

Neurexin 1 (*NRXN1*) Deletions in Schizophrenia

George Kirov^{1,2}, Dan Rujescu³, Andres Ingason^{4,5}, David A. Collier⁶, Michael C. O'Donovan², and Michael J. Owen²

²Medical Research Council Centre for Neuropsychiatric Genetics and Genomics, Department of Psychological Medicine and Neurology, School of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, UK; ³Division of Molecular and Clinical Neurobiology, Department of Psychiatry, University of Munich, Munich, Germany; ⁴Research Institute of Biological Psychiatry, Mental Health Center Sct. Hans, Copenhagen University Hospital, Roskilde, Denmark; ⁵deCODE Genetics, Reykjavik, Iceland; ⁶Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College, London, UK

Introduction

Over the last 2 years, several reports have suggested that submicroscopic chromosomal deletions that disrupt the gene neurexin 1 (*NRXN1*) increase the risk of developing schizophrenia. In this article, we will review the evidence for this association.

NRXN1 encodes NRXN1, a synaptic neuronal adhesion molecule. NRXNs are found presynaptically and are believed to interact with postsynaptic neuroligins (NLGNs) in excitatory and inhibitory synapses in the brain. The structure and possible function of NRXNs and NLGNs have recently been comprehensively reviewed.¹

Vertebrate NRXNs and NLGNs are the only adhesion molecules for which a specifically synaptic function has been demonstrated.¹ Current evidence suggests that NRXNs and NLGNs act transsynaptically to mediate essential signaling between presynaptic and postsynaptic specializations. Evidence from cell culture experiments and the study of mouse knockouts suggest that these molecules are required for synapse function but not for synapse formation, that they influence transsynaptic activation of synaptic transmission; and that their dysfunction impairs the properties of synapses and disrupts neural networks without completely abolishing synaptic transmission. NRXNs and NLGNs probably function by binding to each other and by interacting with intracellular proteins (most notably those with PDZ domain), but the precise mechanisms involved remain unknown.¹

Whatever the mechanism and their relationship to synaptic function, it is clear that mice lacking NRXNs or NLGNs show marked deficits in synaptic transmission.¹

There are 5 NLGN genes and 3 NRXN genes in humans (*NRXN1* [2p16.3], *NRXN2* [11q13], *NRXN3* [14q31]). The 3 NRXN genes each encode an α protein and a β protein from independent promoters. NRXNs have a striking molecular diversity: the messenger RNA can be processed by alternative splicing, giving potentially thousands of distinct protein isoforms.²

There is strong evidence for an involvement of NLGNs and *NRXN1* in autism (reviewed by Kumar and Christian³). Several studies reported an increased rate of deletions or other genetic variants in *NRXN1* in subjects suffering with autism.^{4–11} Additionally, deletions in this gene have been implicated in mental retardation and developmental delay.^{12,13} A number of studies have reported *NRXN1* deletions in cases with schizophrenia, which we will review in more detail.

NRXN1 Deletions in Schizophrenia

The first suggestion that *NRXN1* deletions might confer risk of schizophrenia came from a study by Kirov et al.¹⁴ They used array comparative genome hybridization (array-CGH) to screen the genomes of 93 cases of schizophrenia recruited in Bulgaria with approximately 35 000 probes for chromosomal copy number variants (CNVs) at a resolution of about 80 000 bp. They identified a deletion of *NRXN1* in a female case, which was also present in her affected brother. The deletion spanned the promoter and the first exon of the gene. Neither sibling had signs of premorbid cognitive problems or dysmorphic features. Given that deletions of *NRXN1* were not seen in 372 controls, and at that time there were reports of *NRXN1* deletions and point mutations in autism^{4,5} and one deletion in a subject with mental retardation,¹² the authors hypothesized that deletions in this gene might confer risk to schizophrenia as well as to other neurodevelopmental disorders. Intriguingly, these authors also found a de novo duplication of 1.4Mb of chromosome 15 that involved, among other genes, *amyloid beta A4 precursor protein-binding (APBA2)* gene encoding a protein known as Mint2. Mint2 is a neuronal adaptor protein that binds directly to NRXNs in a PDZ domain-mediated interaction.

¹To whom correspondence should be addressed; tel: +442920687071, fax: +442920687068, e-mail: kirov@cardiff.ac.uk

Table 1. Data From All Available Studies on Deletions in Neurexin 1

Study	All Deletions >100 kb			Deletions >100 kb Disrupting Exons	
	Cases	Controls	Size (kb)	Cases	Controls
Kirov et al ¹⁴	1/93	0/372	250	1/93	0/372
Walsh et al ¹⁵	1/233	0/268	115	1/233	0/268
International Schizophrenia Consortium ¹⁷	4/3391	3/3181	122–291	3/3391	1/3181
Rujescu et al ¹⁶	6/2977	11/33 746	100–419	5/2977	5/33 746
Need et al ¹⁸	3/1073	0/1148	200–418	3/1073	0/1148
Kirov et al ¹⁹	1/471	3/2792	231–631	1/471	3/2792
Ikeda et al. (unpublished data, 2009, see text citation for all authors)	1/560	0/547	161	0/560	0/547
Totals	17/8798 (0.19%)	17/42054 (0.04%) ^a		14/8798 (0.16%)	9/42 054 (0.02%) ^b

Note: All deletions are filtered for >100kb in size

^aFisher Exact test: $P = .000013$, odds ratio = 4.78, 95% confidence interval = 2.44–9.37.

