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# Comparative genome hybridization suggests a role for *NRXN1* and *APBA2* in schizophrenia

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**Copy number variations (CNVs) account for a substantial proportion of human genomic variation, and have been shown to cause neurodevelopmental disorders. We sought to determine the relevance of CNVs to the aetiology of schizophrenia (SZ). Whole-genome, high-resolution, tiling path BAC array comparative genomic hybridization (array CGH) was employed to test DNA from 93 individuals with DSM-IV SZ. Common DNA copy number changes that are unlikely to be directly pathogenic in SZ were filtered out by comparison to a reference dataset of 372 control individuals analyzed in our laboratory, and a screen against the Database of Genomic Variants. The remaining aberrations were validated with Affymetrix 250K SNP arrays or 244K Agilent oligo-arrays and tested for inheritance from the parents. A total of 13 aberrations satisfied our criteria. Two of them are very likely to be pathogenic. The first one is a deletion at 2p16.3 that was present in an affected sibling and disrupts *NRXN1*. The second one is a *de novo* duplication at 15q13.1 spanning *APBA2*. The proteins of these two genes interact directly and play a role in synaptic development and function. Both genes have been affected by CNVs in patients with autism and mental retardation, but neither has been previously implicated in SZ.**

## INTRODUCTION

Schizophrenia (SZ, MIM 181500) is a chronic mental disorder with a life-time risk of ~1% (1). It has a strong genetic component with heritability estimates of up to 85% (2) and a ~10-fold elevated recurrence risk in first-degree relatives (1). Various chromosomal abnormalities have been reported in patients with SZ, but the aetiological relevance of most of these is unclear (3,4). The best established finding concerns the chromosome 22q11 deletion syndrome, also known as DiGeorge/velocardiofacial syndrome, which is associated with SZ in 20–30% of adult carriers (5) and is found in up to 0.6% of patients with SZ (6). This suggests that copy number variation (CNV) in the human genome might be of more general aetiological relevance in this disorder. CNVs have recently been shown to be common in the general population and to account for more genomic differences between individuals than SNPs (7–10).

SZ has certain features that suggest a partially overlapping aetiology with mental retardation (MR) and autism, including a tendency to show delayed development and lower IQ (11), language and communication problems, and a higher rate of minor physical anomalies (12). Sufferers with MR have an increased risk of developing psychotic illness (13), and patients with 22q11 deletion syndrome have increased rates of autism and MR, as well as SZ (14). Both MR and autism are associated with high rates of CNVs elsewhere in the genome (15–19). However, there have been few systematic attempts to detect sub-microscopic CNVs in SZ, apart from two small-scale, low-resolution pilot studies (20,21).

We therefore undertook a systematic search for CNVs in 93 patients with SZ, using high-resolution, tiling path BAC arrays

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and compared the results with those from patients with unrelated disorders obtained using the same platform. Two of the aberrations detected in our study are very likely to be pathogenic: one has arisen *de novo*, and the other one segregates with illness in the family. In addition, the two aberrations encompass genes encoding directly interacting synaptic proteins neither of which has previously been implicated in the pathogenesis of SZ. These novel findings have potentially important implications for understanding the pathogenesis of SZ and suggest common pathogenic mechanisms with autism and MR.

## RESULTS

Altogether, 13 CNVs passed our filtering criteria (see *Materials and Methods*) and were validated with a second platform (Table 1). Demographic and phenotypic details for these patients are presented in Table 2.

Two changes are very likely to be of aetiological relevance. The first is a 1.4 Mb duplication on chromosome 15q13.1. This was confirmed with Affymetrix and Agilent arrays and was found to be absent in the unaffected parents, i.e. it occurred *de novo* (Fig. 1).

Paternity was confirmed by array-based SNP typing. The duplicated interval in this proband contains three genes: the amyloid precursor-binding protein A2 gene (*APBA2*), the Necdin-like gene 2 (*NDNL2*), and the tight junction protein 1 gene (*TJP1*). This region partly overlaps with a larger, 3.95 Mb deletion, identified by Sharp *et al*. (17), as causing MR (Fig. 1B). The Database of Genome Variants reports only a large inversion encompassing these genes. The region is flanked by segmental duplications, known as breakpoints BP3A and BP4 (22), containing low copy repeats (LCRs) with a high sequence homology. The presence of these repeats probably explains the instability of this region. Their positions are shown at the bottom of Figure 1B.

