Copy number variants of schizophrenia susceptibility loci are associated with a spectrum of speech and developmental delays and behavior problems

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Purpose: Recently, molecular cytogenetic techniques have identified novel copy number variants in individuals with schizophrenia. However, no large-scale prospective studies have been performed to characterize the broader spectrum of phenotypes associated with such copy number variants in individuals with unexplained physical and intellectual disabilities encountered in a diagnostic setting. **Methods:** We analyzed 38,779 individuals referred to our diagnostic laboratory for microarray testing for the presence of copy number variants encompassing 20 putative schizophrenia susceptibility loci. We also analyzed the indications for study for individuals with copy number variants overlapping those found in six individuals referred for schizophrenia. **Results:** After excluding larger gains or losses that encompassed additional genes outside the candidate loci (e.g., whole-arm gains/losses), we identified 1113 individuals with copy number variants encompassing schizophrenia susceptibility loci and 37 individuals with copy number variants overlapping those present in the six individuals referred to our laboratory for schizophrenia. Of these, 1035 had a copy number variant of one of six recurrent loci: 1q21.1, 15q11.2, 15q13.3, 16p11.2, 16p13.11, and 22q11.2. The indications for study for these 1150 individuals were diverse and included developmental delay, intellectual disability, autism spectrum, and multiple congenital anomalies. **Conclusion:** The results from our study, the largest genotype-first analysis of schizophrenia susceptibility loci to date, suggest that the phenotypic effects of copy number variants associated with schizophrenia are pleiotropic and imply the existence of shared biologic pathways among multiple neurodevelopmental conditions. ***Genet Med* 2011:13(10):868–880.**

Key Words: *CNV, microarray, deletion, duplication, neurodevelopment*

Schizophrenia is a neuropsychiatric disorder characterized by delusions and hallucinations, paranoia, apathy, anhedonia, social withdrawal, and extensive cognitive impairments.[1] The disorder is relatively common, with a prevalence of 1.1% of the population older than 18 years (National Institute of Mental Health) and a worldwide incidence of approximately 1 in 4000.

The etiology of schizophrenia is not well understood, but it has been established that genetic predisposition is a major determinant of susceptibility to schizophrenia; adoption and twin studies suggest heritability estimates as high as 80%.[2] However, understanding the genetic architecture of a complex disorder such as schizophrenia has been challenging. Conventional genome-wide linkage scans on sib pairs and affected pedigrees have provided limited evidence for strong susceptibility loci, often with limited reproducibility between the various studies.[3,4] More recently, large-scale genotyping of thousands of cases and controls with hundreds of thousands of tagged single-nucleotide polymorphisms throughout the genome has allowed for the examination of common genetic variability associated with complex disease traits. Although such genome-wide association studies (GWAS) have identified common variants associated with complex diseases such as macular degeneration, rheumatoid arthritis, and colon cancer, the results from GWAS of schizophrenia have been less promising.[5–9] Although multiple single-nucleotide polymorphisms have been associated with schizophrenia, none have reached genome-wide significance or could be conclusively implicated in the disease; furthermore, replication of the results from different studies has been limited.[5–9] These data suggest that the heretofore accepted "common-disease/common-variant" model, in which common alleles with low to moderate disease risk may have a cumulative effect,[10] may not be sufficient to explain the genetic factors that cause schizophrenia.

The inconclusive results of GWAS on schizophrenia, combined with the widespread use of higher resolution molecular cytogenetic assays such as microarray-based comparative genomic hybridization (aCGH) for the characterization of individuals with intellectual and physical disabilities, have renewed interest in the search for novel copy number variants (CNVs) associated with schizophrenia. Cytogenetic aberrations have long been associated with schizophrenia.[11] The most well-characterized CNV associated with schizophrenia is the 22q11 microdeletion that results in velocardiofacial syndrome/DiGeorge syndrome (DGS); most individuals with DGS have cognitive deficits of varying severity, and approximately 30% have behavioral abnormalities, including schizophrenia, bipolar disorder, and autism.[12–15] A second reported chromosome abnormality is alteration of the gene *DISC1* on 1q42.2, which was first identified by a balanced translocation t(1;11)(q42;q14) that cosegregated in a large Scottish family with neuropsychiatric diseases including schizophrenia[16–18] and has also recently been implicated in large-scale GWAS.[19] These studies have shown that the genome-wide burden of rare CNVs is significantly greater in individuals with schizophrenia than in healthy controls.[20–22] More recently, recurrent microdeletions at 1q21.1,[23]

