European Journal of Medical Genetics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

NRXN1 deletion syndrome; phenotypic and penetrance data from 34 families

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ARTICLE INFO

Keywords:
NRXN1 Neurexin 1
Copy number variant
2p16.3 microdeletion
Autism spectrum disorder

ABSTRACT

The spectrum of phenotypes associated with heterozygous deletions of neurexin-1 (*NRXN1*) is diverse and includes: autism spectrum disorder, attention deficit hyperactivity disorder, intellectual disability, seizures, schizophrenia, mood disorders and congenital malformations. Reduced penetrance and variable expressivity of deletions in this gene remain a challenge for genetic counselling. We clinically reviewed 67 *NRXN1* deletions from 34 families to document the phenotype and determine odds ratio. Thirty-four probands (5 adults, 29 children (< 16 years)) were initially identified from a cohort clinically referred for arrayCGH. A further 33 *NRXN1* deletions (16 with established phenotype) from the families were identified following cascade screening. Speech and language delay was a consistent clinical presentation. Pedigree analysis of the inherited group revealed numerous untested relatives with a history of mental health and developmental issues, most notably in the *NRXN1β* isoform patients. Our study highlights the complex nature of the *NRXN1* phenotype in this population.

1. Introduction

Neurexins are highly polymorphic presynaptic cell-adhesion molecules that play critical roles in establishing and maintaining synaptic connections (Anderson et al., 2015). They are known to be extensively modified by alternative splicing at transcription level (Anderson et al., 2015). The Neurexin-1 (*NRXN1*: 2p16.3 2:49,918,505-51,225,5755 GRCh38, OMIM 600565) has two independent promoters which generate two classes of messenger RNA (mRNA): the longer mRNA encodes alpha-neurexins and the shorter mRNA encodes beta-neurexins (Ullrich et al., 1995; Tabuchi and Sudhof, 2002). Through complex alternative splicing thousands of isoforms are produced (Ullrich et al., 1995; Tabuchi and Sudhof, 2002; Treutlein et al., 2014). Heterozygous intragenic microdeletions have been described in individuals with autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), intellectual disability (ID), seizures, schizophrenia and bipolar disorder (Ching et al., 2010; Kirov et al., 2014; Bena et al., 2013; Schaaf et al., 2012; Vinas-Jornet et al., 2014). Collectively the frequency of exonic deletions of *NRXN1* has been reported as 0.019% in control populations (Ching et al., 2010). Microdeletions of *NRXN1* can also be inherited from apparently healthy parents (Dabell et al., 2013). It is hypothesised that other polygenic, epigenetic or multifactorial effects play a role in the penetrance and expression of *NRXN1(7)*. A second hit mechanism has been proposed in other copy number variant (CNV) related disorders (Centanni et al., 2015; Hashemi et al., 2015; Girirajan et al., 2010). Limitations in our understanding of the clinical outcome, particularly neurodevelopmental and neuropsychiatric outcomes associated with *NRXN1* deletions pose genetic counselling challenges, particularly in the prediction of a possible neuropsychiatric phenotype. Previous studies have attempted to estimate penetrance of a number of

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https://doi.org/10.1016/j.ejmg.2018.07.015
Received 30 January 2018; Received in revised form 24 June 2018; Accepted 17 July 2018

similar copy number variants (CNVs) including *NRXN1* (Kirov et al., 2014; Rosenfeld et al., 2013; Vassos et al., 2010). Using different methodologies penetrance has been calculated to be between 10.4 and 62.4% for different CNVs, thus further highlighting the importance of disease or CNV specific penetrance calculations to aid in genetic counselling(14). Herein, we provide comprehensive information on 67 individuals with *NRXN1* deletions from 34 families and provide estimate of penetrance.

2. Materials and methods

Cases were identified from January 2014 to December 2015 by database review within our single national genetic referral centre. Additional cases whose deletion was not reported in the national genetic service were identified through the Trinity College Dublin (TCD) Autism and Neurodevelopmental Disorders research group. Probands had a whole genome arrayCGH performed in either the Department of Clinical Genetics Laboratory in Dublin or ViaPath laboratory in London, United Kingdom. Genomic coordinates are given using the Genome Reference Consortium February 2009 build of the human genome (GRCh37/hg 19). All results were validated in an accredited diagnostic laboratory. Indication for arrayCGH testing included neurodevelopmental delay, autism or congenital anomalies.

