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Molecular characterization of *NRXN1* deletions from 19,263 clinical microarray cases identifies exons important for neurodevelopmental disease expression

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Disclosure

The authors have no conflicts of interest to disclose.

Supplementary Material

Supplementary information is available at the *Genetics in Medicine* website.

Abstract

Purpose—The purpose of the current study was to assess the penetrance of *NRXN1* deletions.

Methods—We compared the prevalence and genomic extent of *NRXN1* deletions identified among 19,263 clinically referred cases to that of 15,264 controls. The burden of additional clinically relevant CNVs was used as a proxy to estimate the relative penetrance of *NRXN1* deletions.

Results—We identified 41 (0.21%) previously unreported exonic *NRXN1* deletions ascertained for developmental delay/intellectual disability, significantly greater than in controls [OR=8.14 (95% CI 2.91–22.72), p< 0.0001)]. Ten (22.7%) of these had a second clinically relevant CNV. Subjects with a deletion near the 3′ end of *NRXN1* were significantly more likely to have a second rare CNV than subjects with a 5′ *NRXN1* deletion [OR=7.47 (95% CI 2.36–23.61), p=0.0006]. The prevalence of intronic *NRXN1* deletions was not statistically different between cases and controls (p=0.618). The majority (63.2%) of intronic *NRXN1* deletion cases had a second rare CNV, a two-fold greater prevalence than for exonic *NRXN1* deletion cases (p=0.0035).

Conclusions—The results support the importance of exons near the 5′ end of *NRXN1* in the expression of neurodevelopmental disorders. Intronic *NRXN1* deletions do not appear to substantially increase the risk for clinical phenotypes.

Keywords

NRXN1; copy number variation; genotype-phenotype; variable expression; penetrance

INTRODUCTION

Neurexins are a group of highly polymorphic presynaptic cell adhesion molecules that primarily bind to neuroligins.[1] The three neurexin genes (*NRXN1*, *NRXN2* and *NRXN3*) are highly conserved and undergo extensive alternative splicing[2] to produce thousands of isoforms that appear to be both spatially and temporally regulated.[3,4] The two main isoforms for each neurexin gene, the longer *NRXN-α* and the shorter *NRXN-β*, are transcribed from two independent promoter regions and give rise to proteins with similar C-terminal regions but different N-terminal ectodomains.[5] The *NRXN-α* and *NRXN-β* isoforms both bind to postsynaptic neuroligins and leucine-rich repeat transmembrane proteins, however with varying degrees of affinity.[1,6,7] *NRXN-α* also binds to dystroglycan and cerebellin.[8,9] *NRXN1* (OMIM 600565) is the largest neurexin gene (comprising about 1.1 Mb of genetic sequence)[2] and the one most implicated as a top candidate gene for neurodevelopmental and neuropsychiatric conditions (Figure 1).[10]

Rare exonic deletions overlapping *NRXN1* on chromosome 2p16 were first identified in individuals with autism spectrum disorder (ASD)[11,12] and developmental delay/intellectual disability (DD/ID).[13] Subsequently, such deletions have been identified in individuals with various neurodevelopmental and neuropsychiatric disorders.[14–20] A few exonic *NRXN1* deletions have been identified in controls and the majority of transmitting parents are

reported to be only mildly affected or clinically unaffected.[10,14] Understanding the factors that contribute to this incomplete penetrance is a key goal for clinical genetics.

Exonic *NRXN1* deletions are non-recurrent and are found across the entire length of this large gene.[2,10,21,22] There is conflicting evidence to suggest that the deletion extent may underlie the *NRXN1* phenotypic heterogeneity.[10,21] A recent study found that individuals with ASD are significantly more likely to harbor a rare *de novo* mutation in exons that are under purifying selection (called "critical exons") than their siblings,[23] suggesting that certain sequences within *NRXN1* may be more important for clinical expression than others. Also, there is accumulating evidence to suggest that non-coding regions of the genome, including long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), play an important role in the aetiology of neurodevelopmental disorders.[24,25] Deletions upstream and within introns of *NRXN1* have been identified in disease cases;[14,22,26] however the pathogenicity of these deletions remains unclear.

As the use of genome-wide microarray technology in the prenatal setting increases,[27] so too does the need for improved understanding of the genetic factors that impact the penetrance of *NRXN1* deletions to inform genetic counselling and anticipatory care. We used a large (n=19,263) clinically ascertained cohort from southern Ontario, Canada and 15,264 population-based controls to investigate the penetrance of *NRXN1* deletions. CNV data from high resolution genome-wide microarrays allowed systematic evaluation of the burden of secondary CNVs that we used as a proxy to estimate the relative penetrance of exonic and intronic *NRXN1* deletions.

