ORIGINAL ARTICLE

Recurrent Rearrangements in Synaptic and Neurodevelopmental Genes and Shared Biologic Pathways in Schizophrenia, Autism, and Mental Retardation

Audrey Guilmatre, PhD; Christèle Dubourg, PhD; Anne-Laure Mosca, MD; Solenn Legallic, BSc; Alice Goldenberg, MD; Valérie Drouin-Garraud, MD; Valérie Layet, MD; Antoine Rosier, MD; Sylvain Briault, MD; Frédérique Bonnet-Brilhault, MD, PhD; Frédéric Laumonnier, PhD; Sylvie Odent, MD, PhD; Gael Le Vacon, MD; Géraldine Joly-Helas, MD; Véronique David, MD; Claude Bendavid, MD; Jean-Michel Pinoit, MD; Céline Henry, MD; Caterina Impallomeni, MD; Eva Germano, MD; Gaetano Tortorella, MD; Gabriella Di Rosa, MD; Catherine Barthelemy, MD; Christian Andres, MD; Laurence Faivre, MD, PhD; Thierry Frébourg, MD, PhD; Pascale Saugier Veber, PhD; Dominique Campion, MD, PhD

Context: Results of comparative genomic hybridization studies have suggested that rare copy number variations (CNVs) at numerous loci are involved in the cause of mental retardation, autism spectrum disorders, and schizophrenia.

Objectives: To provide an estimate of the collective frequency of a set of recurrent or overlapping CNVs in 3 different groups of cases compared with healthy control subjects and to assess whether each CNV is present in more than 1 clinical category.

Design: Case-control study.

Setting: Academic research.

Participants: We investigated 28 candidate loci previously identified by comparative genomic hybridization studies for gene dosage alteration in 247 cases with mental retardation, in 260 cases with autism spectrum disorders, in 236 cases with schizophrenia or schizoaffective disorder, and in 236 controls.

Main Outcome Measures: Collective and individual frequencies of the analyzed CNVs in cases compared with controls.

Results: Recurrent or overlapping CNVs were found in cases at 39.3% of the selected loci. The collective frequency of CNVs at these loci is significantly increased in cases with autism, in cases with schizophrenia, and in cases with mental retardation compared with controls ($P < .001$, $P = .01$, and $P = .001$, respectively, Fisher exact test). Individual significance ($P = .02$ without correction for multiple testing) was reached for the association between autism and a 350-kilobase deletion located at 22q11 and spanning the *PRODH* and *DGCR6* genes.

Conclusions: Weakly to moderately recurrent CNVs (transmitted or occurring de novo) seem to be causative or contributory factors for these diseases. Most of these CNVs (which contain genes involved in neurotransmission or in synapse formation and maintenance) are present in the 3 pathologic conditions (schizophrenia, autism, and mental retardation), supporting the existence of shared biologic pathways in these neurodevelopmental disorders.

Arch Gen Psychiatry. 2009;66(9):947-956

Author Affiliations are listed at the end of this article.

THE DEVELOPMENT OF MICROarray-based technologies for comparative genomic hybridization (array-CGH) analysis has enabled the detection of submicroscopic microdeletions or microduplications, also referred to as copy number variations (CNVs). Recently, this approach has been widely used in neurologic and psychiatric disorders, including mental retardation (MR),[1-3] autism spectrum disorders (ASDs),[4-7] and schizophrenia.[8-11] Findings from these studies suggested that several genes involved in similar neurodevelopmental pathways may be associated with these conditions. However, so far only rare structural variants, sometimes present in a single case, have been identified. Therefore, it is difficult to decipher which of these variations are causative, which are risk factors, and which are only rare polymorphisms unrelated to any pathologic phenotype. De novo rearrangements are usually considered pathogenic, but this argument (which is acceptable for rare large

Table 1. Demographic and Clinical Features of the Sample

Study Group	Age, Mean (SD), y	Percentage			Geographic Ancestry
		Male	Familial	Syndromic	
ASDs (n=260)	11.8 (7.6)	80.5	9.6	8.5	France (n=231), Italy (n=29)
MR (n=247)	13.2 (11.6)	64.8	33.6	62.0	Northwestern France
SZ (n=236)	38.1 (11.2)	66.8	23.7	. . .	Northwestern France
Controls (n=236)	39.5 (16.8)	43.8	. . .	. . .	Northwestern France

Abbreviations: ASDs, autism spectrum disorders; ellipses, not applicable; MR, mental retardation; SZ, schizophrenia or schizoaffective disorder.

rearrangements detectable by conventional cytogenetics) should be considered with caution for smaller CNVs, for which a high mutation rate is expected. Indeed, it has been estimated that a de novo segmental deletion occurs in 1 per 8 newborns and a de novo segmental duplication in 1 per 50 newborns, with most of these rearrangements being benign polymorphic variants.[12] Therefore, the disease association of CNVs has to be tested systematically by comparing the frequency of each candidate CNV in cases and in healthy control subjects. Given the low frequency of each CNV, this would require the study of huge series, achievable only in the context of forthcoming meta-analyses. Other problems arise because ascertainment of most of the published samples, initially recruited for linkage studies, is biased toward multiplex cases and because control samples, when present, are generally composed of subjects not screened for the studied pathologic conditions. The objectives of the present study were (1) to provide an estimate of the collective frequency of a set of recurrent or overlapping CNVs in 3 different groups of cases compared with controls and (2) to assess whether each CNV is present in more than 1 clinical category.

METHODS

ASCERTAINMENT AND DIAGNOSES

Cases with schizophrenia and cases with MR were ascertained at University Hospital, Rouen, France, from consecutive hospitalizations in patients with schizophrenia or from consecutive referrals for phenotypic and genetic investigations in patients with intellectual disability. The ASD sample included cases ascertained from consecutive consultations at 4 units specializing in autism diagnosis and evaluation located in Rouen, Tours, and Dijon (France) and in Messina (Italy), as well as cases directly referred by the French Autism Foundation. Controls, all ascertained at University Hospital, Rouen, were screened using a standardized data sheet derived from the Schedule for Affective Disorders and Schizophrenia[13] and were required to be free of any psychotic disorder or MR themselves or in their first-degree relatives. All psychiatric diagnoses were established according to *DSM-IV* criteria following review of case notes and direct examination of cases. The Schedule for Affective Disorders and Schizophrenia[13] was used for the clinical assessment of all cases with schizophrenia or schizoaffective disorder. The Autism Diagnostic Interview–Revised,[14] the Autism Diagnostic Observation Schedule–Generic,[15] or the Childhood Autism Rating Scale[16] was used for 83.0% of cases with ASDs (100.0% of cases having ASDs with CNVs). Evaluation of IQs was performed using standardized neuropsychological tests (ie, validated mental age–appropriate Weschler scales [Wechsler Preschool and Primary Scale of Intelligence, Wechsler Intelligence Scale for Children, or Wechsler Adult Intelligence Scale]).

The schizophrenia group included 189 cases with schizophrenia and 47 cases with schizoaffective disorder. Postmorbid IQs were available for two-thirds of cases with schizophrenia; 18.0% of these cases had an IQ lower than 70. The ASD group included 257 cases with autism and 3 cases with Asperger syndrome. The MR group included 235 cases with MR and 12 cases with developmental language disorder. All cases with MR and two-thirds of cases with ASDs were examined by an experienced clinical geneticist (A.G., V.D.-G., V.L., F.B.-B., S.O., L.F., or G.D.R.) and were screened for fragile X mutation and karyotype abnormalities. Cases with large chromosomal anomalies, fragile X syndrome, or other established syndromes were excluded. Cases with common environmental causes of MR such as fetal alcohol syndrome or birth complications were also excluded. Additional clinical features, including intrauterine or postnatal growth retardation and dysmorphic features or malformations, were present in 8.5% of cases with ASDs and in 62.0% of cases with MR. Demographic characteristics of the sample, including 979 unrelated white non-Hispanic subjects from France or Italy, are summarized in **Table 1**.

After written informed consent, blood samples were drawn from all included participants and whenever possible from parents and affected relatives of cases. Ethics committee approval was obtained from all regions where families were recruited.

