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Title:
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Date:
2018-12-01

Citation:
Brignell, A., St John, M., Boys, A., Bruce, A., Dinale, C., Pigdon, L., Hildebrand, M. S., Amor, D. J. & Morgan, A. T. (2018). Characterization of speech and language phenotype in children with NRXN1 deletions. American Journal of Medical Genetics Part B Neuropsychiatric Genetics, 177 (8), pp.700-708. https://doi.org/10.1002/ajmg.b.32664.

Persistent Link:
https://hdl.handle.net/11343/284665

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Title page

Characterisation of speech and language phenotype in children with *NRXN1* deletions

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ajmg.b.32664

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Short title: Speech and language in *NRXN1* deletions

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Abstract

Neurexin 1 gene (*NRXN1*) deletions are associated with several neurodevelopmental disorders. Communication difficulties have been reported, yet no study has examined specific speech and language features of individuals with *NRXN1* deletions. Here we characterised speech and language phenotypes in 21 children (14 families), aged 1.8 to 17 years, with *NRXN1* deletions. Deletions ranged from 74 to 702 kilobases and consisted mostly of either of exons 1-3 or 1-5. Speech sound disorders were frequent (69%), although few were severe. The majority (57%) of children had difficulty with receptive and/or expressive language, although no homogeneous profiles of deficit were seen across semantic, morphological or grammatical systems. Social language difficulties were seen in over half the sample (53%). All but two individuals with language difficulties also had intellectual disability/developmental delay. Overall, while speech and language difficulties were common, there was substantial heterogeneity in the severity and type of difficulties observed and no striking communication phenotype was seen. Rather the speech and language deficits are likely part of broader concomitant neurodevelopmental profiles (e.g., intellectual disability, social skill deficits). Nevertheless, given the high rate of affectedness, it is important speech/language development is assessed so interventions can be applied during childhood in a targeted and timely manner.

Key words: *NRXN1*, neurexin, speech, language, phenotype, oral-motor, deletion

Introduction

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The neurexins are a family of cell adhesion molecules that connect pre- and postsynaptic neurons at synapses. Neurexin proteins are anchored to the presynaptic membrane, from where they reach into the synaptic cleft and link with their postsynaptic counterparts, the neuroligins. Formation of this neurexin/neuroligin complex is a key step in the formation of the synapse (Reissner, Runkel, & Missler, 2013). Of the three human neurexins, the most extensively studied is neurexin 1, encoded by the *NRXN1* gene on chromosome 2p16.3 3 and existing in many different isoforms (Missler et al., 2003). *NRXN1* plays an important role in neurodevelopment including learning and cognition (Malhotra & Sebat, 2012; Menten et al., 2006). The *NRXN1* gene has two predominant isoforms (*NRXN1-α* and *NRXN1-β*). Both isoforms are highly expressed during foetal cortical development and in one study expression was highest in the prefrontal cortex region (Jenkins et al., 2016). The same study found an increase in expression levels with gestational age, followed by a peak at birth, significant decrease after 3 years of age and stabilisation of expression levels throughout adulthood. The *NRXN1-α* isoform had higher expression in the brains of individuals with bipolar disorder relative to controls and the *NRXN1-β* isoform was found to be more elevated in individuals with schizophrenia compared to controls (Jenkins et al., 2016). The pre-frontal cortex is also a core region for speech and language.

Heterozygous exonic deletions of *NRXN1* have been associated with a range of neurodevelopmental and neuropsychiatric phenotypes (Bucan et al., 2009; Ching et al., 2010; Curran, Ahn, Grayton, Collier, & Ogilvie, 2013; Gregor et al., 2011; Onay