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15q13.3,[21,24] and 15q11.2,[22,24] microduplications at 16p11.2,[25] and CNVs at other genomic loci[26–28] have been associated with schizophrenia in large cohorts by CNV analyses and other molecular studies. The identification of these rare variants supports a "common-disease/rare-variant" (CD-RV) model, which suggests that the heterogeneity of schizophrenia comes from multiple rare variants with high penetrance.[29,30] Some of these CNVs have been reported in a very small number of individuals, and large pedigrees are an exception.

To delineate the clinical significance of CNVs previously suggested to be schizophrenia candidate susceptibility loci, we searched our database for specific CNVs previously associated with schizophrenia in all our patients regardless of the indication for study (IFS). In addition, we examined the genomic alterations detected by microarray in individuals with schizophrenia as an IFS to determine whether there was concordance with the CNVs identified in the first group. Our results suggest that CNVs previously associated with schizophrenia are associated with a diverse spectrum of neurologic deficits.

MATERIALS AND METHODS

Patient ascertainment

Individuals in this study for whom additional clinical information was obtained provided written informed consent using an Institutional Review Board Spokane-approved consent form.

Patient identification—abnormalities encompassing previously reported schizophrenia candidate susceptibility loci

Between March 2004 and February 2010, our laboratory performed microarray testing on 38,779 probands who were referred for unexplained physical and/or intellectual disabilities (IDs) with or without dysmorphic features. All individuals with abnormalities of the same or reciprocal copy number state (i.e., gain or loss) overlapping previously reported schizophrenia candidate susceptibility loci were identified in our database (Tables 1 and 2). These included six previously published recurrent loci and 20 "rare" loci >100 kb that have been identified in large-population studies of individuals with schizophrenia[6,21,24] and for which adequate probe coverage was present on our microarrays. The analysis for the latter set of 20 "rare" loci was restricted to include only the most relevant from multiple population-based studies and adequate sensitivity and specificity of our array platform for those genomic intervals. Abnormalities that were substantially larger than the size of the CNV reported in the literature were excluded from further analysis to eliminate cases in which additional genes outside the candidate locus were disrupted (e.g., whole-arm gains/losses). Clinicians were asked to supply clinical information for all cases with overlapping abnormalities. Parental and prenatal sample analyses were not included.

Patient identification—schizophrenia as an IFS

We searched our database of 38,779 probands for individuals with a CNV and an IFS that included "schizophrenia," either as a primary indication or as part of a family history. We identified six such individuals. Information about how a diagnosis of schizophrenia was made was not obtained (Table 3). We then identified all individuals in our database with overlapping abnormalities of the same or reciprocal copy number state (i.e., gain or loss) using the same criteria as earlier.

