ARTICLE

Phenotypic spectrum and genotype–phenotype correlations of *NRXN1* exon deletions

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Copy number variants (CNVs) and intragenic rearrangements of the *NRXN1* (neurexin 1) gene are associated with a wide spectrum of developmental and neuropsychiatric disorders, including intellectual disability, speech delay, autism spectrum disorders (ASDs), hypotonia and schizophrenia. We performed a detailed clinical and molecular characterization of 24 patients who underwent clinical microarray analysis and had intragenic deletions of *NRXN1*. Seventeen of these deletions involved exons of *NRXN1*, whereas seven deleted intronic sequences only. The patients with exonic deletions manifested developmental delay/intellectual disability (93%), infantile hypotonia (59%) and ASDs (56%). Congenital malformations and dysmorphic features appeared infrequently and inconsistently among this population of patients with *NRXN1* deletions. The more C-terminal deletions, including those affecting the β isoform of neurexin 1, manifested increased head size and a high frequency of seizure disorder (88%) when compared with N-terminal deletions of *NRXN1*.

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INTRODUCTION

Genomic microarray technology has significantly changed the clinical diagnostic approach in children with intellectual disabilities and neurodevelopmental delays. The increasing ability to obtain detailed quantitative copy number information continues to improve the diagnostic yield in patients with common neuropsychiatric disorders, such as intellectual disability (ID), autism spectrum disorders (ASDs), epilepsy and schizophrenia. Chromosomal microarray (CMA) is now considered a first-tier diagnostic test for individuals with developmental disabilities and congenital anomalies.¹ The genetic basis of several clinical syndromes has been uncovered by this approach and novel microdeletion and microduplication syndromes have been identified from clinically heterogeneous cohorts.²

Neurexins

The neurexins are a family of polymorphic cell adhesion molecules and receptors. In mammals, neurexins are encoded by three highly conserved, unlinked genes (*NRXN1*, *NRXN2* and *NRXN3*) each one of which has two independent promoters – resulting in two major isoforms (α and β) for each gene. The α -neurexins are transcribed from a promoter upstream of exon 1, whereas the β -neurexins

are transcribed from a downstream, intragenic promoter. Thus, the β -neurexins are modified and truncated forms of the larger α -neurexins. Aside from variable promoter usage, extensive utilization of alternative splicing leads to the generation of thousands of neurexin isoforms that are displayed on the neuronal cell surface.^{3,4}

Copy number variants (CNVs) of *NRXN1*

The *NRXN1* gene has been shown to have a fundamental role in synaptogenesis and synaptic maintenance, as well as neurotransmitter release and the function of voltage-gated calcium channels in the synapses of brainstem and neocortex.^{5–8} Variants of *NRXN1* have been associated with cognitive impairment,^{9,10} schizophrenia,^{11–15} nicotine dependence,^{16,17} alcohol dependence¹⁸ and ASDs.^{19–23} More recently, patients with smaller, intragenic deletions of the *NRXN1* gene have been identified. Their phenotypes are reported as variable, including ASDs, mental retardation, language delays and hypotonia.²⁴ Both CNVs deleting the entire *NRXN1* gene and multi-exonic deletions of *NRXN1*- α have been described. Neurexin 1- β deletions seem much less common overall.²⁴ Tandem intragenic duplications of *NRXN1*- β sequences in two families were associated to autistic phenotypes and cognitive delays.²³

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This study set out to characterize cases of small, intragenic deletions of *NRXN1* both clinically and molecularly, and to determine whether genotype–phenotype correlations exist within this cohort.

MATERIALS AND METHODS

Human Subjects

Of 8051 patients referred to the Baylor College of Medicine (BCM) Medical Genetics Laboratory (MGL) for array comparative genomic hybridization (aCGH) analysis from August 2009 to September 2010, 22 patients with intragenic rearrangements in *NRXN1* were identified. Two additional patients were recruited who had clinical chromosome microarray testing at different laboratories (patient E2, University of Iowa Cytogenetics Lab, Iowa city, IA, USA; patient E8, Quest Diagnostics, San Juan Capistrano, CA, USA). Following informed consent, approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine, we performed a comprehensive chart review of medical records and neuropsychological testing. Providers were asked to fill out a clinical questionnaire, which is provided as Supplemental Table 4.