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et al., 2016; Rujescu et al., 2009; Vinas-Jornet et al., 2014; Wang & Gong, 2018; Zweier, 2012) and are found in about 1 in 500 samples referred for clinical microarray analysis. However *NRXN1* deletions are frequently inherited from a mildly affected or clinically unaffected parent, and are also found in control populations at a frequency of about 1 in 5000, indicating incomplete penetrance (Ching et al., 2010; Lowther et al., 2017; Schaaf et al., 2012). In a clinically ascertained population with *NRXN1* deletions, intellectual disability is reported to affect the majority (77-92%; Bena et al., 2013; Dabell et al., 2013; Schaaf et al., 2012) with one study reporting 91% of individuals had moderate to severe intellectual disability (Bena et al., 2013). Other commonly co-occurring conditions include autism spectrum disorder (43-65%; Bena et al., 2013; Dabell et al., 2013; Gonzalez-Mantilla, Moreno-De-Luca, Ledbetter, & Martin, 2016; Schaaf et al., 2012), attention deficit hyperactivity disorder (9-41%; Lowther et al., 2017; Schaaf et al., 2012), anxiety (6-7% ; Dabell et al., 2013; Lowther et al., 2017) and schizophrenia (5%; Lowther et al., 2017). Epilepsy has been reported in 14-53% of participants with *NRXN1* deletion (Bena et al., 2013; Dabell et al., 2013; Gonzalez-Mantilla et al., 2016; Schaaf et al., 2012) and hypotonia in 38-47% (Bena et al., 2013; Schaaf et al., 2012). In one study, around 46% of individuals with *NRXN1* deletion presented with dual neurodevelopmental disorder diagnoses (Lowther et al., 2017).

Speech and language difficulties are expected to be present in many of the above-mentioned neurodevelopmental phenotypes, and delays in speech and/or language have been reported consistently in children with *NRXN1* deletions (Bena et

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al., 2013; Ching et al., 2010; Curran et al., 2013; Dabell et al., 2013; Gregor et al., 2011), yet comprehensive evaluation of speech and language phenotypes has not been reported in these children. Here we describe the communication phenotypes in 21 children with exonic deletions of *NRXN1*, including evaluation of speech production (phonology, articulation, dysarthria, childhood apraxia of speech), oromotor structure and function, receptive and expressive language and pragmatic (social) language ability.

Materials and methods

Participants

Participants were recruited through Victorian Clinical Genetics Services (VCGS) in Melbourne, Australia. Forty seven individuals were identified with *NRXN1* deletions through routine diagnostic screening at the VCGS. Testing was performed using either the Illumina Human CytoSNP or Illumina Infinium CoreExome SNP arrays, and analysed using Karyostudio software. Of these individuals, 10 were excluded as they were parents of children who also carried a *NRXN1* deletion, one was excluded because they lived interstate and one was excluded because they had English as a second language. In total 35 individuals were eligible for the study and were invited to participate. Of those eligible, 5 declined participation and 9 were unable to be contacted. Twenty-one participants consented to the study. To our knowledge genetic and phenotypic information from the participants in the current study have not been published previously.

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Measures

Information on the child's birth, medical and developmental history was collected at interview. Information on comorbidities was also obtained from parents (Table I). If a child had completed a language assessment within the past 12 months, we included these results rather than re-administering the tool, as per the requirements of most standardised language tests.

Speech. The Goldman Fristoe Test of Articulation- Second Edition (GFTA-2; Goldman & Fristoe, 2000) was administered to assess children's pronunciation of speech sounds in words. It provides normative data for participants aged 2 to 21 years. Children were classified into phonological disorder or delay (a cognitive-linguistic deficit where children do not understand the sound contrasts of their language), articulation disorder (difficulty with the motor production, or specific lip and tongue placement, in making specific sounds) or mixed. If children demonstrated a marked or seemingly inconsistent speech profile on the GFTA-2 or during conversation, participants were also asked to complete the Inconsistency Test of Diagnostic Evaluation of Articulation and Phonology (DEAP; Dodd, Hua, Crosbie, Holm, & Ozanne, 2002) and the Single Word Test of Polysyllables (Gozzard, Baker, & McCabe, 2006).

A 10 minute conversational sample was also taken and analysed for features of dysarthria. Dysarthria is a neuromuscular speech disorder of neurological origin. Dysarthria may affect one or more systems that are required for speech production

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(e.g. lips, tongue, jaw, pharynx, larynx). An adapted version of the Mayo Clinic Motor Speech Characteristics Rating Scale (Duffy, 2013) was used to rate Motor Speech features, as previously applied to children (Fedorenko et al., 2016; Mei & Morgan, 2011; Morgan & Liegeois, 2010; Morgan et al., 2015). This system rates a range of speech characteristics such as pitch, loudness, voice quality, resonance, respiration, prosody and articulation. An overall rating of dysarthria is also made.

Childhood Apraxia of Speech. The presence of childhood apraxia of speech (CAS), defined as a disorder of speech motor planning/programming, was examined using criteria from previously published studies (Fedorenko et al., 2016; Mei, Anderson, Waugh, Cahill, & Morgan, 2018). Hallmark characteristics of CAS, specifically; (1) inconsistent errors on consonants and vowels in repeated productions of syllables or words; (2) lengthened and disrupted coarticulatory transitions between sounds and syllables and (3) inappropriate prosody were rated using single word naming tasks and a conversational sample.

