RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Investigation of *NRXN1* Deletions: Clinical and Molecular Characterization

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Manuscript Received: 7 August 2012; Manuscript Accepted: 29 October 2012

Deletions at 2p16.3 involving exons of *NRXN1* are associated with susceptibility for autism and schizophrenia, and similar deletions have been identified in individuals with developmental delay and dysmorphic features. We have identified 34 probands with exonic *NRXN1* deletions following referral for clinical microarray-based comparative genomic hybridization. To more firmly establish the full phenotypic spectrum associated with exonic *NRXN1* deletions, we report the clinical features of 27 individuals with *NRXN1* deletions, who represent 23 of these 34 families. The frequency of exonic *NRXN1* deletions among our postnatally diagnosed patients (0.11%) is significantly higher

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Article first published online in Wiley Online Library (wileyonlinelibrary.com): 12 March 2013
DOI 10.1002/ajmg.a.35780

than the frequency among reported controls (0.02%; $P = 6.08 \times 10^{-7}$), supporting a role for these deletions in the development of abnormal phenotypes. Generally, most individuals with *NRXN1* exonic deletions have developmental delay (particularly speech), abnormal behaviors, and mild dysmorphic features. In our cohort, autism spectrum disorders were diagnosed in 43% (10/23), and 16% (4/25) had epilepsy. The presence of *NRXN1* deletions in normal parents and siblings suggests reduced penetrance and/or variable expressivity, which may be influenced by genetic, environmental, and/or stochastic factors. The pathogenicity of these deletions may also be affected by the location of the deletion within the gene. Counseling should appropriately represent this spectrum of possibilities when discussing recurrence risks or expectations for a child found to have a deletion in *NRXN1*.

Key words: neurexin 1; 2p16.3; developmental delay; autism; dysmorphic; schizophrenia; microarray

How to Cite this Article:
Dabell MP, Rosenfeld JA, Bader P, Escobar LF, El-Khechen D, Vallee SE, Dinulos MBP, Curry C, Fisher J, Tervo R, Hannibal MC, Siefkas K, Wyatt PR, Hughes L, Smith R, Ellingwood S, Lacassie Y, Stroud T, Farrell SA, Sanchez-Lara PA, Randolph LM, Niyazov D, Stevens CA, Schoonveld C, Skidmore D, MacKay S, Miles JH, Moodley M, Huillet A, Neill NJ, Ellison JW, Ballif, BC, Shaffer LG. 2013. Investigation of *NRXN1* deletions: Clinical and molecular characterization.
Am J Med Genet Part A 161A:717–731.

INTRODUCTION

Neurexins are neuronal cell-surface proteins that function as cell-adhesion molecules and receptors. They mediate synapse formation through interactions with neuroligins, leucine-rich repeat transmembrane neuronal proteins (LRRTMs), and glutamate receptor delta 2 (GluRδ2) and function at both glutamatergic and GABAergic synapses [Scheiffele et al., 2000; Dean et al., 2003; Boucard et al., 2005; Ko et al., 2009; de Wit et al., 2009; Siddiqui et al., 2010; Uemura et al., 2010]. Humans have three *NRXN* genes that encode over 1,000 isoforms generated from alternative splicing of two different transcripts from each gene, the longer α-neurexins and the shorter β-neurexins [Ullrich et al., 1995; Rowen et al., 2002; Tabuchi and Sudhof, 2002]. The protein isoforms share a common C-terminal transmembrane domain and short cytoplasmic tail. They differ in the extracellular portion, which consists of six laminin/neurexin/sex hormone-binding globulin (LNS) domains and three intervening epidermal growth factor-like (EGF) domains in α-neurexins and only a single LNS domain in β-neurexins; these extracellular domains are responsible for binding with NRXN's interacting partners [Sudhof, 2008]. The various isoforms interact differently with other proteins, and their expression is controlled spatially and temporally in the brain as well as being dependent on neuronal depolarization [Ichtchenko et al., 1995; Ullrich et al., 1995; Patzke and Ernsberger, 2000; Boucard et al., 2005; Chih et al., 2006; Koehnke et al., 2010; Siddiqui et al., 2010; Iijima et al., 2011; Rozic et al., 2011]. Because of their role in signaling, neurexins are thought to play a central role in the brain's ability to process information.

Heterozygous partial deletions, as well as other mutations and disruptions, of *NRXN1* have been reported in association with susceptibility for neurocognitive disabilities, such as autism spectrum disorders (ASDs) [Feng et al., 2006; Szatmari et al., 2007; Kim et al., 2008; Yan et al., 2008; Bucan et al., 2009; Glessner et al., 2009; Guilmatre et al., 2009; Gauthier et al., 2011; Sanders et al., 2011; Soysal et al., 2011; Duong et al., 2012; Gai et al., 2012; Hedges et al., 2012; Liu et al., 2012], intellectual disability (ID) [Friedman et al., 2006; Zahir et al., 2008; Guilmatre et al., 2009; Ching et al., 2010; Wisniowiecka-Kowalnik et al., 2010; Gregor et al., 2011; Schaaf et al., 2012], and schizophrenia [Kirov et al., 2008; Vrijenhoek et al., 2008; Walsh et al., 2008; Guilmatre et al., 2009; Kirov et al., 2009; Need et al., 2009; Rujescu et al., 2009; Ikeda et al., 2010; Magri et al., 2010; Shah et al., 2010; Gauthier et al., 2011; Levinson et al., 2011; Muhleisen et al., 2011; Stewart et al., 2011; Duong et al., 2012] (Table I). Although multiple studies have shown a significantly higher frequency of *NRXN1* defects in patient populations in comparison to control populations, *NRXN1* alterations have also been identified in normal parents and healthy controls [Feng et al., 2006; Bucan et al., 2009; Kirov et al., 2009; Need et al., 2009; Rujescu et al., 2009; Zweier et al., 2009; Ching et al., 2010; Gregor et al., 2011; Levinson et al., 2011; Sanders et al., 2011; Gai et al., 2012; Hedges et al., 2012; Schaaf et al., 2012]. This suggests variable expression or reduced penetrance with other genetic and/or environmental factors influencing the ultimate phenotype and degree of neurocognitive disabilities. Furthermore, homozygous disruption of *NRXN1*-α in humans causes a severe Pitt–Hopkins-like phenotype including severe ID, absent speech, stereotypies, and autistic features; epilepsy and hyperpnea are also seen in some [Zweier et al., 2009; Harrison et al., 2011; Duong et al., 2012]. Mouse models recapitulate some of this phenotype; mice lacking *Nrxn1*-α have decreased excitatory synapses, impaired sensorimotor gating, increased grooming behavior, and impaired nest building and parenting ability but have unaffected learning, memory, and social interactions [Geppert et al., 1998; Etherton et al., 2009].

