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Molecular and Clinical Characterization of 25 Individuals With Exonic Deletions of *NRXN1* and Comprehensive Review of the Literature

Frédérique Béna,[1] Damien L. Bruno,[2,3] Mats Eriksson,[4] Conny van Ravenswaaij-Arts,[5] Zornitza Stark,[2,3] Trijnie Dijkhuizen,[5] Erica Gerkes,[5] Stefania Gimelli,[1] Devika Ganesamoorthy,[2,3] Ann Charlotte Thuresson,[6] Audrey Labalme,[7] Marianne Till,[7] Frédéric Bilan,[8] Laurent Pasquier,[9] Alain Kitzis,[8] Christele Dubourgm,[9] Massimiliano Rossi,[7] Armand Bottani,[1] Maryline Gagnebin,[1] Damien Sanlaville,[7,10] Brigitte Gilbert-Dussardier,[8] Michel Guipponi,[1] Arie van Haeringen,[11] Marjolein Kriek,[11] Claudia Ruivenkamp,[11] Stylianos E. Antonarakis,[1,12] Britt Marie Anderlid,[13,14] Howard R. Slater,[2,3] and Jacqueline Schoumans[14,15]*

[1]Service of Genetic Medicine, Geneva University Hospital, Geneva, Switzerland

[2]Victorian Clinical Genetics Services, Murdoch Childrens Research Institute, Parkville, Victoria, Australia

[3]Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Parkville, Victoria, Australia

[4]Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

[5]Department of Genetics, University of Groningen, University Medical Center Groningen (UMCG), Groningen, The Netherlands

[6]Department of Immunology, Genetics and Pathology, The Rudbeck Laboratory, Uppsala University, Uppsala, Sweden

[7]Service de Cytogénétique Constitutionnelle, Hospices Civils de Lyon, Groupement Hospitalier Est, Lyon, France

[8]Service de Génétique, Centre Hospitalier Universitaire de Poitiers, Poitiers, France;Université de Poitiers, Poitiers, France

[9]Service de Génétique Médicale—Hôpital Sud—CHU Rennes, Rennes, France

[10]INSERM U1028, CNRS UMR5292, Université Claude Bernard Lyon 1, Equipe TIGER, Lyon, France

[11]Center for Human and Clinical Genetics, Leiden University Medical Center (LUMC), Leiden, The Netherlands

[12]Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland

[13]Department of Neuropediatrics, Astrid Lindgren Children's Hospital, Stockholm, Sweden

[14]Department of Molecular Medicine and Surgery, CMM, Karolinska Institutet, Stockholm, Sweden

[15]Department of Medical Genetics, Lausanne University Hospital, Lausanne, Switzerland

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This study aimed to elucidate the observed variable phenotypic expressivity associated with *NRXN1* (Neurexin 1) haploinsufficiency by analyses of the largest cohort of patients with *NRXN1* exonic deletions described to date and by comprehensively reviewing all comparable copy number variants in all disease cohorts that have been published in the peer reviewed literature (30 separate papers in all). Assessment of the clinical details in 25 previously undescribed individuals with *NRXN1* exonic deletions demonstrated recurrent phenotypic features consisting of moderate to severe intellectual disability (91%), severe language delay (81%), autism spectrum disorder (65%), seizures (43%), and hypotonia (38%). These showed considerable overlap with previously reported *NRXN1*-deletion associated phenotypes in terms of both spectrum and frequency. However, we did not find evidence for an association between deletions

Additional supporting information may be found in the online version of this article.

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*Correspondence to:
Jacqueline Schoumans, Department of Medical Genetics, Lausanne University Hospital, 1011 Lausanne, Switzerland.
E-mail: jacqueline.schoumans@unil.ch

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involving the β-isoform of neurexin-1 and increased head size, as was recently published in four cases with a deletion involving the C-terminus of *NRXN1*. We identified additional rare copy number variants in 20% of cases. This study supports a pathogenic role for heterozygous exonic deletions of *NRXN1* in neurodevelopmental disorders. The additional rare copy number variants identified may act as possible phenotypic modifiers as suggested in a recent digenic model of neurodevelopmental disorders.

Key words: **NRXN1; neurexin; exon; deletion; autism; seizures; review**

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INTRODUCTION

Despite wide-spread adoption of genomic microarray analysis in the postnatal diagnostic setting, the frequent finding of novel and rare CNVs, for which there is often no relevant literature available to assist interpretation, continues to pose difficulties for genetic counseling. Added complexity comes from the growing number of pathogenic CNVs that are incompletely penetrant and which have been associated with a wide phenotypic spectrum. Typical of these are rare CNVs comprising genes involved in neurodevelopment, such as *NGLN4X* and *NRXN1*, which have been repeatedly implicated as risk factors for development of various and often diametric neurobehavioral and neuropsychiatric disorders [Menten et al., 2006; Marshall et al., 2008; Malhotra and Sebat, 2012].

In mammals, three neurexin genes *NRXN1* (*OMIM600535*) *NRXN2* (*OMIM600566*), and *NRXN3* (*OMIM600567*) encode a family of highly polymorphic cell surface proteins involved in synapse development and maintenance [Missler and Sudhof, 1998]. Each neurexin gene encodes two major isoforms, a long α-neurexin isoform and a short β-neurexin isoform; each being transcribed from independent promoters located at different positions in the gene. Both transcripts are subject to alternative splicing, thus explaining the observed transcript heterogeneity [Ullrich et al., 1995; Rowen et al., 2002; Gauthier et al., 2011] and resulting variable expression in the brain [Rowen et al., 2002]. Among the three neurexin genes, *NRXN1* is the largest. It contains 24 exons, spanning 1.1 Mb, with the α- and β-isoform promoter sequences located upstream of exon 1 and downstream of exon 17, respectively [Rowen et al., 2002].

Several studies have extensively associated genomic losses involving *NRXN1* with phenotypic abnormality. Heterozygous deletions involving the *NRXN1* promoter and proximal (N-terminal encoding) gene regions have repeatedly been found to confer a high risk of schizophrenia [Consortium IS, 2008; Kirov et al., 2008; Vrijenhoek et al., 2008; Need et al., 2009; Rujescu et al., 2009; Magri et al., 2010; Levinson et al., 2011, 2012; Stewart et al., 2011]. In addition, several groups have implicated *NRXN1* disruption [Kim et al., 2008], point mutations [Marshall et al., 2008; Shah et al., 2010] and genomic deletions in mental retardation (and overlapping neurodevelopmental phenotypes) [Zahir et al., 2008; Guilmatre et al., 2009; Ching et al., 2010; Gregor et al., 2011; Sahoo et al., 2011; Schaaf et al., 2012], autism spectrum disorder (ASD) [Szatmari et al., 2007; Marshall et al., 2008; Bucan et al., 2009; Glessner et al., 2009; Bradley et al., 2010; Wisniowiecka-Kowalnik et al., 2010; Sanders et al., 2011; Hedges et al., 2012; Prasad et al., 2012], epilepsy [Stewart et al., 2011; Moller et al., 2013; Nicholl et al., 2013], Alzheimer's disease [Swaminathan et al., 2011], and other clinical abnormalities such as vertebral anomalies and dysmorphisms [Zahir et al., 2008]. The spectrum of reported mutations and observed phenotypic associations have furthered the suggestion that partial loss of NRXN1α function perturbs normal neurologic development [Zahir et al., 2008]. In support of this are three recent reports describing bi-allelic loss of the α-isoform of *NRXN1* in a sib pair and two unrelated patients manifesting a severe intellectual disability phenotype [Zweier et al., 2009; Harrison et al., 2011; Duong et al., 2012]. The observed phenotype led Zweir et al. and Duong et al. to postulate that bi-allelic loss of NRXN1α function leads to a fully penetrant, severe neurodevelopmental phenotype while the heterozygous deletion likely represents a susceptibility factor for variable cognitive, neurological, and psychiatric disorders. Contradicting this, two recent studies reported 6 and 17 patients, respectively with heterozygous intragenic *NRXN1* deletions with variable sizes, which appear to expand the spectrum of phenotypic severity into that described for bi-allelic defects of *NRXN1* [Gregor et al., 2011; Schaaf et al., 2012].