Oral motor. The Oral and Speech Motor Control Protocol (Robbins & Klee, 1987) was used to assess the structure and function of oro-pharyngeal motor development. This protocol requires the individual to perform a range of oral motor movements that include the lips, jaw, tongue, velopharynx, and larynx during respiration. Structure is rated as normal/abnormal and function is rated as absent, emerging or adult-like. Monosyllabic and polysyllabic repetition rates, maximum phonation time, prosody and pitch are also rated as absent, emerging or adult-like.

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Language. Receptive and expressive language was assessed using the Clinical Evaluation of Language Fundamentals Fourth Edition (CELF-4; Semel, Wiig, & Secord, 2003). The CELF-4 is a standardized assessment of language that generates subtest and index scores. The subtests assess aspects of receptive and expressive language including: vocabulary, concept and categorical development, associations, relationships, grammar, sentence structure and morphology. A standardized score of 85 and above (less than 1 standard deviation below the mean) was considered within the normal range as per the test manual. One child (Participant (P)16, Table I) had a language assessment completed using the Clinical Evaluation of Fundamentals Revised-Preschool (CELF-P2; Wiig, Secord, & Semel, 2006).

The Peabody Picture Vocabulary Test- Fourth Edition (PPVT-4; Dunn & Dunn, 2013) was used to assess receptive vocabulary. This is a standardized, norm-referenced assessment. A standardized score of 85 and above (less than 1 standard deviation below the mean) was considered within the normal range, as per the test manual.

The MacArthur-Bates Communicative Development Inventories (CDIs; Fenson, 1993) were used for children who did not have the language ability to complete the CELF-4 or were below 3 years of age. The CDIs are a parent report instrument that contains questions about receptive and expressive vocabulary, gesture and grammatical complexity. The inventories are standardized for children aged 8-37 months but can be used for older children with developmental delays.

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Social language. The Pragmatics Profile from the CELF-4 (Semel et al., 2003) was used to assess the child's social language ability. This is a parent-completed criterion-referenced checklist. A child is considered to have social communication difficulties if they score an age specified cut off on the tool. This tool was only used for children who were verbal.

Adaptive functioning. The Vineland Adaptive Behavior Scales (VABS; Sparrow, Cicchetti, & Balla, 2005) were used to assess the child's adaptive behaviour skills. This is a parent interview tool that includes subscales of adaptive communication, socialization and daily living skills. An overall general composite score can be derived from the subscales. A standardized score over 85 (less than 1 standard deviation below the mean) was considered within the normal range as per the test manual.

Non-verbal IQ. The block design and matrix reasoning subtests from the Wechsler Abbreviated Scale of Intelligence-Second Edition (WASI-II; Wechsler, 2001) were used to assess non-verbal cognitive ability. The WASI-II is a standardized tool that provides reference norms based on age. A scaled score greater than 7 (less than 1 standard deviation below the mean) was considered within the normal range as per the test manual.

Ethical considerations

Ethical approval was obtained through the Royal Children's Hospital, Melbourne, Human Research Ethics Committee (#27053W).

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Results

Participant characteristics

The sample comprised 21 participants, from 14 families, with a mean age of 9.4 years (SD 3.93; range: 1.8-17). The deletion was *de novo* in three participants. In five participants the father had *NRXN1* deletion, in five the mother had *NRXN1* deletion, and in two participants parents had not been tested. None of the maternal (n=5) or paternal (n=2) carriers were reported to have discrete speech and/or language difficulties. In one family (of eight children) the mother did not have *NRXN1* deletion and the father had not been tested. Five of the eight siblings had *NRXN1* deletion. All deletions affected the alpha isoform. The sample included three sets of siblings with *NRXN1* deletions: one family had three siblings with *NRXN1* deletions (denoted 'Family A'), one had five siblings with *NRXN1* deletions (denoted 'Family B') and one family had two siblings with *NRXN1* deletions (denoted 'Family C'). In two of these families (Family A and Family B) siblings were tested after the proband/probands were identified with *NRXN1* deletion, as compared to being referred independently. All participants had Fragile X syndrome and other copy number changes excluded during SNP microarray analysis and Fragile X PCR sizing. One participant (P1) had additional testing done (trio exome) which did not detect any variant relevant to the participant's phenotype. Participant 21 did not have any further testing beyond that completed for the rest of the sample. Details of the locations of the deletions are provided in Figure 1.