Microarray analysis

Array comparative genomic hybridization

Microarray analyses were performed between March 2004 and February 2010 with an evolving series of genomic microarrays. The successive seven versions of microarrays have increasing coverage of the genome. The version of the array used on a particular patient depended on the date of sample receipt. Version 1.0 of the bacterial artificial chromosome (BAC)-based SignatureChip® (Signature Genomic Laboratories, Spokane, WA) was used from March 2004 until October 2004, version 2.0 until October 2005, version 3.0 until May 2006, version 4.0 until November 2007, and Whole Genome until December 2009. In addition, oligonucleotide-based microarray analysis was performed on some of the individuals reported in this article using a 105K-feature whole-genome microarray (SignatureChip Oligo Solution™, designed by Signature Genomic Laboratories and made by Agilent Technologies), which was in use from November 2007 to April 2009. The SignatureChipOS V2, a 135K-feature oligonucleotide array designed by Signature Genomic Laboratories and made by Roche-Nimblegen (Madison, WI), is currently in use in our laboratory. A comparison of the genomic coverage and content of each microarray platform can be found at http://www.signaturegenomics.com/clone_list.html. Microarray analysis was performed as previously described for BAC[31] and oligo-based[32] arrays. All results were visualized using our laboratory-developed computer software program Genoglyphix (http://www.signaturegenomics.com/genoglyphix.html). Referral to our laboratory is most commonly based on the clinical presentation of developmental delay, dysmorphic features, developmental disabilities such as autism or epilepsy, and/or multiple congenital anomalies. Copy number alterations detected by aCGH were reported according to previously described criteria.[33] Briefly, reported abnormalities included those that were associated with established genomic disorders, were large and affected a significant gene or genes within the critical interval, or were part of a complex rearrangement such as an unbalanced translocation. Smaller abnormalities were reported if they impacted gene content likely to contribute to the patient's phenotype or when the size of the abnormality could not be well defined if run on a BAC array. For oligonucleotide arrays, based on accepted criteria and extensive validation of the proprietary Genoglyphix software, average intensity thresholds of ± 0.300 for intervals containing a minimum of five probes, ± 0.200 for intervals containing 200 probes, and ± 0.100 for intervals containing 500 probes were used to identify aberrant regions.

Fluorescence in situ hybridization analysis

Abnormalities detected by aCGH were visualized by metaphase or interphase fluorescence in situ hybridization using one or more BAC clones determined to be in the abnormal region as described previously.[34,35]

RESULTS

Individuals with CNVs overlapping recurrent and rare schizophrenia susceptibility loci

We analyzed a subset of 20 important and previously implicated schizophrenia susceptibility loci listed in Tables 1 and 2.[6,20,24] Of these loci, 14 are nonrecurrent, and six are recurrent. We identified 78 individuals harboring CNVs across genomic regions/genes implicated in rare instances (Table 1) and 1035 individuals with CNVs of one of six recurrent identified re-

Table 1 Gains and losses identified by our laboratory of schizophrenia candidate loci

Patient	Age	Indication for study	CNV coordinates	Gain/ loss	Inheritance	Other CNVs	TCAG-DGV and CHOP-CNV entries[a]
1q42.2 (chr1:229,084,935–229,084,935)/*DISC1*							
GC48646	4 yr	DD, DF	chr1:229,786,409–229,877,176	Gain	Mat	None	10 gains and 5 losses
GC50776	7 yr	Autism	chr1:229,815,484–230,007,247	Gain	Unknown	None	10 gains and 5 losses
GC53655	0 mo	MCA	chr1:229,277,960–230,007,247	Loss	Unknown	None	10 gains and 5 losses
2p16.3 (chr2:48,648,754–49,319,683)/*NRXN1*							
GC16414	5 yr 6 mo	DD	chr2:47,401,026–49,050,862	Gain	Unknown	None	7 gains and 140 losses
GC22065	2 yr	DD, MCA	chr2:51,079,474–51,993,245	Gain	Unknown	None	5 gains and 580 losses
GC25162	2 yr	Hypotonia and encephalopathy	chr2:50,835,417–51,079,874	Loss	Unknown	None	25 gains and 64 losses
GC26449	9 yr	Multiple disabilities	chr2:50,629,193–51,079,873	Loss	Unknown	13q12.12 loss	25 gains and 60 losses
GC30019	23 yr	DD, seizure disorder	chr2:50,996,352–51,555,512	Loss	Mat	None	27 gains and 68 losses
GC30020	20 yr	DD, DF	chr2:50,996,352–51,555,512	Loss	Mat	None	27 gains and 68 losses
GC36884	6 yr	DD	chr2:50,271,164–50,936,974	Loss	Unknown	None	0 gains and 11 losses
GC36885	7 yr	Autism	chr2:50,271,164–50,936,974	Loss	Unknown	None	0 gains and 11 losses
GC42036	13 yr	Mild MR, lack of coordination, hearing loss	chr2:50,890,607–51,167,935	Loss	Mat	None	25 gains and 58 losses
GC42093	1 mo	Respiratory distress syndrome	chr2:49,050,661–50,996,413	Gain	Pat	None	4 gains and 270 losses
GC43455	4 yr	DD	chr2:51,079,673–51,459,762	Loss	Dn	None	2 gains and 11 losses
GC45066	15 mo	DD	chr2:50,629,393–50,996,413	Loss	Unknown	3p12.3 loss	0 gains and 11 losses
GC46017	21 mo	Disorder of muscle, ligament, and fascia	chr2:51,048,049–51,215,674	Loss	Pat	11q14.1 loss (pat); 14q13.2 loss (mat)	25 gains and 56 losses
GC49850	8 yr	PDD	chr2:51,048,049–51,215,674	Loss	Unknown	None	25 gains and 56 losses
GC51317	11 yr	Autistic disorder	chr2:50,503,145–50,865,069	Loss	Unknown	None	0 gains and 9 losses
GC51717	1 mo	Respiratory distress, sepsis	chr2:50,763,228–50,954,844	Loss	Unknown	None	0 gains and 8 losses
GC52934	2 yr	DD, speech/motor delay	chr2:50,989,149–51,211,695	Loss	Unknown	None	25 gains and 56 losses
GC53505	3 yr	DD, seizure disorder	chr2:51,124,517–51,354,094	Loss	Unknown	6q26 gain	1 gain and 7 losses
GC54622	5 yr	DD, pulmonic stenosis, ASD	chr2:50,179,711–50,372,437	Gain	Unknown	None	0 gains and 0 losses