CANDIDATE GENES AND ANALYSIS BY QUANTITATIVE MULTIPLEX POLYMERASE CHAIN REACTION OF SHORT FLUORESCENT FRAGMENTS

A MEDLINE search using the terms *CNV*, *schizophrenia*, *autism*, and *mental retardation* allowed us to select nonexhaustively a set of 28 loci with microrearrangements characterized by prior array-CGH analyses, often in a single case. This set included major candidate CNV loci identified in cases with ASDs and schizophrenia before April 2008, as well as 8 functionally related CNV loci identified in MR (**Table 2**). Each locus generally contained a single disease–associated CNV, but in some cases, overlapping CNVs with different boundaries had been described in cases. The gene content of these loci ranged from 1 to 28. At each locus, at least 1 candidate gene had been previously suggested in the seminal studies and was retained for the present analysis. Functionally, most of these candidate genes can be classified in 2 main categories related to synapse formation and maintenance or to neurotransmission.

Copy number variation at each locus was assessed by quantitative multiplex polymerase chain reaction (PCR) of short fluorescent fragments (QMPSF), a method based on the simultaneous amplification of several short genomic fragments under quantitative conditions.[44] For each locus,

Table 2. Candidate Regions and Genes Selected for Analysis by Quantitative Multiplex Polymerase Chain Reaction of Short Fluorescent Fragments

Chromosomal Location	Type	Candidate Genes	Pathways	Function	Evidence	Disorder	Selected Sources
2p16.3	Loss	*NRXN1*	Synapse formation and maintenance	Cell adhesion molecule	CR	ASDs, SZ, MR	Friedman et al,[1] 2006; Marshall et al,[4] 2008; Szatmari et al,[7] 2007; Walsh et al,[8] 2008; Kirov et al,[10] 2008; Kim et al,[27] 2008; Zahir et al,[28] 2008
2q12.3-q14.2	Loss/ gain	*DPP10*	Neurotransmission	Dipeptidyl peptidase	CR	ASDs	Marshall et al,[4] 2008
2q33.3-q34	Loss	*ERBB4*	Synapse formation and maintenance	Neuregulin 1 receptor	CR, M	SZ	Walsh et al,[8] 2008
3p26-p25	Loss/ gain	*CNTN4*	Synapse formation and maintenance	Axon-associated cell adhesion molecule	CR, M	ASDs, MR	Dijkhuizen et al,[29] 2006; Roohi et al,[30] 2009
3p26.1-p25.2	Loss	*GRM7*	Neurotransmission	Glutamate receptor	CR	SZ	Walsh et al,[8] 2008
3q26.31	Gain	*NLGN1*	Synapse formation and maintenance	Cell adhesion molecule	CR	MR	Meins et al,[31] 2005
4p14-q21.1	Gain	*GABRG1*, *GABRA4*, *GABRA2*	Neurotransmission	GABA receptor subunits	CR	ASDs	Marshall et al,[4] 2008; Kakinuma et al,[32] 2008
5p13	Loss	*SLC1A3*	Neurotransmission	Excitatory amino acid transporter	CR, M	SZ	Walsh et al,[8] 2008
7q31.1	Loss	*ST7*	Other	Tumor suppressor	CR, M	ASDs	Marshall et al,[4] 2008
7q35-q36	Loss	*CNTNAP2*	Synapse formation and maintenance	Contactin-associated protein	CR, M	ASDs, SZ, MR, TS	Friedman et al,[33] 2008
8p22-p11	Loss	*NRG1*	Synapse formation and maintenance	Signaling protein	CR	MR	Klopocki et al,[34] 2006
8p23	Gain	*DLGAP2*	Neurotransmission	Associated with NMDA receptor and potassium channels	CR	ASDs	Marshall et al,[4] 2008
8q24-qter	Gain	*PTK2*	Neuronal migration and growth	Focal adhesion kinase	CR	SZ	Walsh et al,[8] 2008
11q21	Loss	*DLG2*	Synapse formation and maintenance	Synaptic scaffolding protein	CR	SZ	Walsh et al,[8] 2008
12q14.3	Loss	*GRIP1*	Neurotransmission	Glutamate receptor–interacting protein	CR	MR	Friedman et al,[1] 2006; Menten et al,[3] 2006
15q11-q14	Gain	*GABRA5*, *GABRB3*, *GABRG3*	Neurotransmission	GABA receptor subunits	CR	ASDs, MR	Bolton et al,[35] 2001; Cook et al,[36] 1997
15q13	Gain	*APBA2*	Neurotransmission	Synaptic exocytosis	CR	ASDs, SZ	Christian et al,[6] 2008; Kirov et al,[10] 2008
15q13.3	Loss	*CHRNA7*	Neurotransmission	Acetylcholine receptor	CR	MR	Sharp et al,[37] 2008
16p11.2	Loss/ gain	*DOC2A*	Neurotransmission	Neurotransmitter release regulation	CR	ASDs, SZ	Marshall et al,[4] 2008; Christian et al,[6] 2008; Walsh et al,[8] 2008; Weiss et al,[25] 2008; Kumar et al,[26] 2008
17q21	Loss/ gain	*MAPT*	Other	Microtubule-associated protein	CR	MR	Kirchhoff et al,[22] 2007; Shaw-Smith et al,[24] 2006
22q11	Loss	*PRODH*	Neuromodulation	Proline dehydrogenase	CR, M	ASDs, SZ, MR	Jacquet et al,[17] 2003
22q13	Loss	*SHANK3*	Synapse formation and maintenance	Synaptic scaffolding protein	CR, M	ASDs, MR	Marshall et al,[4] 2008; Durand et al,[18] 2007; Moessner et al,[19] 2007; Wilson et al,[20] 2003
Xp22.3	Loss/ gain	*NLGN4*	Synapse formation and maintenance	Cell adhesion molecule	CR, M	ASDs, TS, MR	Marshall et al,[4] 2008; Chocholska et al,[38] 2006; Kent et al,[39] 2008; Lawson-Yuen et al,[40] 2008; Macarov et al,[41] 2007
Xp11.4	Gain	*TSPAN7*	Neuronal migration and growth	Transmembrane component	CR, M	ASDs, MR	Marshall et al,[4] 2008; Tzschach et al,[21] 2008
Xp11.4	Loss	*CASK*	Synapse formation and maintenance	Synaptic scaffolding protein	CR	MR	Froyen et al,[2] 2007
Xp22.1-p21.3	Loss	*IL1RAPL1*	Other	Interleukin receptor	CR, M	ASDs, MR	Marshall et al,[4] 2008; Jin et al,[42] 2000
Xq25	Gain	*GRIA3*	Neurotransmission	Kainate receptor subunit	CR	MR	Chiyonobu et al,[23] 2007
Xq28	Gain	*MECP2*	Other	Methylated CpG–binding protein	CR, M	MR	Meins et al,[31] 2005; del Gaudio et al,[43] 2006

Abbreviations: ASDs, autism spectrum disorders; CR, chromosomal rearrangement; GABA, γ-aminobutyric acid; M, mutation; MR, mental retardation; NMDA, *N*-methyl-D-aspartate; SZ, schizophrenia or schizoaffective disorder; TS, Tourette syndrome.

amplicons were designed in the coding sequence of selected candidate genes. All assays were grouped in 3 multiplex PCR experiments that included 10 short genomic fragments (range, 100-301 base pair) each. Primer sequences and PCR conditions are summarized in eTable 1 (http://www.archgenpsychiatry.com). DNA fragments generated by QMPSF were separated on a sequencer (ABI Prism 3100; Applied Biosystems, Norwalk, Connecticut), and the resulting fluorescence profiles were analyzed using commercially available software (Gene Scan 3.7 software; Applied Biosystems). For each case, the QMPSF profile was superimposed on that generated from a control by adjusting the same level that the peak obtained for a control amplicon corresponding to a short exonic fragment of the *PBGD* gene. When a CNV was detected, further analyses aiming to confirm and delineate the size of the rearrangements were performed using additional dedicated QMPSF assays (eFigure 1), array-CGH, or fluorescence in situ hybridization analyses.

OLIGONUCLEOTIDE ARRAY-CGH

Oligonucleotide array-CGH was performed using a commercially available array (human genome CGH microarray 4×44K) (Agilent Technologies, Santa Clara, California). This array contains 60-mer oligonucleotide probes (n=44 290) covering the whole genome, with a mean spatial resolution of approximately 30 to 35 kilobases (kb). Eighty-four percent of the probes reside in intragenic regions, and more than 30 000 genes are each represented by at least 1 probe. All experiments were performed using the June 2006 version of the protocol (version 4.0, Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis; Agilent Technologies).