Array Comparative Genomic Hybridization

Patient DNA was analyzed using the Baylor College of Medicine V8 OLIGO clinical genomic microarray, described in Boone *et al.*²⁵ Briefly, this is a custom-designed genomic microarray with both genome-wide coverage and supplemental exonic coverage of ~1700 known or suspected disease genes, including *NRXN1*. All aCGH procedures, including DNA isolation, sample preparation and labeling, array scanning, and data analysis, were performed as in Boone *et al.*²⁵ Patient E2 had clinical chromosome microarray testing at University of Iowa Cytogenetics lab, using a Nimblegen Nimblechip HG18, ultra-high density oligo array with 385 000 oligonucleotide probes ranging from 50–75 mers and average probe spacing of ~6 kb. Patient E8 had clinical chromosome microarray testing at Quest Diagnostics using a ClariSure CGH, a microarray containing more than 3000 bacterial artificial chromosomes (BACs), each in duplicate, and confirmation by fluorescence *in situ* hybridization.

Fluorescence *in situ* hybridization (FISH), PCR, DNA sequencing of long-range PCR products and DNA sequencing of *NRXN1* are discussed in the Supplemental Methods.

All genomic coordinates refer to the March 2006 assembly of the reference genome (NCBI36/hg18). Exon numbering is based on RefSeq transcript NM_01135659.1 (*NRXN1-α*) and RefSeq transcript NM_138735.2 (*NRXN1-β*).

RESULTS

Between August 2009 and September 2010, a total of 8051 patients were referred to MGL for aCGH. The *NRXN1* gene is covered by 212 oligonucleotide probes, with backbone resolution of 10 kb throughout the gene, and much increased resolution (up to one probe every 100 bp or higher) within and flanking exons (Figure 1a). Among 8051 samples, a total of 20 intragenic deletions of *NRXN1* were detected (0.25%), 13 of which included exonic sequences, and 7 were purely intronic. Two additional cases of exonic deletions were recruited (E2 and E8), who had clinical CMA testing at different institutions. Exonic deletions of the 24 total cases varied in size between 17 and 913 kb, deleting between 2 and 13 exons. Deletion breakpoints were non-recurrent, except for individuals E11/E12 and E16/E17, who were siblings. Deletions affecting the N-terminal domain of neurexin 1 are more frequent overall, affecting only the coding region of *NRXN1-α*. Only four cases deleted part of both *NRXN1-α* and *NRXN1-β* (E14–E17; Figure 1b). All cases detected by chromosome microarray analysis were confirmed by a second, independent method, except case E2, for which confirmation was not attempted. FISH analysis using BAC-clones was used for deletions >50 kb, long-range PCR and cloning of the breakpoints was used for deletions <50 kb. Most cases represented isolated *NRXN1* deletions, but two of the exonic deletion patients and three of the intronic deletion patients were found to carry a second CNV (Table 1). The 22 samples submitted to MGL also underwent DNA sequencing in order to evaluate for coding

Figure 1 Intragenic deletions in *NRXN1* identified by exon-targeted aCGH. (a) The α and β isoforms of *NRXN1* are shown. Aligned with these is a plot of array probe density, demonstrating enhanced resolution within and surrounding exons of the gene. The X-coordinate of each dot corresponds to the genomic position midway between two consecutive probes; the Y-coordinate is the genomic distance between these probes. (b) Twenty-four intragenic deletions of *NRXN1*, aligned to the α and β isoforms of *NRXN1*. Exonic deletions are shown in red, intronic deletions in orange. Each box indicates the patient ID (E1–E17 for exonic deletions, I1–I7 for intronic deletion cases). Plotting is based on UCSC Genome Build hg18 and the minimum interval detected to be deleted by aCGH.

