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Familial deletion within NLGN4 associated with autism and Tourette syndrome

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Neurexin 4 (NLGN4) is a member of a cell adhesion protein family that appears to play a role in the maturation and function of neuronal synapses. Mutations in the X-linked *NLGN4* gene are a potential cause of autistic spectrum disorders, and mutations have been reported in several patients with autism, Asperger syndrome, and mental retardation. We describe here a family with a wide variation in neuropsychiatric illness associated with a deletion of exons 4, 5, and 6 of *NLGN4*. The proband is an autistic boy with a motor tic. His brother has Tourette syndrome and attention deficit hyperactivity disorder. Their mother, a carrier, has a learning disorder, anxiety, and depression. This family demonstrates that *NLGN4* mutations can be associated with a wide spectrum of neuropsychiatric conditions and that carriers may be affected with milder symptoms.

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Introduction

Neurexins are a family of adhesion molecules expressed in postsynaptic neurons that interact with neuroligins expressed in presynaptic neurons.^{1–3} The neurexin–neuroligin interaction is hypothesized to play a role in the amount, specificity, and stabilization of synaptic network formation.⁴ Studies of knockout mice for *NLGN1*, 2, and 3 support a role in synapse maturation and function.⁵

The neurexin family consists of three members (*NRXN1*, *NRXN2*, and *NRXN3*), each with a long α and a short β form. Great variation is achieved through alternative splice variants.⁶ Four members of the neuroligin family have been identified in mice (*NLGN1*, *NLGN2*, *NLGN3*, and *NLGN4*), while five members of the neuroligin family have been identified in humans: *NLGN1*, *NLGN2*, *NLGN3*, *NLGN4*, and *NLGN4Y*.^{1,7,8} *NLGN3* and *NLGN4* are both

X-linked, with loci at Xq13 and Xp22.33, respectively. Mutations in these two genes have been reported in eight families that include members with mental retardation and/or pervasive developmental disorders ranging across the spectrum from Asperger syndrome to autism (Table 1).^{9–12} One report also makes note of a carrier mother affected with a learning disorder.¹¹ Furthermore, mutations in *NRXN1* have been detected in four patients with autism.¹³ Several studies, however, have failed to detect a high rate of *NLGN* mutations in autistic patients, suggesting that *NLGN* mutations may only be responsible for a small subset of cases of autism.^{14–17} A study to test for an association between *NLGN4* or *NLGN4Y* and schizophrenia, another neuropsychiatric condition, did not yield a strong link either.¹⁸

We have recently identified a family with a novel *NLGN4* deletion encompassing exons 4, 5, and 6. This mutation is predicted to result in a significantly truncated protein. This family shows a wide spectrum of different neuropsychiatric illnesses associated with the same *NLGN4* mutation. In addition, it demonstrates that female carriers may also be affected in a milder manner than their male children.

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Table 1 Summary of phenotypes associated with *NLGN3* and *NLGN4* mutations

Reference	Patient description	Mutation
Current case	Two brothers: one with autism and a motor tic, and one with TS and ADHD. Carrier mother has LD, depression, and anxiety.	<i>NLGN4</i> : deletion of exons 4, 5, and 6
Jamain (2003) ⁹	Two brothers: one with autism and one with AS. Mother is a carrier.	<i>NLGN3</i> : C→T transition changing a highly conserved arginine residue (R451C)
Laumonnier (2004) ¹⁰	Two brothers: one with typical autism and one with Asperger syndrome. Mother is a carrier.	<i>NLGN4</i> : frameshift mutation 1186insT causing premature termination (D396X)
Yan (2005) ¹¹	Large extended family with 13 affected male subjects: 10 with MR, 2 with MR with autism, and 1 with PDD	<i>NLGN4</i> : 2 bp deletion 1253del(AG) causing a premature stop codon (D429X)
	Two siblings: one sister with severe autism and one brother with LD. Carrier mother has LD.	<i>NLGN4</i> : G99S
	Male subject with mild autism and normal IQ. Mother is a carrier.	<i>NLGN4</i> : K378R
	Male subject with PDD. Two brothers with the variant had normal social function and school performance. Mother is a carrier.	<i>NLGN4</i> : V403M
	Male subject with autism. One carrier sister with PDD, but the other carrier sister was unaffected. Mother is a carrier	<i>NLGN4</i> : R704C
Talebizadeh (2006) ¹²	Female subject with autism	<i>NLGN4</i> : exon 4 deleted (in transcript only)