MATERIALS AND METHODS

Exonic *NRXN1* deletions: clinical cohorts examined

We searched three Province of Ontario accredited clinical cytogenetics laboratory databases for cases with exonic *NRXN1* deletions. These included subjects submitted for clinical microarray testing before January 2015 at the Hospital for Sick Children (n=11,727), Trillium Health Partners (n=6,022), and Hamilton Health Sciences (n=1,514), all located in southern Ontario, Canada. All three laboratories are provincially funded to provide clinical constitutional microarray testing for individuals with DD/ID, ASD and/or multiple congenital anomalies (MCA). The *NRXN1* deletion cases identified in this study therefore have a strong ascertainment bias for these conditions. Participating physicians completed a detailed clinical checklist (Table S1) for each exonic *NRXN1* deletion case based on their own clinical assessment and lifetime chart reviews, where available. For cases without clinical checklist data, ascertainment and demographic data were collected from laboratory requisition forms. We also included three additional cases with an exonic *NRXN1* deletion (P30, P34 and P35; Figure 1) detected by other clinical laboratories in order to increase the overall number of cases with genome-wide CNV data. These cases were not included in *NRXN1* deletion prevalence calculations for the catchment area.

Molecular methods and validation

All exonic *NRXN1* deletions (chr2:50,145,643–51,259,674; hg19) in cases were identified by one of three microarray platforms, the Affymetrix CytoScan® HD Array, the Oxford Gene Technology (OGT) 4×180 CytoSure Oligonucleotide array or the Illumina (formally BlueGnome) 4x180K CytoChip ISCA array. The OGT and Illumina arrays are similar in design with approximately 200–250 oligonucleotide probes (average probe spacing 5.5–7.6 kb) distributed across the *NRXN1* gene, allowing for a minimum deletion detection of 10 kb. The CytoScan® HD array has approximately 650 oligonucleotide probes distributed across *NRXN1*, with similar minimum deletion detection capabilities. All genomic coordinates are given using the Genome Reference Consortium February 2009 build of the human genome (GRCh37/hg 19). Genomic coordinates for the 22 *NRXN1* exons (α1 transcript; NM_004801.4) were obtained from the NCBI RefSeq database. Exonic *NRXN1* deletions ≥100 kb were confirmed using fluorescence *in situ* hybridization (FISH). Smaller deletions were confirmed by qPCR or by a second microarray.

Additional clinically relevant CNVs as a proxy for calculating the relative penetrance of *NRXN1* deletions

All three clinical laboratories classified a deletion overlapping any one or more of the 22 *NRXN1* exons (NM_004801.4) as pathogenic. Therefore, we used the presence of a second clinically relevant CNV as a proxy for estimating the relative penetrance of the *NRXN1* deletions. As previously shown,[28] CNVs with high penetrance (i.e., not identified in control cohorts) are less likely to harbor a second independent large rare CNV.[28] The high-resolution genome-wide CNV data from each *NRXN1* deletion case was investigated for the presence of a second CNV that may be clinically relevant. The clinical interpretation of these second CNVs as pathogenic, likely pathogenic, or as a variant of unknown significance (VUS) was provided by one of the three experienced clinical laboratory directors using the American College of Medical Genetics guidelines for CNV interpretation.[29] Other clinically relevant variants detected by different laboratory tests were not included in our statistical analyses.

Phenotypic data

The clinical checklist completed for each exonic *NRXN1* deletion case is presented in Table S1. Briefly, data were collected on ascertainment features, demographic variables (age, sex), growth parameters (height, weight, head circumference) and growth abnormalities, dysmorphic features, and lifetime developmental, psychiatric, medical, and family history based on clinical assessment and/or lifetime medical chart reviews. If completed, previous clinical genetic testing results were provided. Where known, data on parental phenotypes were collected.

Control cohorts

To examine the prevalence of exonic and intronic *NRXN1* deletions in the general population we used 15,264 controls with CNV data available from high-resolution genome-wide arrays (Table S3). These controls were analyzed on several different array platforms with variable probe spacing. Therefore, similar to previous studies,[30] we only included

exonic and intronic deletions identified by a minimum of two of three CNV calling algorithms (iPattern, PennCNV, ChAS), spanning 5 consecutive array probes and over 10 kb in size. Over 90% of CNVs called using this method validate using other laboratory methods.[30]

Intronic *NRXN1* deletions

Trillium Health Partners was the only clinical laboratory that systematically recorded intronic *NRXN1* deletions. These clinical cases (n=6,022) were analyzed using the Illumina 4x180K CytoChip ISCA array which had even probe spacing across introns. We used two strategies to assess the pathogenicity of intronic *NRXN1* deletions. First, we compared the prevalence of intronic *NRXN1* deletions between clinically referred cases and the 15,254 population-based controls described above. Second, we assessed the prevalence of secondary CNVs among the intronic *NRXN1* deletion cases and compared this to the prevalence for exonic *NRXN1* deletion cases.