Table 1 NRXN1 exon deletions in 17 patients

Patient ID	Deletion	Minimal interval (on chromosome 2)	Minimal size	Confirmation	Inheritance	Other CMA anomalies
E1	Exons 1–2	51108945–51316396	207 kb	FISH	<i>De novo</i>	Dup 17p12 (min 14040843–15363614)
E2	Exons 1–2	51096075–51415269	319 kb	Not attempted	Unknown	None
E3	Exons 1–3	51056636–51167934	111 kb	FISH	<i>De novo</i>	None
E4	Exons 1–4	51036654–51125770	89 kb	FISH	Paternal	None
E5	Exons 1–5	51006222–51167932	161 kb	FISH	Maternal	None
E6	Exons 1–5	50821957–51167934	346 kb	FISH	<i>De novo</i>	None
E7	Exons 1–5	50892598–51125770	233 kb	FISH	Maternal	None
E8	Exons 1–5	51017041–51307360	290 kb	FISH	Unknown	None
E9	Exons 1–5	50753008–51666029	913 kb	FISH	Unknown	None
E10	Exons 4–5	50902643–51036715	134 kb	FISH	Maternal	None
E11	Exons 6–18	50545885–50922836	376 kb	FISH	Maternal	None
E12 ^a	Exons 6–18	50545885–50922836	376 kb	FISH	Maternal	None
E13	Exons 17–18	50545885–50552890	7 kb	PCR and DNA sequencing, actual deletion size 17 kb	Paternal	None
E14	Exons 19–20	50160134–50481919	321 kb	FISH	Maternal	None
E15	Exons 20–24	49999102–50265693	266 kb	FISH	Maternal	Del 16p13.12 (min 12571173–13109315)
E16	Exons 23–24	49885294–50046403	161 kb	FISH	Paternal	None
E17 ^b	Exons 23–24	49885294–50046403	161 kb	FISH	Paternal	None
Summary exonic deletions			Average: 256 kb +/-48 kb (SEM)	Fifteen FISH, 1 PCR and DNA sequencing	Three <i>de novo</i>, six maternal, three paternal, three unknown, two sibling cases	2/17

Abbreviations: Del, deletion; dup, duplication; FISH, fluorescence *in situ* hybridization; kb, kilobases; min, minimum predicted boundary of the deletion by array comparative genomic hybridization; SEM, standard error of the mean.

^aSibling of E11.

^bSibling of E16.

sequence alteration on the second, non-deleted allele. No coding sequence mutations were detected among these samples.

While all intronic deletions for which both parents were available for testing were found to be inherited (four paternal, two maternal), exonic deletions were either inherited (three independent cases paternal, six independent cases maternal) or *de novo* (three cases). The inheritance of four exonic deletion cases and one intronic deletion case remain uncertain, as at least one parent was not available for testing (Table 1). Of the nine parents from whom NRXN1 exonic deletions were inherited, 8 (89%) have a history of learning problems and/or neuropsychiatric disease. Four were reported to have learning problems or intellectual disability, four had a history of psychiatric problems (depression, anxiety), two had a formal diagnosis of ASD and one had a history of epilepsy. One of the nine parents carrying a NRXN1 deletion (father of E4) had no history of neuropsychiatric phenotypes and no history of cognitive impairment or learning deficits.

We compared indications provided at the time of submission for CMA testing among this cohort to the indications provided in the overall cohort of 8051 samples submitted during the study. The most common indications provided among the probands reported herein were: developmental delay/intellectual disability (seven samples), ASDs (five samples) and seizures/epilepsy (six samples). In the overall cohort, developmental delay/intellectual disability was listed as indication in 2881 cases (frequency 1 in 2.79), ASDs in 521 cases (frequency 1 in 15.45) and seizures/epilepsy in 396 cases (frequency 1 in 20.33). This indicates that the frequency of developmental delay/intellectual disability provided as an indication is comparable between all samples submitted during the given timeframe and those with

intragenic deletions of NRXN1 (χ^2 -test, $P = 0.4984$), while ASDs are overrepresented as an indication among NRXN1 deletion cases (χ^2 -test, $P = 0.0042$), and seizures/epilepsy are highly overrepresented (χ^2 -test, $P = 0.0001$).