Abbreviations: ADHD, attention deficit hyperactivity disorder; AS, Asperger syndrome; LD, learning disorder; MR, mental retardation; PDD, pervasive developmental disorder; TS, Tourette syndrome.

Clinical presentation

The proband, a 7-year-old boy, presented to us for evaluation of an underlying genetic cause for autism. He was born by spontaneous vaginal delivery at full term after an uneventful pregnancy without exposures to known teratogens. His early development appeared unremarkable to parents. By the age of 12 months, he was walking on his own and had spoken his first word. He progressed to a vocabulary of approximately 10–15 words; however, around the age of 2 years, his language regressed and his behavior changed. His family had difficulty in making eye contact with him, and he began to show some repetitive movements, including hand waving and placing his fingers in his ears. In addition, he developed a tic disorder consisting of a jerking of the head and arms. An EEG to evaluate seizure activity was normal. Clinical evaluation confirmed that he met DSM-IV criteria for the diagnosis of autism. Cognitively, he was severely retarded.

Medically, the patient has been otherwise relatively healthy, with the exception of recurrent otitis media requiring tympanostomy tubes. Physical examination was notable for a double hair whorl, upslanting palpebral fissures, and a slightly broad, depressed nasal root. Head circumference at the age of 7 years was 53 cm (75th percentile).

The family was of Irish and English ancestry. The patient had one sibling, a 9-year-old brother diagnosed with Tourette syndrome and attention deficit hyperactivity disorder with mild cognitive deficits. He was treated with depakote, risperidol, and cogentin, which reportedly improved the symptoms of Tourette syndrome. The mother has a learning disability, as well as depression and anxiety treated with sertraline. The maternal grandmother has anxiety. The paternal grandfather was deceased with no

suggestive medical or psychiatric history. The father does not have any psychiatric, autistic, or cognitive issues either in himself or in his extended family.

Routine chromosome analysis revealed a normal karyotype of 46, XY. A chromosome microarray (Signature Genomics Laboratory) revealed a small deletion at Xp22.3. No other deletions or abnormalities were detected by the microarray. The deletion was located approximately 1 Mb from the steroid sulfatase locus, leaving this region intact. To further delineate the nature of the deletion, we proceeded with analysis of the *NLGN4* gene located at Xp22.33. This revealed a deletion encompassing exons 4, 5, and 6 (Figure 1a and b). Sequencing across the break point confirmed that the deletion encompasses 756 797 bp (Chr.X 5935194–5179398, UCSC Genome Browser on Human, March 2006 Assembly), and that there are currently no other known genes in the deleted region (Figures 2 and 3). Chromosome analysis of the mother and the father showed normal 46, XX and 46, XY karyotypes, respectively. Further evaluation of the family showed that his brother and mother both carry this deletion. His mother declined further testing to determine X-chromosome inactivation status. The maternal grandmother declined testing secondary to severe fear of needle sticks, medical testing, and doctors. It should be noted that no deletions were detected on testing of samples from 96 healthy controls. In addition, specific testing for exon 5 or 6 deletions was negative on another 288 healthy controls.