In some ID/developmental delay (DD) and ASD cohorts, individuals with *NRXN1* deletions have been reported with a wide variety of additional phenotypic findings, including prominent speech delays, hypotonia, epilepsy, skeletal abnormalities, heart defects, and dysmorphic features [Zahir et al., 2008; Ching et al., 2010; Wisniowiecka-Kowalnik et al., 2010; Gregor et al., 2011; Hedges et al., 2012; Schaaf et al., 2012]. While some correlation may exist between the specific isoform impacted by the deletion and the phenotype [Rujescu et al., 2009; Ching et al., 2010; Wisniowiecka-Kowalnik et al., 2010; Schaaf et al., 2012], phenotypic variability among individuals with similar deletions has prevented clear genotype–phenotype correlations. Additionally, as most reported *NRXN1* deletions have been part of large population screenings of individuals with ASDs or schizophrenia, this

TABLE I. Exonic *NRXN1* Deletions in Published Cohorts

Reference	Population	Only α exons	α and β exons	Unspecified location	Total tested	Frequency of exonic deletions (%)
ID cohorts						
Ching et al. [2010]	Clinical aCGH cases	7[h]	2	0	3,540	0.25
Gregor et al. [2011]	Severe ID; PTHS-like	1	0	0	45	2.22
Friedman et al. [2006]	Idiopathic ID, normal karyotype	1	0	0	100	1.00
Wisniowiecka-Kowalnik et al. [2010]	Clinical aCGH cases	1	1	0	9,000	0.02
Guilmatre et al. [2009]	Idiopathic ID, normal karyotype	1	0	0	247	0.40
Schaaf et al. [2012]	Clinical aCGH cases	8[i]	3[i]	0	8,051	0.14
This study	Clinical postnatal aCGH cases	27[i]	2[i]	0	25,610	0.11
Sum of ID cohorts		**46**	**8**	**0**	**46,593**	**0.12**
ASD cohorts						
Bucan et al. [2009]	AGRE multiplex families	4	0	1	912	0.55
	ACC cohort: ASD cases	0	0	4	859	0.47
Gai et al. [2012]	AGRE families, mostly multiplex	2	0	0	689	0.29
Guilmatre et al. [2009]	ASD cases (mostly autism)	2	0	0	260	0.77
Sanders et al. [2011][a]	SSC simplex ASD families	3	0	0	1,124	0.27
Glessner et al. [2009]	ACC cohort: ASD cases	0	0	3	859	0.35
	AGRE families, mostly multiplex	3	0	0	778	0.39
Szatmari et al. [2007]	AGP families	0	1	0	173	0.58
Sum of ASD cohorts[b]		**9**	**1**	**5**	**3,328**	**0.45**
Schizophrenia cohorts						
Guilmatre et al. [2009]	Schizophrenia/schizoaffective disorder	1	1	0	236	0.85
Walsh et al. [2008]	Schizophrenia/schizoaffective disorder	0	0	0	150	0
	Childhood-onset schizophrenia	0	1	0	83	1.20
Magri et al. [2010]	Schizophrenia cases	1	0	0	172	0.58
Kirov et al. [2008]	Schizophrenia cases	1	0	0	93	1.08
Kirov et al. [2009]	Schizophrenia/schizoaffective disorder	1	0	0	471	0.21
Vrijenhoek et al. [2008][c]	Schizophrenia cases	3	0	0	846	0.35
Need et al. [2009][d]	Schizophrenia cases	0	0	3	1,073	0.28
Stewart et al. [2011]	NIMH cases with schizophrenia & epilepsy	1	0	0	235	0.43
Ikeda et al. [2010]	Japanese schizophrenia cases	0	0	0	519	0
Levinson et al. [2011]	Schizophrenia/schizoaffective disorder	9	1	0	3,945	0.25
ISC [2008]	Schizophrenia cases	3	0	0	3,391	0.09
Rujescu et al. [2009]	Schizophrenia cases	5[j]	0	0	2,977	0.17
Sum of Schizophrenia cohorts[e]		**23**	**3**	**3**	**12,944**	**0.22**
Control cohorts						
Zweier et al. [2009]	Healthy controls	0	0	0	667	0
Guilmatre et al. [2009]	Psychiatrically screened controls	0	0	0	236	0
Glessner et al. [2009]	CHOP pediatric controls	0	0	0	2,519	0
Magri et al. [2010]	Psychiatrically screened controls	0	0	0	160	0
Kirov et al. [2008]	Non-schizophrenia controls: individuals with congenital anomalies, malformations, or ALS	0	0	0	372	0
Kirov et al. [2009][f]	WTCCC study adult controls	2	0	0	2,792	0.07
Vrijenhoek et al. [2008][c]	Dutch psychiatrically screened controls	0	0	0	706	0
Stewart et al. [2011]	NIMH psychiatrically screened controls	0	0	0	191	0
Ikeda et al. [2010]	Japanese psychiatrically screened controls	0	0	0	513	0
Levinson et al. [2011]	Psychiatrically screened controls	1	0	0	3,611	0.03
ISC [2008]	Mostly unscreened adult controls	1	0	0	3,181	0.03
Rujescu et al. [2009]	Unscreened European controls	5	0	0	33,746	0.01
Muhleisen et al. [2011]	Unscreened adult controls	0	0	0	94	0

(*Continued*)

TABLE I. (*Continued*)

Reference	Population	Only α exons	α and β exons	Unspecified location	Total tested	Frequency of exonic deletions (%)
Bucan et al. [2009]	CHOP pediatric controls	0	0	0	1,070	0
	Neurologically normal NINDS adult controls	0	0	0	418	0
	ACC controls (CHOP pediatric controls)	0	0	0	1,051	0
Shaikh et al. [2009]	CHOP healthy pediatric controls	0	0	0	2,026	0
Cooper et al. [2011]	Adult controls	5	0	0	8,328	0.06
Sum of control cohorts[g]		**12**	**0**	**0**	**53,171**	**0.02**

ACC, Autism Case-Control; aCGH, microarray-based comparative genomic hybridization; AGP, Autism Genome Project; AGRE, Autism Genetic Resource Exchange; ALS, amyotrophic lateral sclerosis; ASD, autism spectrum disorder; CHOP, Children's Hospital of Philadelphia; ID, intellectual disability; ISC, International Schizophrenia Consortium; NIMH, National Institute of Mental Health; NINDS, National Institute of Neurological Disorders and Stroke; PTHS, Pitt–Hopkins syndrome; SSC, Simons Simplex Collection; WTCCC, Wellcome Trust Case Control Consortium.
[a]This study only identified de novo abnormalities.
[b]Sum only includes the AGRE and ACC cohorts as reported by Bucan et al. [2009].
[c]Most of these cases and controls were also screened in the study by Rujescu et al. [2009].
[d]Includes the Aberdeen samples studied by the ISC [2008].
[e]Sum only includes the subsets of the populations in Need et al. [2009] and Rujescu et al. [2009] that had not been previously reported.
[f]These controls were also screened in the study by Cooper et al. [2011].
[g]Excluding the overlapping controls noted above, excluding the controls in the study by Kirov et al. [2008] due to phenotypic similarities with our case cohort, and including only the CHOP pediatric controls reported by Shaikh et al. [2009].
[h]Excludes three cases with a deletion in intron 5 predicted to only impact a minor exon, which is not part of any RefSeq transcripts.
[i]Only including single individuals from tested sibling pairs.
[j]Excludes one deletion that was predicted to impact only exon 5; as this exon has been newly aligned to genomic sequence close to exon 4, the deletion is now predicted to be intronic.

can introduce an ascertainment bias into phenotypic characterization, and the full phenotypic implications of these deletions need to be explored further. Here, we report the identification of 34 probands with deletions limited to *NRXN1* exons, tested in our laboratory using microarray-based comparative genomic hybridization (aCGH), and describe the clinical characterization of 27 individuals from 23 of these 34 families with *NRXN1* deletions, adding to the published phenotypic spectrum.