The second potentially pathogenic aberration is a 0.25 Mb deletion on 2p16.3. It was also present in the affected sibling of the proband and in their asymptomatic mother. It spans the promoter and first exon of the neurexin 1 gene (*NRXN1*). Partially overlapping deletions disrupting the same gene have previously been identified in a mentally retarded patient (16) and in two siblings with autism (23) (Fig. 2). No other deletion or duplication involving this gene was found in our control population.

Three of the aberrations (all duplications) on 3q22.3, 9q21.22 and 22q11.23 did not occur in the schizophrenic siblings of the probands. This makes them less likely to be pathogenic. The duplications on 4q35.2 and 15q26.1, and the deletion on 17q22 contain no known genes or ultra-conserved elements. Databases reveal several predicted genes, and we cannot definitively exclude the possibility that these CNVs predispose to SZ, especially the 17q22 deletion, which was seen in the affected mother. One patient carried a 480 kb deletion of chromosome 16p12.2. Although this deletion was not observed in our control sample, overlapping duplications were seen twice. The region contains two potential candidate genes, *EEF2K* and *CDR2*, which have been implicated in intracellular signalling in brain and neuroimmunology,

**Table 1.** CNVs passing our inclusion criteria. Sty and Nsp refer to the type of 250K Affymetrix SNP arrays used for validation

| Proband ID | Aberration | Present in affected relatives? | Family history | Found in parent? | Predicted range (Mb) | Confirmed range (Mb) | Size (kb) | Validation method | Present in controls? | Genes |
|---|---|---|---|---|---|---|---|---|---|---|
| 4289-1 | 1q44 dup | No DNA from sib | Sib MR | Father | 241.48–241.72 | – | 240 | – | Unique | EFCAB2, KIF26B |
| 3108-1 | 2p16.3 del | Sib (+) | Sib SZ | Mother | 51.14–51.32 | 51.10–51.35 | 250 | Nsp | Unique | 5′end of NRXN1 |
| 2207-4 | 3q22.3 dup | Mother (+) Sib (−) | Sib and mother SZ | Mother | 140.11–140.85 | 140.25–140.97 | 720 | Nsp | >50% Unique | FOXL2, BPESC1, MRPS22, RBP2, RBP1, NMNAT3 |
| 4114-1 | 4q35.2 dup | No DNA from sib | Sib SZ | Mother | 189.92–190.86 | 189.86–190.50 | 640 | Nsp | Unique | Nil |
| 1317-1 | 6p21.1 dup | – | Nil | Father | 41.24–41.50 | 41.39–41.60 | 210 | Sty | Unique | TREML4, TREML1, NCR2 |
| 3383-1 | 9q21.12 dup | Sib (−) | Sib SZ | Mother | 70.76–71.15 | 70.89–71.12 | 230 | Sty/ Nsp | Unique | TRPM3 |
| 4263-1 | 15q13.1 dup | – | Nil | *De novo* | 26.94–28.01 | 27.00–28.40 | 1,400 | Sty/Agilent | Unique | APBA2, NDNL2, TJP1 |
| 4203-1 | 15q26.1 dup | – | Nil | Mother | 92.14–92.64 | 92.01–92.50 | 490 | Nsp | Unique | Nil |
| 3363-1 | 16p12.2 del | Mother (+) | Father &mother affective disorders | Mother | 21.81–22.37 | 21.92–22.32 | 400 | Sty | Twice seen as dupl | UQCRC2, EEF2K, POLR3E, CDR2, & 3 hypothetical genes |
| 3381-1 | 17q22 del | Mother (+) | Mother SZ | Mother | 48.22–48.75 | 48.31–48.67 | 360 | Nsp | Unique | Nil |
| 3373-1 | 22q11.22 dup | – | Nil | Mother | 20.6–21.0 | 20.66–20.92 | 260 | Nsp | Unique | PPM1F, TOP3B, VPREB1 |
| 1314-1 | 22q11.22 dup | – | Nil | Mother | 20.6–21.0 | 20.66–20.92 | 260 | Nsp | Unique | PPM1F, TOP3B, VPREB1 |
| 3268-1 | 22q11.23 dup | Sib (−) | Sib SZ | Father | 23.17–24.18 | 23.25–24.16 | 910 | Sty | >50% Unique | UBP1, SNRPD3, GGT1, PIWIL3, RUTBC2, CRYBB3, CRYBB2, LRP5L |