Defects involving *NRXN2* and *NRXN3* are less frequently described in association with phenotypic abnormalities [Gauthier et al., 2011; Vaags et al., 2012] which may in part be due to the rarity of CNVs involving these genes (ISCA; https://www.iscaconsortium.org/).

In the present study we report 25 previously undescribed patients, who were shown by genome-wide microarray analysis to have a deletion involving exonic sequences of *NRXN1*. These patients showed a variable degree of developmental delay (DD), speech and language problems, intellectual disability (ID), autistic-like features, micro- and macrocephaly, growth retardation, and other mild nonspecific features. All but one of the patients carried a heterozygous deletion; the single remaining patient being compound heterozygous for two independent allelic *NRXN1* deletions. This cohort further extends the spectrum of phenotypes associated with *NRXN1* haploinsufficiency. It also highlights the need to

explore the functional effects of *NRXN1* exonic deletions on the different transcripts of neurexin-1, as well as to further study the interaction of other genetic defects (e.g., oligogenic inheritance) and/or multifactorial factors in modifying phenotypic expression.

MATERIALS AND METHODS

Clinical Cohort

Blood samples for DNA extraction were collected from probands and their parents after informed consent. The primary indication for microarray analysis was developmental delay or intellectual disability. Other common indications included abnormal growth, an ASD or one or more congenital abnormalities. Detailed physical evaluation and assessment of the medical history for each patient with an identified *NRXN1* deletion was carried out. This is a multicenter study and the patients were therefore examined by either a clinical geneticist or a pediatrician or both in each specific center.

Molecular Karyotyping by Microarray Analysis

This cohort of *NRXN1* deletion patients was sourced from multiple centers, using a variety of different microarray platform: nine samples were processed on the 244, 180, or 105k arrays using catalogue or custom designs (Agilent Technologies, Santa Clara, CA); two samples on the 180k custom design array (Oxford Gene Technology Begbroke, Oxfordshire, UK); nine samples on the HumanCytoSNP-12 300k array (Illumina, San Diego, CA); and five samples on the 250k NspI/2.7M arrays (Affymetrix, Santa Clara, CA). Microarray processing was carried out according to the manufacturers' recommendations. Analysis of microarray data were achieved using Nexus Copy Number (BioDiscovery, Hawthorne, CA), Genotyping Console 3.0.2 (Affymetrix), DNA analytics (Agilent Technologies), Cytosure interpret (Oxford Gene Technology Begbroke), or KaryoStudio (Illumina) software.

Confirmation and Parental Testing

Where samples were available, parental testing was carried out to investigate inheritance using a number of different methods. Fluorescence in situ hybridization (FISH) analyses were performed according to standard protocols using RP11-BAC probes. MLPA probes were designed to cover the relevant *NRXN1* exonic sequence (s) according to protocols and guidelines from MRC-Holland, Amsterdam, The Netherlands. Quantitative fluorescent (QF)-PCR was carried out according to standard protocols.

SANGER Sequencing

DNA samples from 23 to 25 patients with an identified *NRXN1* deletion were screened for point mutations by unidirectional direct sequencing of coding exons 2–22 of *NRXN1* (NM004801) and all intronic flanking regions using ABI BigDye Terminator Sequencing Kit v.3 (Life Technologies, Grand Island, NY). The sequencing products were separated by electrophoresis using an automated capillary sequencer (ABI 3730; Life Technologies, Grand Island, NY).

Detailed Summary of Previously Published *NRXN1* Findings

All full-length articles in the PubMed database through January 2013 that discussed CNVs and *NRXN1* were considered. Search terms queried included *NRXN1* and one of the following (using Boolean logic): deletion, intragenic, exon, schizophrenia, bipolar, autism, epilepsy, mental retardation, intellectual disability. Reports published in languages other than English or describing poorly characterized genomic imbalances, whole gene duplications, intronic deletions and duplications, translocations, and "isolated" point mutations were not considered. After removing duplicates and irrelevant papers, a total of 30 distinct papers were formally reviewed and summarized. Note, a considerable proportion of the information collected during the review was sourced from the supplementary information that accompanied the online publication; this information is generally not peer-reviewed.

RESULTS

We describe 25 patients with variable exonic deletions of *NRXN1* in chromosome region 2p16.3. Of these, 24 were heterozygous and one was a compound heterozygote expected to result in bi-allelic *NRXN1* loss of function (case 19). The genomic locations of all identified *NRXN1* deletions is depicted in Figure 1 and further details, including the presence of additional findings of possible or known clinical significance, are given in Tables I and II.

The deletions ranged in size from 0.09 to 1.15 megabases (Mb) and all involved exonic sequences of the *NRXN1* gene. The smallest deletion encompassed 2 exons (exons 17 and 18; case 23), whilst one deletion involved almost the entire *NRXN1* coding sequence (case 1). Parental studies showed six deletions to be de novo, and 10 (eight probands) to be inherited from healthy (two paternal and two maternal) or affected parents (two paternal and four maternal). The case with bi-allelic deletion of *NRXN1* (case 19) showed two inherited, yet independent, partly overlapping deletions with a size of 0.18 and 0.40 Mb (Fig. 2); both of the parental heterozygous deletion carriers were phenotypically healthy. For four cases a paternal sample was not available; in all of these the maternal sample did not show the deletion (cases 4, 14, 15, and 18). For cases 6, 8, 11, 13, and 22 both parental samples were unavailable.

An additional copy number variant of possible or known clinical relevance was identified in five (20%) cases (cases 3, 14, 15, 20, and 23; see Table I for details). Notably, one of these (case 3) was found to carry an unlinked de novo intragenic deletion involving the neurexin-3 gene, *NRXN3*.

Detailed clinical information was available for 23 out of 25 patients and for six of the carrier parents. Limited or no clinical data was obtained for patients 8 and 11. Male patients were overrepresented in this cohort (19 males and 6 females) and the majority of the patients were younger than 25 years of age (22 out of 25). The clinical features of the 23 probands are summarized in Table II. Photos of patients 1, 3, 19, and 21 are shown in Figure 3. Assessment of the clinical details identified the following recurrent phenotypic features: moderate to severe ID (91%, 20/22), language delay (81%, 17/21), ASD (65%, 15/23), seizures (43%, 10/23), and hypotonia (38%, 8/21). Facial dysmorphisms were described in

