Identification of rare variants in *KCTD13* at the schizophrenia risk locus 16p11.2

Franziska Degenhardt[a,b], Barbara Heinemann[a,b], Jana Strohmaier[g], Marvin A. Pfohl[a,b], Ina Giegling[h,e], Andrea Hofmann[a,b], Kerstin U. Ludwig[a,b], Stephanie H. Witt[g], Michael Ludwig[d], Andreas J. Forstner[a,b], Margot Albus[i], Sibylle G. Schwab[k], Margitta Borrmann-Hassenbach[f], Leonard Lennertz[c], Michael Wagner[c], Per Hoffmann[a,b,l], Dan Rujescu[h,e], Wolfgang Maier[c], Sven Cichon[a,b,j,l], Marcella Rietschel[g] and Markus M. Nöthen[a,b]

Duplications in 16p11.2 are a risk factor for schizophrenia (SCZ). Using genetically modified zebrafish, Golzio and colleagues identified *KCTD13* within 16p11.2 as a major driver of the neuropsychiatric phenotype observed in humans. The aims of the present study were to explore the role of *KCTD13* in the development of SCZ and to provide a more complete picture of the allelic architecture at this risk locus. The exons of *KCTD13* were sequenced in 576 patients. The mutations c.6G >T and c.598G >A were identified in one patient each. Both mutations were predicted to be functionally relevant and were absent from the 1000 Genomes Project data and the Exome Variant Server. The mutation c.6G >T was predicted to abolish a potential transcription factor-binding site for specifity protein 1. Altered specifity protein 1 expression has been reported in SCZ patients compared with controls. Further studies in large cohorts are warranted to determine the relevance of the two identified mutations. *Psychiatr Genet* 26:293–296

Psychiatric Genetics 2016, **26**:293–296

Keywords: autism spectrum disorder, copy number variants, neurodevelopmental, psychosis, schizoaffective

[a]Institute of Human Genetics, [b]Department of Genomics, Life and Brain Center, [c]Department of Psychiatry and Psychotherapy, [d]Department of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Bonn, [e]Department of Psychiatry, Ludwig-Maximilians-University Munich, [f]Kbo Kliniken des Bezirks Oberbayern, Munich, [g]Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Heidelberg, [h]Department of Psychiatry, University of Halle-Wittenberg, Halle, [i]Isar Amper Klinikum München Ost, kbo, Haar, [j]Institute of Neuroscience and Medicine (INM-1), Structural and Functional Organisation of the Brain, Genomic Imaging, Research Centre Juelich, Juelich, Germany, [k]Faculty of Science, Medicine & Health, University of Wollongong, Wollongong, Australia and [l]Department of Biomedicine, Division of Medical Genetics, University Hospital Basel, University of Basel, Basel, Switzerland

Correspondence to Franziska Degenhardt, MD, Department of Genomics, Life and Brain Center, Institute of Human Genetics, Sigmund-Freud-Straße 25, Bonn 53127, Germany
Tel: +49 228 6885 433; fax: +49 228 6885 401;
e-mail: franziska.degenhardt@uni-bonn.de

Received 17 December 2015 **Revised** 17 May 2016 **Accepted** 19 July 2016

Introduction

Schizophrenia (SCZ) is a common and often severely disabling neuropsychiatric disorder. Patients show a variety of symptoms, including hallucinations, delusions, disorganized speech, affective flattening, and avolition (American Psychiatric Association, 1994). The estimated heritability of SCZ ranges between 60 and 80% (Sullivan *et al.*, 2003; Wray and Gottesman, 2012). Previous research has implicated a small number of rare copy number variants (CNVs) from specific chromosomal regions in the risk of SCZ (Malhotra and Sebat, 2012; Sullivan *et al.*, 2012; Rees *et al.*, 2014).