The table shows whether there was positive family history, and if so, whether the aberration was found in affected members (columns 3 and 4); (−) and (+) refer to the absence or presence of the aberration in affected relatives. The chromosomal position of the aberration, as predicted with CGH and confirmed with Affymetrix/Agilent, and their sizes, are shown in columns 6, 7 and 8. The column 'present in controls' denotes whether this aberration was seen in our set of 372 controls. The final column lists the genes within these intervals.

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**Table 2.** Demographic and illness characteristics of the probands

| ID | Aberration | Gender | Age at onset | Age | School result | SZ type | Clinical features |
|---|---|---|---|---|---|---|---|
| 4289-1 | 1q44 dup | Male | 29 | 31 | Good | Paranoid | Paranoid, depressed |
| 3108-1 | 2p16.3 del | Female | 21 | 24 | Excellent | Paranoid | Thought disorder, voices, paranoid delusions, good remissions |
| 3108-4 (sibling) | 2p16.3 del | Male | 20 | 28 | Good | Disorganized | Bizarre speech, aggression, perplexed, paranoid, intermittent voices, chronic course |
| 2207-4 | 3q22.3 dup | Female | 25 | 32 | Good | Paranoid | Constant voices, odd behaviour, paranoid delusions, partial remissions |
| 4114-1 | 4q35.2 dup | Female | 32 | 42 | Very good | Paranoid | Paranoid, constant voices, paranoid, aggressive, thought disorder, chronic course |
| 1317-1 | 6p21.1 dup | Female | 32 | 32 | Pass | Disorganized | Odd behaviour, lack of communication, self-neglect, bizarre ideas, partial remissions |
| 3383-1 | 9q21.12 dup | Male | 42 | 42 | Pass | Paranoid | Paranoid, hearing commanding voices |
| 4263-1 | 15q13.1 dup | Male | 23 | 43 | Pass | Disorganized | Paranoid, self-neglect, voices, aggressive, odd behaviour, chronic course |
| 4203-1 | 15q26.1 dup | Male | 21 | 32 | Pass | Paranoid | Thought disorder, odd behaviour, paranoid, voices, partial remission |
| 3363-1 | 16p12.2 del | Female | 20 | 28 | Pass | Paranoid | Aggressive, paranoid, severe voices, good remissions |
| 3381-1 | 17q22 del | Male | 27 | 42 | Pass | Paranoid | Paranoid, negative symptoms, constant voices, chronic course |
| 1314-1 | 22q11.22 dup | Female | 12 | 19 | Excellent | Disorganized | Constant voices, aggressive, self-neglect, odd behaviour, chronic course |
| 3373-1 | 22q11.22 dup | Male | 20 | 57 | Very good | Catatonic | Manneristic grimacing, posturing, stupor, thought disorder, self neglect, disorganised behavoiur, chronic course |
| 3268-1 | 22q11.23 dup | Female | 29 | 36 | Good | Paranoid | Thought disorder, voices, manic symptoms, good remissions |

respectively (24,25). The duplications on 1q44, 6p21.1 and 22q11.22 contain genes (Table 1) but occurred in unaffected parents, so their pathogenic status remains uncertain. The 22q11.22 duplication was the only CNV that was seen in two unrelated subjects with SZ. It does not overlap the 22q11 deletion syndrome known to be associated with high risk of SZ (see section *Introduction*).