FLUORESCENCE IN SITU HYBRIDIZATION

Fluorescence in situ hybridization analyses were performed on metaphase spreads obtained from peripheral lymphocytes from the cases. Selected human genomic bacterial artificial chromosome clones were obtained from a distribution center (BACPAC Resources Center, Oakland, California [http://bacpac.chori.org]).

DNA SEQUENCING AND PATERNITY CHECKING

Sequence analysis of the coding exons of the *PRODH* (OMIM_606810) gene was performed using primers and PCR conditions previously described[45] via an automated sequencer (model 3100; Applied Biosystems). Paternity was checked by microsatellite typing.

DETERMINATION OF PLASMA PROLINE LEVEL

Plasma proline levels in cases were determined after overnight fasting. All samples were analyzed using ion exchange chromatography (LC 3000 system; Biotronik, Maintal, Germany).

STATISTICAL ANALYSIS

Categorical variables were compared using the Fisher exact test. Two hypotheses were tested. First, the distribution of the collective set of recurrent or overlapping CNVs found in each disease group of cases was compared with that found in controls (3 tests). Second, the distribution of each recurrent or overlapping CNV present in our population was compared between each disease group of cases and controls (33 tests). *P* values are reported without Bonferroni correction.

RESULTS

DISEASE-ASSOCIATED CNVs

Among 743 cases, the proportion of recurrent or overlapping CNVs identified among 28 selected loci (Table 2) was 11 of 28 (39.3%). Their collective frequency was 10 per 236 cases with schizophrenia (4.2%), 16 per 260 cases with ASDs (6.2%), and 13 per 247 cases with MR (5.3%) vs 1 per 236 controls (0.4%), demonstrating a significant excess of these CNVs in each disease group compared with controls ($P=.01$, $P<.001$, and $P=.001$, respectively, Fisher exact test) (**Table 3**, eTable 2, and eTable 3). None of the cases had more than 1 of the 28 CNVs. Only 1 CNV identical to a previously described disease-associated CNV (ie, a 350-kb deletion located at 22q11 spanning the *PRODH* and *DGCR6* (OMIM_601279) genes)[17] was detected in the control group. This deletion, present in a single control, had a low frequency (1 per 236 controls) similar to that previously reported in Japanese[46] and Canadian[47] populations. Individual significance for the association with ASDs was reached for this *PRODH/DGCR6* deletion (9 per 260 cases with ASDs vs 1 per 236 controls [$P=.02$]).

Among the 4 most prevalent CNVs, 3 (located at 22q11, 16p11, and 15q13) were flanked by known regions of segmental duplication and resulted most likely from a nonallelic homologous recombination mechanism. At the 2p16 locus, the *NRXN1* gene was recurrently disrupted by a set of partially overlapping deletions spanning the promoter and first exons of neurexin 1α or the exons coding for the middle section of this protein, as well as for the proximal region of neurexin 1β. These rearrangements occurred in a region devoid of any segmental duplication and resulted from another mechanism distinct from a nonallelic homologous recombination mechanism.

TRANSMISSION AND COSEGREGATION IN MULTIPLEX SIBSHIPS

Among 27 families in which transmission was tested (69.2% of families with CNVs), 8 CNVs (located at 8p23, 15q11-q13, 15q13, 16p11, and 22q13) had occurred de novo (Table 3). The mean (SD) paternal age was not significantly different between families with de novo and inherited CNVs (27.2 [4.7] vs 30.7 [4.6] years; $P=.19$, Mann-Whitney test). In most families, CNVs were transmitted from an apparently nonaffected (although not clinically or neuropsychologically assessed) parent. This includes a partial duplication of the X-linked *GRIA3* gene, present in a young male case with autism, which was inherited from the nonaffected mother. The 350-kb deletion located at 22q11 spanning the *PRODH/DGCR6* locus was also transmitted in 11 of 11 tested cases. *PRODH* encodes for proline dehydrogenase, and PRODH deficiency is responsible for type 1 hyperprolinemia, a condition often associated with cognitive impairment and with psychotic symptoms.[45] However, hemizygous deletion of the *PRODH* gene is insufficient per se to result in hyperprolinemia, as only 35% to 50% of cases with velocardiofacial syndrome, all bearing a single copy of

Table 3. Recurrent Copy Number Variations (CNVs) and Clinical Features of the Sample

Case/Sex/ Age, y	Diagnosis	Family History	CNV Chromosomal Location	Amplicon Size	Genes Involved	Type	Transmission	Cognitive Features	Associated Clinical Features
113/M/52	SZ	Sporadic	Xp11.4	56 kb	*TSPAN7*	Dup	Unknown	IQ 105	. . .
313/M/32	SZ	Familial	8p23	110 kb	*DLGAP2*	Dup	Unknown	IQ 67	Dysmorphism
185/M/48	SZ	Sporadic	15q13	994 kb	*APBA2*, *TJP1*, *NDNL2*	Dup	Unknown	IQ 73	. . .
144.1/F/42	SZ AFF	Familial	22q11	350 kb	*PRODH*, *DGCR6*	Del	Unknown[a]	IQ 48	. . .
223/M/38	SZ	Sporadic	2p16.3	107 kb	*NRXN1*α exons 1-2	Del	Unknown	IQ 94	. . .
220/M/25	SZ	Unknown	2p16.3	<532 kb	*NRXN1*α, *NRXN1*β	Del	Unknown	NA	. . .
33.1/F/53	SZ	Familial	16p11	500 kb	*DOC2A*, 24 other genes	Dup	Maternally inherited[b]	IQ 56	. . .
136/F/41	SZ	Sporadic	16p11	500 kb	*DOC2A*, 24 other genes	Dup	De novo	IQ 75	. . .
146/M/32	SZ AFF	Familial	16p11	500 kb	*DOC2A*, 24 other genes	Dup	Unknown	IQ 95	. . .
151/M/53	SZ	Sporadic	17q21	627 kb	*MAPT*, 2 other genes	Dup	Unknown	IQ 90	. . .
T35/F/38	ASDs	Sporadic	2p16.3	<427 kb	*NRXN1*α exons 1-2	Del	Paternally inherited	IQ 66	. . .
45 431/M/10	ASDs	Sporadic	2p16.3	107 kb	*NRXN1*α exons 1-2	Del	Maternally inherited	NA	. . .
47 604/M/8	ASDs	Sporadic	22q13	2.26 Mb	*SHANK3*, 28 other genes	Del	De novo	IQ <40	. . .
Si22/M/9	ASDs	Sporadic	22q13	NA	*SHANK3*	Del	De novo	IQ <40	Dysmorphism, epilepsy
60 478/M/15	ASDs	Sporadic	15q11-q13	4 Mb	*GABRA5*, *GABRB3*, *GABRG3*, 17 other genes	Dup	De novo	IQ <40	Epilepsy
T34/M/10	ASDs	Sporadic	Xq25	1.42 Mb	*GRIA3* exons 1-4	Dup	Maternally inherited	NA	. . .
12 746/M/6	HFA	Sporadic	22q11	350 kb	*PRODH*, *DGCR6*	Del	Paternally inherited	IQ 74	. . .
12 452/F/8	ASDs	Sporadic	22q11	350 kb	*PRODH*, *DGCR6*	Del	Paternally inherited	IQ <40	. . .
13 899/M/11	ASDs	Sporadic	22q11	350 kb	*PRODH*, *DGCR6*	Del	Unknown	IQ <40	. . .
44 737/M/7	ASDs	Sporadic	22q11	350 kb	*PRODH*, *DGCR6*	Del	Paternally inherited	IQ <40	. . .
45 435/F/31	ASDs	Sporadic	22q11	350 kb	*PRODH*, *DGCR6*	Del	Unknown	IQ <40	. . .
45 856/M/38	ASDs	Sporadic	22q11	350 kb	*PRODH*, *DGCR6*	Del	Maternally inherited	IQ <40	. . .
46 261/F/11	ASDs	Sporadic	22q11	350 kb	*PRODH*, *DGCR6*	Del	Paternally inherited	NA	. . .
47 766/M/8	ASDs	Sporadic	22q11	350 kb	*PRODH*, *DGCR6*	Del	Paternally inherited	NA	. . .
Si30/F/5	ASDs	Sporadic	22q11	350 kb	*PRODH*, *DGCR6*	Del	Maternally inherited	IQ <40	Dysmorphism, epilepsy
44 813/M/8	HFA	Familial	15q13	3.8 Mb	*APBA2*, *CHRNA7*, 16 other genes	Dup	Maternally inherited	NA	. . .
12 363/M/11	MR	Sporadic	22q11	350 kb	*PRODH*, *DGCR6*	Del	Maternally inherited	Mild MR	Dysmorphism
11 780/M/3	MR	Sporadic	22q11	350 kb	*PRODH*, *DGCR6*	Del	Paternally inherited	Mod MR	Dysmorphism
9680/F/11	MR	Familial	22q11	350 kb	*PRODH*, *DGCR6*	Del	Maternally inherited	Mod MR	Dysmorphism
14 684/M/6	MR	Familial	22q11	350 kb	*PRODH*, *DGCR6*	Del	Maternally inherited	DLD	. . .
11 695.1/F/10	MR	Familial	2p16.3	<427 kb	*NRXN1*α exon 1	Del	Maternally inherited[c]	Mod MR	. . .
14 921/M/4	MR	Familial	16p11	500 kb	*DOC2A*, 24 other genes	Del	De novo	Mild MR	Dysmorphism
10 417/M/14	MR	Familial	16p11	500 kb	*DOC2A*, 24 other genes	Del	Maternally inherited	Mild MR	Intrauterine growth retardation, dysmorphism
13 165/F/4	MR	Sporadic	16p11	500 kb	*DOC2A*, 24 other genes	Dup	Maternally inherited	Mild MR	Dysmorphism
14 390.1/F/9	MR	Familial	16p11	500 kb	*DOC2A*, 24 other genes	Del	De novo[d]	DLD	. . .
13 907/F/9	MR	Sporadic	15q13	1.57 Mb	*CHRNA7*, 5 other genes	Del	Unknown	Mild MR	Microcephaly, dysmorphism
11 919/F/7	MR	Familial	15q13	1,57 Mb	*CHRNA7*, 5 other genes	Del	Unknown	Mild MR	. . .
9930/M/12	MR	Familial	15q13	1,57 Mb	*CHRNA7*, 5 other genes	Del	De novo	Mild MR	Dysmorphism
12 988/M/8	MR	Sporadic	8p23	3 Mb	*DLGAP2*, 23 other genes	Del	De novo	Mild MR	Microcephaly, dysmorphism, epilepsy
011/003/F/84	Control	. . .	22q11	350 kb	*PRODH*, *DGCR6*	Del	Unknown	. . .	. . .