One of these chromosomal regions is 16p11.2. Duplications and deletions in 16p11.2 are particularly interesting as they are implicated in mirrored phenotypes. Deletions in this region are associated with macrocephaly and obesity (Bochukova *et al.*, 2010; Shinawi *et al.*, 2010; Walters *et al.*, 2010; Jacquemont *et al.*, 2011), whereas duplications in 16p11.2 are associated with microcephaly and low BMI (Shinawi *et al.*, 2010; Jacquemont *et al.*, 2011). Furthermore, deletions and duplications in this chromosomal region are established risk factors for developmental delay, intellectual disability, autism spectrum disorder, and epilepsy (Kumar *et al.*, 2008; Weiss *et al.*, 2008; Shinawi *et al.*, 2010; Xiang *et al.*, 2010; Sullivan *et al.*, 2012). However, only duplications in 16p11.2 increase the susceptibility to SCZ (odds ratio ~10; McCarthy *et al.*, 2009; Kirov *et al.*, 2012; Sullivan *et al.*, 2012; Rees *et al.*, 2014; Szatkiewicz *et al.*, 2014).

The CNVs reported to date in 16p11.2 are flanked by segmental duplications and are typically ~600 kb in size, spanning more than 25 genes (Weiss *et al.*, 2008). Several of these are interesting candidate genes for neuropsychiatric phenotypes. Golzio *et al.* (2012) published the results of an analysis in genetically modified zebrafish. The authors generated evidence that *KCTD13* is a major driver of the mirrored neuroanatomical phenotypes of the

0955-8829 Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.

DOI: 10.1097/YPG.0000000000000145

CNVs in 16p11.2 (Malhotra and Sebat, 2012). In zebrafish, overexpression of the *KCTD13* human transcript caused microcephaly, which resembled the microcephaly phenotype associated with the 16p11.2 duplication. In contrast, inhibition of *KCTD13* expression caused a macrocephalic phenotype, which has been associated previously with the 16p11.2 deletion (Golzio *et al.*, 2012; Malhotra and Sebat, 2012). In addition, *KCTD13* is located in one of the 108 genome-wide significant loci reported in the largest SCZ genome-wide association study worldwide (36 989 patients and 113 075 controls; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

The aims of the present study were to explore the role of *KCTD13* in the development of SCZ and to provide a more complete picture of the allelic architecture at the 16p11.2 risk locus. The identification of rarer variants in this gene might provide genetic evidence for the role of *KCTD13* in susceptibility to SCZ. Furthermore, rarer variants with higher penetrance might be more suitable for functional follow-up studies than common variants with small effects.

Methods

The study was approved by the respective ethics committees and all participants provided written informed consent before inclusion. All study procedures were carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants were of German descent according to self-reported ancestry.

Sample description

In total, 576 patients were included. The patients were recruited from consecutive admissions to psychiatric inpatient units in Germany. A lifetime 'best estimate' diagnosis (Leckman *et al.*, 1982) of SCZ according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed., criteria (American Psychiatric Association, 1994) was assigned on the basis of the medical records, family history, and the Structured Clinical Interview (Spitzer *et al.*, 1992) and/or the OPCRIT (McGuffin *et al.*, 1991). Each individual was of German descent according to self-reported ancestry.

Sanger sequencing

Primer design was based on the NCBI37/hg19 reference sequence (Ensembl transcript ID: ENST00000568000). All six coding exons and their flanking sequences (± 30 bp of each exon analyzed) were amplified. Exons 3 and 4 were grouped together in one amplicon. Sanger sequencing was performed in part at Beckman Coulter Genomics (Takeley, UK) and in part at the Institute of Human Genetics in Bonn. The variants identified were confirmed at the Institute of Human Genetics in Bonn by sequencing the complementary strand of a second, independent amplicon. For the verification step, the 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California, USA) was used. The nucleotide sequences obtained were analyzed using SeqMan II (DNASTAR, Madison, Wisconsin, USA). Primer sequences are obtainable upon request.

Analysis of sequence variants

To predict the effect of an amino-acid change on protein function, scores from the following three programs were used: MutationTaster (Schwarz *et al.*, 2010; *http://mutationtaster.org/*); PolyPhen-2, version 2.2.2 (Adzhubei *et al.*, 2010; *http://genetics.bwh.harvard.edu/pph2/*); and SIFT (Ng and Henikoff, 2001; *http://sift.jcvi.org/*). To obtain information on transcription-binding sites that might be altered by the identified mutations, a search was performed of the TRANSFAC public database (Wingender *et al.*, 1996; *http://www.gene-regulation.com/pub/databases.html*).