Statistical analyses

Statistical analyses were performed using SAS software (version 9.2; SAS Institute, Cary, NC). For categorical data we used χ^2 or Fishers exact test, where appropriate. For continuous data we used Welch's t-test. All analyses were two tailed, with statistical significance defined as $p<0.05$. Odds ratios (OR) and 95% confidence intervals were used to assess the prevalence of secondary CNVs between deletions overlapping one or more of exons 1–4 and those overlapping exons ≥5 and the association between the *NRXN1* deletions (exonic and intronic) ascertained from the clinical diagnostic population compared to controls.

RESULTS

Prevalence of exonic *NRXN1* deletions in cases and controls

As of January 2015, a total of 19,263 individuals were submitted for clinical microarray testing across the three participating cytogenetics laboratories. There were 41 (0.21%) unrelated, previously unpublished, probands identified to have deletions overlapping one or more *NRXN1* exons (Figure 1). The prevalence of exonic *NRXN1* deletions for the individual laboratories was 0.33%, 0.26%, and 0.14% (Table S4). The prevalence of exonic *NRXN1* deletions was significantly greater in cases compared to 15,264 controls [OR=8.14 (95% CI 2.91–22.72), $p< 0.0001$)] (Table S3).

Exonic *NRXN1* deletions

Genomic coordinates for all 44 exonic *NRXN1* deletions, including three additional cases with exonic *NRXN1* deletions from laboratories outside the catchment area, are represented in Figure 1. All were non-recurrent, with sizes ranging from 29 kb to 806 kb (median=244 kb). The majority of the deletions (n=32; 72.7%) overlapped at least one or more of exons 1–4 (referred to as a 5′ *NRXN1* deletion hereafter). Ten (22.7%) deletions overlapped exons ≥5 (hereafter termed 3′ *NRXN1* deletions). Two deletions (P28 and P30; Figure 1) that overlapped both the 5′ and 3′ end of *NRXN1* were excluded from analyses comparing 5′ and 3′ *NRXN1* deletions but not the descriptive statistics. This categorization of exons has

been used in previous publications.[10,21] The 5′ *NRXN1* deletions (median size=242 kb) were significantly larger than the 3′ *NRXN1* deletions (median size=83 kb), [t(18.5)=2.55, p=0.019].

The 44 exonic *NRXN1* deletions were inherited (11 maternal, 5 paternal) in 16 (64.0%), *de novo* in nine (36.0%) and unknown in 19 subjects. Of these, 5′ *NRXN1* deletions were inherited in thirteen (61.9%) and *de novo* in 8 (38.1%) subjects; 3′ *NRXN1* deletions were inherited in 3 (75.0%) and *de novo* in 1 (25.0%) subject. Of the 11 transmitting mothers, four were identified as clinically affected: three with mild ID and one with anxiety and depression. Clinical outcomes for the remaining seven transmitting mothers and five transmitting fathers were unknown.

Genome-wide prevalence of additional clinically relevant CNVs

Clinical microarray testing identified 10 (22.7%) exonic *NRXN1* deletion cases with one or more additional clinically relevant CNV (n=14), ranging in size from 38 kb to whole chromosomal anomalies (Table 1). The prevalence of males (n=7/28; 25.0%) with a secondary CNV was similar to that of females (n=3/16; 18.8%). Twelve (85.7%) of these CNVs were classified as VUS and two as pathogenic (Table 1). The two pathogenic CNVs were *de novo*, two of the VUS were paternally inherited and the remaining 10 additional VUS were of unknown inheritance. The prevalence of these secondary CNVs was significantly higher in subjects with a 3′ *NRXN1* deletion (n=7/10; 70.0%) compared to subjects with a 5′ *NRXN1* deletion (n=3/32; 9.4%) [OR=7.47 (95% CI 2.36–23.61), p=0.0006] (Figure 1). Eleven (78.6%) of the fourteen secondary CNVs overlapped one or more genes known to be involved in central nervous system function (Table 1).

Clinical characteristics of exonic *NRXN1* deletion cases

Completed clinical checklists were returned for 21 (47.7%) exonic *NRXN1* deletion cases by referring physicians, with basic ascertainment and demographic data available for the other 23 cases (Table S2). As expected given the criteria for microarray testing, all of the 44 probands (16 female, 28 male) with exonic *NRXN1* deletions were ascertained for DD/ID. Thirty-five (79.5%) were children (median 5.2, range 1–6 years) and nine (20.5%) were adults (median 32.0, range 21–59 years).

