

Molecular characterization of *NRXN1* deletions from 19,263 clinical microarray cases identifies exons important for neurodevelopmental disease expression

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Purpose: The purpose of the current study was to assess the penetrance of *NRXN1* deletions.

Methods: We compared the prevalence and genomic extent of *NRXN1* deletions identified among 19,263 clinically referred cases to that of 15,264 controls. The burden of additional clinically relevant copy-number variations (CNVs) was used as a proxy to estimate the relative penetrance of *NRXN1* deletions.

Results: We identified 41 (0.21%) previously unreported exonic *NRXN1* deletions ascertained for developmental delay/intellectual disability that were significantly greater than in controls (odds ratio (OR) = 8.14; 95% confidence interval (CI): 2.91–22.72; $P < 0.0001$). Ten (22.7%) of these had a second clinically relevant CNV. Subjects with a deletion near the 3' end of *NRXN1* were significantly more likely to have a second rare CNV than subjects with a 5' *NRXN1*

deletion (OR = 7.47; 95% CI: 2.36–23.61; $P = 0.0006$). The prevalence of intronic *NRXN1* deletions was not statistically different between cases and controls ($P = 0.618$). The majority (63.2%) of intronic *NRXN1* deletion cases had a second rare CNV at a prevalence twice as high as that for exonic *NRXN1* deletion cases ($P = 0.0035$).

Conclusions: The results support the importance of exons near the 5' end of *NRXN1* in the expression of neurodevelopmental disorders. Intronic *NRXN1* deletions do not appear to substantially increase the risk for clinical phenotypes.

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Key Words: copy-number variation; genotype–phenotype; *NRXN1*; penetrance; variable expression

INTRODUCTION

Neurexins are a group of highly polymorphic presynaptic cell adhesion molecules that primarily bind to neuroligins.¹ The three neurexin genes (*NRXN1*, *NRXN2*, and *NRXN3*) are highly conserved and undergo extensive alternative splicing² to produce thousands of isoforms that appear to be both spatially and temporally regulated.^{3,4} The two main isoforms for each neurexin gene, the longer *NRXN-α* and the shorter *NRXN-β*, are transcribed from two independent promoter regions and

give rise to proteins with similar C-terminal regions but different N-terminal ectodomains.⁵ The *NRXN-α* and *NRXN-β* isoforms both bind to postsynaptic neuroligins and leucine-rich repeat transmembrane proteins but with varying degrees of affinity.^{1,6,7} *NRXN-α* also binds to dystroglycan and cerebellin.^{8,9} *NRXN1* (OMIM 600565) is the largest neurexin gene (comprising approximately 1.1 Mb of genetic sequence)² and the one most implicated as a top candidate gene for neurodevelopmental and neuropsychiatric conditions (Figure 1).¹⁰

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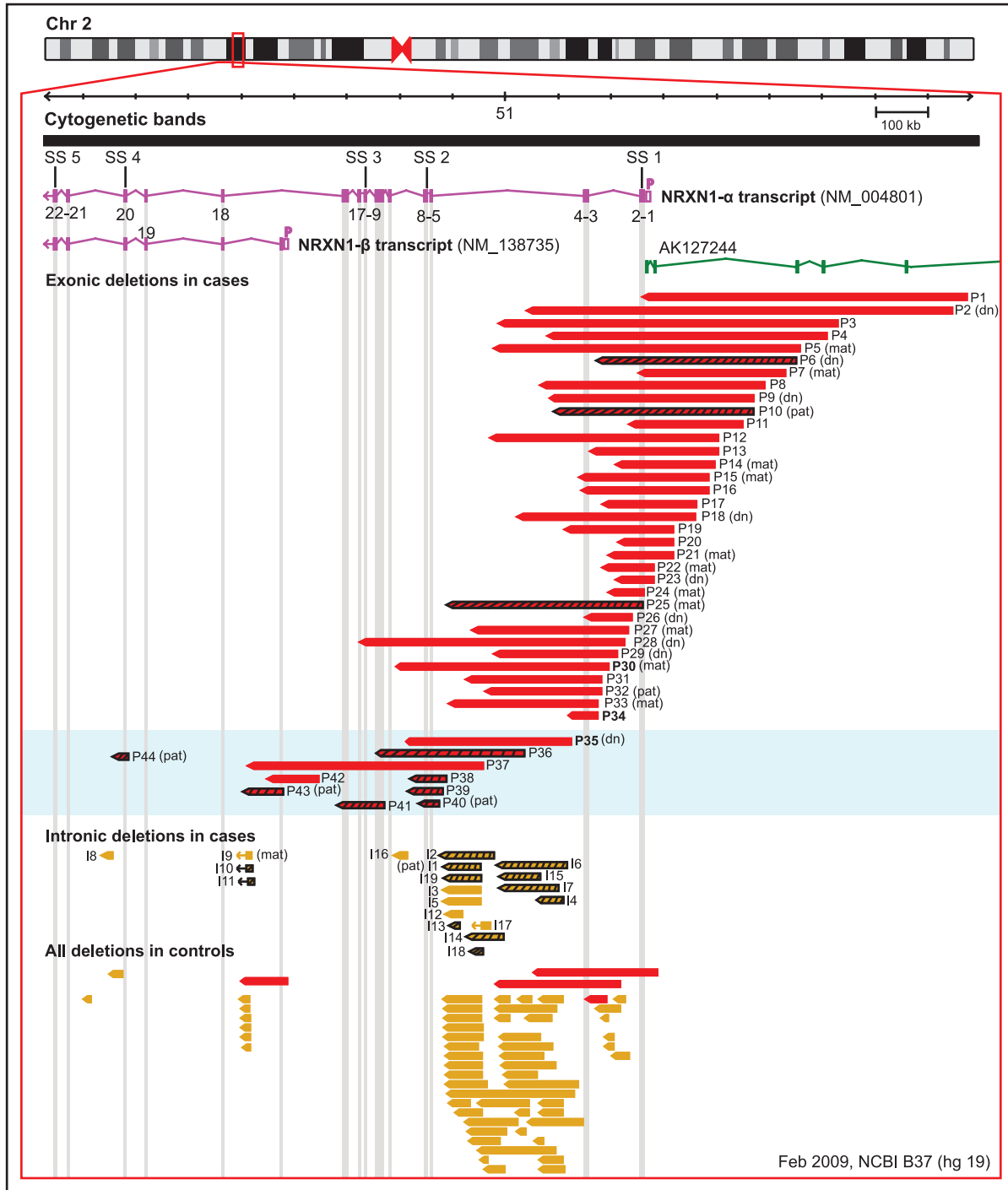