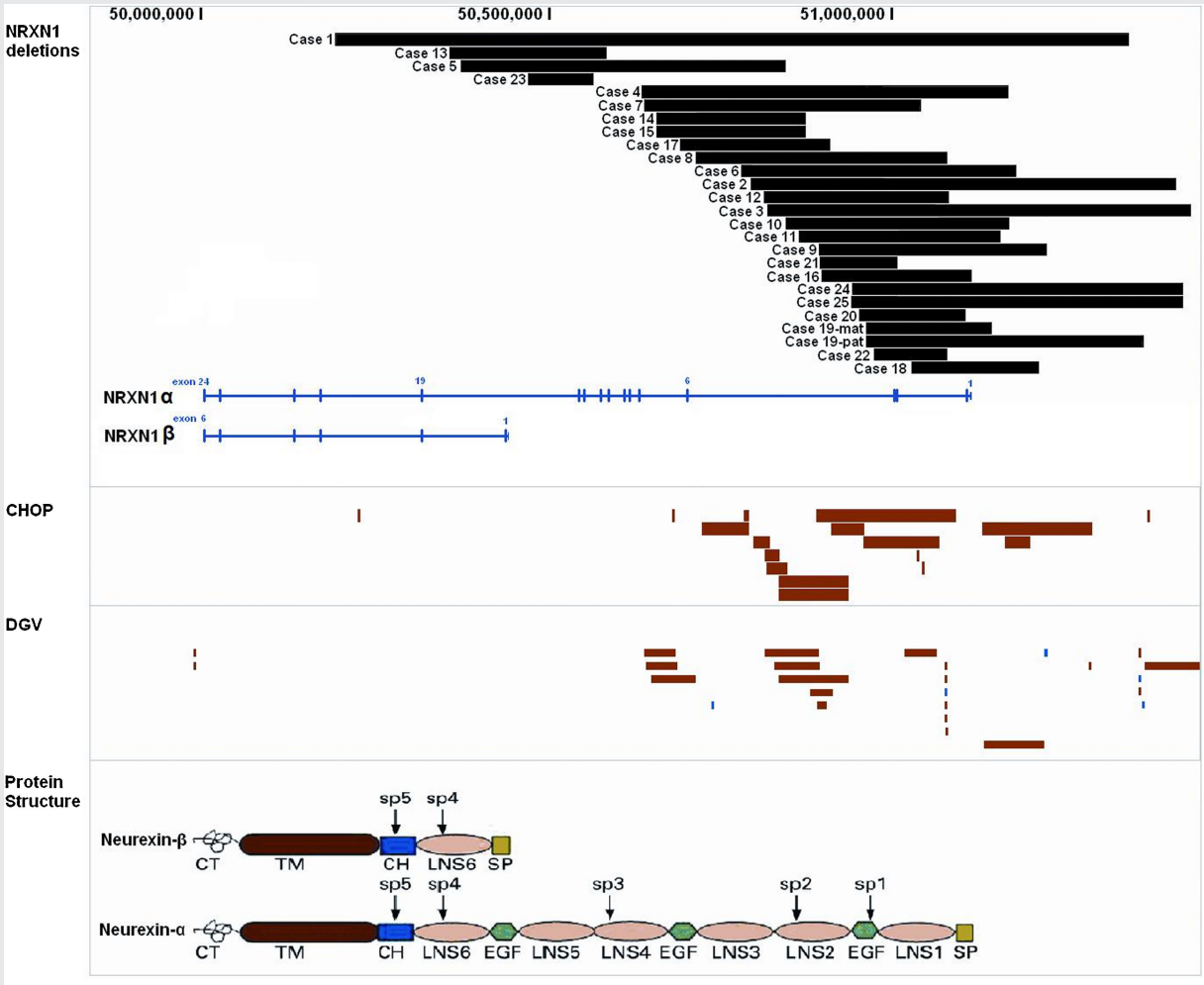

FIG. 1. ***NRXN1*** **exonic deletions identified in 25 individuals (cases 1–25). Upper panel: Black bars indicate the deleted region in each case, with cases ordered according to the start genomic position. Two bars are shown for case 19 with two unique heterozygous deletions inherited from each parent (note, the overlap between these two bars marks the homozygously deleted region in the proband). All breakpoints appear to be unique; there was no evidence of clustering at low copy repeats at the resolution of the microarray analyses. The** ***NRXN1*** **coding region is shown in blue; exons are depicted by vertical lines. Middle panels: CNV data from the Childrens Hospital of Philadelphia (CHOP; deletions are shown in red) and Database of Genomic Variants (DGV; deletions are shown in red and duplications in blue). Lower panel: schemes of the protein structure of the α-Neurexin and β-Neurexin isoforms (CH, carbohydrate binding region; CT cytoplasmic tail; EGF epidermal growth factor-like domains; LNS 1–6 laminin, neurexin, sex hormone binding domains 1–6; SP signal peptide; TM, transmembrane region).**

45% of the cases (10/22) but comparing the facial features did not allow identification of specific recognizable signs. A detailed description of five particularly interesting cases, which serve to highlight the genetic complexities associated with *NRXN1* genotype–phenotype correlation, is included in Supplementary Material.

Previously Published *NRXN1* Findings

A detailed summary of previously published intragenic (exonic) copy number variants in *NRXN1*, in all disease cohorts, is provided in Table III. This review comprises data sourced from 30 peer-reviewed papers and summarizes the details for 119 exonic deletions and 5 exonic (intragenic) duplications that were identified in 123 unrelated individuals. Fifteen of these 30 papers described case–control studies and 12/15 included frequency information for both the case and control cohorts. Of the 124 exonic copy number changes 11 were de novo, 41 were inherited (18 maternal, 12 paternal, 11 not stated) and 19 were of unknown origin. In the remaining 53 cases the origin was not ascertained by the authors or was simply not stated in the report.

Mutational Screening

Twenty-three cases were screened for DNA sequence changes in the coding and flanking intronic regions of *NRXN1*; no pathogenic

TABLE I. Molecular Data for Patients With Exonic Deletions of *NRXN1*

Patient	Genomic location (hg18)	Size (Mb)	NRXN1 region	Isoform of *NRXN1* affected	Array platform	Major phenotypic features	Inheritance	Parental testing	Other finding of possible or known clinical significance[a]
1	50,194,000–51,344,000	1.15	Exons 1–19	α and β	Agilent 244k	Severe ID, ASD	de novo	FISH	None
2	50,797,032–51,412,595	0.61	Exons 1–5	α	Illumina-12–300k	ASD	de novo[b]	MLPA	None
3	50,821,756–51,433,366	0.61	Exons 1–4	α	Agilent 105k	Dysmorphisms, ASD, moderate DD, ID, language delay, seizures	de novo	Agilent 105k and FISH	de novo *NRXN3* deletion (exons 8–12); chr14:78.4–78.7 Mb
4	50,638,683–51,168,031	0.53	Exons 1–9	α	Affymetrix 250k/NspI	DD, hypotonia	Not maternal	Affymetrix 250k/NspI	None
5	50,376,840–50,845,795	0.47	Exons 6–18 (5–17)	α and β	Agilent 180k	ID, seizures, language delay, dysmorphisms	Maternal (affected)	qPCR	None
6	50,783,685–51,180,001	0.40	Exons 1–5	α	Illumina-12–300k	Seizures, ASD, language delay, ID, dysmorphisms	Unknown	Not done	None
7	50,642,229–51,040,803	0.40	Exons 2–9	α	Agilent 180k	Language delay, ASD, ID, DD, dysmorphisms	Maternal (affected)	Agilent 180k	None
8	50,716,777–51,080,338	0.36	Exons 4–5	α	Illumina-12–300k	Osteogenesis imperfecta	Unknown	Not done	None
9	50,894,976–51,223,965	0.33	Exons 1–5	α	Illumina-12–300k	Severe ID, ASD, language delay	Maternal (affected)	Not done	None
10	50,847,740–51,170,975	0.32	Exons 1–5	α	Illumina-12–300k	Language delay, DD, ASD, seizures	de novo[a]	MLPA	None
11	50,867,151–51,157,414	0.29	Exons 1–5	α	Illumina-12–300k	Growth retardation	Unknown	Not done	None
12	50,815,420–51,083,778	0.27	Exons 1–5	α	Affymetrix 250k NspI	Language delay, ASD, hypotonia, DD	Paternal (affected)	MLPA	None
13	50,359,788–50,584,706	0.22	Exons 13–18	α and β	Affymetrix 250k	ID language delay, seizures, hypotonia	Unknown	Not done	None
14	50,658,484–50,874,992	0.22	Exons 4–9	α	Illumina-12–300k	Borderline DD	Not maternal; sibling (case 15) also carries deletion	MLPA	0.9 Mb triplication from 18q23; chr18:72.9–73.7 Mb
15	50,658,484–50,874,992	0.22	Exons 4–9	α	Illumina-12–300k	Borderline DD	Not maternal; sibling (case 14) also carries deletion	MLPA	0.9 Mb triplication from 18q23; chr18:72.9–73.7 Mb
16	50,898,579–51,114,964	0.22	Exons 1–5	α	Affymetrix Cyto-2.7M	Moderate DD, language delay, hypotonia	Maternal	MLPA	None
17	50,693,782–50,909,965	0.22	Exons 6–9	α	Agilent 180k	DD, seizures	de novo	FISH	None
18	51,029,000–51,212,526	0.18	Exons 1–3	α	Agilent 180k	ID, seizures, ASD, DD	Not maternal	Agilent 180k	None
19	50,963,194–51,144,527	0.18	Exons 1–5	α	Agilent 244k	ID, moderate DD, language delay, seizures, ASD, hypotonia	Paternal and maternal each parent carries one of the 2 different deletions	180k OGT custom design and FISH	None
	50,963,194–51,364,465	0.40	Exons 1–5	α					
20	50,953,916–51,105,061	0.15	Exons 4–5	α	OGT custom design 180k	Language delay	de novo	MLPA and FISH	de novo 0.68 Mb deletion at 16p11.2; chr16: 29.5–30.2 Mb
21	50,897,002–51,006,610	0.11	Exons 4–5	α	OGT custom design 180k	Moderate ID, language delay, ASD	Maternal (affected)	OGT Agilent	None
22	50,975,394–51,079,873	0.10	Exons 4–5	α	Agilent 105k	Moderate ID	Unknown	Not done	None
23	50,473,743–50,567,027	0.093	Exons 17–18	α and β	Agilent 105k	ID, language delay, dysmorphisms, seizures, ASD	Paternal	qPCR	Paternally inherited 0.1 Mb deletion in gene *MCC* (5q22.2)
24	50,941,534–51,421,039	0.48	Exons 1–5	α	Illumina-12–300k	ASD, language delay	Paternal (affected)	MLPA	None
25	50,941,534–51,421,039	0.48	Exons 1–5	α	Illumina-12–300k	ASD, language delay	Paternal (affected)	MLPA	None