To maximize the number of patients included in the sequencing step, no controls were sequenced and publically available datasets were used to calculate the allele frequency of the identified variants. The allele frequency of the identified variants was checked in the 1000 Genomes Project data (Abecasis *et al.*, 2010; Total European Ancestry EUR; *http://www.1000genomes.org/*), and the Exome Variant Server (European American population; Exome Variant Server, NHLBI GO Exome Sequencing Project, Seattle, Washington, USA (*http://evs.gs.washington.edu/EVS/*) (November 2015).

Results

High-quality sequencing data were obtained from (i) 552 patients for exon 1; (ii) 554 patients for exon 2; (iii) 563 patients for exons 3, 4, and 5; and (iv) 571 patients for exon 6. Two variants were identified and verified in one patient each: (i) c.6G>T in exon 1 and (ii) c.598G>A in exon 5. These variants were present in neither the 1000 Genomes Project data nor the Exome Variant Server. No additional variants were identified in our sample.

The mutation c.6G>T in exon 1 is a synonymous substitution, which was in-silico predicted to be disease causing (MutationTaster). According to TRANSFAC (Wingender *et al.*, 1996), the alteration in the DNA sequence abolishes a potential transcription factor-binding site for *Sp1*. The nonsynonymous substitution c.598G>A in exon 5 p.Asp200Asn was predicted to be functionally relevant by Polyphen-2 (probably damaging); SIFT (damaging); and MutationTaster (disease causing).

No parental DNA was available to test whether the mutation c.6G>T in exon 1 was inherited or *de novo*. The mutation c.598G>A in exon 5 had not been inherited from the respective patient's mother. However, the patient's brother was shown to carry the same mutation. No paternal DNA was available for testing. The brother of the patient was diagnosed with recurrent major depression, agoraphobia, and an unspecified eating disorder. No information on the head size of the mutation carriers was available.

Discussion

The synonymous mutation c.6G>T was in-silico predicted to abolish a potential transcription factor-binding site for specifity protein 1 (SP1). The zinc finger transcription factor SP1 is located on chromosome 12q13.13 and regulates the expression of a number of genes by binding to GC-rich sequences (Suske, 1999). Several studies have reported altered *SP1* expressions in patients with SCZ compared with controls (Ben-Shachar and Karry, 2007; Fusté *et al.*, 2013; Pinacho *et al.*, 2013, 2014). In a small sample of first-episode psychosis patients, Fusté *et al.* (2013) found reduced SP1 protein levels in mononuclear cells from peripheral blood (Fusté *et al.*, 2013). Ben-Shachar and Karry (2007) carried out postmortem expression analyses in various human brain regions. The authors identified significantly decreased *SP1* messenger RNA levels in the prefrontal cortex and in the striatum, with increased levels in the ventral parieto-occipital cortex and in lymphocytes (Ben-Shachar and Karry, 2007). Pinacho *et al.* (2014) reported significantly increased *SP1* messenger RNA expression levels in the postmortem hippocampus of patients with chronic SCZ (Pinacho *et al.*, 2014).

The mutation c.6G>T is not reported in either the 1000 Genomes Project data or the Exome Variant Server (total of ~4.700 individuals). In the European (non-Finnish) sample ascertained by the Exome Aggregation Consortium (ExAC) (Cambridge, Massachusetts, USA) (*http://exac.broadinstitute.org*), the c.6G>T mutation was detected in 43 of 7838 European individuals (allele frequency = 0.003). Notably, ExAC includes data from the 1000 Genomes Project, the Exome Variant Server [NHLBI GO Exome Sequencing Project, and sequencing studies in patients with psychiatric disorders. The mutation was not detected in the Schizophrenia Exome Sequencing Genebook (Purcell *et al.*, 2014; *http://atgu.mgh.harvard.edu/~spurcell/genebook/genebook.cgi?user=guest&cmd=search-gene&tbox=KCTD13*)], which contains the exome sequencing data of 2536 SCZ patients and 2543 controls.