MATERIALS AND METHODS

Patient Ascertainment

From February 2008 through June 2012 we tested 30,065 (prenatal and postnatal) patients submitted to Signature Genomic Laboratories from the United States and abroad using oligonucleotide-based aCGH platforms that provide coverage over *NRXN1*. The most common indications for study were ID, DD, and/or multiple congenital anomalies. Clinicians were contacted to provide clinical information about their patients carrying exonic *NRXN1* deletions. Either de-identified clinical information was supplied, or informed consent for publication of clinical information and photographs was obtained, according to a protocol approved by the Institutional Review Board-Spokane.

Microarray-Based Comparative Genomic Hybridization

All 2p16.3 deletions were characterized by aCGH using various whole-genome, oligonucleotide-based platforms, depending on the time of testing. Microarray analysis was performed using a 105K-feature platform (SignatureChipOS v1.0, custom-designed by Signature Genomics, manufactured by Agilent Technologies, Santa Clara, CA) or one of two versions of a 135K-feature platform (SignatureChipOS v2.0 and v3.0, custom-designed by Signature Genomics, manufactured by Roche NimbleGen, Madison, WI) as previously described [Ballif et al., 2008; Duker et al., 2010]. Microarray analysis using the SignatureChipOS v3.0 uses a new design with targeted coverage of 245 known genetic syndromes and over 980 gene regions of functional significance (http://www.signaturegenomics.com/disorders_tested.html). Over *NRXN1*, versions 1–3 of the arrays have an average probe spacing of 71, 32.5, and 10 kb, respectively, and a minimum deletion detection threshold of 284, 130, and 40 kb, respectively (Fig. 1).

All genomic coordinates are according to the UCSC March 2006 build of the human genome (hg18). *NRXN1* exons are numbered according to the α1 isoform (NM_004801.4), and genomic coordinates of the exons were obtained from the NCBI Consensus Coding Sequence database (CCDS 54360.1). This most recent alignment differs from that described in earlier case reports [Kim et al., 2008; Zahir et al., 2008; Rujescu et al., 2009; Zweier et al., 2009; Ching et al., 2010; Gregor et al., 2011; Hedges et al., 2012; Schaaf et al., 2012] in that exon 5 was reassigned to genomic sequence near exon 4, instead of being near exon 6 (Fig. 1).

Fluorescence In Situ Hybridization (FISH)

For individuals with exonic *NRXN1* deletions, metaphase FISH was performed to visualize the deletions using previously described methods [Traylor et al., 2009]. When available, parental samples were also tested by FISH; for deletions too small to be visualized by FISH, parental samples were tested using aCGH.

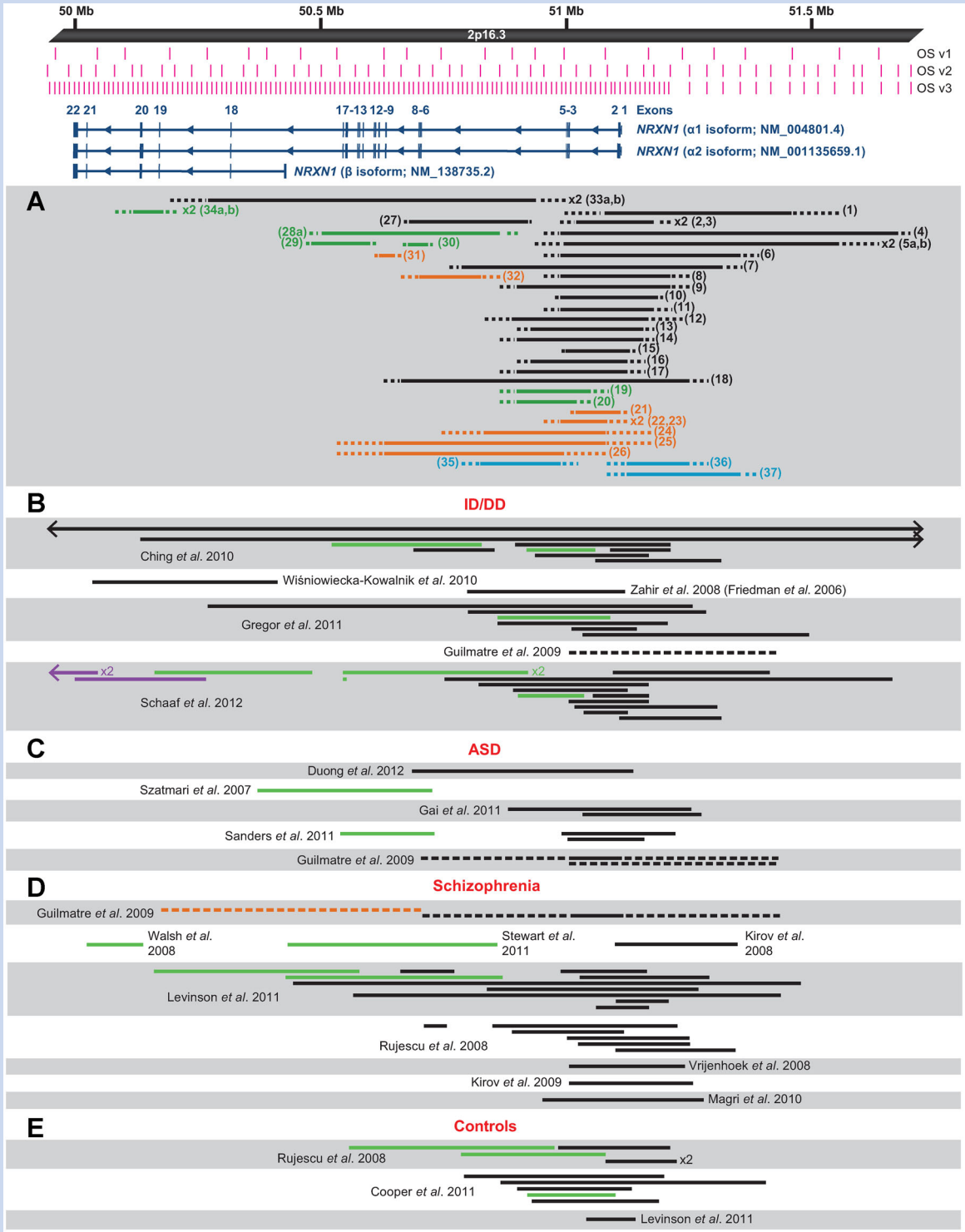

FIG. 1. Overview of exonic *NRXN1* deletions. At the top of the figure is a partial idiogram showing chromosome band 2p16.3, with genomic coordinates corresponding to the hg18 build of the human genome. The three major *NRXN1* transcripts are shown in blue, with vertical pink dashes representing the locations of oligonucleotide probes on the three array versions used. A–E: Horizontal bars represent deletions, with solid bars depicting the minimum deletion sizes and the dashed lines extending through gaps in coverage to show the maximum deletion sizes. Black bars correspond to deletions that likely abolish gene function by either removing the promoter region of *NRXN1*-α or through a frameshift. Green bars represent in-frame deletions. Orange bars have an uncertain effect on the reading frame. Blue bars may or may not be exonic; exons fall within the gap between deleted and nondeleted probes on the array. Purple bars truncate the 3′ end of the gene. Numbers in parentheses correspond to Patient numbers in this article. Deletions were identified in (A) the clinical aCGH cohort reported here, (B) individuals with intellectual disability (ID)/developmental delay (DD), (C) individuals with autism spectrum disorders (ASD), (D) individuals with schizophrenia, and (E) control cohorts.