DD, developmental delay motor; ID, Intellectual disability; ASD, autistic spectrum disorder; *MCC*, Homo sapiens mutated in colorectal cancers (MIM 159350).
[a]A clinically recognized CNV or a rare CNV involving at least one gene which has some evidence in the literature indicating possible relevance to the patient's phenotype.
[b]Apparently de novo (in situ studies not preformed).

TABLE II. Phenotypic Data for Patients With Exonic Deletions of *NRXN1*

Case	BY	Gender	ID	Motor DD	ASD	Language delay	Seizures	Hypotonia	DF	Growth	HC	Hyper activity	Other	Parental phenotype (carrier)
1	2000	M	++	+	+	++	+	+	+	−2.5 SD			Sleep disturbance	
2	2005	M	−	−	+	−	−	−	−	Normal		−		
3	2001	F	++	+	+	++	+	−	+	−2.5 SD	−2.2 SD	+	Angelman like	
4	2008	M	+	+	−	−	−	+	−	Normal	−1 SD			
5	1987	F	+	−	−	+	+	−	+	−1 SD	−1.5 DS	−	Kyphoscoliosis	Learning difficulties, depression
6	2003	M	+	+	+	+	+	+	+	Normal		−	−	
7	1982	M	+	+	+	++	−	NI	+	Normal	Normal	+	Automutilation, aggressive	Moderate ID, possibly autistic-like features
8	1958	M	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	Osteogenesis imperfecta	
9	2003	F	++	−	+	++	−	−	−	Normal		−	−	Anxiety and depression
10	2002	M	+	−	+	+	+	−	+	Normal		−	Periventricular nodular heterotopia, lambdoidsynsostosis, abnormal thumb, micropenis	
11	1996	F	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI		
12	2002	M	NI	+	+	++	−	+	NI	+3 SD			Temporal arachnoidal cyst, celiac	Depression ASD in paternal family
13	1988	M	+	+	−	+	+	+	+	Normal	+2 SD	−	−	
14	2003	M[a]	+	+	−	+	−	−	−	Normal	Normal	−		
15	2002	M[a]	+	+	−	+	−	−	−	Normal	Normal	+		
16	2007	M	+	+	−	+	−	+	+	Normal		−	−	
17	2005	F	+	+	−	NI	+	−	+	Normal		−	Limbstereotypes	
18	1989	M	+	+	+	NI	+	NI	−	Normal		+	Hydrocephalus communicans, hyperacusis, hyperlaxity	
19	2003	F	+	+	+	++	+	+	−	Normal	+1 SD	+		
20	2001	M	−	−	−	++	−	−	−	Normal	Normal	−		
21	2005	M	+	−	+	++	−	−	−	Normal	+1.5 SD	+		ASD
22	1954	M	+	NI	+	−	−	+	−	−3 SD				
23	1998	M	+	+	+	+	+	−	+	Normal		+		
24	2006	M[a]	++	+	+	++	−	−	−	Normal		−	−	Mild autistic features
25	2008	M[a]	+	−	+	−	−	−	−	Normal		−	−	Mild autistic features
			20/22	15/21	15/23	17/21	10/23	8/21	10/22					

BY, birth year; ID, intellectual disability; DD, developmental delay; ASD, autism spectrum disorder; DF, dysmorphic features; HC, head circumference; NI, no information.
[a]siblings.

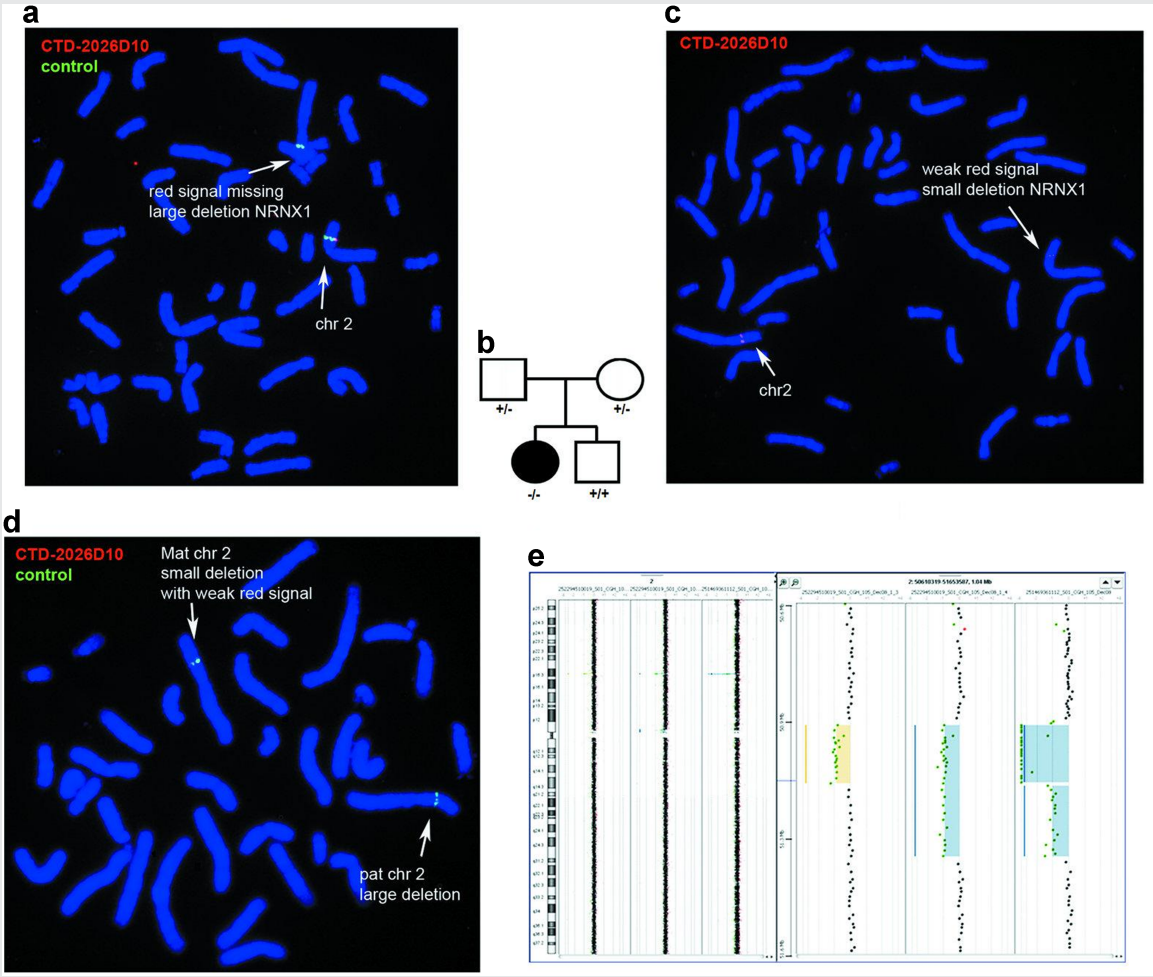

FIG. 2. Confirmation of the compound heterozygous deletion in case 19 by chromosomal 244k Agilent and OGT custom design microarray analysis (e) and parental testing by metaphase FISH (a, c, and d) using clone CTD-2026D10 (red) and a control probe at 2p16.3 (green). Chromosomes 2 are marked with a white arrow. a: Metaphase chromosomes from the father showing absence (i.e., deletion) of the red signal on one of the chromosomes 2. b: Pedigree of the family (normal allele +; deleted allele −). c: Metaphase chromosomes from the mother showing a weak red signal indicating a partial deletion of the probe target region. Note, the control probe has been omitted for better visualization of the weak red signal on the aberrant chromosome. d: Metaphase chromosomes from the proband; one chromosome two has no red signal (inferred as paternal chromosome) and the other chromosome two has a weak red signal (inferred as maternal chromosome). e: Array-CGH profile displaying the 2p16.3 deletions detected in DNA samples from the proband and her parents. A whole-chromosome view is shown in the left panel, whereas the right panel is a zoomed-in view of the *NRXN1* gene region (using DNA analytics software; Agilent technologies). In each panel, the profiles are ordered, mother analyzed with a 180k OGT custom array, father analyzed with a 180k OGT custom array and proband analyzed with a 244k Agilent array (from left to right).

sequence changes were identified in the DNA samples of the 23 investigated patients.