## DISCUSSION

We performed array CGH using high-resolution whole-genome tiling path arrays in 93 patients suffering with SZ. The availability of DNA from the parents of all cases studied enabled us to establish whether the aberrations had occurred *de novo*. Another strength of our study was the use of a large control sample of 372 patients with unrelated disorders which had been examined using the same methodology in the same laboratory. This allowed us to estimate the relevance of any detected aberrations to SZ. Finally, we required putative genomic aberrations to be detected by three or more neighbouring BACs. The use of this stringent '3-clone rule' resulted in a low false positive rate, with just a single aberration not validated (not presented in Table 1). However, this raises the possibility that we have missed smaller aberrations of possible pathogenic significance that spanned only one or two clones.

In our study, the proportion of potentially pathogenic aberrations is much lower than that previously reported in two small studies of SZ using CGH (20,21). This might appear surprising given the much greater resolution of our tiling path method, compared with those used in the previous studies. However, it is likely that several factors specific to our study account for this discrepancy. These include our stringent criteria for CGH data interpretation, the testing of inheritance and co-segregation within the affected families, and especially the use of a large group of controls. In addition, we had access to more data on CNVs that have been reported in the general population since the previous publications. Finally, a recent study (26) could not identify the CNVs reported by Wilson *et al*. (21) when using different technologies in a large sample of SZ patients, and most importantly, in two of the samples analyzed by Wilson *et al*. (21) previously shown to exhibit duplications and deletions of the genes in question.

We found one *de novo* aberration in a sample of 51 sporadic cases: a duplication at 15q13.1. The relative paucity of large *de novo* aberrations in SZ should not be surprising, given that large aberrations, especially *de novo* ones, are associated with the more severe neurodevelopmental phenotypes of MR and autism, and are often accompanied by a variety of more pronounced physical anomalies (16). We specifically excluded patients with IQ $<70$ from our study, given that we wished to establish associations with SZ that are independent of MR.

The two aberrations in our study that are most likely to be pathogenic are the 0.25 Mb deletion of 2p16.3, and the 1.4 Mb *de novo* duplication of 15q13.1. The first of these

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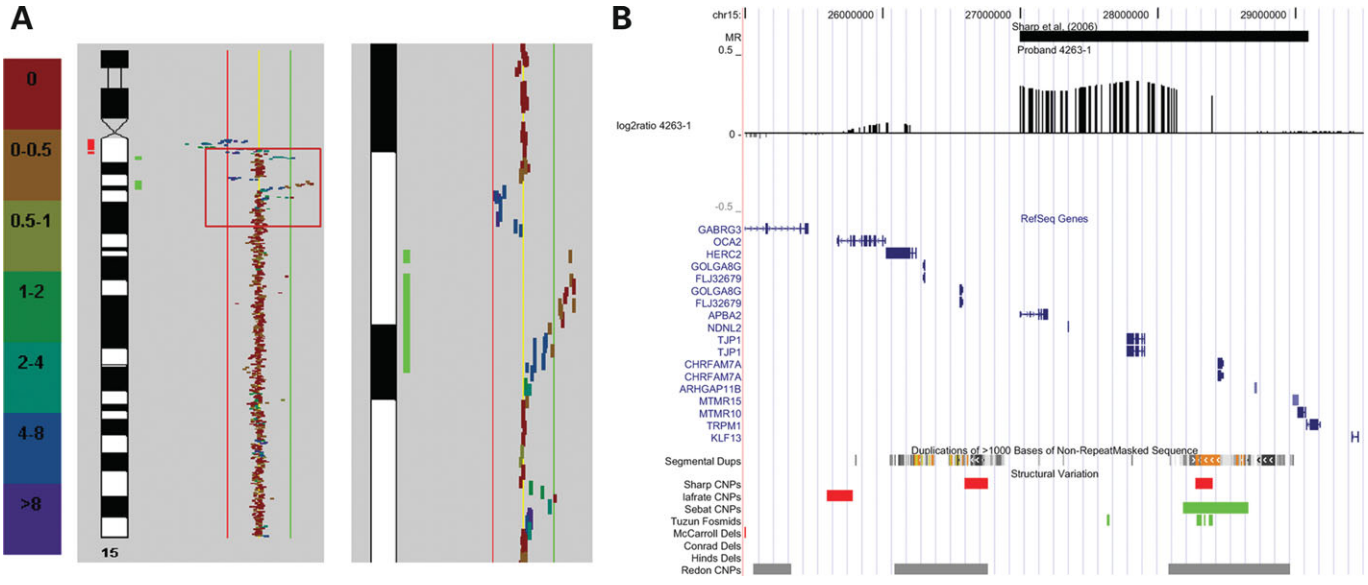