Figure 1 Novel exonic and intronic *NRXN1* deletions identified in cases and controls. The image was modified from the Database of Genomic variants (<http://dgv.tcag.ca>), NCBI Build 37 (hg 19).^{37,38} The two primary *NRXN1* transcripts (α 1 and β 1) are shown in pink; other transcripts are not included. The long noncoding RNA (lncRNA) AK127244 is shown in green. Each of the 22 exons is identified by a number according to the NM_004801.4 transcript. The five splice site (SS 1–5) locations are represented above the *NRXN1-α* transcript. The hollow pink box denoted by a P adjacent to each transcript represents the α promoter and β promoter, respectively. All exonic and intronic deletions (chr2: 50,145,643–51,259,647; hg 19) are represented by solid red and yellow bars, respectively. Deletions with a black grid are subjects that were identified as having a second copy-number variation of potential clinical relevance. Inheritance status of the *NRXN1* deletion is represented in brackets following the patient ID number (d.n., de novo; mat, maternal inheritance; pat, paternal inheritance; blank, unknown). P28 and P30 overlap both the 5' and 3' ends of *NRXN1* and were not included in statistical analyses. P14 and P32 were identified as having maternal uniparental disomy of chromosome 14 and a *RAF 1* mutation, respectively. The light blue box designates subjects with deletions overlapping exons numbered 5 and higher (3' deletion). Case numbers were kept consistent throughout the article, tables, and supplemental documents. Cases P30, P34, and P35 were obtained from other laboratories and are represented in bold font.

Rare exonic deletions overlapping *NRXN1* on chromosome 2p16 were first identified in individuals with autism spectrum disorder (ASD)^{11,12} and developmental delay/intellectual disability (DD/ID).¹³ Subsequently, such deletions have been identified in individuals with various neurodevelopmental and neuropsychiatric disorders.^{14–20} A few exonic *NRXN1* deletions have been identified in controls, and the majority of transmitting parents are reported to be only mildly affected or clinically unaffected.^{10,14} Understanding the factors that contribute to this incomplete penetrance is a key goal for clinical genetics.

Exonic *NRXN1* deletions are nonrecurrent and are found across the entire length of this large gene.^{2,10,21,22} There is conflicting evidence suggesting that the deletion extent may underlie the *NRXN1* phenotypic heterogeneity.^{10,21} A recent study found that individuals with ASD are significantly more likely to harbor a rare de novo mutation in exons under purifying selection (called “critical exons”) than their siblings,²³ suggesting that certain sequences within *NRXN1* may be more important for clinical expression than others. Also, accumulating evidence suggests that noncoding regions of the genome, including long noncoding RNAs (lncRNAs) and microRNAs (miRNAs), play an important role in the etiology of neurodevelopmental disorders.^{24,25} Deletions upstream and within introns of *NRXN1* have been identified in disease cases;^{14,22,26} however, the pathogenicity of these deletions remains unclear.

