deletions are not suggestive of an ASD diagnosis on their own and must instead be working with other common and rare variants in order for an ASD presentation. Family members and deletion carriers may express ASD phenotypic behavior and comorbidities, while other related carriers may not meet the ASD diagnosis and present themselves with another set of symptoms, pointing to the variable NRXN mutation expression even within families.

Limitations

While individual case studies can bring up novel claims on NRXN variation, sample size is incredibly important in identifying consistent NRXN mutations and more population models and case-control studies should be performed considering case studies.

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