Detailed clinical information was obtained on all 24 patients with NRXN1 deletion cases. While clinical details of the seven intronic deletion cases are presented in the online supplement (Supplemental Tables 1 and 2), the 17 cases of exonic NRXN1 deletions are discussed below (Tables 2 and 3): these were 11 boys and 6 girls between ages 5 months and 16 years from various ethnic backgrounds (11 Caucasian, 4 Hispanic, 1 Ashkenazi Jewish, 1 mixed). While growth parameters were within normal limits overall, both average height (34th percentile; SEM = 5.5) and average weight (34th percentile; SEM = 8.7) were slightly decreased when compared with the general population, based on CDC growth charts.²⁴ The average head circumference was on the 60th percentile (SEM = 8.3). Mild dysmorphic features were reported in several patients, but no characteristic facial or physical phenotype was noted across individuals within this cohort (Table 2). Only 2 of the 17 individuals with exonic NRXN1 deletions had a history of congenital anomalies: proband E1, who has a second CNV (dup 17p12, see Table 1) was born with a complex congenital heart defect (double outlet right ventricle, dextro-transposition of the great arteries). Proband E9 had a history of omphalocele, pulmonary hypoplasia, bilateral club feet, scoliosis and a tongue cyst. Brain imaging had been performed on 14 of the 17 patients. Structural brain anomalies were found in 6 of these 14 individuals, but most of these constituted rather unspecific MRI findings, and no pattern of structural brain malformations associated with NRXN1 deletions can be concluded from this cohort.

Table 2 Physical features in 17 patients with *NRXN1* exon deletions

Patient		Age at			Weight	Length	FOC				Congenital	
ID	Deletion	Sex	diagnosis	Ethnicity	%ile	%ile	%ile	Vision	Hearing		anomalies	Dysmorphic features
E1	Exons 1–2	F	16 days	Hispanic	15	10	5	Normal	Normal		Complex CHD (DORV, D-TGA)	Cupped ears, epicanthal folds, long palpebral fissures, flat nasal bridge
E2	Exons 1–2	M	12 years	Caucasian	35	25	65	Normal	Normal		None	Deep set eyes
E3	Exons 1–3	F	3 years	Hispanic	45	85	60	Normal	Normal		None	Low set ears, telecanthus, epicanthal folds
E4	Exons 1–4	M	6 years	Caucasian	65	50	25	Normal	Normal		None	Mild hypertelorism
E5	Exons 1–5	M	7 years	Caucasian	50	50	50	Normal	Normal		None	Epicanthal folds, upslanting palpebral fissures, anteverted nares
E6	Exons 1–5	F	2 months	Caucasian	10	20	75	Normal	Normal		None	None
E7	Exons 1–5	M	2.5 years	Caucasian/ Hispanic	<1	2	53	Normal	Normal		None	None
E8	Exons 1–5	M	5 years	Caucasian	15	12	5	Normal	Normal		None	None
E9	Exons 1–5	M	10 years	Hispanic	<3	3	5	Normal	Normal		Pulmonary hypoplasia, omphalocele, bilateral club feet, scoliosis	Low set, posteriorly rotated ears, bushy, arched eyebrows, wide mouth, high arched palate, dental crowding
E10	Exons 4–5	M	3 years	Hispanic	76	86	10	Normal	Normal		None	None
E11	Exons 6–18	M	13 years	Caucasian	20	25	>98	Normal	Normal		None	None
E12 ^a	Exons 6–18	M	16 years	Caucasian	50	25	98	Normal	Normal		None	None
E13	Exons 17–18	M	5 years	Caucasian	35	43	52	Normal	Normal		None	Epicanthal folds
E14	Exons 19–20	F	5 years	Ashkenazi Jewish	46	55	92	Normal	Normal		None	None
E15	Exons 20–24	M	10 years	Caucasian	25	25	95	Normal	Mild hearing impairment		None	Deep set eyes
E16	Exons 23–24	F	9 years	Caucasian	65	50	>98	Normal	Normal		None	None
E17 ^b	Exons 23–24	F	9 years	Caucasian	25	10	>98	Normal	Normal		None	None
Summary		11 M	6.94 years,	11 Caucasian,	34th %ile	34th %ile	60th %ile	0/17	1/17		2/17	8/17, but no consistent
exonic		6 F	+/-1.05	4 Hispanic,	+/-5.5	+/-6.3	+/-8.3					abnormalities
deletions			(SEM)	1 Ashkenazi Jewish,	(SEM)	(SEM)	(SEM)					
				1 mixed								