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<Figure 1 about here>

Fourteen participants were male. Eight participants (38%) were reported by their parents to have delayed motor milestones. Twenty participants (95%) had been seen by a speech pathologist and seven (33%) had used or were currently using augmentative communication methods (n=3 key word signs, n=4 Picture Exchange Communication System). Eight (38%) of the children scored more than one standard deviation below the mean on an IQ test or had received a diagnosis of developmental delay (i.e. below the average in a developmental quotient) or intellectual disability outside of the study. Of those with below average scores on the VABS (n=8), 5 individuals had concomitant ID/DD. One child had average IQ but low adaptive behaviour and two had average adaptive behaviour and ID/DD. Eight of 20 (40%) participants who had completed the VABS were more than one standard deviation below the mean in adaptive behaviour skills. The mean VABS composite score for the 5 siblings in Family B was within the average range (M 99.4; SD 19.8) and higher than the rest of the sample (M 79.6; SD 24.06). Six participants had confirmed visual problems. Of the whole sample, six (29%) were diagnosed with attention deficit hyperactivity disorder (ADHD), 13 (62%) with autism spectrum disorder (ASD) and three (14%) with epilepsy. Participant characteristics are provided in Table 1. Denominators will vary in the following as we only report on those children who had completed testing for each domain.

<Table I about here>

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Speech and oral motor function

There were mixed findings with regard to the participants' speech. Of the 16 participants who had adequate language and co-operation to complete speech assessments, 5/16 (41%) had no speech difficulties and 11/16 (69%) had some level of speech impairment. Of the children with speech difficulties, 1/11 (9%) had mixed phonological and articulation disorder, 3/11 (27%) had mixed phonological delay and articulation disorder, 2/11 (18%) had mixed phonological delay and disorder, 3/11 (27%) had a phonological delay, 1/11 (9%) had an articulation disorder and 1/11 met criteria for childhood apraxia of speech (Figure 2; Appendix I). The most common error patterns were mild errors commonly seen in the general population, i.e., fronting 'th' to 'f' (n=9) and interdental lisp for 's' (n=5). See Appendix II for further details. Only one child (P2) had been diagnosed with CAS by a speech language pathologist and had striking features of CAS based on the single word naming and conversational tasks. Specific speech features for this participant are described in Appendix III. Some participants had up to two features in one of the three primary CAS criteria (Fedorenko et al., 2016), specifically repetition of sounds and syllables, equal stress, slow rate of speech, altered suprasegmentals.

<Figure 2 about here>

Sixteen children had conversational samples that could be rated for dysarthria. Four of these children (25%) were rated as having subclinical features of dysarthria (i.e. not significant enough to warrant a clinical diagnosis of dysarthria), three (19%) were

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mild and one (6%) was moderate. Specific features of dysarthria are described in Appendix IV. Some participants also displayed a handful of abnormal speech features on the Mayo Clinic Motor Speech Characteristics Rating Scale (Duffy, 2013), which are described in Appendix IV, but these were not sufficient to result in a clinical diagnosis of dysarthria.

Abnormal oral motor function was observed in 11 of the 16 participants (69%) who were able to complete the assessment (Appendix V). The most common difficulties included lip coordination (pucker to smile (5/16; 31%) and interdental (between teeth) placement with the tongue (5/16; 31%)). Tongue fasciculations or tone disruption (which included impairment of tongue function and movement) was noted in 5/16 (31%) participants and 4/16 (25%) had difficulty coordinating tongue lateralisation (sideways movement). One quarter of participants had a high arch palate (4/16; 25%). Difficulties with pitch variation were observed in 4/16 (25%) participants. The majority of participants (11/16; 69%) had difficulty coordinating speech movements for alternating consonant+vowel repetition (e.g. patticake).

Language (receptive, expressive and social)

Twelve of the 21 participants (57%) demonstrated some degree of receptive and/or expressive language delay. Fifteen children completed a standardised assessment of expressive language and 14 standardised assessment of receptive language. Language abilities were highly variable ranging from severely delayed to above average. Of those that could complete formal assessment, 2/14 (14%) had severe receptive

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language delay, 1/14 (7%) moderate, 2/14 (14%) mild and 9/14 (64%) were within the normal range (Appendix VI; Figure 3). Four of 15 children (27%) had severe expressive language delay, 1/15 (7%) moderate and the majority 10/15 (67%) were within the normal range. Receptive vocabulary was within the normal range for 10/16 (63%) participants that completed the PPVT-IV. Three (19%) demonstrated a severe delay in receptive language, 2/16 (13%) moderate and 1/16 (6%) a mild delay. There were no clear profiles of strength or weakness observed in regard to specific language domains (i.e. syntax, semantics and morphology). All but two children (P3, P5) with language delay had concomitant developmental delay or intellectual disability.