As the use of genome-wide microarray technology in the prenatal setting increases,²⁷ so does the need for an improved understanding of the genetic factors that impact the penetrance of *NRXN1* deletions to inform genetic counseling and anticipatory care. We used a large ($n = 19,263$) clinically ascertained cohort from southern Ontario, Canada, and 15,264 population-based controls to investigate the penetrance of *NRXN1* deletions. Copy-number variation (CNV) data from high-resolution genome-wide microarrays allowed systematic evaluation of the burden of secondary CNVs that we used as a proxy to estimate the relative penetrance of exonic and intronic *NRXN1* deletions.

MATERIALS AND METHODS

Exonic *NRXN1* deletions: clinical cohorts examined

We searched three accredited Province of Ontario clinical cytogenetics laboratory databases for cases with exonic *NRXN1* deletions. These included subjects submitted for clinical microarray testing before January 2015 at the Hospital for Sick Children ($n = 11,727$), Trillium Health Partners ($n = 6,022$), and Hamilton Health Sciences ($n=1,514$), all located in southern Ontario, Canada. All three laboratories are provincially funded to provide clinical constitutional microarray testing for individuals with DD/ID, ASD, and/or multiple congenital anomalies. The *NRXN1* deletion cases identified in this study therefore have a strong ascertainment bias for these conditions. Participating physicians completed a detailed clinical checklist (Supplementary Table S1 online) for each exonic *NRXN1* deletion case based on their own clinical assessment and lifetime chart reviews, when available. For cases without clinical checklist data, ascertainment and demographic data were collected from laboratory requisition forms. We also included

three additional cases with an exonic *NRXN1* deletion (P30, P34, and P35; Figure 1) detected by other clinical laboratories to increase the overall number of cases with genome-wide CNV data. These cases were not included in *NRXN1* deletion prevalence calculations for the catchment area.

Molecular methods and validation

All exonic *NRXN1* deletions (chr2:50,145,643–51,259,674; hg19) in cases were identified by one of three microarray platforms: the Affymetrix CytoScan HD Array, the Oxford Gene Technology (OGT) 4x180 CytoSure Oligonucleotide array, or the Illumina (formerly BlueGnome) 4x180K CytoChip ISCA array. The OGT and Illumina arrays are similar in design with approximately 200–250 oligonucleotide probes (average probe spacing, 5.5–7.6 kb) distributed across the *NRXN1* gene, allowing for a minimum deletion detection of 10 kb. The CytoScan HD array has approximately 650 oligonucleotide probes distributed across *NRXN1* with similar minimum deletion detection capabilities. All genomic coordinates are given using the Genome Reference Consortium February 2009 build of the human genome (GRCh37/hg 19). Genomic coordinates for the 22 *NRXN1* exons ($\alpha 1$ transcript; NM_004801.4) were obtained from the NCBI RefSeq database. Exonic *NRXN1* deletions ≥ 100 kb were confirmed using fluorescence *in situ* hybridization (FISH). Smaller deletions were confirmed by quantitative polymerase chain reaction or by a second microarray.

Additional clinically relevant CNVs as a proxy for calculating the relative penetrance of *NRXN1* deletions

All three clinical laboratories classified a deletion overlapping any one or more of the 22 *NRXN1* exons (NM_004801.4) as pathogenic. Therefore, we used the presence of a second clinically relevant CNV as a proxy for estimating the relative penetrance of the *NRXN1* deletions. As previously shown,²⁸ CNVs with high penetrance (i.e., not identified in control cohorts) are less likely to be accompanied by a second, large, rare CNV.²⁸ The high-resolution, genome-wide CNV data from each *NRXN1* deletion case was investigated for the presence of a second CNV that may be clinically relevant. The clinical interpretation of these second CNVs as pathogenic, likely pathogenic, or as a variant of unknown significance (VUS) was provided by one of the three experienced clinical laboratory directors using the American College of Medical Genetics and Genomics guidelines for CNV interpretation.²⁹ Other clinically relevant variants detected by different laboratory tests were not included in our statistical analyses.

Phenotypic data

The clinical checklist completed for each exonic *NRXN1* deletion case is presented in Supplementary Table S1 online. Briefly, data were collected regarding ascertainment features, demographic variables (age, sex), growth parameters (height, weight, head circumference) and growth abnormalities, dysmorphic features, and lifetime developmental, psychiatric, medical, and family history based on clinical assessment and/or lifetime medical chart reviews. If completed, previous clinical genetic

testing results were provided. When known, data regarding parental phenotypes were collected.

Control cohorts

To examine the prevalence of exonic and intronic *NRXN1* deletions in the general population, we used 15,264 controls with CNV data available from high-resolution genome-wide arrays (**Supplementary Table S3** online). These controls were analyzed on several different array platforms with variable probe spacing. Therefore, similar to previous studies,³⁰ we only included exonic and intronic deletions identified by a minimum of two of three CNV calling algorithms (iPattern, PennCNV, ChAS) spanning five consecutive array probes and more than 10 kb in size. More than 90% of CNVs called using this method validate using other laboratory methods.³⁰

Intronic *NRXN1* deletions

Trillium Health Partners was the only clinical laboratory that systematically recorded intronic *NRXN1* deletions. These clinical cases ($n = 6,022$) were analyzed using the Illumina 4x180K CytoChip ISCA array, which had even probe spacing across introns. We used two strategies to assess the pathogenicity of intronic *NRXN1* deletions. First, we compared the prevalence of intronic *NRXN1* deletions between clinically referred cases and the 15,254 population-based controls described. Second, we assessed the prevalence of secondary CNVs among the intronic *NRXN1* deletion cases and compared this to the prevalence for exonic *NRXN1* deletion cases.