^bFisher Exact test: $P = .0000037$, odds ratio = 7.44, 95% confidence interval = 3.22–17.18.

Shortly after this initial report, Walsh et al,¹⁵ also using array-CGH, reported identical twins with early-onset schizophrenia who shared a 115-kb deletion in the gene (disrupting exons in the 3' end) among a total of 233 schizophrenia cases and 268 controls.

Array technology has progressed tremendously since these small early reports, and it is now possible routinely to detect CNVs using microarrays with up to 2 million probes covering the whole genome. Several groups have found deletions of *NRXN1* in patients with schizophrenia using the new technologies. Rujescu et al¹⁶ examined *NRXN1* for CNVs in 2977 schizophrenia patients and 33 746 control subjects from 7 European countries (Iceland, Finland, Norway, Germany, the Netherlands, Italy, and the UK). Sixty-one *NRXN1* deletions, including a de novo deletion, and 5 duplications were detected. Of these, 12 deletions and 2 duplications occurred in schizophrenia cases (0.47%) compared with 49 deletions and 3 duplications (0.15%) among the >10-fold larger control sample ($P = .13$, odds ratio [OR] = 1.73; 95% confidence interval [CI] = 0.81–3.50). There was no common breakpoint, and the CNVs varied in size from 18 to 420 kb. Because the penetrance of *NRXN1* CNVs is likely to vary according to the level of functional impact on the gene, these authors next restricted analysis to CNVs that disrupt exons. Seven were found in cases (0.24%) and five in controls (0.015%). Compared with all CNVs spanning *NRXN1*, those affecting exons were more strongly associated with schizophrenia and had a higher OR ($P = .0027$, OR = 8.97, 95% CI = 1.8–51.9).

A second large collaborative study, the International Schizophrenia Consortium,¹⁷ found 4 deletions in 3391 cases and 3 in 3181 controls from 5 European countries (Sweden, Bulgaria, Ireland, Portugal, and the UK). If we only consider those deletions that disrupt exons, as suggested by Rujescu et al,¹⁶ the rate is 3 vs 1 or approximately 0.09% in cases and 0.03% in controls. No duplications were found.

Need et al¹⁸ reported 3 *NRXN1* deletions in a sample of 1013 cases and 1084 controls of European ancestry and 60 cases and 64 controls of African American ancestry.

Kirov et al¹⁹ examined 471 patients and 2792 controls from the United Kingdom. They found one deletion in *NRXN1* in a case and 3 in controls, giving a 2-fold non-significant increase in the rate in cases (0.2% vs 0.1%). All are likely to disrupt exons.

Finally, M. Ikeda et al. (M. Ikeda, MD, PhD, B. Aleksic, MD, PhD, G. Kirov, MRCPsych, PhD, Y. Kinoshita, MD, PhD, Y. Yamanouchi, MD, PhD, T. Kitajima, MD, PhD, K. Kawashima, MD, PhD, T. Okochi, MD, T. Kishi, MD, PhD, I. Zaharieva, M.J. Owen, FRCPsych, PhD, M.C. O'Donovan, FRCPsych, PhD, N. Ozaki, MD, PhD, N. Iwata, MD, PhD, unpublished data, 2009) tested 560 patients and 547 controls from Japan and found one deletion in a case and none in controls. The deletion was 161 kb long but does not disrupt an exon.

In the analysis described below, we do not include the 4 patients described in the study of Vrijenhoek et al²⁰ as they were included in the study by Rujescu et al.¹⁶

Frequency of *NRXN1* Deletions in Schizophrenia Across All Available Studies

Given that deletions in *NRXN1* are extremely rare and many of the studies are too small to provide statistically meaningful results, we reanalyzed all available data in order to find out whether there is convincing evidence for association. To make the results comparable across all studies, we included only deletions >100 kb, as all studies, apart from one,¹⁶ had used this selection criterion. We provide all the data in table 1 and visualize the exact breakpoints of each deletion on the background of the gene using UCSC tracks (<http://www.genome.ucsc.edu/>) to enable readers to see the relationship between the deletions and exons (figure 1). In total, there were deletions in 17/8789 cases (0.19%) and in 17/42054