Six children did not complete formal language assessment. One child was non-verbal and used a combination of PECS and gesture to communicate (P21). Another child had severe developmental difficulties and was unable to be engaged for the assessment (P7). Two other children were unable to co-operate for formal assessment (P4, P14). Two children aged under 2.5 years had minimal language and were not able to be assessed using standardised tools (P1, P6). Generally there was consistency between developmental level and/or IQ and language ability. All participants who had developmental delay/intellectual disability (n=7) had concomitant language impairment. Three children had normal IQ but delayed language and for 10 participants both language and IQ were within the normal range. One participant had low average IQ and no language delay (P19).

<Figure 3 about here>

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Consistent with an increased risk of ASD, 9/17 (53%) of children with *NRXN1* deletions who had adequate language to complete the CELF Pragmatics Profile had social language difficulties. Social language difficulties may have been present in children who had limited spoken language but the CELF Pragmatics Profile requires a minimum amount of verbal language to be valid. Eight of 21 (38%) children had below average scores on the adaptive socialisation domain of the VABS.

Discussion

This is the first study to provide detailed phenotyping of speech and language in children with *NRXN1* deletion. Speech and language difficulties were common in children with *NRXN1* deletions, but there was substantial heterogeneity in both the type and severity of characteristics expressed. No dominant speech and language patterns were consistently seen in the group. Almost half (10/21) the sample did not demonstrate language difficulties, and about one-third (5/16) did not demonstrate speech difficulties, suggesting significant incomplete penetrance of the *NRXN1* deletion for these phenotypes.

Speech and oral motor function

Children with *NRXN1* deletions presented with varied speech difficulties. Articulation and phonological delays and disorders were present, both in isolation and mixed form. While speech difficulties were more common (69%) in children with *NRXN1* deletion compared with population samples (3.4% in Australian children aged 4 years from the same geographical region; Eadie et al., 2015), the types of speech difficulties were not

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striking and were more in line with typical error profiles seen in the general population. This is in contrast to the strong motor speech profile seen in rare, penetrant, single gene causal mutations such as *FOXP2, GRIN2A, KTM2D* (Morgan, Fisher, Scheffer, & Hildebrand, 2017; Morgan et al., 2015; Turner et al., 2013; Turner et al., 2015). Most children had intelligible speech and only one child had features of CAS. This child also had a moderate dysarthria and his speech (at age 8;10 years) was difficult to understand in conversation. A number of children (n=5) aged over 10 years had mild residual phonological processes that are typically outgrown by 7 years of age. Three of 16 (19%) children presented with mild dysarthric features. Overall the findings suggest that while speech difficulties are common in children with *NRXN1* deletions, affecting around 65%, they tend not to be persistent and severe. Three children had phonological disorder however, the presence of which places these children at greater risk of literacy difficulties (Hayiou-Thomas, Carroll, Leavett, Hulme, & Snowling, 2017). Given this is the first study to investigate speech and language in detail, we are unable to provide phenotypic comparison to previous studies on speech. Similar to the speech profile, abnormalities in oral motor structure and function were common and typically mild, but the type of deficit varied across participants.

Language (receptive, expressive and social)

Language difficulties in children with *NRXN1* deletion were observed in just over half the group, often co-occurring with other developmental disorders such as ASD and intellectual disability. The severity of language delays varied widely. A handful of

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individuals demonstrated above average standard scores in core language while one child remained minimally verbal at 11 years of age. If language delay was present, both receptive and expressive domains were both typically affected. There were no clear profiles of strength or weakness in terms of specific language domains (i.e. syntax, semantics and morphology were affected heterogeneously across the children). The frequency of language difficulties in our study is lower than two other studies that recruited children referred for developmental concerns (Bena et al., 2013; Ching et al., 2010). In these studies, language delays were found in 75% (Ching et al., 2010) and 81% (Bena et al., 2013) of children with *NRXN1* deletion. Differences in our findings may relate to method of recruitment. Our sample contained a number of siblings with *NRXN1* deletion. In Family B, none of the five siblings with *NRXN1* deletions had language delay and all but one had an IQ within the average range. Of those that had neurodevelopmental symptoms, these tended to be mild and the children may not have otherwise been referred for genetic testing if not associated with the original proband. The mean VABS composite score for the 5 siblings was within the average range and was higher than the rest of the sample. Social language difficulties occurred in nine individuals that had verbal language, typically in the presence of an ASD diagnosis. Although it is possible more children had social language difficulties than reported here, as several children were unable to be assessed with the CELF Pragmatics Profile due to with limited language or young age.