Statistical analyses

Statistical analyses were performed using SAS software (version 9.2; SAS Institute, Cary, NC). For categorical data, we used χ^2 or Fisher's exact test, as appropriate. For continuous data, we used the Welch *t*-test. All analyses were two-tailed, with statistical significance defined as $P < 0.05$. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the prevalence of secondary CNVs between deletions overlapping one or more of exons 1–4 and those overlapping exons 5–22 and the association between the *NRXN1* deletions (exonic and intronic) ascertained from the clinical diagnostic population compared with controls.

RESULTS

Prevalence of exonic *NRXN1* deletions in cases and controls

As of January 2015, a total of 19,263 individuals had been submitted for clinical microarray testing across the three participating cytogenetics laboratories. Forty-one (0.21%) unrelated, previously unpublished, probands were identified as having deletions overlapping one or more *NRXN1* exons (**Figure 1**). The prevalences of exonic *NRXN1* deletions for the individual laboratories were 0.33, 0.26, and 0.14% (**Supplementary Table S4** online). The prevalence of exonic *NRXN1* deletions was significantly greater in cases compared with 15,264 controls (OR = 8.14; 95% CI: 2.91–22.72; $P < 0.0001$) (**Supplementary Table S3** online).

Exonic *NRXN1* deletions

Genomic coordinates for all 44 exonic *NRXN1* deletions, including three additional cases with exonic *NRXN1* deletions from laboratories outside the catchment area, are presented in **Figure 1**. All were nonrecurrent, with sizes ranging from 29 to 806 kb (median = 244 kb). The majority of the deletions ($n = 32$; 72.7%) overlapped at least one or more of exons 1–4 (referred to as a 5' *NRXN1* deletion hereafter). Ten (22.7%) deletions overlapped one or more of exons 5–22 (hereafter termed 3' *NRXN1* deletions). Two deletions (P28 and P30; **Figure 1**) that overlapped both the 5' and 3' ends of *NRXN1* were excluded from analyses comparing 5' and 3' *NRXN1* deletions but not from the descriptive statistics. This categorization of exons has been used in previous publications.^{10,21} The 5' *NRXN1* deletions (median size = 242 kb) were significantly larger than the 3' *NRXN1* deletions (median size = 83 kb; $t(18.5) = 2.55$; $P = 0.019$).

The 44 exonic *NRXN1* deletions were inherited (11 maternal, 5 paternal) in 16 (64.0%), de novo in 9 (36.0%), and unknown in 19 subjects. Of these, 5' *NRXN1* deletions were inherited in 13 (61.9%) and de novo in 8 (38.1%) subjects; 3' *NRXN1* deletions were inherited in 3 (75.0%) and de novo in 1 (25.0%) subject. Of the 11 transmitting mothers, 4 were identified as clinically affected: 3 with mild ID and 1 with anxiety and depression. Clinical outcomes for the remaining 7 transmitting mothers and 5 transmitting fathers were unknown.

Genome-wide prevalence of additional clinically relevant CNVs

Clinical microarray testing identified 10 (22.7%) exonic *NRXN1* deletion cases with one or more additional clinically relevant CNVs ($n = 14$) ranging in size from 38 kb to whole chromosomal anomalies (**Table 1**). The prevalence of males ($n = 7/28$; 25.0%) with a secondary CNV was similar to that of females ($n = 3/16$; 18.8%). Twelve (85.7%) of these CNVs were classified as VUS and two were classified as pathogenic (**Table 1**). The two pathogenic CNVs were de novo, two of the VUS were paternally inherited, and the remaining 10 additional VUS were of unknown inheritance. The prevalence of these secondary CNVs was significantly higher in subjects with a 3' *NRXN1* deletion ($n = 7/10$; 70.0%) compared with subjects with a 5' *NRXN1* deletion ($n = 3/32$; 9.4%; OR = 7.47; 95% CI: 2.36–23.61; $P = 0.0006$) (**Figure 1**). Eleven (78.6%) of the 14 secondary CNVs overlapped one or more genes known to be involved in central nervous system function (**Table 1**).