**Figure 1.** *De novo* duplication of 15q13.1. (a) Array-CGH image. Whole chromosome and zoom-in view showing the duplication of 15q13.1 in individual 4203. Cy3/Cy5 intensity ratios of each BAC clone are plotted in a size-dependent manner along the chromosome ideograms by means of the programme CGHPRO (57). Red and green lines indicate the log2ratio thresholds −0.3 (loss) and 0.3 (gain), respectively. Red to blue colour codes indicate BACs with increasing percentage of low copy repeats (LCRs). The colouring scheme is shown on the left, numbers refer to the percentage of the clone covered by LCRs (57), i.e. red clones contain no LCRs. (b) Validation with Affymetrix 250K arrays. Log2 ratios were produced by the CNAT programme and are displayed on the UCSC genome browser. Every SNP is indicated by a short vertical bar (longer bars are within the duplication). There are no SNPs on either side of the duplication, as these regions are flanked by segmental duplications (shown at the bottom of the figure). Figure 1A shows the BAC clones in these regions in a blue colour (they have a high percentage of LCRs). Also shown are the genes in the area. The horizontal bar marked Sharp *et al*. 2006 indicates the region reported in reference (17) where a subject with MR had a partially overlapping, 3.95 Mb deletion.

spans the promoter and exon 1 of *NRXN1* (Fig. 2B). This is the first time that a disruption of *NRXN1* has been reported in SZ. We conclude that this deletion is likely to be pathogenic because it was found in the affected sibling of the proband and because deletions of *NRXN1* have been found in two other neurodevelopmental disorders, MR (16) and autism (23) (Fig. 2B), and rare mutations in this gene are likely to increase the risk for autism (27). Neurexins are encoded by three genes (*NRXN1-3)* which, by the use of two distinct promoters, produce two classes of transcripts, α- and β-neurexin. These, via further alternative splicing, produce thousands of distinct cell-surface proteins (28) that are localized on pre-synaptic terminals. As pre-synaptic cell-adhesion molecules, neurexins bind to postsynaptic cell-adhesion molecules including neuroligins (29). Interestingly, truncating mutations in neuroligin genes have been identified both in cases of autism (30) and MR (31). Neurexins are believed to play an important role in the release of neurotransmitters from pre-synaptic vesicles (32–34), and together with neuroligins are thought to be involved in synapse formation and the use-dependent validation of neural circuits by which the ratio of excitatory to inhibitory neurotransmission is determined (29,34,35). Evidence that the balance of excitatory and inhibitory neurotransmission is abnormal in autism spectrum disorders is accumulating on the basis of several lines of evidence including recent work on mouse models (36,37). Our findings suggest that these proposed pathogenic mechanisms might apply to a wider spectrum of neurodevelopmental abnormalities including SZ.

The fact that the apparently unaffected mother also carried the deletion could reflect incomplete penetrance, which is a feature of other chromosomal abnormalities that are associated with high risk of SZ (5,38). In addition, although the mother has not presented to psychiatric services, she was described as 'odd and neurotic' by the psychiatrist treating her children. Thus it remains possible that she has features of schizotypal personality disorder or one the other sub-clinical conditions found in relatives, and especially parents, that comprise the extended phenotype of SZ (1).