There were 20 (45.5%) subjects who met diagnostic criteria for another (i.e., additional to DD/ID) neurodevelopmental and/or neuropsychiatric condition, often referred to as ‘dual diagnosis’. Including multiple features per subject, the prevalence of these conditions was: ASD or pervasive developmental disorder (n=14; 31.8%), epilepsy/seizures (n=6; 13.6%), ADHD (n=4; 9.1%), anxiety (n=3; 6.8%), Tourette’s syndrome (n=2; 4.5%) and tardive dyskinesia secondary to antipsychotic treatment in two adults with schizophrenia (n=2; 4.5%). There was no significant difference in the prevalence of dual diagnosis, seizures/epilepsy or macrocephaly between subjects with a 5′ *NRXN1* deletion compared to subjects with a 3′ *NRXN1* deletion (data not shown).

In contrast to the prominent neurodevelopmental phenotype, congenital anomalies were identified in only four (9.1%) cases: two with tetralogy of Fallot (TOF), one of whom also had a tracheoesophageal fistula and imperforate anus, a third with cryptorchidism, and a

fourth with a small atrial septal defect. All four of these subjects had a 5′ *NRXN1* deletion and none were identified to have a rare second CNV. However, one TOF case was identified to have a *RAF1* sequence mutation causing Noonan syndrome (OMIM 611553).

Eleven (25.0%) cases had additional clinical genetic testing that was found to be normal, including karyotype, fragile X syndrome, 22q11.2 deletion syndrome, Prader-Willi syndrome, muscular dystrophy gene panel and a gene panel for progressive myoclonic epilepsy. Two long contiguous stretches of homozygosity on chromosome 14 (identified on follow-up to be maternal uniparental disomy of chromosome 14) were identified in a single subject (P14; Figure 1).

Intronic *NRXN1* deletions

We identified 19 of 6,022 (0.32%) cases submitted for clinical microarray testing and 55 of 15,264 (0.36%) controls to have an intronic *NRXN1* deletion (Figure 1). In contrast to the prevalence of exonic *NRXN1* deletions identified in this laboratory (0.33% [OR 12.67 (95% CI 4.33–37.08), p<0.0001], there was no significant difference in the prevalence of intronic *NRXN1* deletions between cases and controls [OR 0.88 (95% CI 0.52–1.48), p=0.618)]. These intronic *NRXN1* deletions ranged in size from 11 kb to 134 kb (median=38 kb), significantly smaller than the 44 exonic deletions (p<0.0001). The majority (n=14; 73.7%) of the intronic deletions were located in the large intron 5 (Figure 1). Inheritance data are limited as most of the intronic deletions were not included in clinical reports; one case had follow-up and was found to be paternal in origin. Several of the intronic deletions were recurrent, including a 70.6 kb deletion in intron 5 and an 11.1 kb deletion in intron 18. Twelve (63.2%) of the 19 intronic *NRXN1* deletion cases had a second reportable CNV on clinical microarray (Table 2), a significantly greater prevalence than for the exonic *NRXN1* deletion cases [OR=2.59 (95% CI 1.37–4.91); p=0.004].

Of these 19 (11 female, 8 male) unrelated cases with intronic *NRXN1* deletions, 17 (89.5%) were children (median=5.0, range 0.5–10 years) and two were adults (Table S2). Sixteen (84.2%) were ascertained for DD/ID and/or ASD and three (6.8%) for MCA (n=1), CHD (n=1) and absent radius/thumb. Of the 16 cases with DD and/or ASD, ten (62.5%) had a second rare CNV that overlapped one or more genes involved in central nervous system function (Table 2).

DISCUSSION

This is the largest study characterizing exonic and intronic *NRXN1* deletions to date. Our aim was to use data from a large clinical population-based sample to systematically investigate factors affecting the penetrance of deletions overlapping this large gene. In addition to confirming the predominantly neuropsychiatric phenotypic expression of pathogenic CNVs overlapping *NRXN1*, novel results support the importance of the genomic extent of these deletions, including the particular region involved and overlapped exons.

Penetrance of exonic *NRXN1* deletions

We report on 44 novel exonic *NRXN1* deletion cases ascertained for DD/ID (Table S2). We assessed penetrance using multiple factors, both those used previously (prevalence in cases

versus controls and the ratio of *de novo* versus inherited deletions) and those novel to this study (prevalence of secondary CNVs and classification as pathogenic or VUS). We found that the 0.21% prevalence of exonic *NRXN1* deletions in this clinical population was over 8-fold greater than in controls and that 34% of these deletions were identified to be *de novo*, supporting the relatively high penetrance of these deletions for clinically important phenotypes. About one in every four cases with an exonic *NRXN1* deletion had one or more other rare CNVs reported on clinical microarray (Table 1). Their distribution amongst the cases indicated a relatively lower penetrance of 3′ *NRXN1* deletions.

