Neuroscience Research 127 (2018) 53–60

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Review article

Neurexins and neuropsychiatric disorders

Enas Kasem[a], Taiga Kurihara[a], Katsuhiko Tabuchi[a,b,*]

[a] Department of Molecular & Cellular Physiology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, 390-8621 Japan
[b] Institute for Biomedical Sciences, Interdisciplinary Cluster for Cutting Edge Research, Shinshu University, Matsumoto 390-8621, Japan

ARTICLE INFO

Article history:
Received 17 April 2017
Received in revised form
24 September 2017
Accepted 3 October 2017
Available online 6 December 2017

Keywords:
Synapse
Neurexins
Attention deficit hyperactivity disorder
Autism spectrum disorders
Intellectual disability
Pitt-Hopkins syndrome
Schizophrenia

ABSTRACT

Neurexins are a family of presynaptic single-pass transmembrane proteins that act as synaptic organizers in mammals. The neurexins consist of three genes (NRXN1, NRXN2, and NRXN3), each of which produces a longer α- and shorter β-form. Genomic alterations in NRXN genes have been identified in a wide variety of neuropsychiatric disorders, including autism spectrum disorders (ASD), schizophrenia, intellectual disability (ID), and addiction. Remarkably, a bi-allelic deficiency of NRXN1 was recently linked to Pitt-Hopkins syndrome. The fact that some mono-allelic functional variants of NRXNs are also found in healthy controls indicates that other genetic or environmental factors affect the penetrance of NRXN deficiency. In this review, we summarize the common research methods and representative results of human genetic studies that have implicated NRXN variants in various neuropsychiatric disorders. We also summarize studies of rodent models with NRXN deficiencies that complement our knowledge of human genetics.

Contents

1. Introduction 54
2. Human genetic studies of NRXNs in neuropsychiatric disorders 54
   2.1. NRXN gene structure and strategies for identifying genomic deficiencies 54
   2.2. Autism spectrum disorders (ASD) 54
   2.3. Pitt-Hopkins syndrome 56
   2.4. Schizophrenia 56
   2.5. Intellectual disability 56
   2.6. Other neuropsychiatric disorders 57
3. Nrxn rodent models 57
   3.1. Behaviors of mouse and rat models deficient in α- or β-Nrxn 57
   3.2. β-Nrxn triple-knockout mice 58
   3.3. Nrxn3 knock-in/knockout mice 58
4. Conclusions 58
Authors' contributions 58
Conflict of interest 59
Acknowledgments 59
References 59

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorders; CGH, comparative genomic hybridization; CHO, carbohydrate attachment; CNV, copy number variations; DGAP, developmental genome anatomy project; FISH, fluorescent in situ hybridization; GWAS, genome-wide association studies; ID, intellectual disability; LNS, laminin/neurexin/sex hormone–binding globulin; NRXN, human neurexin gene; Nrxn, rodent neurexin gene; NSID, non-syndromic intellectual disability; PPI, prepulse inhibition; PTHS, Pitt-Hopkins syndrome; QMPSF, quantitative multiplex PCR of the short fluorescent fragments; SNP, single nucleotide polymorphisms; WGSA, hole-genome sampling analyses.

* Corresponding author at: Department of Molecular & Cellular Physiology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, 390-8621 Japan.
E-mail address: ktabuchi@shinshu-u.ac.jp (K. Tabuchi).

https://doi.org/10.1016/j.neures.2017.10.012
0168-0102/

1. Introduction

Neurexins, one of the best-studied families of synaptic organizers, consist of three genes in mammals (NRXN1, NRXN2, and NRXN3). Each gene produces two distinct forms of single-pass transmembrane proteins, both of which localize to presynaptic terminals, by two independent promoters. The α-Neurexins, the longer form, are produced by 5′ promoters. The proteins contain a tandem array of six laminin/neurexin/sex hormone–binding globulin (LNS) domains that are split by the insertion of three epidermal growth factor (EGF)-like repeats between the first and second, third and fourth, and fifth and sixth LNS domains, and are followed by a carbohydrate attachment (CHO) site, a transmembrane region, a protein 4.1–binding site, and a class II PDZ–binding motif at the carboxy-terminus (Fig. 1). The β-Neurexins are transcribed by another promoter predicted to be located in the middle of the gene. α-Neurexins and β-Neurexins share the exons that encode the last LNS domain to the carboxy terminus, with the exception of a β-specific first exon (Fig. 1) (Baig et al., 2017; Cao and Tabuchi, 2017). NRXNs have five canonical alternative splice sites in the extracellular region (Rowen et al., 2002; Tabuchi and Sudhof, 2002), and a sixth splice site has been identified in NRXN1 and NRXN3 (Fig. 1) (Treutlein et al., 2014). This combination of exon-insertion splice sites produces ∼4000 structural variants of the Neurexin proteins, and this structural diversity determines the Neurexins' binding specificities to extracellular ligands, such as neuroligins, leucine-rich repeat transmembrane proteins (LRRTMs), cerebellins, and C1q-like proteins (Boucard et al., 2005; de Wit et al., 2009; Ko et al., 2009; Matsuda and Yuzaki, 2011; Matsuda et al., 2016; Siddiqui et al., 2010; Tabuchi and Sudhof, 2002; Uemura et al., 2010; Um et al., 2016).