(*Continued*)

Table 1 *Continued*

Patient	Age	Indication for study	CNV coordinates	Gain/ loss	Inheritance	Other CNVs	TCAG-DGV and CHOP-CNV entries[a]
2q31.2-q31.3 (chr2:179,643,864–182,145,339)/*UBE2E3,ITGA4,CERKL*							
GC24230	4 yr	DD; t(1;2) (p32.3;q32.2)	chr2:181,676,882–182,629,684	Loss	Unknown	None	3 gains and 17 losses
GC30150[b]	6 yr 6 mo	DD, behavior problems, difficulty focusing, insensitivity to pain	chr2:180,880,359–183,050,227	Loss	Unknown	None	30 gains and 123 losses
GC24978	2 yr 5 mo	DD, DF, MCA	chr2:181,786,766–182,100,518	Gain	Dn	None	0 gains and 0 losses
7q21.11 (chr7:77,451,259–79,099,281)/*MAGI2*							
GC28418	3 yr	DD, microcephaly	chr7:77,892,575–78,307,306	Gain	Mat	None	0 gains and 0 losses
GC41692	3 yr	Not specified	chr7:77,416,989–77,796,903	Gain	Pat	None	0 gains and 1 loss
GC47730	5 yr	DD	chr7:77,625,668–77,670,776	Loss	Unknown	None	0 gains and 0 losses
7q35q36.1 (chr7:144,990,091–148,376,428)/*CNTNAP2*							
GC47218	1 mo	MCA	chr7:146,082,279–146,294,234	Loss	Unknown	None	0 gains and 11 losses
GC33731	8 yr	DD, DF	chr7:147,152,042–147,413,975	Loss	Unknown	None	0 gains and 1 loss
GC43660	15 yr	Not specified	chr7:145,313,965–145,476,481	Loss	Unknown	3q12.2 gain, 16p13.3 loss (*CREBBP*-RTS locus)	1 gains and 2 losses
GC55055	7 yr	DD	chr7:145,237,002–145,540,982	Loss	Unknown	None	1 gains and 2 losses
7q36.1 (chr7:151,069,763–151,531,755)/*PRKAG3, MLL3*							
GC10032	12 yr 5 mo	Central auditory processing disorder	chr7:150,752,371–151,663,649	Gain	Mat	None	12 gains and 22 losses
GC25195	4 yr	VCFS—significantly affected	chr7:151,071,240–151,385,729	Gain	Mat	22q11.21 loss	0 gains and 10 losses
GC25747	10 yr 6 mo	DD, autism	chr7:150,219,239–152,619,553	Gain	Unknown	None	73 gains and 262 losses
GC30217	6 yr 7 mo	DD	chr7:151,225,901–151,575,197	Gain	Unknown	None	8 gains and 6 losses
GC31208	7 yr	Central auditory processing disorder	chr7:151,071,240–151,385,729	Gain	Mat	13q12.1 loss (pat)	0 gains and 10 losses
GC24368	3 yr	DD	chr7:151,071,240–151,385,729	Loss	Dn	2p25.3 gain (dn)	0 gains and 10 losses
8p12 (chr8:31,616,809–32,742,100)/*NRG1*							
GC53746	15 mo	Hypotonia, DD, microcephaly	chr8:32,103,740–32,465,428	Gain	Unknown	None	0 gains and 5 losses
8q24.3 (chr8:142,025,432–142,393,948)/*PTK2, E1F2C2*							
GC7522	3 yr	DD, DF, short stature	chr8:141,375,124–143,213,811	Gain	Unknown	None	283 gains and 88 losses
GC26910	6 yr	Mild MR, DF, hypotonia	chr8:141,793,142–142,145,556	Loss	Unknown	None	58 gains and 0 losses