The effect of genomic position on penetrance of exonic *NRXN1* deletions

Similar to previous studies,[10,21,22,31] the majority of the exonic *NRXN1* deletions identified in clinical cases overlapped the promoter and the first few exons of the *NRXN1-α* transcript. For subjects with deletions overlapping the 3′ end of *NRXN1* there was an over 7-fold increased likelihood of having a second clinically relevant CNV compared to subjects with a 5′ *NRXN1* deletion (Figure 1). Further, none of the additional rare CNVs identified in the 5′ *NRXN1* deletion subjects were classified as pathogenic, demonstrating the relative importance of 5′ *NRXN1* deletions over 3′ *NRXN1* deletions for the expression of clinical phenotypes.

A potential explanation for the higher penetrance of 5′ *NRXN1* deletions may be that these deletions directly overlap or indirectly influence the lncRNA AK127244 that is adjacent to the promoter of *NRXN1-α* (Figure 1). There is accumulating evidence to suggest that AK127244 may play a role in the etiology of neuropsychiatric disorders.[26,32,33] This includes the identification of two deletions that overlap this lncRNA (and not *NRXN1*) in a child with borderline IQ and early onset schizophrenia.[32] A recent report also described five ASD cases with deletions overlapping AK127244.[26] The biological function of AK127244 has yet to be elucidated. However, a significant proportion of lncRNAs are expressed in the brain and have important roles in neurodevelopmental processes.[34]

There was little evidence that additional phenotypic features were indicators of higher penetrance in individuals with exonic *NRXN1* deletions in this study. This could be due in part to a high phenotypic floor effect present in clinically recruited subjects. It is interesting to note that the two adults with comorbid ID and schizophrenia in this study had deletions overlapping the 5′ end of *NRXN1*. Similarly, in the largest study of *NRXN1* deletions in schizophrenia to date there were 10 exonic *NRXN1* deletions identified, the majority of which overlapped the 5′ end of *NRXN1*.[14] Also, two cases from this study had a deletion overlapping AK127244 but not *NRXN1*,[14] providing support for the possible role of this lncRNA in the etiology of schizophrenia.

In contrast to our findings supporting the reduced penetrance of 3′ *NRXN1* deletions, the limited number of these deletions reported in the literature had previously led to the hypothesis that they are associated with severe phenotypes, including prenatal lethality.[31] To further investigate if deletions overlapping the 3′ end of *NRXN1* are associated with prenatal lethality we examined ten studies of miscarriages and/or stillbirths (see refs 6 and 17–26 in Rosenfeld et al.)[35] for rare CNVs overlapping *NRXN1*. Among >900 products of conception there was only one paternally inherited 95 kb deletion overlapping *NRXN1-α*

(exons 2–4) identified.[35] We also identified one deletion overlapping the *NRXN1-β* promoter among 15,254 controls. These data provide further support for the increased penetrance of 5′ relative to 3′ *NRXN1* deletions.

Interpretation of intronic *NRXN1* deletions

We identified 19 novel cases with an intronic *NRXN1* deletion, 16 with DD/ID and/or ASD and three with a congenital anomaly. Our group used two strategies to assess the penetrance of intronic *NRXN1* deletions. First, we compared the prevalence of intronic *NRXN1* deletions between cases and population-based controls and detected no significant difference. Indeed, 18 of the 19 intronic *NRXN1* deletions identified in cases had breakpoints similar to those seen in controls (Figure 1). Second, we determined that intronic *NRXN1* deletion cases were two-fold more likely to harbour a second clinically relevant CNV compared to exonic *NRXN1* deletion cases. Therefore, intronic *NRXN1* deletions appear unlikely to substantially increase the risk for a neurodevelopmental disorder and/or MCA.

However, seven (36.8%) of the intronic *NRXN1* deletion cases, each with severe phenotypes had no additional rare CNV reported on clinical microarray. One of these seven *NRXN1* deletions overlapped intron 9 (Case I16; Figure 1), which had no corresponding deletion identified in controls. Using the VISTA enhancer browser (https://enhancer.lbl.gov)[36] we identified a known enhancer element (hs1348) located 37 kb upstream of the intron 9 deletion, which if perturbed could potentially alter the transcriptional levels of *NRXN1* and thus increase the penetrance of this deletion.

Advantages and limitations

There are several advantages to this study. We used data from three clinical laboratories to compile the largest cohort of clinically referred individuals with exonic and intronic *NRXN1* deletions assembled to date. Our systematic approach to the detection and interpretation of additional rare CNVs allowed us to use the burden of these secondary CNVs as a proxy for determining the relative penetrance of *NRXN1* deletions. We employed robust methods for CNV detection, to evaluate the prevalence of intronic *NRXN1* deletions in cases and controls which had previously been ignored due to the use of different CNV calling algorithms and reporting practices across clinical laboratories.