The nonsynonymous substitution c.598G>A in exon 5 (p.Asp200Asn) is not reported in the 1000 Genomes Project data or the Exome Variant Server. In the European (non-Finnish) ExAC sample, the mutation c.598G>A was identified in 3 of 33 154 individuals (allele frequency = 0.00005). In the Swedish SCZ exome-sequencing study, this mutation was identified in one patient and in one control (Purcell *et al.*, 2014; *http://atgu.mgh.harvard.edu/~spurcell/genebook/genebook.cgi?user=guest&cmd=search-gene&tbox=KCTD13*). At the protein level, the G>A substitution causes an exchange of the charged acidic amino-acid aspartic acid to the polar uncharged amino acid asparagine. Moreover, Asp200 is strictly conserved at its corresponding position in *KCTD13* as far down as *Danio rerio* (data from Swiss-Prot).

To date, exome sequencing data from eight studies analyzing de-novo mutations in more than 850 patients with SCZ have been published (Girard *et al.*, 2011; Xu *et al.*, 2011, 2012; Gulsuner *et al.*, 2013; Fromer *et al.*, 2014; Guipponi *et al.*, 2014; McCarthy *et al.*, 2014; Kranz *et al.*, 2015). None of these studies reported a mutation in *KCTD13*.

The present study has three main limitations. First, the sequencing of *KCTD13* was restricted only to patients. During the project design phase, we opted to sequence *KCTD13* in as many patients as possible, rather than reducing the number of patients to cover the cost of sequencing controls. Second, we could not determine the phenotype of the mutation carriers identified in ExAC. This hampers the interpretation of the allele frequency reported for the two mutations identified in the present analyses. In particular, information on the variant carriers' head size and their mental wellbeing would have improved the data interpretation. Third, we focused our analysis on exonic variants and therefore cannot rule out the presence of phenotypically relevant mutations in regulatory regions. Furthermore, *KCTD13* is an interesting candidate gene on the basis of a study in genetically modified zebrafish (Golzio *et al.*, 2012). Therefore, we cannot rule out that genetic variants in a/several other genes located in 16p11.2 contribute toward the neuropsychiatric phenotype observed among human CNV carriers. Future studies sequencing all genes located in 16p11.2 are warranted to obtain more information on their relevance to disease pathogenesis.

The lack of an association between single base pair mutations in *KCTD13* and SCZ, both in the present study and in the previously published exome sequencing data, may indicate that rare point mutations in this gene do not contribute toward the genetic architecture of SCZ, or alternatively, that mutations in this gene are extremely rare. Our study generated no strong evidence for the involvement of damaging mutations in *KCTD13* in the development of SCZ. Therefore, the relevance of the identified rare mutations in *KCTD13* remains unclear. Further studies in large, independent cohorts are now warranted.

Acknowledgements

The authors thank all patients for participating in this study. They also thank the NHLBI GO Exome Sequencing Project and its ongoing studies, which produced and provided exome variant calls for comparison: the Lung GO Sequencing Project (HL-102923), the WHI Sequencing Project (HL-102924), the Broad GO Sequencing Project (HL-102925), the Seattle GO Sequencing Project (HL-102926), and the Heart GO Sequencing Project (HL-103010). And they would also like to thank the Exome Aggregation Consortium and the groups that provided exome variant data for comparison. A full list of contributing groups can be found at *http://exac.broadinstitute.org/about*. Furthermore, they thank Rainald Mössner for the provision of DNA samples.

This study was funded by the German Federal Ministry of Education and Research (BMBF) through: (i) the Integrated

Genome Research Network (IG) MooDS (Systematic Investigation of the Molecular Causes of Major Mood Disorders and Schizophrenia; grant 01GS08144 to Markus M. Nöthen and Sven Cichon; grant 01GS08147 to Marcella Rietschel) under the auspices of the National Genome Research Network plus (NGFNplus); and (ii) the Integrated Network IntegraMent (Integrated Understanding of Causes and Mechanisms in Mental Disorders; grant 01ZX1314G and 01ZX1314A), under the auspices of the e:Med Programme. Markus M. Nöthen received support from the Alfried Krupp von Bohlen und Halbach-Stiftung, and is a member of the DFG-funded Excellence Cluster ImmunoSensation. Marcella Rietschel was supported by the 7th Framework Programme of the European Union (ADAMS project, HEALTH-F4-2009-242257; CRESTAR project, HEALTH-2011-1.1-2). Franziska Degenhardt and Barbara Heinemann received support from the BONFOR Programme of the University of Bonn, Germany.