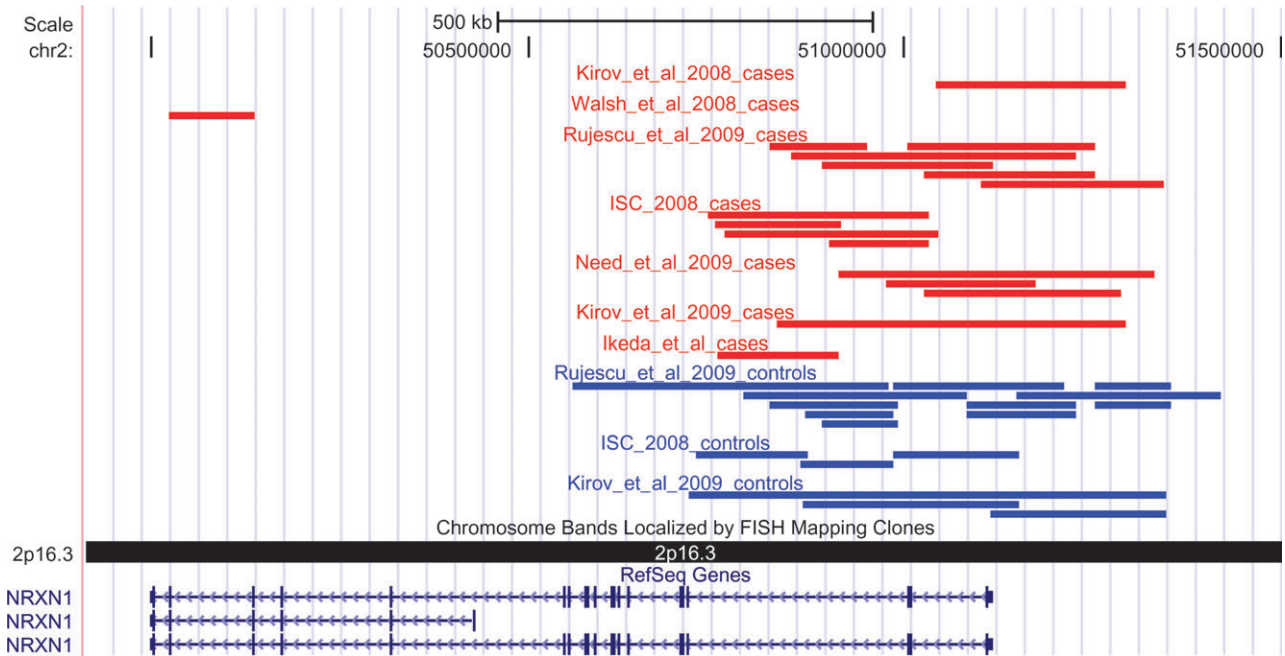


Fig. 1. Copy Number Variants in *NRXN1* in All Studies, Filtered for >100 kb. The shorter form of the gene encodes for the NRXN1 β form, while the longer one encodes for the NRXN1 α form. Red bars are deletions in cases ($n = 8798$); blue are those in controls ($n = 42\,054$).

controls (0.04%), a highly significant excess (Fisher Exact test: $P = .000013$, OR = 4.78, 95% CI = 2.44–9.37). Re-analysis for deletions >100kb that only disrupt exons reveals a similar result ($P = .000037$, OR = 7.44, 95% CI = 3.22–17.18; table 1).

Conclusions

The findings of studies to date provide strong evidence that deletions of *NRXN1* confer a substantial increase in risk of schizophrenia. However, some degree of caution is still warranted. In the context of their rarity, there have still been relatively few subjects studied, and there are technical difficulties associated with detecting CNVs of variable size. Much of the available control data in our analysis came from the Icelandic sample reported by Rujescu et al.¹⁶ Given these controls are derived from a large population sample with a good phenotypic information, the rate of *NRXN1* deletions observed is very likely to be an accurate representation in control populations. Moreover, it is also similar to the estimates from the majority of the other smaller studies. Nevertheless, the rarity of *NRXN1* deletions means that the overall findings are quite strongly dependent upon that single study. There is therefore a need to study an even greater number of cases and controls in independent samples, using high-resolution platforms, which can detect smaller CNVs.

These findings at *NRXN1* should be seen in the context of a number of recent demonstrations that risk of schizophrenia is increased by CNVs.²¹ In particular,

there is very convincing evidence that large recurrent deletions affecting 1q21.1, 15q11.2, 15q13.3, and 22q11.2 confer risk.^{17,19} All 4 deletions contain multiple genes, and it might not be straightforward to understand which genes and pathways are implicated in risk of psychosis. In contrast, the deletions described in the present article only disrupt *NRXN1*. These findings could therefore represent a decisive step toward identifying a specific pathway implicated in the pathogenesis of schizophrenia.

Another key area of future research will be work aimed at understanding the nature of the pleiotropic effects that are associated with those CNVs that confer risk of schizophrenia. Interestingly, all the risk CNVs seem not only to associate with variable neurodevelopmental phenotypes, particularly autism, and variable degrees of cognitive dysfunction but can also occur in the absence of an apparent neurodevelopmental phenotype.^{22,23} The highly variable outcomes associated with these genomic abnormalities suggest that more detailed understanding of the mechanisms involved might point the way to new therapeutic opportunities.

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