The second abnormality, that is likely to be pathogenic, is the *de novo* duplication of 15q13.1. This lies telomeric of *HERC2* and is distal to the Prader–Willi critical region and to the duplications of 15q11–q13 that are also observed in 1–3% of cases with autism (39,40). To our knowledge, this is the first time that this genomic region has been implicated in SZ. Although, interestingly, the duplicated region lies within the region duplicated in idic(15) marker chromosomes which are associated with autism (40). The duplication in our case involves three genes: *NDNL2, TJP1* and *APBA2. NDNL2* encodes a protein of unknown function, that is expressed in all human tissues tested, with highest expression in testis (41). TJP1, also known as zonula occludens protein 1 (ZO-1), is located on a cytoplasmic membrane surface of vertebrate intercellular tight junctions. The role of ZO-1 in the CNS is poorly understood and most available data on this protein are focused around its role in the formation of tight junctions between epithelial cells (42). However, there is evidence suggesting a role for it in synaptogenesis since it has been localized to puncta adhaerentia junctions formed between mossy fibre terminals and the shaft of hippocampal CA3 dendrites to which it is recruited by its association with the calcium-independent cell adhesion molecule nectin and the actin-binding protein afadin (43).

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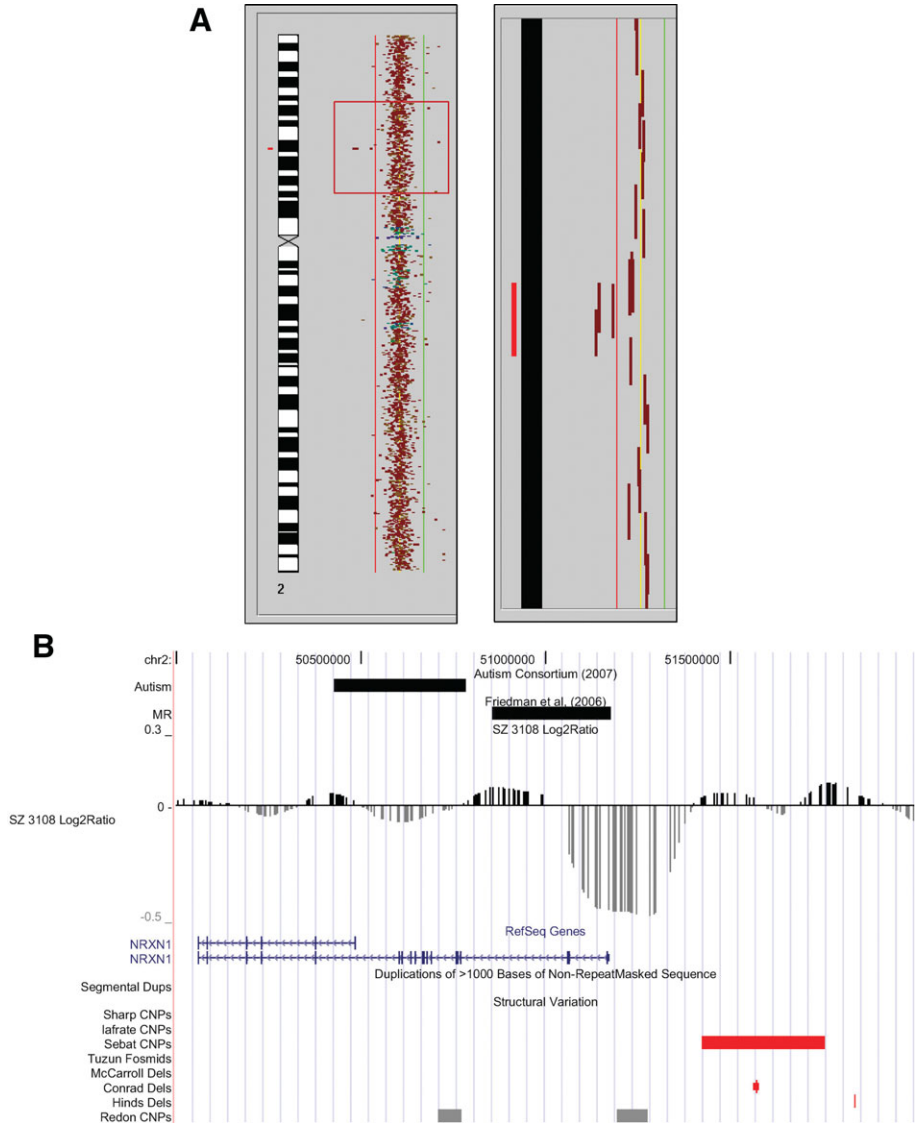


**Figure 2.** Deletion on 2p16.3. (a) Array-CGH image (see legend to Fig. 1A). (b) Validation with Affymetrix 250K array (see legend to Fig. 1B). The only gene in the region is *NRXN1*. The two horizontal bars indicate the regions reported in references (16) and (23), where deletions disrupting the same genes have been reported in MR and autism, respectively.