Patients were examined and detailed phenotypic information was gathered. Psychiatric diagnoses in adult patients were based on self-reported known clinical diagnoses. A comprehensive family history was also ascertained. This data was collected in a proforma along with other genetic testing results, magnetic resonance imaging (MRI) of the brain and electroencephalogram (EEG) reports. Due to the broad spectrum of phenotypes, additional genetic tests were undertaken as part of the work up of developmental delay (DD) in most patients prior to the diagnosis, a summary of which is shown in supplemental data.

First degree relatives of those with a deletion were offered cascade arrayCGH screening as were first degree relatives of those who were found to carry the deletion following initial cascade screening. Ascertainment of cases is described in Fig. 1. An odds ratio was calculated from the cascade screening using a 2 × 2 contingency table (http://vassarstats.net/odds2x2.html).

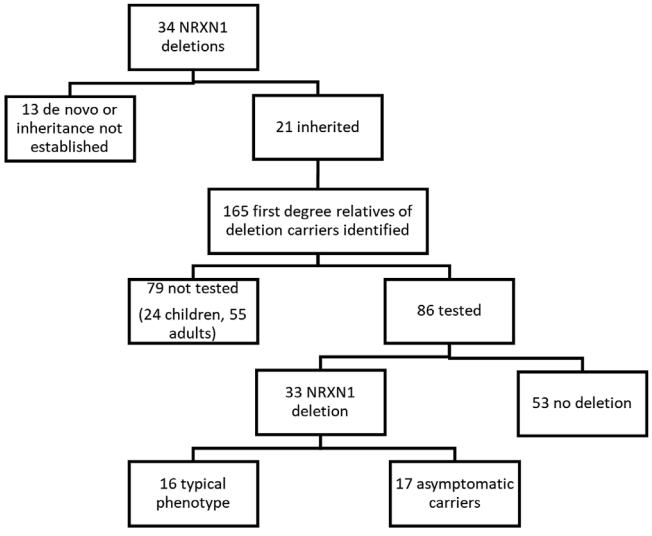

Fig. 1. Flowchart of case ascertainment. 34 cases originally identified with 165 relatives identified for cascade screening, 86 of whom were tested. Of those, 33 were identified as having *NRXN1* deletion, 16 with a typical phenotype. Typical phenotype was defined as psychiatric disorders, neurodevelopmental disorders, intellectual disability, epilepsy. 53 had normal arrayCGH with 5 of these having typical symptoms giving and OR of 0.11.

3. Results

Thirty-four probands were identified. Of those, 21 were inherited, 11 paternally and 10 maternally. Detailed phenotype information and genomic data is presented in Tables 1 and 2 and Fig. 2. Table 1 is a breakdown of diagnoses and demographic data of each patient. Table 2 is a breakdown of intronic vs extronic genotype-phenotype correlation. Examples of complex pedigrees are shown in Fig. 3. The majority (29/34) of probands had array CGH performed due to DD. The remaining 5/34 presented initially with a congenital malformation (CM). However, DD was documented in 4/5 later in childhood, the delay not being obvious as infants.

Between the period of January 2014 and December 2015, 2954 diagnostic array CGH tests were processed in the Department of Clinical Genetics laboratory for various clinical reasons with 17 deletions containing the *NRXN1* gene identified (17/34 excluded due to detection at an outside laboratory). This gives a frequency, in this clinically referred population, of 0.57%.

3.1. Cascade screening

From the 21 inherited cases, 71 first degree relatives were identified for cascade screening (Fig. 1). Of those relatives that had screening arrayCGH performed, 24 were identified as carrying a *NRXN1* deletion, 13 had a typical phenotype. Additional cascade screening was offered to first degree relatives of those individuals who tested positive (94) in the initial round of screening (total of 162 offered cascade screening). Nine further family members were identified as carrying deletions bringing the total number identified via cascade screening to 33.

Twenty-four healthy children and 55 adults who were identified for screening did not proceed. Twenty of the adults who declined testing (36%) were reported to have mental health or psychiatric issues. The uptake of cascade screening, after removing the untested 24 healthy children from the calculation was 86/141 (60.9%). The odds ratio comparing symptomatic cases of NRXN1 deletion carriers to symptomatic cases without NRXN1 deletions is 0.11 [0.03–0.34, CI 95%].