Abbreviations: ASDs, autism spectrum disorders; Del, deletion; DLD, developmental learning disorder; Dup, duplication; ellipses, not applicable; HFA, high-functioning autism; kb, kilobases; Mb, megabases; Mod, moderate; MR, mental retardation; NA, not assessed; SZ, schizophrenia; SZ AFF, schizoaffective disorder.

[a] Deletion also present in a sibling with schizophrenia.

[b] Duplication present in a case with schizophrenia and in a healthy sibling and absent in a sibling with schizoaffective disorder.

[c] Deletion present in the proband and in an affected sibling (maternally derived).

[d] Deletion not present in a sibling with mental retardation.

Table 4. Genotypes and Plasma Proline Levels of the Cases Bearing the *PRODH* Deletion

Case/Sex/ Age, y	Diagnosis	Plasma Proline Level, μmol/L[a]	Genotype	Predicted *PRODH* Residual Activity, %[b]
144.1/F/42	SZ AFF	**538**	Del/**R453C**+R185W	2
144.2/F/39	SZ	**338**	Del/Q19P	30
12 452/M/6	ASDs	**287**	Del/R185W	25
12 746/F/8	HFA	214	Del/Q19P+*A58T*	≤30
13 899/M/11	ASDs	**312**	Del/Q19P	≤25
44 737/M/7	ASDs	**329**	Del/*T275N*+V427M	≤20
45 435/F/31	ASDs	**487**	Del/R185W+Q19P	≤25
45 856/M/38	ASDs	NA	Del/Q19P+*P30S*	≤30
46 261/F/11	ASDs	**243**-283	Del/R185W+Q19P	≤25
47 766/M/8	ASDs	NA	Del/WT	50
Si30/F/5	ASDs	**422-1883**	Del/**R453C**+Q19P+*A58T*+V427M	≤2
12 363/M/11	MR	**512**	Del//R185W	25
11 780/M/3	MR	**NA**	Del/R185W+Q19P	≤25
9680/F/11	MR	**299**	Del/Q19P+*P30S*	≤30
14 684/M/6	MR	238	Del/R185W+Q19P	≤25

Abbreviations: ASDs, autism spectrum disorders; HFA, high-functioning autism; NA, not assessed; MR, mental retardation; SZ, schizophrenia; SZ AFF, schizoaffective disorder.

[a] Abnormal plasma proline values are in boldface. Abnormal fasting plasma proline values are as follows: age older than 18 years (>377 μmol/L for men and >316 μmol/L for women), age 5 to 18 years (>270 μmol/L), and age younger than 5 years (>235 μmol/L).

[b] Evaluated for each genotype according to the functional data published by Bender et al.[49] Mutations with severe effect on *PRODH* activity are in boldface, mutations with moderate effect are in roman font, and mutations with unknown effect are in italics.

PRODH, exhibit hyperprolinemia.[45,48] Indeed, a reduction of more than 50% of the enzymatic activity is generally required to produce hyperprolinemia.[45] Therefore, the presence of a mutation affecting enzyme activity[49] on the second allele is necessary. To examine this issue, the remaining *PRODH* allele was sequenced in all cases bearing the 350-kb deletion, and the plasma proline level was assessed whenever possible. As summarized in **Table 4**, 14 of 15 cases harbored a genotype predicted to result in a reduction of at least 70% of enzymatic activity. Among 12 cases from whom the plasma proline level was assessed, 9 had mild to severe hyperprolinemia, and 3 had plasma proline levels at the upper boundary of normal values.

Cosegregation of the CNV with pathologic conditions was examined in 4 multiplex families in which DNA from affected siblings was available. In family 144 (Tables 3 and 4), the 2 sibs with schizophrenia or schizoaffective disorder harbored the *PRODH/DGCR6* deletion. In family 11 695 (Table 3), the 2 MR sibs harbored the 2p16.3 deletion. In family 14 390 (Table 3), the 16p11 deletion was present in the proband with developmental language disorder but not in his sib with MR. In family 33 (Table 3), the 16p11 duplication was present in 2 sibs with schizophrenia and in a nonaffected sibling but was absent in a third sibling with schizoaffective disorder (eFigure 2).

DISEASE SPECIFICITY

Combining the results of the present study and previous findings, none of the observed rearrangements were disease specific, with the possible exception of the maternally derived 15q13 duplication associated with ASDs. The 22q11[17] and 2p16[1] deletions were found in the 3 conditions, whereas the 22q13 deletion in 2 cases with ASDs had already been described in ASDs and in MR.[4,18-20] The 16p11 and 8p23 rearrangements previously described in ASDs[4,5] were found in cases with schizophrenia, and the 16p11 rearrangement was found in cases with MR. The Xp11.4 duplication spanning the *TSPAN7* gene, previously described in MR and in ASDs,[4,21] was found in a case with schizophrenia, as well as the 17q21 duplication previously described in a patient with MR.[22] Two different-sized 15q13 duplications that included *APBA2* were found in 1 case with ASDs and in 1 case with schizophrenia. A partial duplication of the *GRIA3* gene, including the promoter region and the exons coding for the proximal region of GRIA3, was detected in a single case with autism. Although slightly different based on its size and the number of duplicated exons, this partial duplication is reminiscent of that recently reported in a patient with MR.[23] At the 8p23 locus, gain and loss of material were found, as well as at the 16p11 and 17q21 loci, as recently described.[22,24,25] This suggests that dosage-sensitive genes, whose expression is finely tuned, are located within these rearranged segments.