Abbreviations: CHD, congenital heart defect; DORV, double outlet right ventricle; D-TGA, dextro-transposition of the great arteries; F, female; FOC, fronto-occipital circumference; M, male; SEM, standard error of the mean.

^aSibling of E11.

^bSibling of E16.

A wide range of neurodevelopmental and neuropsychiatric phenotypes was present among patients with exonic *NRXN1* deletions (Table 3, and Supplemental Table 5). Attainment of developmental milestones was delayed with an average age of independent sitting of 9.4 months (SEM = 0.8 month), walking at 17.5 month (SEM = 1.7 month) and first word spoken at 23.6 month (SEM = 4.0 month). Information about intellectual development and schooling was available for 14 patients, of which 13 (93%) had a history of intellectual disability and/or requirement of special education. One proband (E2) with deletion of exons 1 and 2 had formal testing with a full scale IQ of 116. ASDs were reported in 10 of 17 patients, with three individuals diagnosed with autistic disorder, three with pervasive developmental disorder, not otherwise specified (PDD-NOS) and four with a general diagnosis of ASDs. An additional two probands were reported to have autistic features, but had not undergone formal testing at the time of enrollment, and one proband carried a diagnosis of sensory integration disorder.

A history of seizures was reported in 9 of 17 patients (53%). Five patients had a history of generalized tonic-clonic seizures, four a history of absence seizures and one proband experienced atonic drop attacks in addition of absence epilepsy. Eight individuals had been

formally evaluated with electroencephalography (EEG), five of whom were found to have an abnormal EEG (see Table 3 for details).

Interestingly, seizures were more commonly reported in probands that had deletions of the more C-terminal exons of *NRXN1* when compared with those with N-terminal deletions. Of the 10 patients with deletions within the first five exons of the gene, only one (E2) had a history of absence seizures. On the other hand, all patients with deletions affecting exons 6 and higher had a history of epilepsy. The difference in seizure incidence between these two groups is statistically significant (χ^2 -test, two-tailed P -value = 0.0254).

A second phenotype that seemed to discriminate between probands ascertained with C-terminal deletions was macrocephaly. The average percentile for head circumference of all patients with N-terminal deletions (affecting the first 5 exons of the gene, probands E1–E10) was 38.3 (SEM = 8.2), while the average percentile for patients with C-terminal deletions (exons 6 and higher, probands E11–E17) was 90.6 (SEM = 6.5). The mean Z-score for head circumference was -0.53 among the patients with N-terminal deletions (SEM = 0.29) and 2.33 among patients with C-terminal deletions (SEM = 0.60). This was statistically significant by unpaired t -test (two-tailed P -value = 0.0003, see Figure 2).