(*Continued*)

Table 1 *Continued*

Patient	Age	Indication for study	CNV coordinates	Gain/ loss	Inheritance	Other CNVs	TCAG-DGV and CHOP-CNV entries[a]
9p24.3p24.2 (chr:2,013,316–3,118,374)/*SMARCA2, VLDLR*							
GC12715	9 yr	DD, autistic features	chr9:2,922,366–4,437,068	Gain	Pat	None	5 gains and 276 losses
GC13067	8 yr	Short stature	chr9:2,814,583–3,104,871	Gain	Mat	None	0 gains and 2 losses
GC14448	10 yr	Not specified	chr9:2,814,583–3,104,871	Gain	Pat	None	0 gains and 2 losses
GC29843	19 yr	DD	chr9:2,084,860–2,205,240	Gain	Unknown	None	0 gains and 13 losses
GC32511	6 yr 6 mo	DD, DF	chr9:2,484,815–3,162,020	Gain	Unknown	None	2 gains and 15 losses
GC34537	1 yr 4 mo	MCA	chr9:1,447,605–2,351,750	Gain	Unknown	None	5 gains and 59 losses
GC36936	3 yr 5 mo	MCA	chr9:2,284,737–2,816,582	Gain	Unknown	None	2 gains and 13 losses
GC38752	16 yr 7 mo	DD, DF	chr9:1,447,605–2,674,273	Gain	Unknown	None	6 gains and 59 losses
9p24.2 (chr9:3,104,250–3,544,339)/*RFX3*							
GC6738	3 yr 7 mo	DD, postnatal growth retardation	chr9:188,707–3,535,195	Loss	Unknown	None	82 gains and 670 losses
GC13968	3 yr	DD	chr9:3,223,206–3,409,890	Loss	Dn	None	0 gains and 0 losses
GC15750[c]	5 yr	ID, behavior problems	chr9:3,358,032–3,535,195	Loss	Pat	None	0 gains and 1 loss
GC19203	12 yr	DD, functional age 8–9 yr; mild/minimal PDD; epilepsy, generalized, starting at 8yr, no myoclonus or atonic events; no prolonged febrile seizures	chr9:2,284,737–5,809,060	Loss	Unknown	None	42 gains and 791 losses
GC15568	21 yr	MR, DD	chr9:2,284,737–3,535,195	Loss	Dn	None	2 gains and 16 losses
GC19494	6 yr	DD	chr9:2,922,366–3,409,890	Loss	Unknown	None	0 gains and 2 losses
GC44180	5 yr	DD	chr9:3,244,763–3,309,778	Loss	Pat	None	0 gains and 0 losses
GC44467	20 mo	DD, DF	chr9:3,129,007–3,492,815	Gain	Mat	1p36.32 gain (pat)	0 gains and 0 losses
11p13 (chr11:33,260,889–33,540,102)/*HIPK3*							
GC18855	4 yr	Microcephaly, hypotonia	chr11:33,205,943–33,593,825	Gain	Dn	19p13.3 gain (dn)	3 gains and 0 losses
GC36516	3 yr	PDD, stereotype behaviors, high pain tolerance	chr11:32,964,767–33,519,066	Gain	Pat	1p36.33 loss (dn)	4 gains and 2 losses
GC39077	6 yr	DD, Poland anomaly	chr11:32,964,767–33,475,540	Gain	Dn	None	4 gains and 2 losses
GC40992	9 mo	DD, macrocephaly	chr11:33,074,409–33,405,033	Gain	Mat	None	3 gains and 0 losses