However, the 15q13.1 gene of greatest apparent interest is *APBA2* which encodes the amyloid precursor-binding protein A2. APBA2 has also been named Mint2 (Munc18 interacting protein 2). Remarkably, APBA2 (Mint2) interacts directly with neurexins. Mint2 is a neuronal adaptor protein that binds directly to neurexins in a PDZ domain-mediated interaction as part of a multi-protein complex including Munc 18, Cask, syntaxin 1 and other members of the SNARE complex that likely functions as an intermediate in neurotransmitter synaptic vesicle docking/fusion (44,45). Indeed, mice lacking both neuron-specific Mint isoforms (Mints 1 and 2) exhibit a decline in spontaneous neurotransmitter release, lowered synaptic strength and enhanced paired-pulse facilitation, suggesting abnormalities of pre-synaptic neurotransmitter release (46). There are a number of lines of evidence, including data from genetic association studies, pointing to SZ being a disorder of the synapse (47). More specifically, abnormalities have been observed in the expression of a number of pre-synaptic markers including various proteins associated with neurotransmitter release (47,48). Our findings suggest that disruption of pre-synaptic mechanisms might be fundamental to the pathophysiology of SZ and suggest that further work might fruitfully focus upon those processes in which NRXN1, MINT2 and associated proteins participate.

Although the statistical support for association of the two putative pathogenic CNVs is necessarily weak given their rarity, the case for their involvement in pathogenesis is strengthened considerably by the fact that they alter copy number of two directly interacting proteins, NRXN1 and APBA2/MINT2, and by the fact that chromosomal abnormalities disrupting the same genes have been reported in both autism and MR. The apparently pleiotropic effect of *NRXN1* deletions and 15q13.1 duplications is of considerable interest. As described in the *Introduction* section, SZ, autism and MR

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share a number of phenotypic features, and all three disorders have a neurodevelopmental basis. Similarly, the chromosome 22q11 deletion syndrome is also associated with increased risk of MR, SZ and autism (14) demonstrating that the same deletion can cause these distinct phenotypes which share certain features. In addition, recent work suggests that autism and SZ might have a more substantial overlap in genetic susceptibility than hitherto believed (49). Our work adds to the evidence that there is a profound commonality in the aetiology and pathogenesis of these disorders and suggests this might involve disruption of neurexins and associated proteins with consequences for synaptic development and function. Further studies of the relevant genes and proteins in all three disorders are called for.

Although autism and SZ share a number of features, they are very distinct phenotypes and are unlikely to be confused. The variable expressivity of *NRXN1* deletions and 15q13.1 duplications, as well as the lack of an overt psychiatric phenotype seen in the mother of the siblings with *NRXN1* deletions, suggest that modifying and compensatory mechanisms are likely to be at work. These might be genetic, environmental or stochastic. While it is premature to speculate further on their nature, the variable outcomes associated with these genomic abnormalities suggest that more detailed understanding the mechanisms involved might point the way to new therapeutic opportunities.

Our findings also suggest that larger studies seeking CNVs in SZ should be conducted. The discovery of further *de novo* variants should be based upon the study of parent–proband trios, and the recent availability of arrays with >1 million probes (e.g. Affymetrix 6.0 array and Illumina human1M BeadChip) should enable much smaller pathogenic aberrations to be identified. It is likely that such studies will identify further genes of potential pathogenic relevance. It is also possible that common copy number polymorphisms (CNPs) will turn out to play a role in conferring susceptibility to SZ as they do in other conditions (50). These studies will require the development of novel platforms capable of highly reliable 'genotyping quality' calling, as well as the use of large patient and control samples.