Table 3 Neurological and psychiatric phenotypes in 17 patients with NRXN1 exon deletions

Patient ID	Deletion	Sex	Age at diagnosis	Motor coordination				First word	Regression	Autism	ADHD	IQ/DQ	Seizures	EEG	MRI brain
				Hypotonia	deficits	Sitting	Walking								
E1	Exons 1–2	F	16 days	Present	–	9 months	N/A	N/A	–	No formal testing	–	Unknown	–	None	Frontal subdural hygroma, mild ventriculomegaly
E2	Exons 1–2	M	12 years	–	–	Unknown	10 months	18 months	–	Sensory integration disorder	Yes	FS IQ 116	Absence	Normal	Atrophic changes of the hippocampus
E3	Exons 1–3	F	3 years	Present	Present	Unknown	10 months	2 years old	Language regression at age 2 years old	PDD-NOS	–	Delayed in all categories, except for adaptive behavior (below average).	–	None	Minimal periventricular white matter changes
E4	Exons 1–4	M	6 years	Mild	–	6 months	13 months	18 months	Regression after 18 months	Autism Spectrum Disorder	–	Unknown	–	None	Normal
E5	Exons 1–5	M	7 years	Present	Present	8 months	16 months	12 months	Regression at age 2 years old	Autistic features	Yes	ID (clinical impression)	–	None	None
E6	Exons 1–5	F	2 months	Present	–	N/A	N/A	N/A	N/A	N/A	N/A	Unknown	–	None	Normal
E7	Exons 1–5	M	2.5 years	Present	–	9 months	18 months	12 months	–	Autistic features	–	Cogn 85, motor 76, lang 62	–	None	None
E8	Exons 1–5	M	5 years	–	Present	8 months	18 months	2 years old	–	PDD-NOS	Yes	No formal testing, but requires special education	GTCS during infancy	Normal	Normal
E9	Exons 1–5	M	10 years	Present	–	18 months	2 years old	2 years old	–	–	Yes	Moderate ID, adaptive behavior score 58	–	Suggestive of global brain dysfunction	Normal
E10	Exons 4–5	M	3 years	–	–	8 months	14 months	18 months	–	Autism Spectrum Disorder	Yes	Gross motor 79, fine motor 51, Lang 72, social 49	–	None	Normal
E11	Exons 6–18	M	13 years	–	–	Unknown	20 months	2 years old	–	Autism Spectrum Disorder	–	No formal testing, but requires special education	Absence	Not available	None
E12 ^a	Exons 6–18	M	16 years	–	–	Unknown	14 months	12 months	–	Autism Spectrum Disorder	–	Mild to moderate ID (clinical impression)	GTCS	Right frontal spike activity	Normal
E13	Exons 17–18	M	5 years	–	Present	10 months	16 months	18 months	–	–	Yes	Verbal 91, spatial 61, nonverbal 60	GTCS	Bifrontal and central spike and wave	Normal
E14	Exons 19–20	F	5 years	–	–	Unknown	12 months	6 months	–	Autistic disorder	–	Gross motor 38, fine motor 89, receptive lang 38, expressive lang 53	Absence	Normal	Bilateral perisylvian pachygyria. Symmetric white matter volume loss, thinning of the corpus callosum
E15	Exons 20–24	M	10 years	Mild	–	Unknown	Unknown	Unknown	–	PDD-NOS	Yes	FS IQ 61, verbal 85, performance 57	Absence and atonic drop attack	Frontocentral generalized spike and slow wave activity	Normal

Table 3 (Continued)

Patient ID	Deletion	Sex	Age at diagnosis	Motor coordination deficits			Walking	First word	Regression	Autism	ADHD	IQ/DQ	Seizures	EEG	MRI brain
				Sitting	Hypotonia	Age at coordination deficits									
E16	Exons 23–24	F	9 years	12 months	–	–	3 years old	5 years old	–	Autistic disorder	–	No formal testing, but requires special education	GTCS	Poorly organized beta frequency. Background with poor variability and reactivity.	Prominent Virchow–Robin spaces
E17 ^b	Exons 23–24	F	9 years	6 months	–	–	2 years old	5 years old	–	Autistic disorder	–	No formal testing, but requires special education	GTCS	None	Focal areas of white matter hyperintensity, prominent Virchow–Robin spaces
Summary exonic deletions		11 M 6 F	6.94 years, +/-1.05 (SEM)	4/17 9.4 months +/-0.8 (SEM)	8/17	4/17	17.5 months +/-1.7 (SEM)	23.6 months +/-4.0 (SEM)	3/17	10/17 with ASD, plus 2/17 with autistic features	7/17	14/15	9/17, 4 absence, 5 GTCS	5/8 abnormal	6/14 abnormal, but no consistent abnormalities