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Our sample appeared to be representative of children with *NRXN1* deletion and was consistent with previous study findings with regard to the variability in the clinical characteristics and co-occurring neurodevelopmental disabilities (Bena et al., 2013; Ching et al., 2010; Curran et al., 2013). In our sample 38% had intellectual disability or developmental delay, 62% ASD, 29% ADHD and 14% had epilepsy. These frequencies are higher than those reported in some studies (e.g. Curran et al., 2013) but generally lower compared to studies that have used selected samples of children with developmental delay or intellectual disability (e.g. Bena et al., 2013; Dabell et al., 2013; Schaaf et al., 2012). The exception was with ASD which was similar to that found in studies using selected samples. Adaptive behaviour across all domains was below average in 40% of the 20 participants that completed the VABS and 40% of the participants demonstrated delays in the communication and social domains of the VABS.

Speech and language difficulties are a prominent feature in children with *NRXN1* deletions, yet the substantial variability and lack of specificity around deficits hint that *NRXN1* does not underpin a specific neurobiological pathway relating only to speech and language. Rather, expression of the speech and language phenotype may result from a complex interaction between environmental and genetic factors (e.g. genetic modifiers) beyond *NRXN1* deletion. This hypothesis is supported by the findings in Family C. Both siblings in Family C had *NRXN1* deletions in the same location yet presented with vastly different phenotypes. One sibling presented with generally age appropriate language and IQ with mild speech difficulties and the other presented as

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non-verbal with severe intellectual disability. The variable presentation of *NRXN1* is also consistent with the high frequency of *NRXN1* deletions in controls (Ching et al., 2010). This is in contrast to other genetic conditions that have more specific speech phenotypes (e.g. *FOXP2* and *GRIN2A*), which are not typically observed in controls. Thus, the lack of penetrance both in siblings and parents and the inconsistency in phenotypic expression suggest *NRXN1* deletion may explain only part of the neurodevelopmental and speech and language difficulties observed. Moreover, while *NRXN1* deletions are a risk factor for neuropsychiatric and neurodevelopmental disorders, and are enriched (2 to 3%) in many of these disorders, they are also found in around 0.5% of healthy controls (Kirov, 2015). This may further explain the incomplete penetrance observed in some families.

The speech and language profile in individuals with *NRXN1* deletion may also be a relatively non-specific consequence of intellectual disability and ASD. In the current study, with the exception of two participants, all those who had language difficulties had concomitant intellectual disability. Previous studies also support a correlation between ID, ASD and language difficulties (e.g. Levy et al., 2010; Liao et al., 2015). It would also be interesting to test this hypothesis in a cohort of individuals who have *NRXN1* deletions without intellectual disability, such as in a cohort of patients with idiopathic generalized epilepsy (e.g. Moller et al. 2013). Currently, children with isolated speech and language difficulties typically do not get SNP microarray testing in the geographic region the children were recruited from, therefore our participants are biased with ID/DD/ASD. Increased genetic screening in children with isolated

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communication difficulties by methods capable of detecting *NRXN1* deletions, such as SNP microarray may help clarify this association.

Clinical implications

Information we have provided about the type and severity of speech and language characteristics seen across the group may assist families to better understand the prognosis of speech and language in *NRXN1* deletion. Findings cannot yet guide diagnosis however, in that distinct speech and language profiles were elusive. Given the heterogeneous profile seen, a detailed speech and language assessment would be required for referred cases to identify specific needs and monitor or intervene as appropriate.

Acknowledgements

Funding was provided by National Health and Medical Research Council (NHMRC) Practitioner Fellowship #1105008 (AM); NHMRC Centre of Research Excellence in Speech and Language Neurobiology #1116976 awarded to AM, MSH, DA. NHMRC Career Development Fellowship #1063799 awarded to MSH. We wish to thank Christiane Zweier, Friedrich Alexander-University Erlangen Nürnberg, for her review of the manuscript and insightful comments.

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