Clinical characteristics of exonic *NRXN1* deletion cases

Completed clinical checklists were returned for 21 (47.7%) exonic *NRXN1* deletion cases by referring physicians, with basic ascertainment and demographic data available for the other 23 cases (**Supplementary Table S2** online). As expected, given the criteria for microarray testing, all of the 44 probands (16 female, 28 male) with exonic *NRXN1* deletions were ascertained for DD/ID. Thirty-five (79.5%) were children (median, 5.2; range, 1–6 years) and 9 (20.5%) were adults (median, 32.0; range, 21–59 years).

Table 1 Additional clinically relevant CNVs identified in 10 of 44 exonic NRXN1 deletion subjects

ID ^a	Main clinical features	NRXN1 deletion	Cytoband	CNV	Start (hg 19)	Size (kb)	No. of genes	Inheritance	Protein-coding candidate genes ^b	Clinical laboratory classification
P44	ID, ASD, ADHD, ODD, anxiety, TS	Exon 20	X chr (47, XXX)	Gain	—	—	—	De novo	Various, including <i>FMR1</i> , <i>MECP2</i> , <i>SYN1</i>	Pathogenic
P43	DD	β promoter	Yp11.32-p11.2	Gain	10,863	4,459	—	De novo	Various, including <i>NLGN4Y</i>	Pathogenic
			Yq11.21-q12	Loss	14,630,081	44,700	—			
P41	DD	Exons 10–17	1q43	Gain	236,929,252	613	3	Unknown	<i>MTR</i> , <i>RYR2</i>	VUS
			15q13.1-q13.2 ^c	Loss	28,940,069	184	9		<i>APBA2</i> , <i>CHRFAM7A</i> , <i>TJPI</i>	VUS
P40	DD	Exons 5–8	11p11.2-p11.12	Gain	48,088,592	831	8	Paternal	None	VUS
P39	DD, ASD	Exons 5–8	1q23.3	Gain	160,927,546	428	23	Unknown	<i>USF1</i> , <i>PVRL4</i> , <i>PFDN2</i> , <i>PPOX</i> , <i>B4GALT3</i> , <i>ADAMTS4</i> , <i>NDUFS2</i> , <i>NR1B3</i> , <i>PCP4L1</i> , <i>MPZ</i> , <i>SDHC</i>	VUS
P38	DD/ID, motor and speech delay, PDD, anxiety, failure to thrive	Exons 5–8	4q35.2	Loss	188,355,766	383	1	Unknown	None	VUS
P36	DD, motor and speech delay	Exons 5–10	19q13.43	Loss	57,656,482	794	3	Unknown	None	VUS
P13	DD, behavior problems	Exons 1–2	16p13.3	Gain	6,679,225	38	1	Unknown	None	VUS
P10	DD, speech delay	α promoter and exons 1–4	8p23.3	Gain	843,413	750	4	Paternal	<i>DLGAP2</i>	VUS
P6	DD, behavioral problems, hypotonia, bilateral sensorineural loss	α promoter and exons 1–2	1p22.1	Gain	92,179,826	526	6	Unknown	<i>TGFBF3</i>	VUS
			3q29 ^d	Loss	192,404,455	164	2	Unknown	<i>FGF12</i>	VUS
			7q31.2-q31.31	Gain	117,382,934	1,700	3	Unknown	<i>CTTNBP2</i>	VUS
			Xp22.23	Gain	1,588,945	777	4	Unknown	<i>P2RY8</i> , <i>ASMT</i>	VUS

ADHD, attention-deficit hyperactivity disorder; ASD, autism spectrum disorder; CNV, copy-number variation; DD, developmental delay; ID, intellectual disability; MCA, multiple congenital anomalies; ODD, oppositional defiant disorder; PDD, pervasive developmental disorder; TS, Tourette syndrome; VUS, variant of unknown significance.

^aThe subject IDs match those found in Figure 1. ^bProtein-coding genes known to be expressed and/or implicated in nervous system function or cardiac function based on literature search. ^cThis deletion is distal to the Prader-Willi/Angelman syndrome region but proximal to the 15q13.3 deletion syndrome region (OMIM 612001). ^dDoes not overlap the 3q29 microdeletion syndrome region associated with schizophrenia (OMIM 609425).

Table 2 Additional clinically relevant CNVs identified in 12 of 19 intronic NRXN1 deletion subjects