Abbreviations: ADHD, attention deficit hyperactivity disorder; Cogn, cognitive; DQ, developmental quotient; F, female; FS, IQ; full scale intelligence quotient; GTCS, generalized tonic-clonic seizures; ID, intellectual disability; IQ, intelligence quotient; Lang, language; M, male; N/A, not applicable; ODD, oppositional defiant disorder; PDD-NOS, pervasive developmental disorder, not otherwise specified; SEM, standard error of the mean; –, not present.

^aSibling of E11.

^bSibling of E16.

Figure 2 Box plot of Z-scores for head circumference of individuals with N- and C-terminal exon deletions of *NRXN1*. Comparing the N-terminal cases of *NRXN1* deletions (involving the first five exons) and the C-terminal cases of *NRXN1* deletions (exons six and higher) reveals a significant difference in head size between the two groups (unpaired *t*-test, $P=0.0003$). Y-coordinates correspond to the Z-score for head circumference (sex and age matched).

DISCUSSION

Chromosome microarray analysis is now considered a first-tier test for individuals with intellectual disability and ASDs.¹ It has been suggested that the detection rate using high-resolution chromosome microarrays among unexplained cases of intellectual disabilities and neurodevelopmental or neuropsychiatric phenotypes is between 7 and 20%,²⁷ depending on the cohort. Exon-targeted microarrays, with increased density of coverage within the coding regions of disease-associated genes, may further increase this diagnostic yield.²⁵ We report a total of 24 cases of intragenic *NRXN1* deletions, with deletion sizes varying between 17 and 913 kb.

Deletions and loss-of-function point mutations of *NRXN1* have been linked to autism, schizophrenia and intellectual disability.^{13–15,21,23} More recently, intragenic rearrangements of *NRXN1* have been described and associated with a wide spectrum of developmental disorders.^{23,24} There is a significantly higher prevalence of *NRXN1* deletions among clinical samples when compared with control populations. In the reported cohort, the incidence of intragenic *NRXN1* deletions was 20/8051 among clinically referred cases (0.25%), which is quasi identical to the rate reported by Ching *et al* (9/3450; ie, 0.25%).²⁴ The frequency of exonic deletions of *NRXN1* among control populations is 10/51 939 (0.019%).²⁴ Similar findings have been reported for schizophrenia populations, where incidence of *NRXN1* deletions >100 kb among individuals with schizophrenia has been determined 0.19% (17/8798) vs 0.04% (17/42 054) among controls.¹³

The largest previously reported cohort of individuals with *NRXN1* deletions includes nine individuals with whole-gene or multiple exon deletions, and three individuals with deletions in intron 5. In that cohort, the most common symptoms included cognitive impairment (5/12), language delay (9/12), ASDs (5/12) and hypotonia (4/12).²⁴ Although multiple publications suggest *NRXN1* deletions to be pathogenic, little is known about the prevalence and pathogenicity

of *NRXN1* duplications or about intronic CNVs in the *NRXN1* gene. Intragenic, frame-shifting duplications would represent an exception, but only one such case has been described.²³ No genotype–phenotype correlations for *NRXN1* CNVs have been suggested, likely due to the fairly small number of cases identified.

Here, we describe the largest cohort of individuals with intragenic deletions of *NRXN1* reported to date, provide detailed clinical and phenotypic information, and, for the first time, propose some genotype–phenotype correlation for exonic *NRXN1* deletions. Similar to previous reports, developmental delay and intellectual disability (12/13), ASDs (10/17) and hypotonia (8/17) represent some of the most common phenotypes observed among those with exonic deletions of *NRXN1*. In addition, attention deficit hyperactivity disorder (ADHD) is reported in 7 of 17 patients. The inheritance of the reported exonic deletion cases was delineated in 12 independent cases. Of these, 3 (25%) were found to be *de novo*. In a large meta-analysis, Rees *et al*²⁸ calculated the *de novo* rate of exonic *NRXN1* deletions to 22%, which is remarkably similar.