A limitation of this study is that the prevalence of individual clinical features would necessarily be influenced by the ascertainment bias inherent in clinically referred cases. This tends to overestimate DD/ID and underestimate other features. The prevalence of *NRXN1* deletions was about an order of magnitude different between each clinical laboratory, reaching statistical significance between the Hospital for Sick Children (0.14%) and Trillium Health Partners (0.33%) [OR 2.29 (95% CI 1.19–4.39), p=0.012)] (Table S3). This may be due to differences in indications for referral, with the Hospital for Sick Children and Hamilton Health Sciences servicing children with the most severe and intractable neurological disorders from this catchment area. Given that some *NRXN1* deletions are identified in control subjects,[14,31] it is possible that the prevalence of these deletions is highest at Trillium Health Partners since it is a community-based hospital that services

individuals with comparatively milder clinical phenotypes. The resolution of the microarrays used to define the *NRXN1* deletion breakpoints and detect genome-wide structural variants limited us to those >10 kb that could influence phenotypic expression.

Only one control cohort (OPGP; Table S3) was systematically screened for neurodevelopmental and/or neuropsychiatric conditions. Control subjects with *NRXN1* exonic or intronic deletions may thus have had mild and/or subclinical symptoms. This could have reduced the effect size of the case-control results. We did not have access to the individual SNP data for the *NRXN1* deletion cases or the controls and as a result were unable to genetically confirm that each individual was unrelated to any other. However, given that none of the exonic *NRXN1* deletions identified in cases or controls had similar breakpoints it is unlikely that these individuals were related. Further, only two of the eight control cohorts (Table S3) were ascertained from the same catchment area as our cases and they included adults only. This makes it unlikely that the intronic *NRXN1* deletion cases (89% children) were the same individuals as those among the controls.

Future directions

The major challenge moving forward will be to determine how genetic and non-genetic factors converge to explain the variable expression and incomplete penetrance of exonic and intronic *NRXN1* deletions. Examination of the genes overlapped by additional rare CNVs as well as applying next generation sequencing to detect variants within the coding and non-coding regions of the genome in subjects with a *NRXN1* deletion may serve as a key step towards identifying novel pathways to disease expression. Interestingly, none of the second rare CNVs identified in this study have been previously reported in combination with an exonic *NRXN1* deletion,[10,21,22,31] suggesting that a large *NRXN1* deletion consortium may be required to compile enough cases to identify novel biological patterns among the additional variants.

Conclusions

The expression of exonic *NRXN1* deletions appears to be primarily neuropsychiatric, with DD/ID often comorbid with another neuropsychiatric condition. The results of this study suggest that deletions near the 5′ end of *NRXN1* have higher penetrance, potentially related to perturbation of the lncRNA AK127244 located adjacent to the *NRXN1-α* promoter. Subjects with a 3′ *NRXN1* deletion had a 7-fold increased likelihood of having a second rare CNV detected by clinical microarray, supporting reduced penetrance for these deletions. There is insufficient evidence as yet to suggest that all intronic *NRXN1* deletions are benign.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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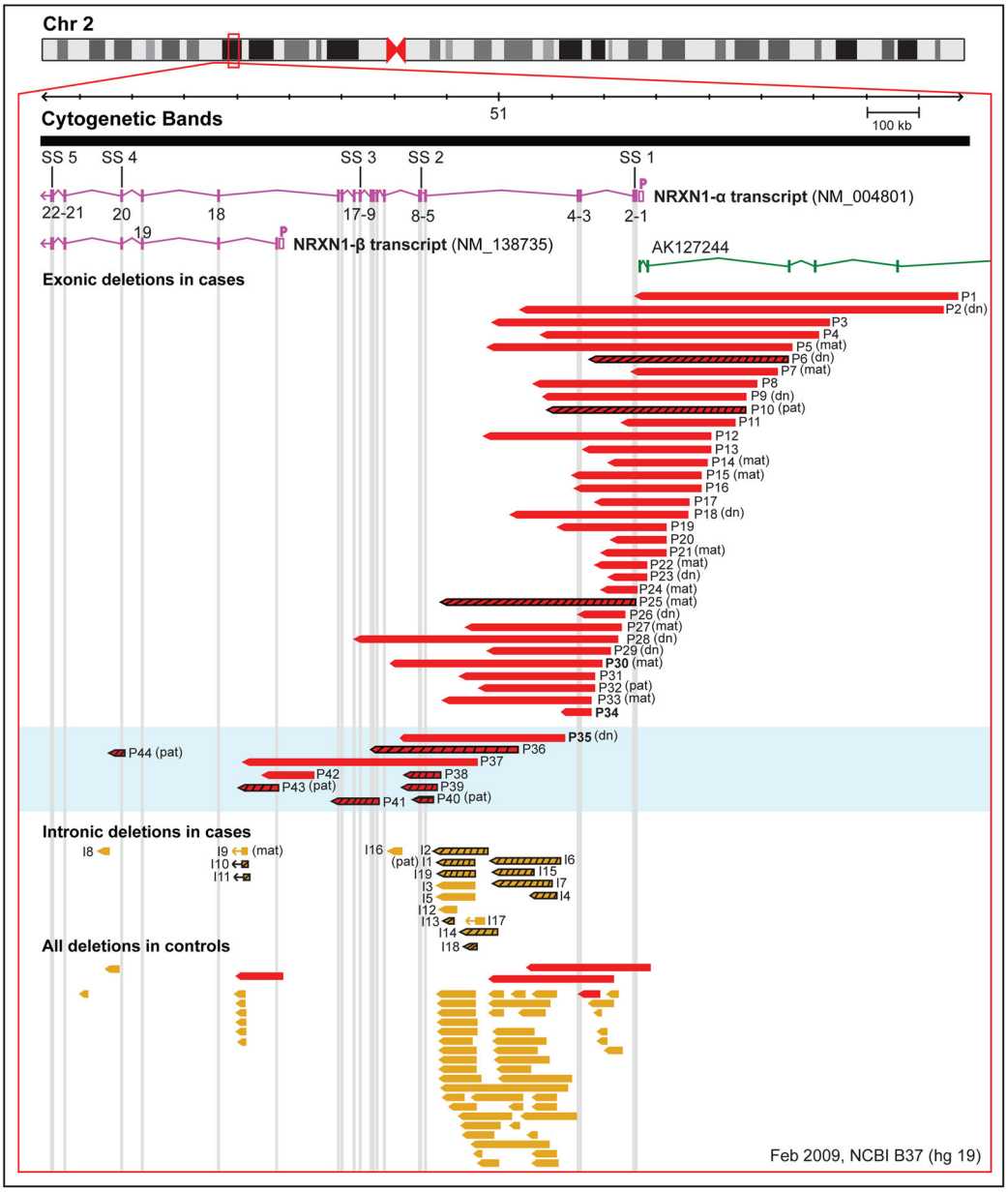