3.2. NRXN1β isoform deletion

Within our cohort we identified six patients with a deletion affecting the *NRXN1β* isoform (cases 17,21,28,29,30,31). This isoform is of interest in the context of neurodevelopmental disorders as it primarily binds to neuroligin, a key binding partner implicated in synaptic specification, maturation and synaptic plasticity, that are critical processes in neurodevelopment (Reissner et al., 2013). Rare loss of function mutations in neuroligins have been reported in ASD (Jamain et al., 2003). The phenotypes in patients with *NRXN1β* deletions included mild to moderate ID (5/6), language delay (5/6), seizures (1/6), and ASD (4/6). Two were *de novo* and three were inherited from parents with self-reported mental health issues and in one case heritability could not be determined.

4. Discussion

In this report we have described 34 cases of *NRXN1* deletions identified from a cohort of patients referred as part of a routine assessment for developmental delay with a further 33 identified from cascade screening. The variable expressivity and incomplete penetrance of *NRXN1* deletions that we and others have observed represent a challenge for providing genetic counselling to patients and families who are carriers of the deletion. Penetrance rates are difficult to accurately estimate due to poor uptake of cascade. It was not possible to ascertain well operationalised diagnoses for the mental disorders in the carriers and the relatives identified for screening who refused testing. These CNVs (and others that impact brain disorders) are increasingly recognised as conferring risks for neuropsychiatric disorders but have not

Table 1
Phenotype/Genotype correlation in *NRXN1* deletions in originally identified probands.

ID	Age Gender	2p16.3 Start-stop (hg19)	Size (kb)	α/β isoform Intronic	Inheritance	ASD	Learning Disability	Speech and Language Delay	Seizures	Others
1	10y M	50,138,031–50,214,776	77	α/β	NA	+	+	+	–	ADHD Hallucinations
2	8y4m F	50,463,521–50,505,665	42	α/β	*De novo*	+ +	+ +	+	–	
3	4y M	50,483,652–50,495,891	12	Intronic	Mat First cousin patient 4, 5	+	+	+	–	
4	13y M	50,483,652–50,495,891	12	Intronic	Mat First cousin patient 3, 5	+	+ +	+ +	–	Pectus excavatum ADHD
5	2y F	50,483,652–50,495,891	12	Intronic	Mat First cousin patient 3, 4	–	+	+	–	Gross motor delay
6	11y4m M	50,566,968–50,897,061	330	α/β	*De novo*	–	+	+	+	Anal stenosis Hypotonia
7	24y F	50,690,984–50,870,064	118	α	*De novo*	+ +	+ + +	+ + +	+	
8	5y10m M	50,709,538–50,760,000	50	α	*De novo*	NA	+	+	–	–
9	5y4m M	50,881,995–50,947,729	61	Intronic	Mat (aff)	+	+ +	+	+	
10	2y9m F	50,881,995–50,999,825	117	Intronic	Pat	–	+ +	+ +	+	Sensorineural hearing loss
11	11y3m F	50,937,444–51,166,725	230	α	Mat	–	+	+	+	Hypotonia
12	7y8m M	50,947,670–50,964,907	17	Intronic	NA	+	+ +	+	–	
13	1y6m F	50,957,455–51,021,511	64	Intronic	Mat	NR	NR	+	–	Tetralogy of Fallot
14	11y8m M	50,957,455–51,251,557	294	Intronic	Pat (aff)	+ +	+ + +	+ +	–	
15	4 y M	50,964,848–51,212,338	247	α	Pat	NA	NA	NA	NA	IUGR Right aortic arch Dysplastic ear
16	9y7m M	50,964,848–51,251,557	287	α	*De novo*	+	+	+	–	Pyloric stenosis Hypotonia
17	5y M	50,968,453–51,043,557	75	Intronic	Pat	NA	+	+ +	–	
18	7y F	50,968,453–51,260,612	292	Intronic	Mat	+	+	+	–	SNHL
19	18y F	50,982,113–51,446,873	465	α	Pat (aff)	+	+ +	+	–	Psychosis
20	1m F	51,013,626–51,066,637	53	Intronic	NA	–	–	–	+	Low set ears Contractures Ventriculomegaly
21	13y M	51,057,824–51,142,908	85	Intronic	Mat	+ +	+ + +	+ +	–	
22	1y8m M	51,066,578–51,100,471	34	Intronic	Mat	NR	NR		–	Microcephaly
23	6y F	51,083,410–51,172,182	89	α	Pat	+	+	+ +	–	Short stature
24	1 y M	51,083,410–51,201,469	118	α	Mat	NR	NR		–	Aortic stenosis Cleft Lip and Palate
25	8y10m M	51,098,578–51,114,116	15	Intronic	Pat	–	+	+	–	
26	8y1m F	51,109,690–51,314,430	205	α	*De novo*	–	+ +	+	–	
27	20y M	51,122,091–51,314,430	192	α	*De novo*	+ +	–	+		
28	7y10m F	51,122,091–51,382,872	261	α	Pat	+	+ +	+	–	
29	4y3m M	51,122,091–51,606,257	484	α	Pat (affected)	+	+	+	–	
30	20y M	51,137,071–51,314,430	177	α	De novo	+ +	+ +	+	–	Wilm's Tumour
31	21y M	51,148,508–51,251,557	103	α	De novo	+	+ +	+ +	+	
32	7y10m M	51,153,052–51,260,612	108	α	NA	+	+ + +	+ + +	–	
33	3 y F	51,212,279–51,237,059	25	Intronic	Pat	NA	–	–	–	Tetralogy of Fallot