DISCUSSION

This study describes the largest series of patients with exonic deletions of *NRXN1* reported to date, and through detailed molecular and clinical analyses of this and comparable, previously published patient cohorts has helped elucidate the observed incomplete penetrance and variable expressivity associated with *NRXN1* haploinsufficiency.

The majority of patients in this series (24/25) carried a heterozygous deletion involving exclusively part of the coding sequence of the *NRXN1* gene. These exonic deletions are located discretely within the *NRXN1* gene, and appear to cluster at the promoter and first exons of the α-isoform of neurexin-1; a trend that has been reported previously [Ching et al., 2010; Schaaf et al., 2012]. A key question that arises from this observation is whether the particular phenotypic manifestations as well as the phenotypic severity in patients with a *NRXN1* deletion correlate with involvement of the respective isoforms of *NRXN1* (i.e., NRXN1α and NRXN1β). It is therefore noteworthy that NRXN1α is highly diffuse along

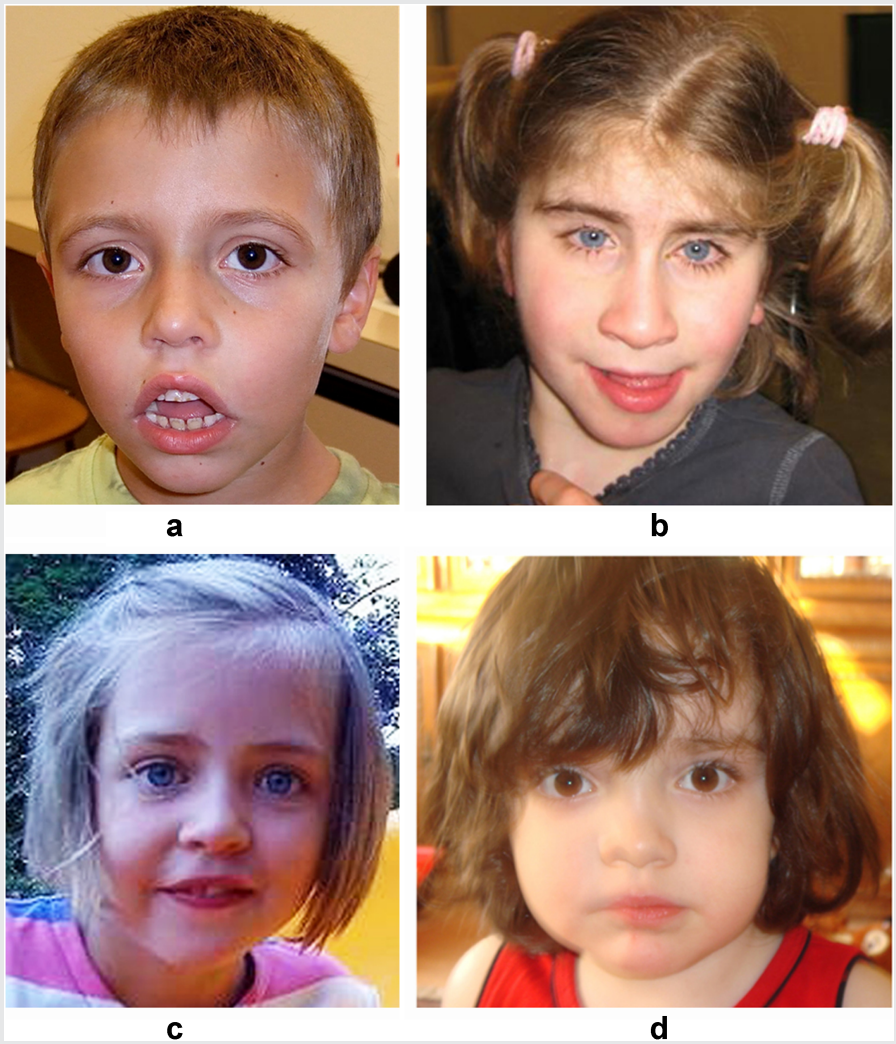

FIG. 3. Photographs of patients 1, 3, 19, and 21.

developing axons, whereas NRXN1β is strictly anchored at terminals through binding to postsynaptic ligands [Fu and Huang, 2010]. Defects that involve NRXN1β appear to be far rarer compared to those involving only NRXN1α; only 15 of the 96 *NRXN1* exonic copy number changes (for which genomic coordinates were accessible) involve both α- and β-isoforms of *NRXN1* (Table III). A single nonsense mutation [Awadalla et al., 2010] and two putative structural missense variants [Feng et al., 2006] that involve NRXN1β have also been described. Within our cohort we identified four patients with a deletion affecting the NRXN1β isoform (cases 1, 5, 13, and 23). The recurrent phenotype in these patients comprised of moderate to severe ID (4/4), language delay (4/4), seizures (4/4), motor developmental delay (3/4), ASD (2/4), and hypotonia (2/4). While the major characteristics of the patients with a heterozygous deletion involving only the NRXN1α isoform included ID (88%, 14/16), language delay (73%, 11/15), ASD (65%, 11/17), motor developmental delay (62%, 10/16), hypotonia (33%, 5/15), and seizures (25%, 4/16). There was no difference in inheritance pattern between the two groups: the deletion of the NRXN1β isoform was inherited once from a normal parent, once from an affected parent and was once shown to be de novo. While the deletion of the NRXN1α isoform was inherited twice from a normal parent, in six cases from an affected parent and was in five cases shown to be de novo.

Schaaf et al. [2012] observed more frequently macrocephaly and seizures in patients with deletions affecting the NRXN1β isoform (Patients E14–E17 in). In the present series, macrocephaly was present in only one (case 13) out of four cases with a deletion affecting the NRXN1β isoform, whereas seizures were present in all four cases. Moreover, macrocephaly did not appear to demarcate deletions involving the α- and β-isoforms of *NRXN1* as macrocephaly was present in one out of 12 cases with an N-terminal deletion as well as in case 19 with the compound heterozygous deletion of *NRXN1*. Thus, this study does not show evidence for an association between deletions involving the β-isoform of neurexin-1 and increased head size.