ID ^a	Main clinical features	NRXN1 deletion	Cytoband	CNV	Start (hg 19)	Size (kb)	No. of genes	Inheritance	Protein-coding candidate genes ^b	Clinical laboratory classification
I8	DD	Intron 21	3q27.1-q27.2	Loss	184,027,899	1,300	4	Unknown	EIF4G, CLCN2, CHRD, EPHB3, SENP2	VUS
I11 ^c	ASD, heart defect, hip hypoplasia, clubfoot, absent radius	Intron 18	1q21.1 ^d	Loss	144,986,396	998	20	Unknown	PZDK1	Pathogenic
I10	DD	Intron 18	4p16.2	Gain	3,185,517	155	3	De novo	HIT	VUS
I6	DD	Intron 5	1p21.2-p21.1	Gain	101,742,523	554	1	Unknown	OLFM3	VUS
I2 ^c	DD	Intron 5	3p13	Loss	71,041,637	406	1	Unknown	FOXP1	Likely pathogenic
I1	DD, microcephaly	Intron 5	6q22.31-q23.2	Loss	125,993,504	5,900	22	Maternal	LAMA2	VUS
I13 ^c	MCA	Intron 5	Mosaic trisomy chr 9	Gain	—	—	~800	Unknown	Various, including SETX	Pathogenic
I15	DD, ASD	Intron 5	11p13	Gain	33,008,222	557	6	Unknown	None	VUS
I4	Absent radius and thumb	Intron 5	15q13.1-q13.2	Gain	28,859,279	1,500	4	Unknown	APBA2	VUS
I14 ^c	DD	Intron 5	15q11.2	Loss	22,669,082	998	5	Unknown	CYFIP1, NIPA2, NIPA1	Pathogenic
I17	DD	Intron 5	16p13.13	Loss	10,556,892	297	4	Paternal	None	VUS
I19	DD	Intron 5	11p15.5	Gain	1,222,378	318	4	Unknown	BRSK2	VUS

ASD, autism spectrum disorder; CNV, copy-number variation; DD, developmental delay; MCA, multiple congenital anomalies; VUS, variant of unknown significance.

^aThe subject IDs match those found in Figure 1. ^bProtein-coding genes known to be expressed and/or implicated in nervous system function or cardiac function based on literature search. ^cSubjects with CNVs classified as pathogenic. All other CNVs were classified as a variant of unknown significance. ^dOverlaps susceptibility locus for thrombocytopenia-absent radius syndrome (OMIM 274000).

Twenty (45.5%) subjects met diagnostic criteria for another (i.e., in addition to DD/ID) neurodevelopmental and/or neuropsychiatric condition, often referred to as “dual diagnosis.” Including multiple features per subject, the prevalences of these conditions were as follows: ASD or pervasive developmental disorder ($n = 14$; 31.8%), epilepsy/seizures ($n = 6$; 13.6%), ADHD ($n = 4$; 9.1%), anxiety ($n = 3$; 6.8%), Tourette syndrome ($n = 2$; 4.5%), and tardive dyskinesia secondary to antipsychotic treatment in two adults with schizophrenia ($n = 2$; 4.5%). There was no significant difference in the prevalence of dual diagnosis, seizures/epilepsy, or macrocephaly between subjects with a 5′ NRXN1 deletion compared to subjects with a 3′ NRXN1 deletion (data not shown).

In contrast to the prominent neurodevelopmental phenotype, congenital anomalies were identified in only four (9.1%) cases: two with tetralogy of Fallot (TOF), one of whom also had a tracheoesophageal fistula and imperforate anus; one with cryptorchidism; and one with a small atrial septal defect. All four of these subjects had a 5′ NRXN1 deletion and none were identified to have a rare second CNV. However, one TOF case was identified to have a RAF1 sequence mutation causing Noonan syndrome (OMIM 611553).

Eleven (25.0%) cases had additional clinical genetic testing with normal results with respect to karyotype, fragile X syndrome, 22q11.2 deletion syndrome, Prader-Willi syndrome, muscular dystrophy gene panel, and a gene panel for progressive myoclonic epilepsy. Two long contiguous stretches of homozygosity on chromosome 14 (identified on follow-up to be maternal uniparental disomy of chromosome 14) were identified in a single subject (P14; Figure 1).

Intronic NRXN1 deletions

We identified 19 of 6,022 (0.32%) cases submitted for clinical microarray testing and 55 of 15,264 (0.36%) controls with an intronic NRXN1 deletion (Figure 1). In contrast to the prevalence of exonic NRXN1 deletions identified in this laboratory (0.33%; OR, 12.67; 95% CI: 4.33–37.08; $P < 0.0001$), there was no significant difference in the prevalence of intronic NRXN1 deletions between cases and controls (OR 0.88; 95% CI: 0.52–1.48; $P = 0.618$). These intronic NRXN1 deletions ranged in size from 11 to 134 kb (median = 38 kb), which is significantly smaller than the 44 exonic deletions ($P < 0.0001$). The majority ($n = 14$; 73.7%) of the intronic deletions were located in the large intron 5 (Figure 1). Inheritance data are limited because most of the intronic deletions were not included in clinical reports; one case underwent follow-up and was found to be paternal in origin. Several of the intronic deletions were recurrent, including a 70.6-kb deletion in intron 5 and an 11.1-kb deletion in intron 18. Twelve (63.2%) of the 19 intronic NRXN1 deletion cases had a second reportable CNV on clinical microarray (Table 2), which is a significantly greater prevalence than for the exonic NRXN1 deletion cases (OR = 2.59; 95% CI: 1.37–4.91; $P = 0.004$).