(*continued on next page*)

Table 1 (*continued*)

ID	Age Gender	2p16.3 Start-stop (hg19)	Size (kb)	α/β isoform Intronic	Inheritance	ASD	Learning Disability	Speech and Language Delay	Seizures	Others
34	4y M	51,237,000–51,260,612	24	α	De novo	+	+	+	–	

Y = years, Pat = paternally inherited, Mat = maternally inherited, NA = Not available, + = present, + + = marked - = absent. SNHL = sensori-neural hearing loss.

Table 2
Further Genotype/Phenotype data in the originally identified probands.

Phenotype (%)	Exonic deletions %(n = 23)	Intronic deletions %(n = 11)
Global delay (83%)	83%(19/23)	81% (9/11)
Speech delay (94%)	95% (22/23) 1 NA	90% (10/11) 1 NA
Intellectual disability (80%)	87% (21/23)	63% (7/11)
Mild	6	3
Moderate	8	4
Severe	7	–
Autistic spectrum disorder (60%)	4 NA 70% (16/23)	5 NA 36% (4/11)
Seizures (17%)	25% (5/23)	–
Head circumference	21% (4/23)	45% (5/11)
Normal	16% (2/23)	–
> 90th centile < 2nd centile	16% (2/23)	18% (2/11)
Congenital Heart Defect 15%	4% (1/23) Aortic stenosis 4% (1/23) Patent ductus arteriosus 4% (1/23) Right aortic arch	18% (2/11) Tetralogy of Fallot
Other birth defects	8% (2/23)sensorineural hearing loss 1 Cleft Lip and Palate	

E = Exon, IN = Intron, Mat = maternally inherited, Pat = paternally inherited, NA = Not available, ASD = autism spectrum disorder.

been well studied to date. Our estimate of penetrance is 46% but it may be an underestimate or indeed an overestimate. A more accurate estimate is that of odds ratio which we calculated to be 0.11.

Schaaf et al. as well as Dabell et al. previously reported heterozygous intragenic *NRXN1* deletions in ID, DD and seizure cohorts (Schaaf et al., 2012; Dabell et al., 2013). Enrichment among adult schizophrenia cohorts was studied by Kirov and colleagues and Vassos and colleagues (Kirov et al., 2014; Vassos et al., 2010). A higher frequency of 0.60% of all *NRXN1* deletion CNVs was observed in our clinically referred population compared to 0.028% reported frequency among cases with developmental delay, ASD or congenital malformation. This could be attributed to smaller sample size in our cohort of clinically referred cases (~3000 cases) and most deletions cluster at the promoter and first exons of the alpha-isoform of neurexin-1; a trend that has been reported previously (Ching et al., 2010; Schaaf et al., 2012).

Two further limitations to this study included younger age of the index cases and relatively small cohort size. The majority of index cases were children and therefore it was not appropriate to calculate prevalence or penetrance for adult onset neuropsychiatric conditions, e.g. psychosis or mood disorders which typically present in the third or fourth decades. Because of the lack of data on the frequency of the deletion in each phenotype and the complex nature of such conditions it is difficult to estimate penetrance accurately.