TABLE II. Possible Exonic *NRXN1*-Specific Deletions Identified in Our Clinical aCGH Cohort

Patient	Sex	Age at testing	Indication for study	Inheritance	Other abnormality	Platform	Genomic coordinates (hg18)	Deleted exons (potentially deleted)
5′ deletions involving only *NRXN1-α*								
1	M	4 y	DD	DN	None	OS v1	chr2: 51079673–51459761	1–2 (1–5)
2	M	4 y	DD, muscle weakness	Unk	None	OS v2	chr2: 51020497–51178152	1–2 (1–5)
3	M	3 y	DD, epilepsy	Unk	arr 5q14.2q14.3 (82692609–88073704) × 1[a]	OS v2	chr2: 51020497–51178152	1–2 (1–5)
4	M	23 y	ASD	Pat	None	OS v2	chr2: 50989149–51675291	1–5
5a	M	23 y	DD, epilepsy	Mat	None	OS v1	chr2: 50996352–51555511	1–5
5b	M	20 y	DD, DF		None			
6	M	10 y	DD, DF, short stature	Pat	None	OS v2	chr2: 50989149–51354094	1–5
7	M	11 y	DD	Unk	None	OS v2	chr2: 50788065–51318184	1–5
8	M	2 y	DD (language and motor)	Unk	None	OS v2	chr2: 50989149–51211695	1–5
9	M	6 y	DD, DF	DN	None	OS v2	chr2: 50900500–51211695	1–5
10	F	8 y	PDD	Unk	None	OS v3[c]	chr2: 50990905–51189246	1–5
11	M	Prenatal	Cystic hygroma, hydrops, micrognathia	Pat	None	OS v2	chr2: 50989149–51178152	1–5
12	M	13 y	Mild ID, HL	Mat	None	OS v1	chr2: 50890607–51167934	1–5
13	M	7 y	DD, epilepsy	Unk	None	OS v2	chr2: 50928021–51157870	1–5
14	F	0 m	CHD	Pat	None	OS v2	chr2: 50900500–51157870	1–5
15	F	12 y	Epilepsy	Unk	None	OS v3	chr2: 50999149–51130917	1–5
16	F	5 y	DD/ID, hypotonia, DF	Unk	None	OS v2	chr2: 50928021–51124577	1–5
17	M	6 y	DD	Unk	None	OS v2	chr2: 50900500–51124577	1–5
18	M	56 y	Severe ID, PDD	Unk	None	OS v2	chr2: 50664886–51250425	1–8 (1–9)
19	M	22 y	DD, ASD	Unk	None	OS v2	chr2: 50900500–51020557	3–5
20	F	0 m	MCA	Unk	None	OS v2	chr2: 50900500–51049751	3–5
21	M	21 m	DD, DF	Pat	arr 11q14.1 (84007950–84169251) × 1 pat[b]	OS v3[c]	chr2: 51020434–51109974	2 (1–2)
22	M	6 y	DD, ASD	Unk	arr 10q11.22q11.23 (49062854–52062367) × 3[b]	OS v2	chr2: 50989209–51084499	3–5 (1–5)
23	M	Prenatal	Cystic hygroma, shortened upper limbs, fetal demise	Pat	None	OS v2	chr2: 50989149–51084499	3–5 (1–5)
24	F	12 y	Hypotonia, encephalopathy	Unk	None	OS v1	chr2: 50835616–51079733	3–5 (1–5)
25	F	9 y	Multiple disabilities	DN	arr 13q12.12 (22464761–23808883) × 1 pat[b]	OS v1	chr2: 50629393–51079733	3–9 (1–17)
Other deletions only in *NRXN1-α*								
26	F	15 m	DD	Unk	arr 3p12.3 (76485892–79401678) × 1[b]	OS v1	chr2: 50629393–50996412	6–10 (3–17)
27	M	Prenatal	Abnormal ultrasound	Unk	None	OS v3	chr2: 50680324–50921132	6–8
28a	M	11 y	ASD	DN	None	OS v2	chr2: 50503145–50865069	6–17
29	M	12 y	DF, ASD	Mat	None	OS v3	chr2: 50480647–50600362	13–17
30	F	5 y	PDD	Unk	None	OS v3	chr2: 50680324–50720466	6–8
31	F	5 y	Encephalopathy	Unk	None	OS v3	chr2: 50620135–50650670	9 (9–11)
32	F	3 m	MCA	Unk	None	OS v2	chr2: 50702475–50825788	6 (6–8)
Deletions of *NRXN1-α* and *NRXN1-β* exons								
33a	F	6 y	DD	Unk	None	OS v1	chr2: 50271164–50936973	6–18
33b	M	7 y	Autism		None			
34a	F	15 y	DD, ASD	Unk	None	OS v2	chr2: 50118810–50179711	19–20
34b	F	7 y	ADHD		None			

(*Continued*)

TABLE II. (*Continued*)

Patient	Sex	Age at testing	Indication for study	Inheritance	Other abnormality	Platform	Genomic coordinates (hg18)	Deleted exons (potentially deleted)
Unclear deletions (may or may not be exonic)								
35	F	0 m	CHD	Unk	None	OS v2	chr2: 50825728–50989209	(3–5)
36	F	2 y	DD, short stature	Pat	arr 22q11.23 (22080929–23321669) × 3 pat[b]	OS v2	chr2: 51124517–51250425	(1–2)
37	F	3 y	DD, epilepsy	Unk	None	OS v2	chr2: 51124517–51354094	(1–2)

ADHD, attention deficit/hyperactivity disorder; ASD, autism spectrum disorder; CHD, congenital heart defect; DD, developmental delay; DF, dysmorphic features; DN, de novo; F, female; HL, hearing loss; ID, intellectual disability; M, male; m, months; Mat, maternal; MCA, multiple congenital anomalies; Pat, paternal; PDD, pervasive developmental disorder; Unk, unknown; y, years.
[a]This deletion involves *MEF2C* and is clinically significant.
[b]The clinical significance of this copy number alteration is unclear.
[c]Case originally ascertained on bacterial artificial chromosome-based array.

RESULTS

Molecular Analysis

Among the 30,065 patients tested on oligonucleotide-based arrays, 35 have deletions limited to exons of *NRXN1*, including three pairs of affected siblings. Two additional *NRXN1* deletions were initially identified in patients tested on bacterial artificial chromosome-based arrays (Patients 10 and 21). These deletions were subsequently better characterized using oligonucleotide-based aCGH; however, these two patients are not included in calculations of the frequency of *NRXN1* deletions ascertained by oligonucleotide-based aCGH. Finally, three additional patients' deletions may or may not include *NRXN1* exons, due to the exons' presence in a gap between deleted and non-deleted oligonucleotide probes (in Patients 35–37; Fig. 1). Five patients have secondary copy number alterations of unclear clinical significance, while a sixth has a clinically significant 5q14 deletion including *MEF2C* (Table II). Among the 34 unrelated exonic *NRXN1* deletions, the majority (25; in Patients 1–25) are clustered over the 5′ region and first four coding exons (2–5), which are part of only *NRXN1*-α transcripts. While those deletions including the transcription start site of *NRXN1*-α are predicted to abolish expression of this isoform, deletions involving only exons 3–5 are predicted to result in an in-frame deletion. We found seven deletions (in Patients 26–32) limited to exons specific to *NRXN1*-α in the middle of the gene; at least three of these are also predicted to be in-frame. Only two unrelated deletions (in Patients 33 and 34) involve the 3′ end of the gene, interrupting the *NRXN1*-β transcript in addition to *NRXN1*-α; one of these deletions, present in two siblings, is also predicted to be in-frame (in Patients 34a,b; Fig. 1).