Human genetic studies have implicated NRXNs in a wide variety of neuropsychiatric disorders, and NRXN genomic alterations are frequently identified in autism spectrum disorders (ASD) and schizophrenia. Indeed, all of the NRXN family members are listed in public databases of risk genes for ASD and associated disorders, such as the SFARI gene (https://sfari.org/) and AutismKB (http://autismkb.cbi.pku.edu.cn/) datasets, with high scores (Table 1). Notably, it was recently discovered that a bi-allelic NRXN1 deficiency is linked to Pitt-Hopkins syndrome, in which autism is a core feature. In this review, we summarize the research strategies and results of representative human genetic studies of ASD, schizophrenia, and other disorders in which NRXNs have been implicated. We also summarize studies of Nrxn-deficient rodent models that are relevant to neuropsychiatric disorders that have been linked to NRXN.

2. Human genetic studies of NRXNs in neuropsychiatric disorders

2.1. NRXN gene structure and strategies for identifying genomic deficiencies

NRXNs are among the largest human genes. NRXN1 spans 1,108.4 kb with 24 exons, NRXN2 spans 106.4 kb with 23 exons, and NRXN3 spans 1,618.5 kb with 24 exons (Table 1) (Tabuchi and Sudhof, 2002). Exon 18 of NRXN1 and exon 17 of NRXN2 and NRXN3 are β- specific first exons. NRXN1, NRXN2, and NRXN3 are located at 2p16.3, 11q13.1, and 14q24-q31.1 in the human chromosomes, respectively (Table 1).

Genomic deficiencies can be identified from human samples by karyotyping, fluorescent in situ hybridization (FISH), array comparative genomic hybridization (array-CGH), quantitative PCR (qPCR), and the sequencing of target genes. Studies often combine these techniques. Array-CGH is used for genome-wide association studies (GWAS), also called whole-genome sampling analyses (WGSA), which search over the entire genome in different individuals and are often chosen as the first line of screening. Resolving an array-CGH depends on the number of probes. For instance, the Agilent 244 K array-CGH (G4411B, Agilent Technologies, Palo Alto, CA) contains 236,381 oligonucleotide probes on the slide, of which 130 probes cover the 1.11 Mb NRXN1 genomic region, making the average interprobe space within 8.6 kb. Thus, it can detect microdeletions as small as 43 kb (Ching et al., 2010). Oligo probes designed to detect single nucleotide polymorphisms (SNPs) are also used for array-CGH, and can detect copy number variations (CNVs) as well as SNPs. Array-CGH data are deposited in a public database, where they can be used for large sample studies and other extended analyses by researchers using up-to-date computer programs. Deficient loci identified by array-CGH are further analyzed by gene-specific candidate-driven approaches. Multiplex qPCR (qPCR with single- or multiple-pair primers) is used to detect the precise regions of CNVs. Sequence analysis is sometimes the only way to identify small de novo or rare mutations. To save time and cost, researchers generally focus on the exons and splice junctions of target genes for sequencing. These gene-specific, candidate-driven approaches are also used for first screenings.

Before discussing NRXN variants, it is helpful to distinguish between common and rare variants. A common variant is a polymorphism that is shared with more than 5% of all populations and appears to have occurred relatively early in human evolution and to have been transferred through generations (Bourgeron, 2015). Thus, common variants are generally less toxic and are sometimes excluded from results by comparison with controls. In contrast, a rare variant, such as a de novo mutation, is identified only from small populations. These mutations have a greater chance of being deleterious and are therefore more likely to be of interest.

Table 1
Characteristics of human Neurexin genes.

	NRXN1	NRXN2	NRXN3
Chromosome band	2p16.3	11q13.1	14q24-q31.1
Size of gene	1,108.4 kb	106.4 kb	1618.5 kb
α-coding exons	exons 1–17, 20–24	exons 1–16, 18–23	exons 1–16, 18–24
β-coding exons	exons 18–24	exons 17–23	exons 17–24
SFARI score[a]	2	4	3
AutismKB score[b]	28	16	3

[a] SFARI scoring: (1) high confidence, (2) strong confidence, (3) suggestive evidence, (4) minimal evidence, (5) hypothesized, (S) syndromic, (NA). not available.
[b] Higher AutismKb scores indicate a stronger association with ASD.

2.2. Autism spectrum disorders (ASD)

The first evidence that NRXNs might be linked to ASD was the discovery of two rare missense mutations in NRXN1β (p.S14L and p.T40S) when exon 1 of all three β-NRXNs was sequenced for 131 Caucasian and 61 African-American patients with ASD; these mutations were not identified in 535 healthy controls (Table 2) (Feng et al., 2006). The mutations were found within the coding region of NRXN1β's signal sequence, and presumably affect the processing or proper localization of the protein. Next, an Autism Genome Project Consortium research group screened 1181 families having at least two ASD individuals, and found two ASD siblings with a 300-kb hemizygous deletion in the exons of NRXN1α, apparently due to a gonadal mosaicism of the unaffected father. The deletion was identified using a 10 K SNP array and confirmed by qPCR (Table 2) (Szatmari et al., 2007). The screening also identified two NRXN1 SNPs that were positively associated with transmission to ASD patients (Szatmari et al., 2007). Kim and colleagues karyotyped 200 samples from the Developmental Genome Anatomy Project (DGAP), and identified an insertion disrupting exon 5 of NRXN1α