(*Continued*)

Table 1 *Continued*

Patient	Age	Indication for study	CNV coordinates	Gain/ loss	Inheritance	Other CNVs	TCAG-DGV and CHOP-CNV entries[a]
11q14.1 (chr11:82,843,705–85,015,962)/*DLG2*							
GC8406	8 mo	DF, speech delay	chr11:84,032,781–84,154,190	Loss	Unknown	None	0 gains and 8 losses
GC33254	22 yr	DD, DF	chr11:84,097,090–84,461,881	Loss	Unknown	15q13.2q13.3 gain	0 gains and 23 losses
GC41800	17 yr	Myopathy	chr11:84,032,781–84,222,602	Loss	Unknown	None	0 gains and 18 losses
GC43330	3 yr	DD	chr11:83,806,353–83,940,575	Loss	Pat	2q13 loss	0 gains and 2 losses
GC46017	21 mo	Disorder of muscle, ligament, and fascia	chr11:84,007,950–84,169,251	Loss	Pat	2p16.3 loss (pat), 14q13.2 loss (mat)	0 gains and 8 losses
GC53207	5 yr	Hypochondriasis	chr11:84,032,781–84,193,841	Loss	Unknown	None	0 gains and 8 losses
18p11.31p11.23 (chr18:7,070,926–7,565,943)/*LAMA1, ARHGAP28, PTPRM*							
GC23345	6 yr	Not specified	chr18:7,329,402–7,649,289	Gain	Unknown	None	0 gains and 3 losses
GC26813	0 mo	Hypoplastic left heart syndrome	chr18:7,329,402–7,649,289	Gain	Unknown	None	0 gains and 3 losses
GC30619	19 mo	Encephalopathy	chr18:7,329,402–7,649,289	Gain	Unknown	5q14.3 loss	0 gains and 3 losses
GC35063	18 mo	Lack of coordination, delayed milestones	chr18:4,660,755–7,536,206	Gain	Mat	None	56 gains and 223 losses
GC40425	4 mo	DD	chr18:6,776,579–7,372,339	Gain	Mat	None	29 gains and 7 losses
GC44238	11 mo	DD, anomalies of skull and face bones	chr18:6,672,974–7,041,982	Gain	Mat	10q23.1 loss (*NRG3*) (mat), additional 18p11.31 gain (mat)	2 gains and 3 losses
19q13.41-q13.42 (chr19:59,045,962–59,363,706)/*NLRP12, MYADM, PRKCG, CACNG6-7-8*							
GC5116	10 yr	DD	chr19:57,807,296–61,304,890	Gain	Unknown	None	>500 gains and >500 losses
GC21640	9 mo	Features consistent with PHACES syndrome	chr19:59,218,314–61,271,527	Gain	Unknown	None	>500 gains and >500 losses
GC40121	4 yr	Autistic disorder	chr19:58,930,106–59,157,057	Gain	Unknown	None	2 gains and 53 losses
GC37334	1 mo	Right midshaft femur fracture failure	chr19:59,237,251–59,294,810	Loss	Pat	None	26 gains and 199 losses

[a]TCAG-DGV data from: http://projects.tcag.ca/variation/; CHOP-CNV data from: http://cnv.chop.edu/.
[b]Parental features, Father: MR, schizophrenia and Mother: MR, bipolar disorder, epilepsy.
[c]Parental features, Father has history of anxiety and depression.
ASD, atrial septal defect; DD, developmental delay; DF, dysmorphic features; dn, de novo; ID, intellectual disability; mat, maternal; MCA, multiple congenital anomalies; MR, mental retardation; pat, paternal; PDD, pervasive developmental delay; RTS, Rubinstein-Taybi syndrome; VCFS, velocardiofacial syndrome.