TABLE III. Detailed Summary of Previously Published *NRXN1* Findings

Study type	Phenotype(s) studied	*NRXN1* variant	*NRXN1* transcript	Frequency of *NRXN1* findings in study	Patient study ID	Sex	Size in kilobases	Genomic coordinates (hg18)	CNV origin	# Cases studied*	# Controls studied*	Frequency of *NRXN1* findings in controls (%)	Secondary CNVs (hg18)	Refs.
Case series	Broad neurodevelopmental and behavioral phenotypes and multiple congenital abnormalities	15 deletions and 1 intragenic duplication	3 α and β (incl. duplication), 12 α only	16/1,150	GC25162, GC26449, GC30019, GC30020, GC36884, GC36885, GC42036, GC43455, GC45066, GC46017, GC49850, GC51317, GC51717, GC52934, GC53505, (GC54622-intragenic duplication)	Not stated	244, 451, 559, 559, 666, 666, 277, 380, 367, 168, 168, 362, 192, 223, 230, 193	chr2:50,835,417–51,079,874, chr2:50,629,193–51,079,873, chr2:50,996,352–51,555,512, chr2:50,996,352–51,555,512, chr2:50,271,164–50,936,974, chr2:50,271,164–50,936,974, chr2:50,890,607–51,167,935, chr2:51,079,673–51,459,762, chr2:50,629,393–50,996,413, chr2:51,048,049–51,215,674, chr2:51,048,049–51,215,674, chr2:50,503,145–50,865,069, chr2:50,763,228–50,954,844, chr2:50,989,149–51,211,695, chr2:51,124,517–51,354,094, chr2:50,179,711–50,372,437 (gain)	4 inherited, 1 de novo, 11 unknown	1,150 individuals referred for CMA	NA	NA	GC26449 had a 1.34 Mb deletion in 13q12.12 and GC45066 had a 2.9 Mb deletion in 3p12.3 and GC46017 had a CNV at 11q14.1 (*DLG2* locus)	Sahoo et al. [2011]
Case series	Broad neurodevelopmental and behavioral phenotypes and multiple congenital abnormalities	1 whole gene deletion and 8 exonic deletions	2 α and β, 7 α only	9/3,540	Patients 1–9	Not stated	5,077, 3,923, 315, 231, 139, 257, 122, 305, 164	chr2:46,938,685–52,015,885, chr2:50,128,256–54,050,713, chr2:50,897,002–51,212,385, chr2:50,936,914–51,167,934, chr2:50,920,082–51,059,469, chr2:51,059,410–51,316,396, chr2:51,090,504–51,212,385, chr2:50,522,892–50,827,767, chr2:50,689,280–50,853,329	5 inherited, 2 de novo, 2 unknown	3,540 individuals referred for CMA	51,939 literature controls (multiple sources)	10/51,939 (0.02)	Not stated	Ching et al. [2010]
Case series	Broad neurodevelopmental and behavioral phenotypes and multiple congenital abnormalities	17 exonic deletions (2 sibling cases)	13 α only, 4(3) α and β	14/8,051 (E2, E8 and E17 excluded)	E1–E17 (E2, E8, and E17 excluded)	11 males and 6 females	207, 319, 111, 89, 161, 346, 233, 290, 913, 134, 376, 376, 7,321,266,161,161	chr2:51,108,945–51,316,396, chr2:51,096,075–51,415,269, chr2:51,056,636–51,167,934, chr2:51,036,654–51,125,770, chr2:51,006,222–51,167,932, chr2:50,821,957–51,167,934, chr2:50,892,598–51,125,770, chr2:51,017,041–51,307,360, chr2:50,753,008–51,666,029, chr2:50,902,643–51,036,715, chr2:50,545,885–50,922,836, chr2:50,545,885–50,922,836, chr2:50,545,885–50,552,890, chr2:50,160,134–50,481,919, chr2:49,999,102–50,265,693, chr2:49,885,294–50,046,403, chr2:49,885,294–50,046,403	7 maternal, 3 paternal, 3 de novo, 1 unknown (E2, E8, and E17 excluded)	NA	NA	NA	Not stated	Schaaf et al. [2012]

(*Continued*)

TABLE III. (*Continued*)

Study type	Phenotype(s) studied	*NRXN1* variant	*NRXN1* transcript	Frequency of *NRXN1* findings in study	Patient study ID	Sex	Size in kilobases	Genomic coordinates (hg18)	CNV origin	# Cases studied*	# Controls studied*	Frequency of *NRXN1* findings in controls (%)	Secondary CNVs (hg18)	Refs.
Case report	Developmental delay, unusual autistic-like behaviors, multiple vertebral anomalies and an unusual facial appearance	1 exonic deletion	α only	NA	NA	M	321	chr2:50,799,281–51,120,644	de novo	NA	NA	NA	Not stated	Zahir et al. [2008]
Case report	Intellectual disability (key phenotypic feature)	6 exonic deletions (no defect in nondeleted allele)	1 α and β (duplication), 5 α only	NA	N1–N6	4 males and 2 females	348, 987, 133, 425, 229, 462	chr2:50,860,393–51,208,000, chr2:50,270,203–51,257,206, chr2:51,011,745–51,144,527, chr2:50,800,974–51,286,171, chr2:50,861,527–51,090,563, chr2:51,033,865–51,496,143	2 maternal, 3 paternal, 1 de novo	NA	NA	NA	N3 had a paternally inherited duplication from 21q22.3 (44,534,530–44,820,473) and a maternally inherited duplication from Xp22.33 (0,000,001–2,710,316); N4 had a maternally inherited deletion in 15q26.1 (88,028,337–88,072,545) and a maternally inherited duplication from 16q12.1 (50,773,658–51,135,179)	Gregor et al. [2011]
Case/control	Mental retardation, ASD, schizophrenia	3 exonic deletions	3 α only	3/743	T35 (autism), 45,431 (autism), 11,695 (mental retardation)	Not stated	427, 729, 427	chr2:51,006,556–51,433,167, chr2:50,704,195–51,433,167, chr2:51,006,556–51,433,167	2 maternal, 1 paternal	743 (total); 247 cases with mental retardation, 260 cases with ASD, 236 cases with schizophrenia	236	0/236 (0)	Not stated	Guilmatre et al. [2009]
Case/control	ASD	2 exonic deletions	Not stated	2/676	78,391, L384	1 male and 1 female	13, 18	Not stated	1 maternal and 1 paternal	676 ASD	1,000	Not stated	1q23.1 (21.7 kb) loss *OR6K2*; 5p15.33 (56.2 kb) gain not in any gene;5q34 (35.3 kb) loss not in any gene;11p12 (16.5 kb) loss not in any gene;12q21.32 (13.6 kb) loss *MGAT4C*;18q12.1 (58.4 kb) loss not in any gene	Prasad et al. [2012]
Case/control	ASD	11 deletions (8 unrelated cases)	Not stated	4/859 and 7(4 unrelated)/1,336 [8/2,195]	ACC: 1211_004, 2367_004, 15062_68740, NIMH_157-1155-001_06C58406A; AGRE: AU041105, AU041106, AU054203, AU1210303, AU1495303, AU1495304	Not stated	Not stated	Not stated	Not stated	859 ASD cases from the ACC cohort & 1,336 ASD cases from the AGRE cohort	2,519 (1,409 ACC discovery controls and 1,110 AGRE replication controls)	0/2,519 (0)	Not stated	Glessner et al. [2009]
Case/control (array only)	ASD	1 exonic deletion	α only	1/168	Not stated	M	70	chr2:50,948,094–51,013,968	Not stated	168 ASD affected	149 (array only)	1/149 (6.7%)	Not stated	Hedges et al. [2012]

(*Continued*)