Among the 86 relatives tested, five of eight siblings with LD and ASD had a normal genomic arrayCGH. This supports the recent exome

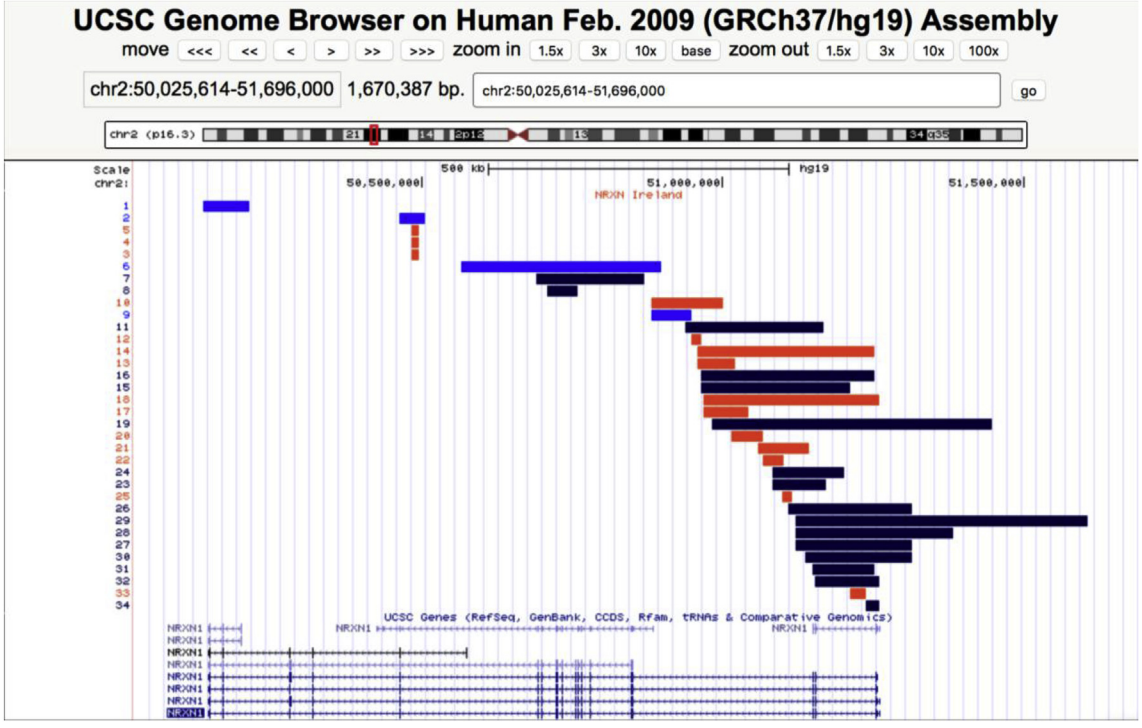

Fig. 2. Genomic co-ordinates of cases on USCS. Blue represents the α/β isoform, navy represents the α isoform and red represents the intronic isoform. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