Inheritance of the exonic *NRXN1* deletions was determined in 13 families. Of these, four deletions (31%) are apparently de novo, and nine (69%) are inherited, three maternally and six paternally (Table II). An additional two sets of affected siblings have the same *NRXN1* deletion, but parental samples were not available for testing. Additionally, one healthy mother was identified to have an exonic *NRXN1* deletion (involving exons 1–2 and potentially exons 1–5), which was not present in her affected child.

Among the 3,247 probands tested on the v3.0 oligonucleotide-based array, which has the densest coverage over *NRXN1*, ten have deletions limited to intron 5 of the gene. A single patient tested on the v2.0 oligonucleotide-based array also has a deletion limited to this intron (data not shown). These were excluded from further clinical analysis due to unknown effects on gene expression and protein function.

Clinical Analysis

The indications for study in the 37 individuals with exonic *NRXN1* deletions vary and include ID/DD, congenital anomalies, and epilepsy. Approximately one-third (10/31) of probands older than 1 year were referred with an ASD. Ages at testing varied, ranging from prenatal (three samples referred for ultrasound anomalies) to 56 years (a man with severe ID who lives in a group home) (Table II).

Detailed clinical information is given for 27 patients, including three sets of siblings (identical twins 28a,b, two affected brothers and their healthy sister 5a–c, and maternal half-sisters 34a,b; Table III). Deletions in these 27 individuals range in size from 40 to 586 kb, and only the deletions in Patients 34a,b include *NRXN1*-β exons. There is wide phenotypic variability among patients, but at least four features are shared by the majority (Table IV). Of the 24 patients older than 3 months, 21 have DD; Patient 5c has dyslexia but normal development and is an A/B student, and Patients 30 and 34b have behavioral issues but normal development. Speech delays are prominent, with three patients older than 2 years showing absent or minimal speech. Of the 24 patients older than 3 months, 19 have abnormal behaviors, of whom 10 have confirmed diagnoses of ASD. Twenty patients have some degree of dysmorphic features (Fig. 2). Eight parents of these patients also carry the *NRXN1* deletion, including a mother with bipolar disease, a mother with a short attention span, a father with learning disabilities and short stature, a father with type 1 diabetes who is otherwise healthy, and at least three healthy parents; one carrier father holds an advanced degree. In one family (5a–c), the healthy mother carries the deletion, while the father has a

TABLE III. Clinical Features of Individuals With Exonic *NRXN1* Deletions

Patient	Exons deleted (potentially deleted)	Age; sex	Percentiles: height, weight, OFC	Neurological features	Behavioral features	Dysmorphic features	Ophthalmologic features	Skeletal/joint features	Other anomalies	Family history
1	1–2 (1–5)	5 y; M	98th, >98th, >98th	Global DD; hypotonia	Autistic behaviors	Prominent brow; upslanting PF; large & protruding ears; wide nasal tip; inverted nipples	Astigmatism; strabismus	Genu valgum; mild pes planus	Asthma; lactose/gluten intolerance; abnormal urine flow	NS
2	1–2 (1–5)	3.5 y; M	10–25th, 25th, 25th	Global DD; hypotonia; Conductive HL due to fluid	Anxiety	Tall & broad forehead; deep-set eyes; bulbous nasal tip; depressed columella; broad philtrum (Fig. 2A)	—	—	History of FTT; asthma; food allergies; hyperflexibility; GERD; constipation	Mother (no del) has duplicated reproductive system; father (nt) has DD, asthma/allergies, hypotonia
5a	1–5	23 y; M	25–50th, 25–50th, 75–98th	Profound ID (anoxic brain injury during surgery); nonverbal	—	High nasal bridge; bitemporal narrowing; bulbous nasal tip; macroglossia; high palate	Strabismus	—	Sinus and AV node dysfunction; cutaneous 2–5 finger syndactyly; mild 2–3 toe syndactyly	Patients 5a–c are siblings; maternal (del) & paternal family history of ADHD; father with LD
5b	1–5	20 y; M	25–50th, 25–50th, 50–98th	IQ 65; SD; bilateral ptosis	ADHD	Bitemporal narrowing; high nasal bridge; bulbous nasal tip; pointed chin; high palate	Strabismus; wears glasses	—	Sinus and AV node dysfunction; cutaneous 2–4 finger syndactyly; mild 2–3 toe syndactyly	
5c	1–5	16 y; F	WNL	Dyslexia; nl development	—	—	—	—	—	
6	1–5	9 y; M	<3rd, 15th, 50th	Borderline ID; poor articulation	PDD-NOS	Mild midface hypoplasia; broad/short nails (Fig. 2B)	Mild myopia	Hypoplastic phalanges; pseudoarthrosis of clavicle	FTT; allergies; mild eczema	Father (del) with LD, short stature, knee problems
7	1–5	10 y; M	75th, >97th, 75th	DD; IQ in 80s	ODD; ADHD; bipolar	—	Strabismus		Cryptorchidism; asthma and allergies	Father (nt) with bipolar, asthma, & alcoholism
8	1–5	27 m; M	25th, <3rd, <3rd	DD; moderate expressive SD	Adaptive behavior deficits	NS	—	—	Early history of GERD	Mother (nt) with significant SD
9	1–5	5 y; M	25–50th, 10–25th, 10–25th	Mild DD; speech impediment; hypotonia	Repetitive mannerisms, improved	Small face; midface hypoplasia; flat nasal bridge; low-set & prominent ears	—	—	Cryptorchidism; Raynaud's phenomenon	Paternal family history of LD
10	1–5	8 y; F	27th, 25th, 80th	IQ 76; SD; tic disorder; hypotonia	PDD-NOS; ADHD	Prominent forehead; deep-set eyes; upslanting PF; prominent nose with broad tip; high palate; maxillary hypoplasia; underbite	Retinopathy	Ligamentous laxity; hammertoes	—	Adopted
11	1–5	Infant; M	NS	NS	NA	Prominent eyes; ridged coronal suture; micrognathia; webbed neck	NS	NS	Septated cystic hygroma; hydrops; peripheral branch pulmonic stenosis; deceased (sepsis)	Father (del) is healthy
12	1–5	13 y; M	50–75th, 50–75th, 50th	Mild ID; SD; hypotonia; HL (due to infection)	ADD	Deep-set eyes; short PF; midface hypoplasia; bulbous nose; irregular teeth; protruding & malformed ears	Strabismus	—	Allergies	Mother (del) has bipolar disease; paternal family history of LD
14	1–5	3 m; F	<3rd, 3rd, 3rd–10th	Hypotonia; white matter injury	NA	Upslanting PF; narrow nose	—	—	FTT; HRH, atrial septal defect, absent pulmonary valve, tricuspid stenosis, pulmonary atresia; grade II VUR; chronic lung disease; hepatomegaly; small gall bladder; hyperbilirubinemia	NS
16	1–5	5 y; F	50th, 25–50th, <3rd	DD; hypotonia	—	Epicanthal folds; wide nasal bridge; minor hypertrichosis	—	Mildly short fourth metacarpals	—	Non-contributory
17	1–5	6 y; M	10–25th, 25–50th, 50–75th	Global DD; has 3–4-word sentences; hyperintense signal in splenium of CC	—	—	—	—	Unilateral cryptorchidism	Three brothers (nt) with SD
18	1–8 (1–9)	57 y; M	<3rd, 10th, 25–50th	Severe ID; childhood seizures	PDD; self-injurious	Large chin; narrow palate	Congenital cataract; anisocoria	Brachydactyly	—	Non-contributory