Previously, in four studies, a total of seven individuals with *NRXN1* deletions were reported to have a history of seizures. One was an individual with a whole-gene deletion of *NRXN1*.²⁴ Gregor *et al*²⁹ reported a total of six heterozygous intragenic *NRXN1* deletions, three of which had seizures. However, one of their patients (N4) had additional CNVs at 15q26 (deletion) and 16q12 (duplication), and another proband (N5) was the offspring of a consanguineous mating. In a different study, a *NRXN1* deletion carrier was reported to have had one single seizure as a child.²¹ Lastly, Harrison *et al*³⁰ recently reported on two sisters with compound heterozygous deletions of *NRXN1* (one affecting the promoter and exons 1–5, and the second one deleting exons 20 and 21). Both sisters had severe, early-onset epilepsy.

In our study, 9 of 17 patients are affected with epilepsy or have a history of seizures. Four individuals have absence seizures, and five have generalized tonic–clonic epilepsy. Most interestingly, epilepsy is a consistent feature of individuals with C-terminal intragenic deletions. Only 2 of 10 individuals with N-terminal deletions (within the first five exons of *NRXN1*) were reported to have a history of seizures, while all seven patients with C-terminal deletions have epilepsy. One might speculate that this is because of C-terminal deletions affecting other neurexin 1 isoforms, considering the extensive use of alternative splicing, which has been reported for the neurexin genes.³¹ Notably, within this given cohort, all four patients with deletions affecting *NRXN1*-β (patients E14–E17) have epilepsy.

A second phenotype associated with C-terminal deletions, but not with N-terminal deletions of *NRXN1*, is macrocephaly. Comparing the head sizes of all 17 individuals with exonic deletions, there is a significant difference in head size. Defining macrocephaly as a head circumference at or above the 95th percentile for age, five of seven patients with C-terminal deletions are affected, whereas none of the individuals with N-terminal deletions meets this criterion.

Future studies will show whether these genotype–phenotype correlations are consistent across various cohorts. As for most, if not all, neuropsychiatric disorders associated with CNVs, there is considerable variability of expressivity of symptoms, and only larger cohorts will uncover strong genotype–phenotype relationships.

Detailed molecular studies would be warranted to unravel how certain exonic deletions may affect various splice forms of the neurexin proteins and how that affects neuronal connectivity, excitability and function.

Our study leaves the question of whether intronic deletions of *NRXN1* may be pathogenic unanswered. One could imagine that

certain intronic deletions affect splicing or delete promoter sequences of respective isoforms. However, none of the intronic deletions reported herein affects known splice sites or promoter sequences of neurexin 1 (*NRXN1*) isoforms. Furthermore, all seven cases of intronic *NRXN1* deletions are inherited (except for one, for which the father is not available for study), and of the six carrier parents, only one is reported to manifest neuropsychiatric symptoms (anxiety and depression). This may suggest that intronic deletions of *NRXN1* are not pathogenic *per se*, or at least with relatively low penetrance, operating in a multi-factorial milieu to increase risk for developmental and neuropsychiatric phenotypes.

In summary, we report the clinical and molecular phenotypes of 24 patients with intragenic CNVs of the *NRXN1* gene. Exonic deletions of *NRXN1* are associated with developmental delay, intellectual disability of various degrees, ASDs, hypotonia and ADHD. Deletions of C-terminal exons of *NRXN1* associate with increased head size and epilepsy within our cohort.

CONFLICT OF INTEREST

Drs Schaaf, Brown, Patel, Stankiewicz and Cheung are faculty members of the Department of Molecular and Human Genetics at Baylor College of Medicine, which derives revenue from the chromosomal microarray analysis offered in the Medical Genetics Laboratory. The remaining authors declare no conflict of interest.

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Supplementary Information accompanies the paper on European Journal of Human Genetics website (<http://www.nature.com/ejhg>)