Conflicts of interest

There are no conflicts of interest.

References

Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA, *et al.*, 1000 Genomes Project Consortium (2010). A map of human genome variation from population-scale sequencing. *Nature* **467**:1061–1073.

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, *et al.* (2010). A method and server for predicting damaging missense mutations. *Nat Methods* **7**:248–249.

American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental Disorders*. Washington, DC: American Psychiatric Association.

Ben-Shachar D, Karry R (2007). Sp1 expression is disrupted in schizophrenia; a possible mechanism for the abnormal expression of mitochondrial complex I genes, NDUFV1 and NDUFV2. *PLoS One* **2**:e817.

Bochukova EG, Huang N, Keogh J, Henning E, Purmann C, Blaszczyk K, *et al.* (2010). Large, rare chromosomal deletions associated with severe early-onset obesity. *Nature* **463**:666–670.

Fromer M, Pocklington AJ, Kavanagh DH, Williams HJ, Dwyer S, Gormley P, *et al.* (2014). De novo mutations in schizophrenia implicate synaptic networks. *Nature* **506**:179–184.

Fusté M, Pinacho R, Meléndez-Pérez I, Villalmanzo N, Villalta-Gil V, Haro JM, Ramos B (2013). Reduced expression of SP1 and SP4 transcription factors in peripheral blood mononuclear cells in first-episode psychosis. *J Psychiatr Res* **47**:1608–1614.

Girard SL, Gauthier J, Noreau A, Xiong L, Zhou S, Jouan L, *et al.* (2011). Increased exonic de novo mutation rate in individuals with schizophrenia. *Nat Genet* **43**:860–863.

Golzio C, Willer J, Talkowski ME, Oh EC, Taniguchi Y, Jacquemont S, *et al.* (2012). KCTD13 is a major driver of mirrored neuroanatomical phenotypes of the 16p11.2 copy number variant. *Nature* **485**:363–367.

Guipponi M, Santoni FA, Setola V, Gehrig C, Rotharmel M, Cuenca M, *et al.* (2014). Exome sequencing in 53 sporadic cases of schizophrenia identifies 18 putative candidate genes. *PLoS One* **9**:e112745.

Gulsuner S, Walsh T, Watts AC, Lee MK, Thornton AM, Casadei S, *et al.* (2013). Spatial and temporal mapping of de novo mutations in schizophrenia to a fetal prefrontal cortical network. *Cell* **154**:518–529.

Jacquemont S, Reymond A, Zufferey F, Harewood L, Walters RG, Kutalik Z, *et al.* (2011). Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. *Nature* **478**:97–102.

Kirov G, Pocklington AJ, Holmans P, Ivanov D, Ikeda M, Ruderfer D, *et al.* (2012). De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. *Mol Psychiatry* **17**:142–153.

Kranz TM, Harroch S, Manor O, Lichtenberg P, Friedlander Y, Seandel M, *et al.* (2015). De novo mutations from sporadic schizophrenia cases highlight important signaling genes in an independent sample. *Schizophr Res* **166**:119–124.

Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, *et al.* (2008). Recurrent 16p11.2 microdeletions in autism. *Hum Mol Genet* **17**:628–638.

Leckman JF, Sholomskas D, Thompson WD, Belanger A, Weissman MM (1982). Best estimate of lifetime psychiatric diagnosis: a methodological study. *Arch Gen Psychiatry* **39**:879–883.

Malhotra D, Sebat J (2012). CNVs: harbingers of a rare variant revolution in psychiatric genetics. *Cell* **148**:1223–1241.

McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S, *et al.* (2009). Microduplications of 16p11.2 are associated with schizophrenia. *Nat Genet* **41**:1223–1227.