## MATERIALS AND METHODS

We selected 45 male and 48 female unrelated proband–parent trios from our sample of ~600 Bulgarian SZ trios recruited as described previously (51). Diagnoses in probands and their siblings were made according to DSM-IV criteria, following assessment by a psychiatrist using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (52) which has been validated for use in the Bulgarian language by one of its authors, and hospital notes. Parents were interviewed about history of psychiatric illness in the family. We deliberately chose a combination of severe sporadic cases ($n = 51$) and cases with an affected first-degree relative ($n = 42$, of whom 22 had a sibling with SZ) in order to increase the chances of detecting *de novo* and recurrent aberrations, respectively. In all cases, IQ was >70. The mean age of probands was 33.8 years (SD = 10.1, range 13–57 years). The mean age at onset of psychotic symptoms was 22.1 years (SD = 6.6, range 11–44 years). All patients and their parents received information and signed a consent form for participation in genetic studies. Ethics committee approval was obtained from all regions where families were recruited.

CNVs have recently been shown to be common in the general population as pointed out in the *Introduction* section (7–10). We needed to filter out common CNVs, which have been reported in the general population, thus making them less likely to be pathogenic in SZ. Therefore we compared our findings in SZ with those in the Database of Genomic Variants (http://projects.tcag.ca/variation/) and with data from 372 control DNA samples. These were samples collected from patients affected with disorders that we consider unrelated to SZ, which were analyzed in the same laboratory using the same methodology. DNA of patients affected with the following conditions served as controls: congenital heart disease ($n = 105$), Noonan syndrome ($n = 23$); cleft lip/palate ($n = 12$); Opitz syndrome BBBG ($n = 15$); malformations of the pituitary gland ($n = 66$); or thyroid gland ($n = 76$); amyotrophic lateral sclerosis ($n = 75$).

Proband DNAs were hybridized against pooled DNA from 50 sex-matched healthy controls from Bulgaria. Pools were produced by taking equimolar amounts of DNA from each individual. Array CGH was performed as described previously (53). Briefly, sonicated patient and reference DNA was labelled by random priming (Bioprime Array CGH, Invitrogen, Carlsbad, CA) with Cy3 and Cy5 (Amersham Biosciences, Piscataway, NJ), respectively, and hybridized onto a tiling path BAC array, consisting of ~36 000 BAC clones obtained from several sources (54–56), as described on our website (http://www.molgen.mpg.de/~abt_rop/molecular_cytogenetics/). All protocols are also provided on that website. Details concerning this platform have been submitted to the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/; GLP: 5000 and 5114). On average 33–35 500 BAC clones per array could be analyzed, providing an average functional resolution of 150–200 kb. For the analysis and visualization of array CGH data, our software package CGHPRO (57) was employed. For the assessment of copy number gains and losses, we used conservative log2 ratio thresholds of 0.3 and −0.3, respectively. Deviant signal intensity ratios involving three or more neighbouring BAC clones were considered to be potentially pathogenic, unless they were covered by more than one known DNA copy number variant, as listed in the Database of Genomic Variants (http://projects.tcag.ca/variation/) or covered by >50% of their length at least once in our reference set of 372 samples. All genomic coordinates reported in this work are based on May 2004 UCSC assembly (build 35). The content of LCRs in the BAC clones was determined with a search against the Segmental Duplication Database (http://humanparalogy.gs.washington.edu/). A colour scheme indicating the percentage of LCR content of each clone is employed by the software and this is shown on the left in Figure 1A. Briefly, red clones have no LCRs, whereas blue clones are rich in LCRs and tend to be associated with common CNVs and also with genomic disorders.

CNVs passing our inclusion criteria were validated with Affymetrix 250K SNP arrays (Affymetrix, Santa Clara, CA) and/or Agilent 244K oligonucleotide arrays (Agilent, Santa

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Clara, CA), following the manufacturers' protocols. For patients with validated CNVs, both parents were tested to distinguish familial from *de novo* aberrations. Paternity was examined on the basis of SNP genotypes in the Affymetrix arrays. Where available, affected siblings of probands with CNVs were also examined.

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*Conflict of Interest statement*. None declared.

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