Of these 19 (11 females, 8 males) unrelated cases with intronic NRXN1 deletions, 17 (89.5%) were children (median = 5.0; range 0.5–10 years) and 2 were adults (Supplementary Table S2 online). Sixteen (84.2%) were ascertained for DD/ID

and/or ASD and 3 (6.8%) were ascertained for multiple congenital anomalies ($n = 1$), CHD ($n = 1$), and absent radius/thumb. Of the 16 cases with DD and/or ASD, 10 (62.5%) had a second rare CNV that overlapped one or more genes involved in central nervous system function (Table 2).

DISCUSSION

This is the largest study characterizing exonic and intronic *NRXN1* deletions to date. Our aim was to use data from a large, clinical, population-based sample to systematically investigate factors affecting the penetrance of deletions overlapping this large gene. In addition to confirming the predominantly neuropsychiatric phenotypic expression of pathogenic CNVs overlapping *NRXN1*, novel results support the importance of the genomic extent of these deletions, including the particular region involved and the exons overlapped.

Penetrance of exonic *NRXN1* deletions

We report 44 novel exonic *NRXN1* deletion cases ascertained for DD/ID (Supplementary Table S2 online). We assessed penetrance using multiple factors, both those used previously (prevalence in cases versus controls and the ratio of de novo versus inherited deletions) and those novel to this study (prevalence of secondary CNVs and their classification as pathogenic or VUS). We found that the 0.21% prevalence of exonic *NRXN1* deletions in this clinical population was more than eightfold greater than in controls, and that 34% of these deletions were identified as being de novo, supporting the relatively high penetrance of these deletions for clinically important phenotypes. Approximately one in every four cases with an exonic *NRXN1* deletion had one or more other rare CNVs reported on clinical microarray (Table 1). Their distribution among the cases indicated a relatively lower penetrance of 3' *NRXN1* deletions.

The effect of genomic position on penetrance of exonic *NRXN1* deletions

As in previous studies,^{10,21,22,31} the majority of the exonic *NRXN1* deletions identified in clinical cases overlapped the promoter and the first few exons of the *NRXN1- α* transcript. For subjects with deletions overlapping the 3' end of *NRXN1*, there was a more than sevenfold higher likelihood of having a second clinically relevant CNV compared with subjects with a 5' *NRXN1* deletion (Figure 1). Furthermore, none of the additional rare CNVs identified in the 5' *NRXN1* deletion subjects was classified as pathogenic, demonstrating the relative importance of 5' *NRXN1* deletions over 3' *NRXN1* deletions for the expression of clinical phenotypes.

A potential explanation for the higher penetrance of 5' *NRXN1* deletions may be that these deletions directly overlap or indirectly influence the lncRNA AK127244 that is adjacent to the promoter of *NRXN1- α* (Figure 1). Accumulating evidence suggests that AK127244 plays a role in the etiology of neuropsychiatric disorders.^{26,32,33} This includes the identification of two deletions that overlap this lncRNA (and not

NRXN1) in a child with borderline IQ and early-onset schizophrenia.³² A recent report also described five ASD cases with deletions overlapping AK127244 (ref. 26). The biological function of AK127244 has yet to be elucidated. However, a significant proportion of lncRNAs are expressed in the brain and have important roles in neurodevelopmental processes.³⁴

There was little evidence that additional phenotypic features were indicators of higher penetrance in individuals with exonic *NRXN1* deletions in this study. This could be due in part to a high phenotypic floor effect present in clinically recruited subjects. It is interesting that the two adults with comorbid ID and schizophrenia in this study had deletions overlapping the 5' end of *NRXN1*. Similarly, in the largest study of *NRXN1* deletions in schizophrenia to date, 10 exonic *NRXN1* deletions were identified, the majority of which overlapped the 5' end of *NRXN1*.¹⁴ Also, two cases from that study had a deletion overlapping AK127244 but not *NRXN1*,¹⁴ providing support for the possible role of this lncRNA in the etiology of schizophrenia.

In contrast to our findings supporting the reduced penetrance of 3' *NRXN1* deletions, the limited number of these deletions reported in the literature had previously led to the hypothesis that they are associated with severe phenotypes, including prenatal lethality.³¹ To further investigate whether deletions overlapping the 3' end of *NRXN1* are associated with prenatal lethality, we examined 10 studies of miscarriages and/or stillbirths (see refs. 6 and 17–26 in Rosenfeld et al.³⁵) for rare CNVs overlapping *NRXN1*. Among more than 900 products of conception, only one paternally inherited 95-kb deletion overlapping *NRXN1- α* (exons 2–4) was identified.³⁵ We also identified one deletion overlapping the *NRXN1- β* promoter among 15,254 controls. These data provide further support for the increased penetrance of 5' relative to 3' *NRXN1* deletions.