(*Continued*)

TABLE III. (*Continued*)

Patient	Exons deleted (potentially deleted)	Age; sex	Percentiles: height, weight, OFC	Neurological features	Behavioral features	Dysmorphic features	Ophthalmologic features	Skeletal/joint features	Other anomalies	Family history
21	2 (1–2)	21 m; M	50–75th, 50–75th, NS	DD; absent speech; HL ("glue ear")	—	Round cranial vault; displaced hair whorl; deep-set eyes; midface hypoplasia	—	Arm & leg rhizomelia; leg length discrepancy	—	Father (del) has type 1 diabetes
22	3–5 (1–5)	6 y; M	90–97th, 90–97th, 90–97th	Global DD; posturing; toe walking	ASD	—	—	—	—	Mother (nt) with anxiety; maternal & paternal family history of OCD
23	3–5 (1–5)	Fetus; M	NS	NA	NA	NS	NS	Limbs fixed in flexion	Large cystic hygroma; demise at 15w gestation	Father (del) is healthy
26	6–10 (3–17)	12 m; F	10–25th, 5th, 50th	DD; hypotonia; myoclonic choreiform movements; nl EEG	Short attention span	Midface hypoplasia; depressed nasal bridge; low posterior hairline; short neck; hypo-/hyperpigmentation patterns (Fig. 2C–D)	Strabismus	—	—	Mother (nt) with LD
28a	6–17	10 y; M	10–25th, 10–25th, 25–50th	DD; minimal speech; generalized seizure disorder	Autism	Midface hypoplasia; underbite; widely spaced teeth; eversion of upper lip; prominent ears	—	—	Cardiomegaly, resolved (twin-twin transfusion); allergies; anemia	Patients 28a & b are identical twins; father (no del) with ADHD; paternal family history of seizures
28b	6–17	10 y; M	25–50th, 5–10th, 25–50th	DD; single seizure	PDD	Midface hypoplasia; underbite; flattened nose; extra maxillary incisor	—	—	Cardiomyopathy, resolved (twin-twin transfusion); allergies; asthma; constipation	
29	13–17	12 y; M	5th, 70th, 45th	Global DD; IQ 47; hypotonia; abnormal periventricular white matter; tortuous optic nerves	Autism	Frontal hair upsweep; facial hemangioma; flat midface; wide nasal bridge; bulbous nose; small jaw; supernumerary incisor; posteriorly rotated ears; hypoplastic nails (Fig. 2E–G)	Esotropia	Pes planus; tapered fingers	FTT in infancy; heart murmur; echo; laryngeal stenosis; inguinal hernias; asthma	Mother (del) healthy though has short attention span; maternal family history of LD; maternal and paternal family history of neuropsychiatric disease
30	6–8	5 y; F	50–75th, 75–90th, 25–50th	—	PDD; ADD; aggression	Midface hypoplasia; short nose; micrognathia; high palate; smooth philtrum	Blepharo-phimosis	Brachydactyly, especially thumbs; pes planus	Generalized lymphedema; constipation	Mother (nt) with anxiety; paternal cousin (nt) with Asperger
32	6 (6–8)	15 m; F	50th, 97th, >97th	Low-average motor skills; hypotonia	Tantrums	Plagiocephaly; broad forehead; short nose; smooth philtrum; micrognathia; thin upper lip; earlobe creases	Blepharo-phimosis	Overlapping 3rd/4th toes	FTT; diastasis recti	Mother (nt) with LD; father (nt) with ADHD & depression
34a	19–20	15 y; F	62nd, 96th, >98th	Moderate ID; hypotonia; nl head CT	ASD; OCD; ADHD; anxiety; aggression; residential placement	Long prominent nose; small & narrow PF	—	Joint hyper-mobility; pes planus	—	Patients 34a & b are maternal half-sisters
34b	19–20	7 y; F	45th, 36th, >98th	nl intelligence; nl head MRI	ADHD	—	—	Joint hyper-mobility	—	

ADD, attention deficit disorder; ADHD, attention deficit/hyperactivity disorder; ASD, autism spectrum disorder; AV, atrioventricular; CC, corpus callosum; CT, computed tomography; DD, developmental delay; del, deletion; echo, echocardiogram; EEG, electroencephalogram; F, female; FTT, failure to thrive; GERD, gastroesophageal reflux disease; HL, hearing loss; HRH, hypoplastic right heart; ID, intellectual disability; IQ, intelligence quotient; LD, learning disability; M, male; m, months; MRI, magnetic resonance imaging; NA, not applicable; nl, normal; NS, not specified; nt, not tested; OCD, obsessive-compulsive disorder; ODD, oppositional defiant disorder; OFC, occipitofrontal circumference; PDD, pervasive developmental disorder; PDD-NOS, pervasive developmental disorder not otherwise specified; PF, palpebral fissures; SD, speech delay; VUR, vesicoureteral reflux; w, weeks; WNL, within normal limits; y, years; —, feature absent.

TABLE IV. Summary of Features Seen in Individuals with Exonic *NRXN1* Deletions

		α-only deletions			
Phenotypic feature	**α/β deletions[a]**	**Previous reports[b]**	**This study**	**Total**	**%**
Short stature	0/6	4/20	3/23	7/49	14
Tall stature	0/6	0/20	1/23	1/49	2
Underweight/FTT	1/7	2/21	6/23	9/51	18
Overweight	0/7	1/21	2/23	3/51	6
Microcephaly	0/6	1/20	2/22	3/48	6
Macrocephaly	4/6	4/20	2/22	10/48	21
DD/ID	7/9	22/25	20/22	49/56	88
Speech delay	6/8	17/22	17/21	40/51	78
Hypotonia	2/9	12/27	10/23	24/59	41
Hearing loss	1/9	2/28	3/23	6/60	10
Seizures	5/8	8/25	4/23	17/56	30
Brain abnormalities	3/7	4/14	4/6	11/27	41
ASD diagnosis	6/9	9/22	9/21	24/52	46
Autistic features or other behavior problems	9/9	21/25	17/22	47/56	84
Strabismus	0/9	1/26	7/22	8/57	14
Dysmorphic features	4/9	18/26	19/23	41/58	71
Deep-set eyes	1/9	2/26	4/23	7/58	12
Epicanthal folds	1/9	5/26	1/23	7/58	12
Broad/low nasal bridge	0/9	5/26	6/23	11/58	19
Broad/bulbous nose	2/9	3/26	7/23	12/58	21
Midface hypoplasia	1/9	2/26	10/23	13/58	22
Wide mouth	1/9	5/26	0/23	6/58	10
Pointed chin	1/9	3/26	1/23	5/58	9
Ear anomalies	1/9	6/26	8/23	15/58	26
Cardiac defects or dysfunction	1/9	4/28	7/24	12/61	20
Skeletal anomalies	0/9	8/28	6/24	14/61	23
Renal anomalies	0/9	1/28	1/23	2/60	3
Genitourinary anomalies	0/9	2/28	4/23	6/60	10
Asthma or allergies	0/9	1/28	9/23	10/60	17

ASD, autism spectrum disorder; DD, developmental delay; FTT, failure to thrive; ID, intellectual disability.
[a]Patients 34a,b and probands previously reported [Ching et al., 2010; Wisniowiecka-Kowalnik et al., 2010; Schaaf et al., 2012].
[b]Probands previously reported [Zahir et al., 2008; Ching et al., 2010; Wisniowiecka-Kowalnik et al., 2010; Gregor et al., 2011; Schaaf et al., 2012] and mother previously reported [Duong et al., 2012].

history of learning disabilities. Among the patients without parental testing performed, at least six have parents with learning disabilities or psychiatric disease.