COMORBIDITY

From a phenotypic viewpoint, 3 of 9 cases with schizophrenia bearing a candidate CNV had mild MR in an IQ assessment obtained after the onset of their psychotic symptoms (Table 3). No premorbid IQ was available for any case. Although postmorbid IQ likely constitutes an underestimation of the premorbid level of cognitive functioning in cases with schizophrenia, cognitive deficits manifest by severe learning disorders and failure to follow normal schooling were already noted in these 3 cases during childhood before the onset of their psychotic symptoms, supporting the comorbid diagnosis of MR and schizophrenia. In cases with potential MR (case 144.1 [Table 3] and cases 313 and 33.1 [Table 3]), psychotic features appeared at ages 25, 18, and 27 years, respec-

tively, and included prominent positive symptoms such as persecutory delusions, thought insertions, delusions of being controlled, and auditory hallucinations with voices making insulting statements. The 3 cases had marked behavioral disorders such as aggressiveness and psychomotor agitation. Mood instability and suicidal ideation were present in case 144.1. The 3 cases were considered good responders to atypical neuroleptic drug therapy. Initial symptoms gradually declined, and the course of the disease was marked by social isolation, blunted affects, and loosening of associations. These cases are living in long-term institutions.

Except for 2 cases who had normal cognitive functioning (high-functioning autism), all tested CNV-bearing cases with autism had IQs in the range of MR, although case T35 had only mild cognitive dysfunction (Table 3). In 2 cases with high-functioning autism (cases 12 746 [Tables 3 and 4] and 44 813 [Table 3], aged 6 and 8 years, respectively), onset was in the first year of life, when the parents noticed few gestures, almost no babbling, and poor shifting gazes. Subsequently, the cases acquired spoken language, although with significant delay. They are able to carry on conversation, carry out commands, imitate, and dress and groom themselves. They participate in public school with adapted educative programs. However, they remain impaired in their communicative and social skills, and their use of language is often inappropriate. Both cases have developed ritualistic behaviors and show restricted patterns of interest.

OTHER CNVs

In addition to the set of CNVs tested for recurrence in this study, 2 reciprocal rearrangements, previously unassociated with any psychiatric condition, were incidentally observed at 2 loci. Both were benign polymorphisms. A common CNV reciprocal to the expected one (ie, a 350-kb duplication) was found at the 22 q11 locus in 6 of 236 controls, as well as in 7 of 236 cases with schizophrenia, 4 of 260 cases with ASDs, and 9 of 247 cases with MR. Another overlapping reciprocal CNV (ie, a 490-kb duplication) was detected at the *CHRNA7* locus located on chromosome 15q13.3. This CNV was present in 2 of 236 controls, in 1 of 236 cases with schizophrenia, in 1 of 260 cases with ASDs, and in 1 of 247 cases with MR and was unrelated to any pathologic condition (eTable 2).

COMMENT

After a first wave of CNV discovery by array-CGH analyses in neuropsychiatric disorders, this study for the first time (to our knowledge) examines the involvement of a limited number of candidate loci in large samples of cases with different clinical diagnoses. Two strengths of our study design are (1) the inclusion of controls carefully screened for the studied pathologic conditions and of series of cases mostly ascertained through consecutive admissions or consultations and, therefore, (2) the inclusion of cases belonging to simplex or multiplex families. Given the expected rarity of each variant, our first goal was not to test the association of every individual CNV with schizophrenia, ASDs, or MR but to determine whether these variants were collectively more frequent in cases with these diseases than among controls. This aim was successfully achieved, and we were able to obtain suggestive statistical significance for the association between the 350-kb deletion located at 22q11 and ASDs. This deleted segment, located within the chromosomal region deleted in velocardiofacial syndrome (a contiguous gene syndrome known to be associated with a high frequency of MR, ASDs, and psychosis), contained the 2 genes *PRODH* and *DGCR6*. Although we cannot exclude an involvement of *DGCR6* in the neuropsychiatric phenotype of the cases bearing this CNV, previous work from our group strongly suggests that *PRODH* is the prime candidate.[45] It was previously shown that hyperprolinemia, resulting from partial or total inactivation of this enzyme, (1) may lead to MR and autism in patients with type 1 hyperprolinemia,[45] (2) is a risk factor for schizoaffective disorder,[50] and (3) is inversely correlated with IQ in velocardiofacial syndrome.[45] Herein, we show that all cases except 1 harboring this deletion were compound heterozygotes, also bearing mutations affecting enzymatic activity on the second allele. This resulted in a loss of at least 70% of the predicted PRODH residual activity in 14 of 15 assessed cases and resulted in hyperprolinemia in 9 of 12 assessed cases.

Second, we show that de novo CNVs and CNVs inherited from an apparently healthy parent can be found in cases. For transmitted CNVs, the mode of inheritance of the disease was recessive in some cases (eg, hyperprolinemia related to the 22q11 deletion) or implied the transmission of an X-linked gene (*GRIA3*) by a woman to her son. Consistent with findings in previous studies,[4,8,26] the 16p11 rearrangements were inherited from an apparently nonaffected parent in 3 families. These CNVs, whose estimated frequency in the Icelandic population was 5 per 18 834 (0.03%) for the duplication and 2 per 18 834 (0.01%) for the deletion,[25] should be considered risk factors rather than fully causative variations. The presence of affected siblings that do not share the CNV, already noted in a previous study,[26] does not necessarily rule out the causative implication of these CNVs but raises the question of intrafamilial genetic heterogeneity. This hypothesis, which is plausible for these frequent disorders that are often characterized by assortative mating, remains speculative because the parents and their relatives were not psychiatrically or cognitively assessed in these families.

Third and most important, our study confirms and extends recent evidence suggesting that many candidate CNVs are not disease specific but are involved in the expression of different behavioral phenotypes, including MR, ASDs, and schizophrenia. This implies the existence of shared biologic pathways in these 3 neurodevelopmental conditions. These pathways chiefly affect synapse formation and maintenance, as well as neurotransmission (with a special emphasis on glutamate and γ-aminobutyric acid). The dysfunction of specific neuronal networks underlying the particular symptoms of each clinical condition most likely depends on additional genetics, epigenetics, and environmental factors that remain to be characterized. From a clinical point of view, despite the

diversity of categorical diagnoses, many cases harboring these CNVs shared some clinical features: one-third of cases with schizophrenia and 83.3% of cases with autism having CNVs had a level of cognitive functioning in the range of MR. This is in accord with previous studies showing that point prevalence of schizophrenia is increased by a factor of 3 in cases with intellectual disabilities[51] and that 50% of cases with autism have MR.[52] However, the following 2 caveats should be noted: (1) because attention and communication are markedly impaired in children with autism, assessment of their IQs (even performance IQs in nonverbal cases) is unreliable, and (2) these results were not obtained in a single community–based population but in 3 disease groups ascertained according to different schemes, a factor whose effect is difficult to appreciate but which is likely to have implications related to the phenotypic severity in these cases.

Fourth, targeted procedures for CNV analysis such as the QMPSF method is a cost-effective alternative to array-CGH for the screening of candidate loci in large case-control cohorts. We plan to conduct extensive resequencing of these candidate genes to further validate their role in these conditions.

Since our submission of this article for publication, additional studies[9,11,53,54] have been published documenting shared CNVs between MR, ASDs, and schizophrenia.