type	studied	*NRXN1* variant	transcript	study	Patient study ID	Sex	Size in kilobases	(hg18)	CNV origin	studied*	studied*	in controls (%)	Secondary CNVs (hg18)	Refs.
Case/control	ASD	9 exonic deletions	α only (AGRE)	9(5 AGRE and 4 replication cohort)/1,771	AGRE cases: AU1495, AU1210, AU0918, AU0515, AU0411	Not stated	20, 152, 241, 373, 439, 134, 162, 256, 534	Not stated	Not stated	1,771 ASD subjects	2,539 (total)	0/2,539 (0)	Not stated	Bucan et al. [2009]
Case report	ASD	1 exonic deletion and 2 intragenic duplications	2 α and β (duplications, 1 α only	NA	Probands 1–3	1 male and 2 females	390, 194, 331	chr2:50,028,806–50,418,802 (deletion), chr2:50,006,408–50,200,141 (duplication), chr2:50,106,298–50,437,268 (duplication)	2 maternal, 1 unknown	NA	NA	NA	Patient 3 had a duplication from 14q24.2 (72,232,861–72,696,109) which was inherited from the healthy mother	Wisniowiecka-Kowalnik et al. [2010]
Case series	ASD	1 exonic deletion	α only	1/196	AS049	F	355	chr2:50,371,853–50,727,153 (converted)	de novo (paternal gonadal mosaicism)	196 ASD cases from 173 families	NA	NA	Not stated	Szatmari et al. [2007]
Case series	ASD	3 exonic deletions and 1 intragenic duplication	4 α only	4/1,124	14068_1180, 13017_223, 13153_1703, 13037_463	3 males and 1 female	184, 191, 232, 155	chr2:50,493,827–50,677,835 (duplication), chr2:50,539,877–50,730,546, chr2:50,990,306–51,222,043, chr2:51,002,576–51,157,742	4 de novo	1,124 ASD cases	NA	NA	Not stated	Sanders et al. [2011]
Case/control	ASD	1 exonic deletions	α only	1/427	MM0063-003	F	79	chr2:50,722,055–50,801,053 (converted)	Inherited	427 ASD cases	1,652 (total)	0/1,652 (0)	Not stated	Marshall et al. [2008]
Case series	Attention deficit hyperactivity disorder	1 exonic deletion	α only	1/440	153 (DNA ID)	Not stated	185 (approximate)	chr2:50,925,000–51,110,000 (only approximate breakpoints given)	Not stated	440 parent–child trios	NA	NA	Not stated	Bradley et al. [2010]
Case series	Epilepsy (not isolated)	1 exonic deletion (exon 1 of NRXN1beta transcript)	α only	1/247	Case 21	F	66	chr2:50,735,529–50,801,204	Paternal	247 cases with epilepsy and its common co-morbidities referred for CMA	NA	NA	426 kb deletion in 6q22.2	Nicholl et al. [2013]
Case/control	Idiopathic generalized epilepsy	5 exonic deletions	5 α only	5/1,569	L1748, KK2361, 8P1844, 1,696, 247oA	2 males and 3 females	200, 480, 600, 60, 110	chr2:51.03–51.23, chr2:50.83–51.31, chr2:50.93–51.53, chr2:51.00–51.06, chr2:50.90–51.01 (only approximate breakpoints given)	1 maternal (affected), 2 paternal (unaffected), 2 de novo	1,569 patients with IGE	6,201	2/6,201 (0.03)	Not stated	Moller et al. [2013]
Case/control	Schizophrenia	3 large deletions that encompassed the 3′ end of NRXN1	α and β	3/1,013	Not stated	Not stated	200, 260, 420	Not stated	Not stated	1,013 schizophrenia cases	2,462 (total)	Not stated	Not stated	Need et al. [2009]
Case/control	Schizophrenia	1 deletion	α only	1/93	3108-1	F	250 (approximate)	chr2:51.10–51.35 (only approximate breakpoints given)	Maternal	93 individuals with DSM-IV SZ	372	0/372 (0)	Not stated	Kirov et al. [2008]
Case/control	Schizophrenia	6 exonic deletions and 1 intragenic duplication	1 α and β (duplication), 6 α only	7/2,977	Not stated	4 males and 3 females	243, 375, 226, 45, 227, 248, 137 (duplication)	chr2:51,101,161–51,344,213, chr2:50,850,456–51,225,851, chr2:50,890,216–51,116,653, chr2:50,711,199–50,756,435, chr2:51,024,962–51,251,873, chr2:51,002,576–51,250,922, chr2:50,071,499–50,208,992	Not stated	2,977 schizophrenia patients	33,746	5/33,746 (0.01) (exonic deletions only)	Not stated	Rujescu et al. [2009]
Case series	Schizophrenia	1 exonic deletion	α only	1/2,461	AU49-3	Not stated	100 (approximate)	50.7 and 50.8 Mb (only approximate breakpoints given)	Unknown	2,461 individuals from 631 pedigrees	NA	NA	Not stated	Levinson et al. [2012]
Case/control	Schizophrenia	1 exonic deletion	α only	1/172	Not stated	Not stated	328	chr2:50,952,424–51,280,162	Not stated	172 patients with schizophrenia	160	Not stated	Not stated	Magri et al. [2010]
Case/control	Schizophrenia	3 exonic deletions	3 α only	3/3,391	Not stated	Not stated	Not stated (see Supplementary Fig. 2 of publication)	Not stated (see Supplementary Fig. 2 of publication)	Not stated	3,391 patients with schizophrenia	3,181	1/3,181 (0.03)	Not stated	Consortium IS [2008]
Case/control	Schizophrenia	3 exonic deletions	α only	3/806	Patients 2, 7, 9	Male	389, 22, 230	Only provided for Patient 9 (chr2:51,063,670–51,300,517)	Not stated	806 (total) patients with deficit schizophrenia	706	0/706 (0)	Not stated	Vrijenhoek et al. [2008]

(*Continued*)

TABLE III. (*Continued*)

Study type	Phenotype(s) studied	*NRXN1* variant	*NRXN1* transcript	Frequency of *NRXN1* findings in study	Patient study ID	Sex	Size in kilobases	Genomic coordinates (hg18)	CNV origin	# Cases studied*	# Controls studied*	Frequency of *NRXN1* findings in controls (%)	Secondary CNVs (hg18)	Refs.
Case series	Schizophrenia (patients with NRXN1 deletions reported more learning problems and seizures	11 exonic deletions	2 α and β, 9 α only	11/7,556	Not stated	Not stated	419, 441, 1,035, 870, 109, 431, 174, 263, 98, 107, 106	chr2:50,160,749–50,579,961, chr2:50,429,732–50,870,834, chr2:50,444,159–51,479,444, chr2:50,566,914–51,437,353, chr2:50,663,502–50,772,471, chr2:50,838,998–51,269,510, chr2:50,989,423–51,163,646, chr2:51,028,138–51,290,966, chr2:51,042,070–51,140,515, chr2:51,061,058–51,168,210, chr2:51,102,300–51,208,012	Not stated	7,556 (total) subjects with schizophrenia or schizoaffective disorder	NA	NA	Not stated	Levinson et al. [2011]
Case/control	Schizophrenia and idiopathic epilepsy (in combination)	1 exonic deletion	α only	1/235	NIMH ID:147-2403-001	F	429	chr2:50,579,352–51,008,023	Not stated	235 subjects with both schizophrenia and idiopathic epilepsy	191	0/191 (0)	Not stated	Stewart et al. [2011]
Case/control	Late-onset Alzheimer's disease (AD)	4 deletions	Not stated	4/501 (AD participants only)	Not stated	Not stated	Not stated	Not stated	Not stated	501	184	0/184 (0)	Not stated	Swaminathan et al. [2011]
Case series	Not relevant	1 Biallelic loss-of-function deletion and a nonsense point mutation c.2936c > g, p.S979X	α only	1/179	P3	F	113	chr2:51,001,003–51,113,677	Inherited from healthy parent	179 patients	667	0/667 (0)	Not stated	Zweier et al. [2009]
Case report	Not relevant	Compound heterozygous deletion (79 and 287 Kb)	α only	NA	NA	F	79 and 287	chr2:50,214,717–50,293,739 and chr2:51,008,023 –51,294,599	79 Kb maternal; 287 Kb paternal	NA	NA	NA	Paternally inherited 742 kb duplication from 5q35.1 (168,866,009–169,607,847)	Harrison et al. [2011]
Case report	Not relevant	1 Biallelic loss-of-function deletion and a nonsense point mutation c.2880-1G > A, IVS14-1G > A	α only	NA	NA	M	451	chr2:50,666,395–51,117,016 (converted)	Maternal (sub-diagnostic autistic traits)	NA	NA	NA	Not stated	Duong et al. [2012]

ASD was present in 73% of the cases with an N-terminal *NRXN1* deletion in our cohort. This is interesting in light of the finding of putative structural missense variants affecting the N-terminus of *NRXN1* in two autistic individuals [Feng et al., 2006; Kim et al., 2008].