Statistical Analysis

A comparison of the frequency of exonic deletions of *NRXN1* in our postnatal patient population undergoing aCGH testing to a sum of control individuals reported in the literature (Table I) shows an enrichment of deletions in our postnatal patient population (29/25,610 [excludes siblings] vs. 12/53,171; one-tailed $P = 6.08 \times 10^{-7}$, Fisher exact test; odds ratio = 5.0 [2.6–9.8, 95% confidence interval]). Given the rarity of *NRXN1*-β deletions, a case/control comparison for these deletions did not show a significant enrichment, despite the fact that no such deletions have been identified in these control cohorts (2/25,610 vs. 0/53,171; one-tailed $P = 0.11$, Fisher exact test).

DISCUSSION

We have identified exonic *NRXN1*-specific deletions in 0.11% of postnatal patients referred to Signature Genomics for aCGH testing. This frequency is significantly enriched when compared to previously reported control cohorts ($P = 6.08 \times 10^{-7}$), providing additional support for the pathogenicity of these deletions. We report detailed phenotypic features for 27 individuals with deletions, adding to the published phenotypic spectrum seen in patients with *NRXN1* deletions (Table III). As would be predicted for a gene that primarily has a role in neurodevelopment and neurologic function, the major phenotypic overlap among all reported cases is neurobehavioral, though this is subject to an ascertainment bias, given that such phenotypes are common reasons for microarray testing. Our data support previous reports that deletions of *NRXN1* are associated with developmental delays, abnormal behaviors, and variable mild dysmorphic features (Table IV, Fig. 2). Given the

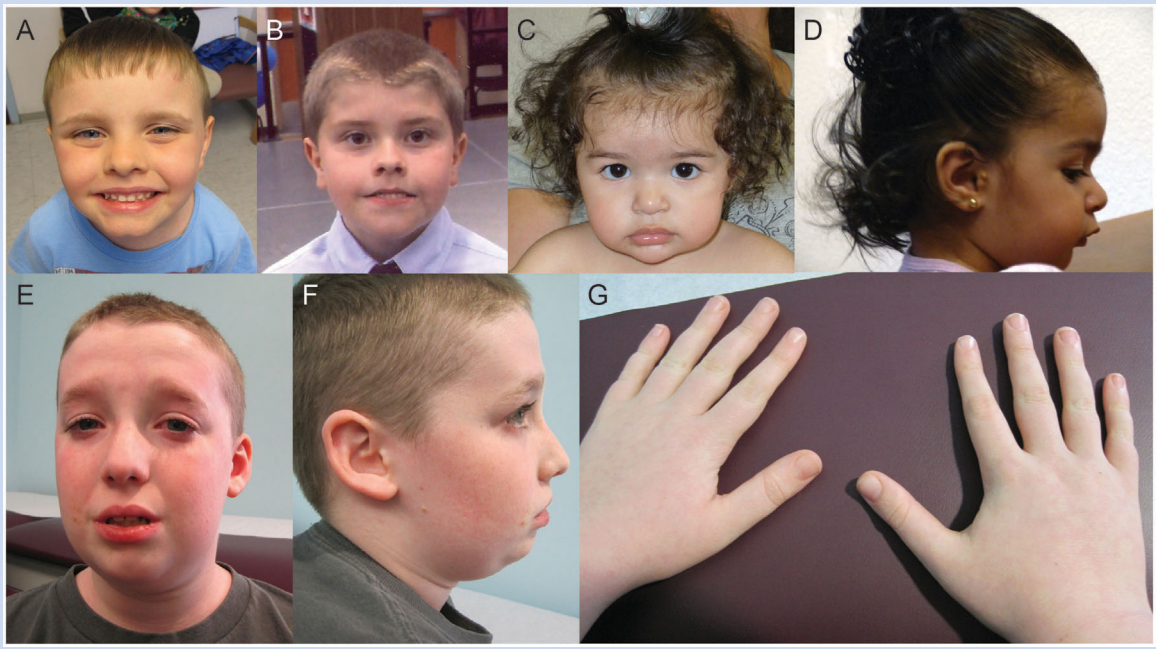

FIG. 2. Features of individuals with *NRXN1* deletions. A: Patient 2 at 5 years. Note broad forehead, deep-set eyes, bulbous nose, depressed columella, and broad philtrum. B: Patient 6 at 11 years. Note mild midface hypoplasia. C,D: Patient 26 at 12 months (C) and 17 months (D). Note depressed nasal bridge, midface hypoplasia, and short neck. E–G: Patient 29 at 12 years. Note frontal hair upsweep, flat midface, wide nasal bridge, bulbous nose, small jaw, posteriorly rotated ears, hypoplastic nails, and tapered fingers.

prevalence of speech delays and abnormal behaviors, it is logical that *NRXN1* has been identified as an autism susceptibility locus [Szatmari et al., 2007; Kim et al., 2008; Bucan et al., 2009; Glessner et al., 2009; Boone et al., 2010; Gai et al., 2012; Hedges et al., 2012], and our data support this association with ten individuals with a formal diagnosis of an ASD. Seizures have been reported in approximately one-third of individuals with *NRXN1* deletions and DD (Table IV); given the presence of epilepsy in individuals with homozygous loss of *NRXN1*-α [Zweier et al., 2009; Harrison et al., 2011; Duong et al., 2012], it is reasonable that haploinsufficiency could predispose to seizures, as well. Despite *NRXN1*'s widespread expression and possible roles in development outside of the nervous system [Saito et al., 2011], congenital anomalies do not appear to be common with *NRXN1* deletions. Of those reported, skeletal and heart anomalies are the most prevalent (Table IV). Skeletal anomalies range from vertebral anomalies in two previously reported individuals [Zahir et al., 2008; Ching et al., 2010] to minor bony changes such as those seen in six of our patients (Patients 1, 6, 17, 18, 21, and 30). Heart defects, both structural and cardiomyopathies, were present in seven of our patients (including two sets of siblings) and in five previously reported cases [Zahir et al., 2008; Ching et al., 2010; Schaaf et al., 2012]. In addition, whereas nine of our patients have asthma and/or allergies, only one other individual with a *NRXN1* deletion has been reported with asthma [Zahir et al., 2008]. Though there has been no other study implicating *NRXN1* deletions with asthma or allergies, some studies suggest a connection between autism and asthma and other inflammatory or autoimmune disorders [Becker, 2007]. Additional studies are required to understand the potential connection between *NRXN1* deletions and congenital anomalies and asthma.