Submitted for Publication: August 5, 2008; final revision received February 6, 2009; accepted February 23, 2009.
Author Affiliations: Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 614, Institut Hospitalo-Universitaire de Recherche Biomédicale (Drs Guilmatre, Mosca, Frébourg, Saugier Veber, and Campion and Ms Legallic), Department of Research, Centre Hospitalier de Saint Etienne du Rouvray (Drs Le Vacon and Campion), and Department of Genetics, University Hospital (Drs Goldenberg, Drouin-Garraud, Joly-Helas, Frébourg, and Saugier Veber), Rouen, Unité Mixte de Recherche 6061, Centre National de Recherche Scientifique, University of Rennes I (Drs Dubourg, David, and Bendavid), and Department of Genetics, University Hospital (Dr Odent), Rennes, Department of Genetics, Groupe Hospitalier du Havre, Le Havre (Dr Layet), Centre de Ressources Autisme de Haute Normandie, Saint Etienne du Rouvray (Dr Rosier), Department of Genetics, University Hospital, and Unité Mixte de Recherche 930 Centre National de Recherche Scientifique, Orléans (Dr Briault), INSERM Unité 930, University Hospital Bretonneau, University François-Rabelais (Drs Bonnet-Brilhault, Laumonnier, and Barthelemy), and INSERM Unité 619 (Dr Andres), Tours, and Department of Genetics (Drs Mosca and Faivre) and Centre de Ressources Autisme de Bourgogne, Childrens Hospital (Drs Pinoit and Henry), University Hospital, Dijon, France; and Department of Medical and Surgical Pediatrics, University Hospital, Messina, Italy (Drs Impallomeni, Germano, Tortorella, and Di Rosa).
Correspondence: Dominique Campion, MD, PhD, Institut National de la Santé et de la Recherche Médicale, Unité 614, Institut Hospitalo-Universitaire de Recherche Biomédicale, 22 Blvd Gambetta, 76000 Rouen, France (dominique.campion@univ-rouen.fr).

Author Contributions: Dr Campion had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Financial Disclosure: None reported.
Funding/Support: This study was supported by the Fondation de France, by Collaborative Biologic Resources From the Autism Foundation (RBCFA), and by the French Autism Foundation. Dr Guilmatre received a fellowship from Region Haute Normandie, and Dr Mosca received a fellowship from the Académie Nationale de Médecine.
Role of the Sponsors: We acknowledge the usefulness of the available resources from the RBCFA and from the families involved in the RBCFA. RBCFA is a program of the French Autism Foundation, which is recognized to be of public usefulness, was created by parents of autistic patients, and whose scientific director is Sylvain Briault, MD, PhD, researcher at the INSERM.
Additional Contributions: Technical support for the transcriptomic platform was provided by OUEST Genopole and by Genethon. We thank our colleagues who helped to identify patients, as well as the patients and their families for their participation.
Additional Information: The eTables and eFigures are available at http://www.archgenpsychiatry.com.

REFERENCES

1. Friedman JM, Baross A, Delaney AD, Ally A, Arbour L, Armstrong L, Asano J, Bailey DK, Barber S, Birch P, Brown-John M, Cao M, Chan S, Charest DL, Farnoud N, Fernandes N, Flibotte S, Go A, Gibson WT, Holt RA, Jones SJ, Kennedy GC, Krzywinski M, Langlois S, Li HI, McGillivray BC, Nayar T, Pugh TJ, Rajcan-Separovic E, Schein JE, Schnerch A, Siddiqui A, Van Allen MI, Wilson G, Yong SL, Zahir F, Eydoux P, Marra MA. Oligonucleotide microarray analysis of genomic imbalance in children with mental retardation [published correction appears in *Am J Hum Genet*. 2006;79(6):1135]. *Am J Hum Genet*. 2006;79(3):500-513.
2. Froyen G, Van Esch H, Bauters M, Hollanders K, Frints SG, Vermeesch JR , Devriendt K, Fryns JP, Marynen P. Detection of genomic copy number changes in patients with idiopathic mental retardation by high-resolution X-array-CGH: important role for increased gene dosage of *XLMR* genes. *Hum Mutat*. 2007;28(10):1034-1042.
3. Menten B, Maas N, Thienpont B, Buysse K, Vandesompele J, Melotte C, de Ravel T, Van Vooren S, Balikova I, Backx L, Janssens S, De Paepe A, De Moor B, Moreau Y, Marynen P, Fryns JP, Mortier G, Devriendt K, Speleman F, Vermeesch JR. Emerging patterns of cryptic chromosomal imbalance in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of published reports. *J Med Genet*. 2006;43(8):625-633.
4. Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, Thiruvahindrapduram B, Fiebig A, Schreiber S, Friedman J, Ketelaars CE, Vos YJ, Ficicioglu C, Kirkpatrick S, Nicolson R, Sloman L, Summers A, Gibbons CA, Teebi A, Chitayat D, Weksberg R, Thompson A, Vardy C, Crosbie V, Luscombe S, Baatjes R, Zwaigenbaum L, Roberts W, Fernandez B, Szatmari P, Scherer SW. Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet*. 2008;82(2):477-488.
5. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, Leotta A, Pai D, Zhang R, Lee YH, Hicks J, Spence SJ, Lee AT, Puura K, Lehtimäki T, Ledbetter D, Gregersen PK, Bregman J, Sutcliffe JS, Jobanputra V, Chung W, Warburton D, King MC, Skuse D, Geschwind DH, Gilliam TC, Ye K, Wigler M. Strong association of de novo copy number mutations with autism. *Science*. 2007;316(5823):445-449.
6. Christian SL, Brune CW, Sudi J, Kumar RA, Liu S, Karamohamed S, Badner JA, Matsui S, Conroy J, McQuaid D, Gergel J, Hatchwell E, Gilliam TC, Gershon ES, Nowak NJ, Dobyns WB, Cook EH Jr. Novel submicroscopic chromosomal abnormalities detected in autism spectrum disorder. *Biol Psychiatry*. 2008;63(12):1111-1117.
7. Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ, Vincent JB, Skaug JL, Thompson AP, Senman L, Feuk L, Qian C, Bryson SE, Jones MB, Marshall CR, Scherer SW, Vieland VJ, Bartlett C, Mangin LV, Goedken R, Segre A, Pericak-Vance MA, Cuccaro ML, Gilbert JR, Wright HH, Abramson RK, Betan-

cur T, Bourgeron T, Gillberg C, Leboyer M, Buxbaum JD, Davis KL, Hollander E, Silverman JM, Hallmayer J, Lotspeich L, Sutcliffe JS, Haines JL, Folstein SE, Piven J, Wassink TH, Sheffield V, Geschwind DH, Bucan M, Brown WT, Cantor RM, Constantino JN, Gilliam TC, Herbert M, Lajonchere C, Ledbetter DH, Lese-Martin C, Miller J, Nelson S, Samango-Sprouse CA, Spence S, State M, Tanzi RE, Coon H, Dawson G, Devlin B, Estes A, Flodman P, Klei L, McMahon WM, Minshew N, Munson J, Korvatska E, Rodier PM, Schellenberg GD, Smith M, Spence MA , Stodgell C, Tepper PG, Wijsman EM, Yu CE, Rogé B, Mantoulan C, Wittemeyer K, Poustka A, Felder B, Klauck SM, Schuster C, Poustka F, Bölte S, Feineis-Matthews S, Herbrecht E, Schmötzer G, Tsiantis J, Papanikolaou K, Maestrini E, Bacchelli E, Blasi F, Carone S, Toma C, Van Engeland H, de Jonge M, Kemner C, Koop F, Langemeijer M, Hijmans C, Staal WG, Baird G, Bolton PF , Rutter ML, Weisblatt E, Green J, Aldred C, Wilkinson JA, Pickles A, Le Couteur A, Berney T, McConachie H, Bailey AJ, Francis K, Honeyman G, Hutchinson A, Parr JR, Wallace S, Monaco AP, Barnby G, Kobayashi K, Lamb JA, Sousa I, Sykes N, Cook EH, Guter SJ, Leventhal BL, Salt J, Lord C, Corsello C, Hus V, Weeks DE, Volkmar F, Tauber M, Fombonne E, Shih A, Meyer KJ; Autism Genome Project Consortium. Mapping autism risk loci using genetic linkage and chromosomal rearrangements [published correction appears in *Nat Genet*. 2007;39(10):1285]. *Nat Genet*. 2007;39(3):319-328.

8. Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, Nord AS, Kusenda M, Malhotra D, Bhandari A, Stray SM, Rippey CF, Roccanova P, Makarov V, Lakshmi B, Findling RL, Sikich L, Stromberg T, Merriman B, Gogtay N, Butler P, Eckstrand K, Noory L, Gochman P, Long R, Chen Z, Davis S, Baker C, Eichler EE, Meltzer PS, Nelson SF, Singleton AB, Lee MK, Rapoport JL, King MC, Sebat J. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science*. 2008;320(5875):539-543.
9. International Schizophrenia Consortium. Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature*. 2008;455(7210):237-241.
10. Kirov G, Gumus D, Chen W, Norton N, Georgieva L, Sari M, O'Donovan MC, Erdogan F, Owen MJ, Ropers HH, Ullmann R. Comparative genome hybridization suggests a role for *NRXN1* and *APBA2* in schizophrenia. *Hum Mol Genet*. 2008;17(3):458-465.
11. Xu B, Roos JL, Levy S, van Rensburg EJ, Gogos JA, Karayiorgou M. Strong association of de novo copy number mutations with sporadic schizophrenia. *Nat Genet*. 2008;40(7):880-885.
12. Lupski JR. Genomic rearrangements and sporadic disease. *Nat Genet*. 2007;39(7)(suppl):S43-S47.
13. Fyer AJEJ, Manuzza S, Klein DF. *Schedule for Affective Disorders and Schizophrenia*. Paris, France: INSERM Ed; 1989.
14. Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview–Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord*. 1994;24(5):659-685.
15. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, Pickles A, Rutter M. The Autism Diagnostic Observation Schedule–Generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord*. 2000;30(3):205-223.
16. Schopler E, Reichler RJ, DeVellis RF, Daly K. Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). *J Autism Dev Disord*. 1980;10(1):91-103.
17. Jacquet H, Berthelot J, Bonnemains C, Simard G, Saugier-Veber P, Raux G, Campion D, Bonneau D, Frebourg T. The severe form of type I hyperprolinaemia results from homozygous inactivation of the *PRODH* gene. *J Med Genet*. 2003;40(1):e7 http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=12525555. Accessed April 9, 2009.
18. Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, Nygren G, Rastam M, Gillberg IC, Anckarsäter H, Sponheim E, Goubran-Botros H, Delorme R, Chabane N, Mouren-Simeoni MC, de Mas P, Bieth E, Rogé B, Héron D, Burglen L, Gillberg C, Leboyer M, Bourgeron T. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat Genet*. 2007;39(1):25-27.
19. Moessner R, Marshall CR, Sutcliffe JS, Skaug J, Pinto D, Vincent J, Zwaigenbaum L, Fernandez B, Roberts W, Szatmari P, Scherer SW. Contribution of *SHANK3* mutations to autism spectrum disorder. *Am J Hum Genet*. 2007;81(6):1289-1297.
20. Wilson HL, Wong AC, Shaw SR, Tse WY, Stapleton GA, Phelan MC, Hu S, Marshall J, McDermid HE. Molecular characterisation of the 22q13 deletion syndrome supports the role of haploinsufficiency of *SHANK3/PROSAP2* in the major neurological symptoms. *J Med Genet*. 2003;40(8):575-584.
21. Tzschach A, Chen W, Erdogan F, Hoeller A, Ropers HH, Castellan C, Ullmann R, Schinzel A. Characterization of interstitial Xp duplications in two families by tiling path array CGH. *Am J Med Genet A*. 2008;146A(2):197-203.
22. Kirchhoff M, Bisgaard AM, Duno M, Hansen FJ, Schwartz M. A 17q21.31 microduplication, reciprocal to the newly described 17q21.31 microdeletion, in a girl with severe psychomotor developmental delay and dysmorphic craniofacial features. *Eur J Med Genet*. 2007;50(4):256-263.
23. Chiyonobu T, Hayashi S, Kobayashi K, Morimoto M, Miyanomae Y, Nishimura A, Nishimoto A, Ito C, Imoto I, Sugimoto T, Jia Z, Inazawa J, Toda T. Partial tandem duplication of *GRIA3* in a male with mental retardation. *Am J Med Genet A*. 2007;143A(13):1448-1455.
24. Shaw-Smith C, Pittman AM, Willatt L, Martin H, Rickman L, Gribble S, Curley R, Cumming S, Dunn C, Kalaitzopoulos D, Porter K, Prigmore E, Krepischi-Santos AC, Varela MC, Koiffmann CP, Lees AJ, Rosenberg C, Firth HV, de Silva R, Carter NP. Microdeletion encompassing *MAPT* at chromosome 17q21.3 is associated with developmental delay and learning disability. *Nat Genet*. 2006;38(9):1032-1037.
25. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T, Platt OS, Ruderfer DM, Walsh CA, Altshuler D, Chakravarti A, Tanzi RE, Stefansson K, Santangelo SL, Gusella JF, Sklar P, Wu BL, Daly MJ; Autism Consortium. Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med*. 2008;358(7):667-675.
26. Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, Gilliam TC, Nowak NJ, Cook EH Jr, Dobyns WB, Christian SL. Recurrent 16p11.2 microdeletions in autism. *Hum Mol Genet*. 2008;17(4):628-638.
27. Kim HG, Kishikawa S, Higgins AW, Seong IS, Donovan DJ, Shen Y, Lally E, Weiss LA, Najm J, Kutsche K, Descartes M, Holt L, Braddock S, Troxell R, Kaplan L, Volkmar F, Klin A, Tsatsanis K, Harris DJ, Noens I, Pauls DL, Daly MJ, MacDonald ME, Morton CC, Quade BJ, Gusella JF. Disruption of neurexin 1 associated with autism spectrum disorder. *Am J Hum Genet*. 2008;82(1):199-207.
28. Zahir FR, Baross A, Delaney AD, Eydoux P, Fernandes ND, Pugh T, Marra MA, Friedman JM. A patient with vertebral, cognitive and behavioural abnormalities and a de novo deletion of *NRXN1*α. *J Med Genet*. 2008;45(4):239-243.
29. Dijkhuizen T, van Essen T, van der Vlies P, Verheij JB, Sikkema-Raddatz B, van der Veen AY, Gerssen-Schoorl KB, Buys CH, Kok K. FISH and array-CGH analysis of a complex chromosome 3 aberration suggests that loss of *CNTN4* and *CRBN* contributes to mental retardation in 3pter deletions. *Am J Med Genet A*. 2006;140(22):2482-2487.
30. Roohi J, Montagna C, Tegay DH, Palmer LE, DeVincent C, Pomeroy JC, Christian SL, Nowak N, Hatchwell E. Disruption of contactin 4 in three subjects with autism spectrum disorder. *J Med Genet*. 2009;46(3):176-182.
31. Meins M, Hagh JK, Gerresheim F, Einhoff E, Olschewski H, Strehl H, Epplen JT. Novel case of dup(3q) syndrome due to a de novo interstitial duplication 3q24-q26.31 with minimal overlap to the dup(3q) critical region. *Am J Med Genet A*. 2005;132A(1):84-89.
32. Kakinuma H, Ozaki M, Sato H, Takahashi H. Variation in GABA-A subunit gene copy number in an autistic patient with mosaic 4 p duplication (p12p16). *Am J Med Genet B Neuropsychiatr Genet*. 2008;147B(6):973-975.
33. Friedman JI, Vrijenhoek T, Markx S, Janssen IM, van der Vliet WA, Faas BH, Knoers NV, Cahn W, Kahn RS, Edelmann L, Davis KL, Silverman JM, Brunner HG, van Kessel AG, Wijmenga C, Ophoff RA, Veltman JA. *CNTNAP2* gene dosage variation is associated with schizophrenia and epilepsy. *Mol Psychiatry*. 2008;13(3):261-266.
34. Klopocki E, Fiebig B, Robinson P, Tönnies H, Erdogan F, Ropers HH, Mundlos S, Ullmann R. A novel 8 Mb interstitial deletion of chromosome 8p12-p21.2. *Am J Med Genet A*. 2006;140(8):873-877.
35. Bolton PF, Dennis NR, Browne CE, Thomas NS, Veltman MW, Thompson RJ, Jacobs P. The phenotypic manifestations of interstitial duplications of proximal 15q with special reference to the autistic spectrum disorders. *Am J Med Genet*. 2001;105(8):675-685.
36. Cook EH Jr, Lindgren V, Leventhal BL, Courchesne R, Lincoln A, Shulman C, Lord C, Courchesne E. Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. *Am J Hum Genet*. 1997;60(4):928-934.
37. Sharp AJ, Mefford HC, Li K, Baker C, Skinner C, Stevenson RE, Schroer RJ, Novara F, De Gregori M, Ciccone R, Broomer A, Casuga I, Wang Y, Xiao C, Barbacioru C, Gimelli G, Bernardina BD, Torniero C, Giorda R, Regan R, Murday V, Mansour S, Fichera M, Castiglia L, Failla P, Ventura M, Jiang Z, Cooper GM, Knight SJ, Romano C, Zuffardi O, Chen C, Schwartz CE, Eichler EE. A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. *Nat Genet*. 2008;40(3):322-328.
38. Chocholska S, Rossier E, Barbi G, Kehrer-Sawatzki H. Molecular cytogenetic analysis of a familial interstitial deletion Xp22.2-22.3 with a highly variable phenotype in female carriers. *Am J Med Genet A*. 2006;140(6):604-610.
39. Kent L, Emerton J, Bhadravathi V, Weisblatt E, Pasco G, Willatt LR, McMahon R, Yates JR. X-linked ichthyosis (steroid sulphatase deficiency) is associated with increased risk of attention deficit hyperactivity disorder, autism and social communication deficits. *J Med Genet*. 2008;45(8):519-524.
40. Lawson-Yuen A, Saldivar JS, Sommer S, Picker J. Familial deletion within *NLGN4* associated with autism and Tourette syndrome. *Eur J Hum Genet*. 2008;16(5):614-618.
41. Macarov M, Zeigler M, Newman JP, Strich D, Sury V, Tennenbaum A, Meiner V. Deletions of *VCX-A* and *NLGN4*: a variable phenotype including normal intellect. *J Intellect Disabil Res*. 2007;51(pt 5):329-333.