Figure 1. Novel exonic and intronic *NRXN1* deletions identified in cases and controls

The image was modified from the Database of Genomic variants (http://dgv.tcag.ca), NCBI Build 37 (hg 19).[37,38] The two primary *NRXN1* transcripts (α1 and β1) are shown in pink; other transcripts are not included. The long non-coding RNA (lnRNA) AK127244 is shown in green. Each of the 22 exons is identified by a number according to the NM_004801.4 transcript. The five splice site (SS 1-5) locations are represented above the *NRXN1-α* transcript. The hollow pink box denoted by a P adjacent to each transcript represents the α and β promoter, respectively. All exonic and intronic deletions (chr2: 50,145,643–51,259,647; hg 19) are represented by solid red and yellow bars, respectively. Deletions with a black grid are subjects that were identified to have a second CNV of potential clinical

relevance. Inheritance status of the *NRXN1* deletion is represented in brackets following the patient ID number (d.n., *de novo*; mat, maternal inheritance; pat, paternal inheritance; blank, unknown). P28 and P30 overlap both the 5′ and 3′ ends of *NRXN1* and were not included in statistical analyses. P14 and P32 were identified to have maternal uniparental disomy of chromosome 14 and a *RAF 1* mutation, respectively. The light blue box designates subjects with deletions overlapping exons ≥5 (3′ deletion). Case numbers were kept consistent throughout the manuscript, tables and supplemental documents. Cases P30, P34 and P35 were obtained from other laboratories and are represented in bold font.

Table I

Additional clinically relevant CNVs identified in 10 of 44 exonic *NRXN1* deletion subjects