Interpretation of intronic *NRXN1* deletions

We identified 19 novel cases with an intronic *NRXN1* deletion: 16 with DD/ID and/or ASD and 3 with a congenital anomaly. Our group used two strategies to assess the penetrance of intronic *NRXN1* deletions. First, we compared the prevalence of intronic *NRXN1* deletions between cases and population-based controls and detected no significant difference. Indeed, 18 of the 19 intronic *NRXN1* deletions identified in cases had breakpoints similar to those seen in controls (Figure 1). Second, we determined that intronic *NRXN1* deletion cases were twice as likely to harbor a second clinically relevant CNV compared with exonic *NRXN1* deletion cases. Therefore, intronic *NRXN1* deletions appear unlikely to substantially increase the risk for a neurodevelopmental disorder and/or multiple congenital anomalies.

However, seven (36.8%) of the intronic *NRXN1* deletion cases, each with a severe phenotype, had no additional rare CNV reported on clinical microarray. One of these seven *NRXN1* deletions overlapped intron 9 (case I16; Figure 1), which had no corresponding deletion identified in controls. Using the VISTA enhancer browser (<https://enhancer.lbl.gov>),³⁶ we identified a known enhancer element (hs1348) located 37 kb

upstream of the intron 9 deletion that, if perturbed, could potentially alter the transcriptional levels of *NRXN1* and thus increase the penetrance of this deletion.

Advantages and limitations

There are several advantages to this study. We used data from three clinical laboratories to compile the largest cohort of clinically referred individuals with exonic and intronic *NRXN1* deletions assembled to date. Our systematic approach to the detection and interpretation of additional rare CNVs allowed us to use the burden of these secondary CNVs as a proxy for determining the relative penetrance of *NRXN1* deletions. We used robust methods for CNV detection to evaluate the prevalence of intronic *NRXN1* deletions in cases and controls that had previously been ignored due to the use of different CNV calling algorithms and reporting practices across clinical laboratories.

A limitation of this study is that the prevalence of individual clinical features would necessarily be influenced by the ascertainment bias inherent in clinically referred cases. This tends to overestimate DD/ID and underestimate other features. The prevalence of *NRXN1* deletions was approximately an order of magnitude different between each clinical laboratory, reaching statistical significance between the Hospital for Sick Children (0.14%) and Trillium Health Partners (0.33%) (OR 2.29; 95% CI: 1.19–4.39; $P = 0.012$) (**Supplementary Table S3** online). This may be due to differences in indications for referral, with the Hospital for Sick Children and Hamilton Health Sciences servicing children with the most severe and intractable neurological disorders from this catchment area. Given that some *NRXN1* deletions are identified in control subjects,^{14,31} it is possible that the prevalence of these deletions is highest at Trillium Health Partners because it is a community-based hospital that services individuals with comparatively milder clinical phenotypes. The resolution of the microarrays used to define the *NRXN1* deletion breakpoints and detect genome-wide structural variants limited us to those >10 kb that could influence phenotypic expression.

Only one control cohort (OPGP; **Supplementary Table S3** online) was systematically screened for neurodevelopmental and/or neuropsychiatric conditions. Thus, control subjects with an exonic or intronic *NRXN1* deletion may have had mild and/or subclinical symptoms. This could have reduced the effect size of the case-control results. We did not have access to the individual SNP data for the *NRXN1* deletion cases or the controls, we were therefore unable to genetically confirm that each individual was unrelated to any other. However, given that the exonic *NRXN1* deletions identified in cases or controls had no similar breakpoints, it is unlikely that these individuals were related. Furthermore, only two of the eight control cohorts (**Supplementary Table S3** online) were ascertained from the same catchment area as our cases, and they included only adults. This makes it unlikely that the intronic *NRXN1* deletion cases (89% children) were the same individuals as those among the controls.

Future directions

The major challenge moving forward will be to determine how genetic and nongenetic factors converge to explain the variable expression and incomplete penetrance of exonic and intronic *NRXN1* deletions. Examining the genes overlapped by additional rare CNVs as well as applying next-generation sequencing to detect variants within the coding and noncoding regions of the genome in subjects with a *NRXN1* deletion may serve as key steps toward identifying novel pathways to disease expression. Interestingly, none of the second rare CNVs identified in this study has previously been reported in combination with an exonic *NRXN1* deletion,^{10,21,22,31} suggesting that a large *NRXN1* deletion consortium may be required to compile enough cases to identify novel biological patterns among the additional variants.

Conclusions

The expression of exonic *NRXN1* deletions appears to be primarily neuropsychiatric, with DD/ID often comorbid with another neuropsychiatric condition. The results of this study suggest that deletions near the 5' end of *NRXN1* have higher penetrance, potentially related to perturbation of the lncRNA AK127244 located adjacent to the *NRXN1*- α promoter. Subjects with a 3' *NRXN1* deletion had a sevenfold increased likelihood of having a second rare CNV detected by clinical microarray, supporting reduced penetrance for these deletions. There is insufficient evidence to suggest that all intronic *NRXN1* deletions are benign.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/gim>

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DISCLOSURE

The authors declare no conflict of interest.

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