42. Jin H, Gardner RJ, Viswesvaraiah R, Muntoni F, Roberts RG. Two novel members of the interleukin-1 receptor gene family, one deleted in Xp22.1-Xp21.3 mental retardation. *Eur J Hum Genet*. 2000;8(2):87-94.
43. del Gaudio D, Fang P, Scaglia F, Ward PA, Craigen WJ, Glaze DG, Neul JL, Patel A, Lee JA, Irons M, Berry SA, Pursley AA, Grebe TA, Freedenberg D, Martin RA, Hsich GE, Khera JR, Friedman NR, Zoghbi HY, Eng CM, Lupski JR, Beaudet AL, Cheung SW, Roa BB. Increased *MECP2* gene copy number as the result of genomic duplication in neurodevelopmentally delayed males. *Genet Med*. 2006; 8(12):784-792.
44. Casilli F, Di Rocco ZC, Gad S, Tournier I, Stoppa-Lyonnet D, Frebourg T, Tosi M. Rapid detection of novel *BRCA1* rearrangements in high-risk breast-ovarian cancer families using multiplex PCR of short fluorescent fragments. *Hum Mutat*. 2002; 20(3):218-226.
45. Raux G, Bumsel E, Hecketsweiler B, van Amelsvoort T, Zinkstok J, Manouvrier-Hanu S, Fantini C, Brévière GM, Di Rosa G, Pustorino G, Vogels A, Swillen A, Legallic S, Bou J, Opolczynski G, Drouin-Garraud V, Lemarchand M, Philip N, Gérard-Desplanches A, Carlier M, Philippe A, Nolen MC, Heron D, Sarda P, Lacombe D, Coizet C, Alembik Y, Layet V, Afenjar A, Hannequin D, Demily C, Petit M, Thibaut F, Frebourg T, Campion D. Involvement of hyperprolinemia in cognitive and psychiatric features of the 22q11 deletion syndrome. *Hum Mol Genet*. 2007;16(1):83-91.
46. Ohtsuki T, Tanaka S, Ishiguro H, Noguchi E, Arinami T, Tanabe E, Yara K, Okubo T, Takahashi S, Matsuura M, Sakai T, Muto M, Kojima T, Matsushima E, Toru M, Inada T. Failure to find association between *PRODH* deletion and schizophrenia. *Schizophr Res*. 2004;67(1):111-113.
47. Zogopoulos G, Ha KC, Naqib F, Moore S, Kim H, Montpetit A, Robidoux F, Laflamme P, Cotterchio M, Greenwood C, Scherer SW, Zanke B, Hudson TJ, Bader GD, Gallinger S. Germ-line DNA copy number variation frequencies in a large North American population. *Hum Genet*. 2007;122(3-4):345-353.
48. Goodman BK, Rutberg J, Lin WW, Pulver AE, Thomas GH. Hyperprolinaemia in patients with deletion (22)(q11.2) syndrome. *J Inherit Metab Dis*. 2000;23 (8):847-848.
49. Bender HU, Almashanu S, Steel G, Hu CA, Lin WW, Willis A, Pulver A, Valle D. Functional consequences of *PRODH* missense mutations. *Am J Hum Genet*. 2005; 76(3):409-420.
50. Jacquet H, Demily C, Houy E, Hecketsweiler B, Bou J, Raux G, Lerond J, Allio G, Haouzir S, Tillaux A, Bellegou C, Fouldrin G, Delamillieure P, Ménard JF, Dollfus S, D'Amato T, Petit M, Thibaut F, Frébourg T, Campion D. Hyperprolinemia is a risk factor for schizoaffective disorder. *Mol Psychiatry*. 2005;10(5):479-485.
51. Cooper SA, Smiley E, Morrison J, Allan L, Williamson A, Finlayson J, Jackson A, Mantry D. Psychosis and adults with intellectual disabilities: prevalence, incidence, and related factors. *Soc Psychiatry Psychiatr Epidemiol*. 2007;42(7): 530-536.
52. Fombonne E. The epidemiology of autism: a review. *Psychol Med*. 1999;29(4): 769-786.
53. Stefansson H, Rujescu D, Cichon S, Pietiläinen OP, Ingason A, Steinberg S, Fossdal R, Sigurdsson E, Sigmundsson T, Buizer-Voskamp JE, Hansen T, Jakobsen KD, Muglia P, Francks C, Matthews PM, Gylfason A, Halldorsson BV, Gudbjartsson D, Thorgeirsson TE, Sigurdsson A, Jonasdottir A, Jonasdottir A, Bjornsson A, Mattiasdottir S, Blondal T, Haraldsson M, Magnusdottir BB, Giegling I, Möller HJ, Hartmann A, Shianna KV, Ge D, Need AC, Crombie C, Fraser G, Walker N, Lonnqvist J, Suvisaari J, Tuulio-Henriksson A, Paunio T, Toulopoulou T, Bramon E, Di Forti M, Murray R, Ruggeri M, Vassos E, Tosato S, Walshe M, Li T, Vasilescu C, Mühleisen TW, Wang AG, Ullum H, Djurovic S, Melle I, Olesen J, Kiemeney LA, Franke B, Sabatti C, Freimer NB, Gulcher JR, Thorsteinsdottir U, Kong A, Andreassen OA, Ophoff RA, Georgi A, Rietschel M, Werge T, Petursson H, Goldstein DB, Nöthen MM, Peltonen L, Collier DA, St Clair D, Stefansson K. Large recurrent microdeletions associated with schizophrenia. *Nature*. 2008;455(7210):232-236.
54. Mefford HC, Sharp AJ, Baker C, Itsara A, Jiang Z, Buysse K, Huang S, Maloney VK, Crolla JA, Baralle D, Collins A, Mercer C, Norga K, de Ravel T, Devriendt K, Bongers EM, de Leeuw N, Reardon W, Gimelli S, Bena F, Hennekam RC, Male A, Gaunt L, Clayton-Smith J, Simonic I, Park SM, Mehta SG, Nik-Zainal S, Woods CG, Firth HV, Parkin G, Fichera M, Reitano S, Lo Giudice M, Li KE, Casuga I, Broomer A, Conrad B, Schwerzmann M, Räber L, Gallati S, Striano P, Coppola A, Tolmie JL, Tobias ES, Lilley C, Armengol L, Spysschaert Y, Verloo P, De Coene A, Goossens L, Mortier G, Speleman F, van Binsbergen E, Nelen MR, Hochstenbach R, Poot M, Gallagher L, Gill M, McClellan J, King MC, Regan R, Skinner C, Stevenson RE, Antonarakis SE, Chen C, Estivill X, Menten B, Gimelli G, Gribble S, Schwartz S, Sutcliffe JS, Walsh T, Knight SJ, Sebat J, Romano C, Schwartz CE, Veltman JA, de Vries BB, Vermeesch JR, Barber JC, Willatt L, Tassabehji M, Eichler EE. Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. *N Engl J Med*. 2008;359(16):1685-1699.