We find significant overlap in phenotypic severity between case 19 with compound heterozygous deletions of *NRXN1* and the four previously reported patients with bi-allelic defects in *NRXN1* [Zweier et al., 2009; Harrison et al., 2011; Duong et al., 2012]. The key phenotype in these cases comprised of moderate to severe DD/ID (5/5) with no speech (5/5), early onset seizures (4/5), gastroesophageal reflux (3/5), obstipation (3/5), and motor developmental delay (5/5). The previous reports of bi-allelic *NRXN1* defects include two sisters with inherited, compound heterozygous deletions [Harrison et al., 2011], a female patient with a heterozygous deletion in *NRXN1* on one allele and a nonsense mutation on the other [Zweier et al., 2009] and a 33-year-old man with a 0.45 Mb deletion on one allele and a point mutation predicted to be deleterious on the other [Duong et al., 2012]. Both Harrison et al. and Zweier et al. conclude that dosage of "defective *NRXN1* alleles" correlates with type and severity of neurodevelopmental and neuropsychiatric phenotypes. Consistent with this notion is the absence of phenotypic abnormality in the carrier parents in three families (including the parents of case 19). However, the extension of the spectrum of phenotypic severity in patients with heterozygous *NRXN1* deletions into that described for bi-allelic defects of *NRXN1* recently by Gregor et al. [2011] challenges this interpretation. Instead, Gregor et al. make the suggestion that the variable expressivity and incomplete penetrance may be explained by the action of additional genetic factors, such as that described recently for recurrent 16p12.1 microdeletions [Girirajan et al., 2010]. In this model, *NRXN1* haploinsufficiency predisposes to neuropsychiatric phenotype(s) but only leads to abnormalities in the presence of one or more additional genetic lesions.

In order to investigate the possibility of an unmasked mutation in the intact *NRXN1* allele, which could help explain the observed clinical complexities in this patient cohort and others [Zweier et al., 2009; Gregor et al., 2011; Schaaf et al., 2012], DNA samples from 23 out of 25 patients were screened by SANGER sequencing. These analyses did not detect any pathogenic sequence changes in the coding sequence or exon–intron boundaries of *NRXN1*.

The frequent finding of inheritance of a "susceptibility CNV" from an apparently phenotypically normal parent, of which there is a burgeoning number described in the literature [Lee and Scherer, 2010; Vassos et al., 2010; Grayton et al., 2012], poses significant issues for interpretation, reporting, and genetic counseling in both the postnatal and prenatal diagnostic settings. The assessment of CNV inheritance status should be considered in light of these clinical complexities and in recognition that this information may be helpful in determining risk estimates for individual "susceptibility CNVs." In this series, parental studies showed six deletions to have arisen de novo and 10 to have been inherited from a carrier parent. Of the carrier parents, four were reported to be phenotypically normal (cases 16, both parents of cases 19 and 23) and five (cases 5, 7, 12, 21, 24, and 25) showed phenotypic features within the associated *NRXN1*-haploinsufficiency spectrum. Three of these parents (mothers of cases 5, 7, and 21) were reported with learning disabilities and autistic features. Both parents of case 12 (paternally inherited deletion) had a history of psychiatric problems and were treated for depression and the father of the siblings, cases 24 and 25, had mild autistic features.

The finding of an additional aberration(s) of possible or known clinical significance in five (20%) cases (cases 3, 14, 15, 20, and 23) prompted consideration of a possible digenic, oligogenic, or multifactorial cause for the expression of phenotypic abnormalities in patients with heterozygous *NRXN1* deletions. Of particular interest was the discovery of two separate de novo, exonic deletions of NRXN1α and NRXN3α in a patient (case 3) with severe ID, no speech and ASD, but no congenital abnormalities. A single report exists in the literature associating *NRXN3* haploinsufficiency with clinical abnormality [Vaags et al., 2012]. Vaags et al. identified four ASD-affected individuals with an exonic deletion in *NRXN3*. Two of the probands inherited their deletion from a phenotypically normal parent, one was inherited from a father with subclinical autism, and one was de novo in the proband. Not surprisingly, the segregation pattern in these families suggests incomplete penetrance and variable expressivity for *NRXN3* deletions analogous to that seen for *NRXN1* deletions. We propose that the effect of additive loss-of-function in this synaptic pathway has contributed to the phenotypic severity in this individual (i.e., case 3).

Case 20, presenting with language delay but otherwise normal development, carried in addition to NRXN1α deletion a pathogenic 16p11.2 deletion (chr16:29,560,500–30,240,082; genomic build hg18). Both deletions were found to be de novo by metaphase FISH analysis of parental samples. Despite the phenotypes associated with each of these pathogenic deletions, the patient demonstrated a relatively mild phenotype with normal cognition and motor development but severe speech delay.

In addition to the deletions in these two cases, which were considered of known or suspected pathogenicity, two further cases showed a CNV of unclear significance (2 siblings with a 860 Kb triplication in 18q23 and a single case with a 100 Kb deletion in 5q22.2) (see Table I). The frequency of additional CNVs in our cohort of patients with *NRXN1* exonic deletions appears to support the hypothesis of digenic and/or multifactorial models for neuropsychiatric disease proposed by Girirajan et al. [2010]. Comparison of the de novo rate of mutation (as determined here and in previous studies [Rees et al., 2011; Schaaf et al., 2012]) and the burden of "second hits" in these patients to those reported for several recurrent "susceptibility CNVs" places *NRXN1* exonic deletions somewhere in the spectrum between 15q13.3 and 16p12.1 deletions [Girirajan et al., 2010]. Collectively, these data suggest that the presence or absence of additional genetic lesions may contribute to variable expressivity and incomplete penetrance in the population of patients with a heterozygous *NRXN1* exonic deletion.

Additional evidence to support the pathogenicity of *NRXN1* deletions comes from comparison of the frequency of deletions in clinical cases vs. controls [Glessner et al., 2009; Cooper et al., 2011; Hedges et al., 2012]. Overall, statistically significant enrichment has been observed for *NRXN1* deletions in ASD-affected versus unaffected individuals [Glessner et al., 2009; Hedges et al., 2012]. Analyses of patient cohorts manifesting broader clinical phenotypes have also found evidence to support the *NRXN1* haploinsufficiency

phenotype [Ching et al., 2010; Cooper et al., 2011; Schaaf et al., 2012].

The summary presented here (Table III) represents the most comprehensive resource of *NRXN1* exonic CNVs published to date. The inclusion of patients with diverse phenotypes spanning disease cohorts is particularly noteworthy. It is evident from this summary that too few studies report the presence/absence of additional CNVs (only six studies made note of presence/absence of any additional CNVs and these were poorly described; Table III). As such, the frequency of additional CNVs in reported cohorts of *NRXN1* cases is almost certainly an underestimate and a comparison with that obtained in the present study would be at best inappropriate. The frequency of de novo events in published cases was approximately 21% (Table III), which is a similar figure to that obtained in the present study (6/16, 38%). The lack of phenotypic information for carrier parents is a serious limitation that prohibits a deeper analysis of both penetrance and segregation patterns in ''*NRXN1* families.'' Several case–control studies have been published and 12 of these provide details of *NRXN1* findings in the control group. Studies describing large numbers of cases and controls have found enrichment of *NRXN1* findings in cases versus controls that have reached statistical significance. However a meta-analysis of the *P* values and confidence intervals is made difficult by the fact that control samples/data are shared across some of the studies and that analyses span multiple experimental techniques, samples, and sample sizes.

The enrichment of *NRXN1* findings in cases versus controls and the phenotype with high recurrence of some features, albeit non-specific like epilepsy and ASD, underscores the clinical relevance of CNVs involving *NRXN1*. They at least should be considered as risk factors for neurodevelopmental disorders, like other CNVs with incomplete penetrance. Nonetheless, counseling of such CNVs remains a challenge, especially when the phenotype is atypical or includes congenital malformations. In such cases the geneticist should remain alert for other or contributing factors that may explain the phenotype. If a *NRXN1* deletion is found during prenatal diagnosis the clinical phenotype cannot be predicted. Unfortunately, a precise estimation of the risk for ID, speech delay, ASD, and/or epilepsy in the fetus is not possible with the hitherto available data. Large series of well-phenotyped controls are necessary for that purpose.

CONCLUSION

In summary, this study describes the largest series of patients with *NRXN1* exonic deletions reported to date. Our data reinforce the recurrent link between developmental, neuropsychiatric, and cognitive phenotypes associated with *NRXN1* haploinsufficiency and suggest that the variability of expressivity of these and other associated phenotypes may be underpinned by functional involvement of different neurexin-1 transcripts and by contribution of additional rare genomic variants.

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