Materials and methods

Genomic DNA was extracted from blood using the Puregene kit (Gentra, Minneapolis, MN, USA) as per

manufacturer's instructions. The *NLGN4* primers were designed to specifically amplify the *NLGN4* copy on the X chromosome (Xp22.32–p22.31). A homologous *NLGN* gene is found on the Y chromosome (*NLGN4Y*, Yq11.221). All seven exons were amplified in nine amplicons (exons 2, 5, and 6 are amplified in two segments). PCR was performed using the AmpliTaq Gold with GeneAmp PCR kit (Roche, Branchburg, NJ, USA), as per the manufacturer's instructions. PCR products were verified by agarose gel electrophoresis. Amplification abnormalities were confirmed in a second independent PCR amplification. Cycle sequencing was performed using the Big Dye Terminator kit, v1.1 (Applied Biosystems, Foster City,

CA, USA), as per the manufacturer's instructions. Cycle sequencing products were purified using the CleanSeq magnetic bead purification kit (Agencourt, Beverly, MA, USA). Purified products were run on an Applied Biosystems 3730 automated fluorescent sequencer.

Discussion

Neuroligins are an intriguing study target for neuropsychiatric conditions due to their potential role in synapse function and neuron-to-neuron recognition. Lack of expression of specific neuroligins could affect neuronal interactions within the synaptic network, leading to consequent neuropathology.

Figure 2 NLGN4 deletion junction. Primer sets of varying distances downstream of exon 3 were used to narrow down the breakpoint junction. Amplification across the deletion junction showed a sequence of ...aaaagac^TCCTCAT..., which represents a deletion of 756 797 bp (Chr.X 5935194-5178398).

Figure 1 PCR amplifications of *NLGN4* exons 1–6. (a) Exons 1–6 were amplified in parallel (two separate preparations from the same sample marked as A and B) for the proband, and a control case with a whole gene deletion of *NLGN4* was used. The control is a male subject with a large deletion confirmed by FISH studies on chromosome X that encompasses *NLGN4*. Exons 2, 5, and 6 were amplified in two segments due to their size. A sample from a healthy subject containing a normal copy of *NLGN4* was used for the positive control lane. The water sample contained no DNA template, serving as a negative control for contamination. Cross-amplification was seen in some segments for the proband (exon 5 segment 2 and exon 6 segment 1) due to a homologous *NLGN* copy on the Y chromosome (*NLGN4Y*). (b) Confirmation that these cross-amplified products were in fact *NLGN4Y* was demonstrated by direct sequencing of these products. NL4-E06U2 is the control sequence for the upstream read of exon 6 segment 1 of *NLGN4*. The sequence obtained from the exon 6 segment 1 PCR products from the proband's sample showed the *NLGN4Y* sequence fingerprint (see boxed area), confirming that there was cross-amplification of the Y copy due to the deletion of X copy. The fingerprint is based on a number of single-nucleotide differences between *NLGN4* and *NLGN4Y*. The same was found for both exon 5 segment 2 products (data not shown).

Figure 3 UCSC Genome Browser illustrating deleted region. The figure displays information about the genomic DNA for the deleted region of Chr.X 5935194-5178398. No other known RefSeq genes, other than exons 4, 5, and 6 of the NLGN4 gene are present (arrow).

This family's varied neuropsychiatric phenotypes suggest that mutations in *NLGN4* may be associated with a wider clinical spectrum than previously described, including depression, anxiety, and tic disorders. Their varied phenotypes, all associated with the same mutation, suggest that epigenetic factors play a role in determining presentation. This also suggests that derangement of core elements of synapse function may be associated with multiple neuropsychiatric conditions.

Gene dosage may be integral to neuroligin function as suggested by the mother's relatively mild symptoms (as compared with her sons) of a learning disorder, anxiety, and depression. Knowledge of the mother's X-chromosome inactivation status would be valuable in this interpretation, but was unfortunately not available. This may have important genetic counseling implications, as carrier mothers with mild symptoms may be at risk to have severely affected offspring.

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Conflict of interest

The authors have no conflict of interest to report.

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