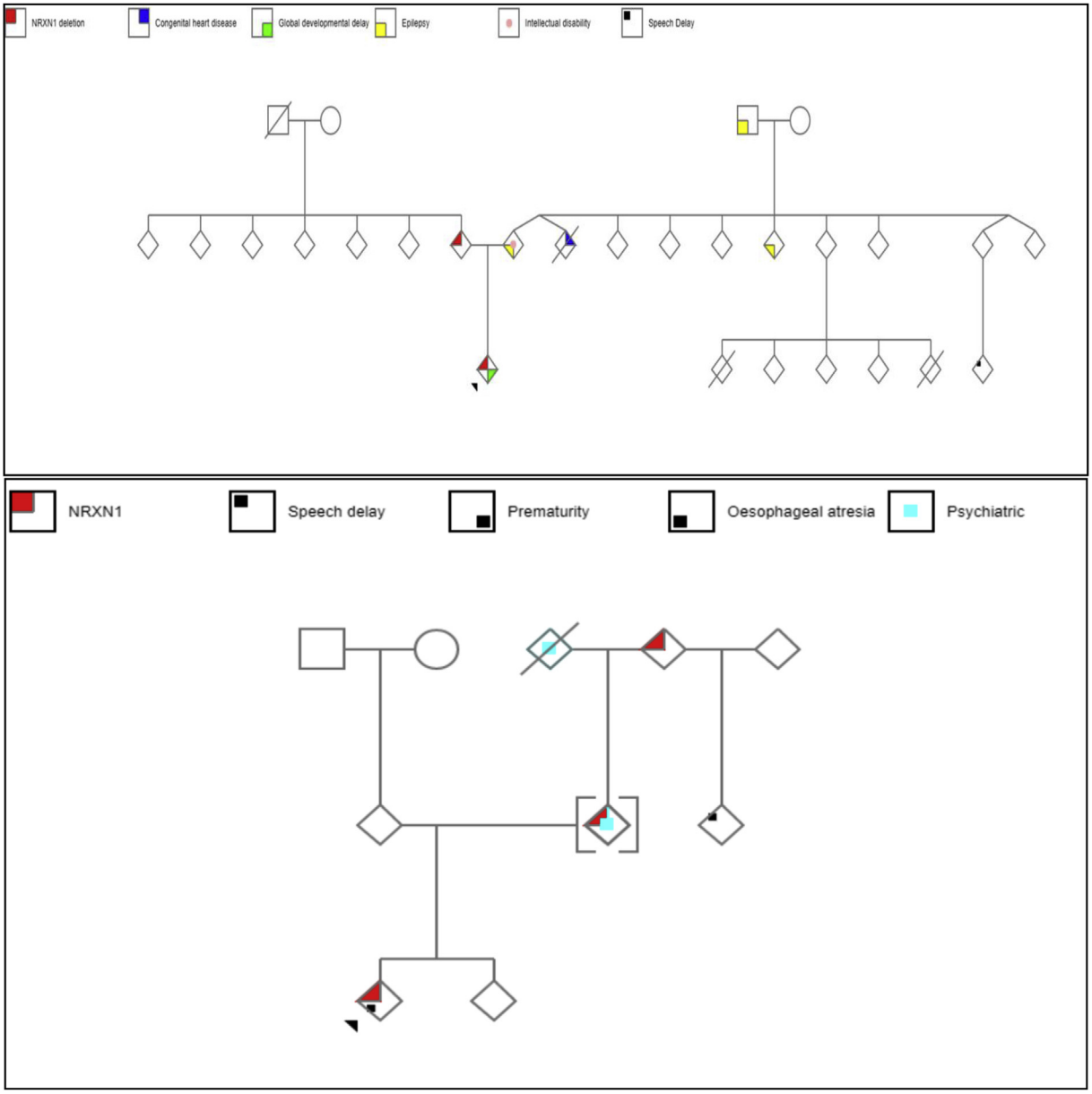

Fig. 3. Pedegree examples of complex family histories typical of the families enrolled in this study. Shown here are families 9 and 10.

sequencing data confirming heterogeneity of ASD where 70% of affected siblings with ASD have different genetic mechanisms underlying their autism (Ching et al., 2010). Next generation sequencing (whole exome and whole genome sequencing) is now being considered to identify rare single nucleotide polymorphisms in candidate ASD genes (Sener et al., 2016).

Counselling for *NRXN1* deletions remains challenging due to heterogeneous phenotypic presentations and associations with adult onset phenotypes. Estimates provided here are helpful in providing further data on penetrance in *NRXN1* deletions. Genetic counselling is important in a number of clinical situations, most importantly for parents with one affected child who wish to make further reproductive choices. However, increasingly antenatal array testing is being implemented in some countries and therefore accurate data is required in order to make informed decisions.

Larger population based and clinical cohort studies are required to extend our understanding of the impact of *NRXN1* deletions on clinical outcome. Population based studies are required to provide accurate estimates of *NRXN1* deletions in the general population. In depth clinical studies that fully evaluate the clinical impact of *NRXN1* deletions, with an emphasis on developmental and neuropsychiatric outcomes are required to provide more reliable data from which to accurately estimate penetrance. For adult onset disorders such as psychosis and schizophrenia, longitudinal follow up studies will be required to estimate these outcomes for patients referred in childhood. Many patients will access information regarding *NRXN1* deletions through online resources and will note that adverse outcomes such as psychosis and severe mood disorders are associated with carrier status. This may have a negative impact and create anxiety about future risks which could be alleviated if more accurate estimates of risk were available.

5. Conclusion

In this paper we have highlighted the significant genetic heterogeneity in *NRXN1* deletions, the complexity of penetrance of deletions in *NRXN1* and the difficult in genetic counselling of these families. The true impact of all the reported deletions on clinical outcome will also require longitudinal studies to identify and quantify the percentage of

deletion cases that present with phenotypes later in life and further genetic and molecular characterisation to interrogate genotype-phenotype interactions. This study adds to the growing literature regarding the role of *NRXN1* deletions in risk for developmental and neuropsychiatric outcomes.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmg.2018.07.015.

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