McCarthy SE, Gillis J, Kramer M, Lihm J, Yoon S, Berstein Y, *et al.* (2014). De novo mutations in schizophrenia implicate chromatin remodeling and support a genetic overlap with autism and intellectual disability. *Mol Psychiatry* **19**:652–658.

McGuffin P, Farmer A, Harvey I (1991). A polydiagnostic application of operational criteria in studies of psychotic illness. Development and reliability of the OPCRIT system. *Arch Gen Psychiatry* **48**:764–770.

Ng PC, Henikoff S (2001). Predicting deleterious amino acid substitutions. *Genome Res* **11**:863–874.

Pinacho R, Villalmanzo N, Roca M, Iniesta R, Monje A, Haro JM, *et al.* (2013). Analysis of Sp transcription factors in the postmortem brain of chronic schizophrenia: a pilot study of relationship to negative symptoms. *J Psychiatr Res* **47**:926–934.

Pinacho R, Valdizán EM, Pilar-Cuellar F, Prades R, Tarragó T, Haro JM, *et al.* (2014). Increased SP4 and SP1 transcription factor expression in the postmortem hippocampus of chronic schizophrenia. *J Psychiatr Res* **58**:189–196.

Purcell SM, Moran JL, Fromer M, Ruderfer D, Solovieff N, Roussos P, *et al.* (2014). A polygenic burden of rare disruptive mutations in schizophrenia. *Nature* **506**:185–190.

Rees E, Walters JT, Georgieva L, Isles AR, Chambert KD, Richards AL, *et al.* (2014). Analysis of copy number variations at 15 schizophrenia-associated loci. *Br J Psychiatry* **204**:108–114.

Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**:421–427.

Schwarz JM, Rödelsperger C, Schuelke M, Seelow D (2010). MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods* **7**:575–576.

Shinawi M, Liu P, Kang SH, Shen J, Belmont JW, Scott DA, *et al.* (2010). Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. *J Med Genet* **47**:332–341.

Spitzer RL, Williams JB, Gibbon M, First MB (1992). The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. *Arch Gen Psychiatry* **49**:624–629.

Sullivan PF, Kendler KS, Neale MC (2003). Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* **60**:1187–1192.

Sullivan PF, Daly MJ, O'Donovan M (2012). Genetic architectures of psychiatric disorders: the emerging picture and its implications. *Nat Rev Genet* **13**:537–551.

Suske G (1999). The Sp-family of transcription factors. *Gene* **238**:291–300.

Szatkiewicz JP, O'Dushlaine C, Chen G, Chambert K, Moran JL, Neale BM, *et al.* (2014). Copy number variation in schizophrenia in Sweden. *Mol Psychiatry* **19**:762–773.

Walters RG, Jacquemont S, Valsesia A, de Smith AJ, Martinet D, Andersson J, *et al.* (2010). A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. *Nature* **463**:671–675.

Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, *et al.* (2008). Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med* **358**:667–675.

Wingender E, Dietze P, Karas H, Knüppel R (1996). TRANSFAC: a database on transcription factors and their DNA binding sites. *Nucleic Acids Res* **24**:238–241.

Wray NR, Gottesman II (2012). Using summary data from the Danish national registers to estimate heritabilities for schizophrenia, bipolar disorder, and major depressive disorder. *Front Genet* **3**:118.

Xiang B, Zhu H, Shen Y, Miller DT, Lu K, Hu X, *et al.* (2010). Genome-wide oligonucleotide array comparative genomic hybridization for etiological diagnosis of mental retardation: a multicenter experience of 1499 clinical cases. *J Mol Diagn* **12**:204–212.

Xu B, Roos JL, Dexheimer P, Boone B, Plummer B, Levy S, *et al.* (2011). Exome sequencing supports a de novo mutational paradigm for schizophrenia. *Nat Genet* **43**:864–868.

Xu B, Ionita-Laza I, Roos JL, Boone B, Woodrick S, Sun Y, *et al.* (2012). De novo gene mutations highlight patterns of genetic and neural complexity in schizophrenia. *Nat Genet* **44**:1365–1369.