ID[a]	Main clinical features	*NRXN1* deletion	Cytoband	CNV	Start (hg 19)	Size (kb)	# of genes	Inheritance	Protein-coding candidate genes[b]	Clinical laboratory classification
P44	ID, ASD, ADHD, ODD, anxiety, TS	Exon 20	X chr (47, XXX)	Gain	-	-	-	*de novo*	Various, including *FMR1, MECP2, SYN1*	Pathogenic
P43	DD	β promoter	Yp11.32-p11.2	Gain	10,863	4,459	-	*de novo*	Various, including	Pathogenic
			Yq11.21-q12	Loss	14,630,081	44,700	-		*NLGN4Y*	
P41	DD	Exons 10–17	1q43	Gain	236,929,252	613	3	Unknown	*MTR, RYR2*	VUS
			15q13.1-q13.2[c]	Loss	28,940,069	184	9		*APBA2, CHRFAM7A, TJP1*	VUS
P40	DD	Exons 5-8	11p11.2-p11.12	Gain	48,088,592	831	8	Paternal	None	VUS
P39	DD, ASD	Exons 5-8	1q23.3	Gain	160,927,546	428	23	Unknown	*USF1, PVRL4, PFDN2, PPOX, B4GALT3, ADAMTS4, NDUFS2, NR1I3, PCP4L1, MPZ, SDHC*	VUS
P38	DD/ID, motor and speech delay, PDD, anxiety, failure to thrive	Exons 5-8	4q35.2	Loss	188,355,766	383	1	Unknown	None	VUS
P36	DD, motor and speech delay	Exons 5-10	19q13.43	Loss	57,656,482	794	3	Unknown	None	VUS
P13	DD, behaviour problems	exons 1-2	16p13.3	Gain	6,679,225	38	1	Unknown	*RBFOX1*	VUS
P10	DD, speech delay	αP and exons 1-4	8p23.3	Gain	843,413	750	4	Paternal	*DLGAP2*	VUS
P6	DD, behavioural problems, hypotonia, bilateral sensorineural loss	αP and exons 1-2	1p22.1	Gain	92,179,826	526	6	Unknown	*TGFBR3*	VUS
			3q29[d]	Loss	192,404,455	164	2	Unknown	*FGF12*	VUS
			7q31.2-q31.31	Gain	117,382,934	1,700	3	Unknown	*CTTNBP2*	VUS
			Xp22.23	Gain	1,588,945	777	4	Unknown	*P2RY8, ASMT*	VUS

CNV, copy number variation. #, number; VUS, variant of unknown significance; DD, developmental delay; ASD, autism spectrum disorder; PDD, pervasive developmental disorder; ID, intellectual disability; ADHD, attention deficit hyperactivity disorder; ODD, oppositional defiant disorder; TS, Tourette's syndrome; MCA, multiple congenital anomalies.

[a] The subject ID's match those found in Figure 1.

[b] Protein coding genes known to be expressed and/or implicated in nervous system function or cardiac function based on literature search.

[c] This deletion is distal to the Prader-Willi/Angelman syndrome region but proximal to the 15q13.3 deletion syndrome region (OMIM 612001).

[d] Does not overlap the 3q29 microdeletion syndrome region associated with schizophrenia (OMIM 609425).

Table II

Additional clinically relevant CNVs identified in 12 of 19 intronic *NRXN1* deletion subjects

ID[a]	Main clinical features	*NRXN1* deletion	Cytoband	CNV	Start (hg 19)	Size (kb)	# of genes	Inheritance	Protein-coding candidate genes[b]	Clinical laboratory classification
I8	DD	Intron 21	3q27.1-q27.2	Loss	184,027,899	1,300	4	Unknown	*EIF4G, CLCN2, CHRD, EPHB3, SENP2*	VUS
I11[c]	ASD, heart defect, hip hypoplasia, clubfoot, absent radius	Intron 18	1q21.1[d]	Loss	144,986,396	998	20	Unknown	*PDZK1*	Pathogenic
I10	DD	Intron 18	4p16.2	Gain	3,185,517	155	3	*de novo*	*HTT*	VUS
I6	DD	Intron 5	1p21.2-p21.1	Gain	101,742,523	554	1	Unknown	*OLFM3*	VUS
I2[c]	DD	Intron 5	3p13	Loss	71,041,637	406	1	Unknown	*FOXP1*	Likely pathogenic
I1	DD, microcephaly	Intron 5	6q22.31-q23.2	Loss	125,993,504	5,900	22	Maternal	*LAMA2*	VUS
I13[c]	MCA	Intron 5	Mosaic trisomy chr 9	Gain	-	-	~800	Unknown	Various, including *SETX*	Pathogenic
I15	DD, ASD	Intron 5	11p13	Gain	33,008,222	557	6	Unknown	None	VUS
I4	Absent radius and thumb	Intron 5	15q13.1-q13.2	Gain	28,859,279	1,500	4	Unknown	*APBA2*	VUS
I14[c]	DD	Intron 5	15q11.2	Loss	22,669,082	998	5	Unknown	*CYFIP1, NIPA2, NIPA1*	Pathogenic
I17	DD	Intron 5	16p13.13	Loss	10,556,892	297	4	Paternal	None	VUS
I19	DD	Intron 5	11p15.5	Gain	1,222,378	318	4	Unknown	*BRSK2*	VUS

CNV, copy number variation; #, number; VUS, variant of unknown significance; DD, developmental delay; ASD, autism spectrum disorder; MCA, multiple congenital anomalies.

[a] The subject ID's match those found in Figure 1.

[b] Protein coding genes known to be expressed and/or implicated in nervous system function or cardiac function based on literature search.

[c] Subjects with CNVs classified as pathogenic. All other CNVs were classified as a variant of unknown significance.

[d] Overlaps susceptibility locus for Thrombocytopenia-Absent radius syndrome (OMIM 274000).