The location of a deletion within *NRXN1* affects how the deletion impairs transcription, translation, and/or protein structure, and this may ultimately affect phenotypic expression. Because we sought to investigate only deletions that would negatively affect *NRXN1* function, we only considered exonic deletions to be likely pathogenic; intronic deletions were excluded from clinical analyses. Intronic deletions may not always be benign, as some studies have shown *NRXN1* intronic sequences to be important to control proper gene splicing [Iijima et al., 2011]. Additionally, there has been one deletion immediately upstream of the gene reported to segregate with ASDs in a family [Ching et al., 2010]. However, this approach of only considering exonic deletions is supported by previous analyses that have found significant enrichment in schizophrenia when considering exonic *NRXN1* copy number variations (CNVs) but not when including intronic ones [Rujescu et al., 2009]. A more recent study found inheritance from unaffected parents was more common with intronic than with exonic deletions [Schaaf et al., 2012]. Also, studies of other genes, such as *SOX5*, provide a precedent for benign intronic variation in a disease-causing gene [Lamb et al., 2012]. Additionally, given the high, and frequently variable, resolution of many arrays used to study published control

cohorts, it was not possible to conduct a meaningful case–control comparison for intronic deletions using our aCGH population; our arrays (especially the earlier versions with less-dense *NRXN1* coverage) do not have the necessary probe density to identify small intronic deletions.

The vast majority of exonic deletions in cases, as well as all deletions in controls, have involved only *NRXN1*-α exons (Fig. 1). This difference is intriguing and could suggest that *NRXN1*-β deletions have more severe phenotypic impacts and possibly a tendency to lethality. Alternatively, there may be genomic instability or an increased recombination rate predisposing the 5′ end of the gene to deletions [Rujescu et al., 2009]. Because of the limited number of cases reported in the literature with *NRXN1*-β deletions, it is currently unclear if these deletions have a more severe phenotypic impact (Fig. 1, Table IV), though multiple generations in a single family have been described with such a deletion [Wisniowiecka-Kowalnik et al., 2010], and one of two siblings carrying such a deletion in our study has attention deficit/hyperactivity disorder, macrocephaly, and normal intelligence. The α- and β-isoforms interact differently with their binding partners at the synapse [Boucard et al., 2005; Chih et al., 2006], so it is feasible that loss of both isoforms would lead to additional loss of protein functions. If any functional redundancy between the various neurexin α- and β-isoforms exists, it is not complete, given the abnormal phenotypes in mice and humans with homozygous loss of *Nrxn1*-α/*NRXN1*-α [Geppert et al., 1998; Etherton et al., 2009; Zweier et al., 2009]. One study has suggested that the deletions that involve *NRXN1*-β exons or *NRXN1*-α exons in the middle of the gene (exons 6–17) are associated with larger head size and more frequent seizures [Schaaf et al., 2012]. Though our sample size is limited, our data provide some support for this; four individuals with deletions including the middle of the gene (Patients 18, 26, 28a, and 28b) are the only ones with histories of seizures or abnormal movements. While the two siblings with deletions of *NRXN1*-β (Patients 34a,b) have macrocephaly, only one of the seven individuals with *NRXN1*-α deletions including the middle of the gene is macrocephalic, while the rest have average or below average head sizes. One possible explanation for this pattern of increased phenotypic severity with deletions in the middle and 3′ end of the gene is that they all disrupt *NRXN1*-β expression, even though the centrally located deletions do not extend to the transcription start site of the β-isoform. A second possible explanation is a dominant negative effect. Deletion of exons 6–17 is predicted to be in-frame. The individuals reported by Schaaf et al. [2012] with epilepsy and deletions within the middle of the gene also had in-frame deletions (Fig. 1), so it is possible that an altered NRXN1-α protein that lacks one or several of its extracellular LNS and EGF domains is produced. However, Patient 18's deletion extends to include the *NRXN1*-α promoter region, which would lead to an absent protein product, not a protein with a dominant negative effect. Given the severity of Patient 18's phenotype, it is also possible that there is a mutation on the second allele or elsewhere in the genome contributing to his disease. It has also been suggested that alternate, minor isoforms of the gene may exert a dominant negative effect [Rujescu et al., 2009]. Further research into these deletions' impacts on gene expression and protein function could help resolve this question.

The variable phenotypes in individuals with *NRXN1* deletions, in addition to the reduced penetrance, pose challenges for counseling about prognosis or recurrence risks, especially when such a deletion is identified prenatally or in a young child. One important consideration when providing counseling is that the individuals reported in the literature likely represent the severe end of a phenotypic spectrum. Some of these individuals may have secondary factors that influence their severe expression, and if a *NRXN1* deletion is found in an individual with severe anomalies, it may be appropriate to look for further etiological explanations or diagnoses [Gregor et al., 2011]. For example, Patient 5a is likely more severely affected than his siblings due to an anoxic brain injury, while in other cases secondary factors could be genetic, including a mutation in the non-deleted allele [Duong et al., 2012]. When trying to counsel about neurodevelopmental disorders, the odds ratios, or relative risks, from population studies may be useful. For example, one study suggested that exonic *NRXN1* deletions or duplications may give an ~10-fold increased risk for schizophrenia, which is less than that for 22q11.21 microdeletions [Rujescu et al., 2009]. The odds ratio calculated from our postnatal patient population is 5.0, with a confidence interval of 2.6–9.8. This number may have minimal utility, given the wide spectrum of phenotypes represented in our patient population. Instead, examining the difference between a sum of ASD cases with *NRXN1* deletions in the literature (15/3,328, 0.45%; Table I) and the reported control cohorts (12/53,141, 0.02%) gives an odds ratio of 20.0 (9.4–42.9, 95% confidence interval, Fisher exact test), suggesting a possible ~20-fold increase in risk for an ASD when an exonic *NRXN1* deletion is present. With current estimates of ~1% for the prevalence of ASD [Fombonne, 2005; CDCP, 2009], this would lead to an estimated ~20% risk for ASD with exonic *NRXN1* deletions, though this estimate could be modified further for counseling by other factors known to have an influence on the development of an ASD, such as family history or sex of the child (i.e., using a starting prevalence of ASD of 1/70 males and 1/315 females [CDCP, 2009] gives a 29% risk for males and a 6% risk for females). Counseling for these deletions should include a description of the phenotypic spectrum, noting that healthy individuals may carry these deletions and phenotypes can vary, even within the same family, like Patients 5a–c or 34a,b described here.

In summary, we provide information on the molecular characterization of *NRXN1* CNVs identified in a large, clinical aCGH testing population as well as detailed phenotypic information for a subset. Combining these data with other reports in the literature shows that the most common features associated with *NRXN1* exonic deletions are developmental delays, especially in speech, abnormal behaviors, and mild dysmorphic features (Table IV). Epilepsy is present in a subset of these individuals, and the location of the deletion may influence this manifestation. Congenital anomalies, such as skeletal anomalies and heart defects, may be present and may be influenced by additional factors, both genetic and environmental. In some individuals, phenotypic manifestations may be subclinical or absent. As we continue to examine the human genome for CNVs on an increasingly finer scale, studies such as these are useful for establishing genotype–phenotype correlations for small, intragenic abnormalities, which will ultimately aid in improved counseling and clinical care.

ACKNOWLEDGMENTS

We thank Allen Lamb (Signature Genomic Laboratories) for his critical input early in the discussion of this manuscript. We also thank Erin Dodge and A. Michelle Caldwell (Signature Genomic Laboratories) for their critical editing, formatting of the manuscript, and assistance with figure creation.

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