Table 2 Summary of recurrent CNVs associated with schizophrenia identified by our laboratory

Region	Microdeletion/ microduplication	No. of individuals identified	Inheritance				Average age at diagnosis (yr)	Age range (yr)	Indications for study
			De novo	Maternal	Paternal	Unknown			
1q21.1	Microdeletion	118	12	18	15	73	7.5	0.2–41.0	DD, autism, FTT, DF, seizures, CHD, polydactyly, microcephaly (del), macrocephaly (dup)
	Microduplication	113	7	19	11	76	8.7	0.1–38.5	
15q11.2	Microdeletion	85	0	2	1	82	8.4	0.2–38.4	DD, DF, autism, seizures
	Microduplication	63	0	2	0	61	8.2	0.2–39.8	
15q13.3	Microdeletion	69	5	12	6	46	6.6	0.1–19.7	DD, autism, DF, seizures, hypotonia, OCD, CHD
	Microduplication	44	1	7	7	29	7.8	0.2–41.9	
16p11.2	Microdeletion	98	27	10	0	61	8.9	0.3–31.8	DD, speech/language delay, behavioral problems, autism/ASD, DF, seizure disorder
	Microduplication	59	6	11	6	36	9.1	0.7–25.3	
16p13.11	Microdeletion	32	3	5	6	18	6.6	0.5–30.0	DD, ASD, DF, MCA, epilepsy, seizure disorder
	Microduplication	74	2	14	16	42	7.8	0.7–38.6	
22q11.2	Microdeletion	186	38	4	4	140	7.1	0.2–50.1	DD, behavioral abnormalities, DF, MCA, CHD, FTT, autism, hypocalcemia, seizure disorder, postaxial polydactyly, clubfeet
	Microduplication	94	10	21	12	51	9.2	0.8–43.3	

ASD, autism spectrum disorder; CHD, congenital heart defect; DD, developmental delay; DF, dysmorphic features; FTT, failure to thrive; MCA, multiple congenital anomalies; OCD, obsessive compulsive disorder.

gions—1q21.1, 15q11.2, 15q13.3, 16p11.2, 16p13.11, and 22q11.2 (Table 2).

Table 1 presents inheritance, age at diagnosis, and copy number state of CNV for the individuals in each of the rare loci analyzed in this cohort. Examination of the IFS for these individuals showed a spectrum of neurologic deficits, including developmental and speech delays, behavioral problems, autism/autism spectrum disorder (ASD), and seizure disorders. None of the individuals had an IFS of schizophrenia, although medical records were not obtained to completely exclude the diagnosis. Of these individuals, the inheritance was de novo in seven, maternal in 14, paternal in 12, and unknown in 45 because parents were not tested. The age range for this group was from 1 month to 23 years; the majority ($n = 60$) were at or below 19 years of age.

Thirteen individuals had a second potentially significant CNV (Table 1). Notably, two individuals with CNVs at the *NRXN1* locus (2p16.3) had an additional loss: GC26449 had a 1.34 Mb loss at 13q12.12 and GC45066 had a 2.9 Mb loss at 3p12.3. One individual (GC43660) with a deletion overlapping the *CNTNAP2* locus at 7q35q36.1 had an additional abnormality, a 322 kb loss at 16p13.3 that encompassed *CREBBP*, associated with Rubinstein-Taybi syndrome. One individual (GC25195) with a gain of material from 7q36.1 also had a 2.5 Mb deletion at the 22q11.2 (DGS-velocardiofacial syndrome) region. Three individuals with CNVs at 11q14.1 (*DLG2* locus) had an additional abnormality: one (GC33254) had a 1.3 Mb duplication at the 15q13.2q13.3 microdeletion syndrome locus, which includes *CHRNA7*, one (GC43330) had a 1.7 Mb loss at 2q13 that includes at least five OMIM genes, and the third (GC46017) had an additional 160 kb loss at 2p16.3 that overlaps the *NRXN1* gene. Two individuals with CNVs at 18p11.31p11.23 (*LAMA1*, *ARHGAP28*, and *PTPRM*) had additional significant aberrations; the first case (GC30619) had a 1.73 Mb loss at 5q14.3 that encompassed *MEF2C*, deletions of which have been associated with mental retardation and epilepsy[36–38]; the second (GC44238) had a 468 kb loss at 10q23.1 that overlapped *NRG3* and a 520 kb gain at 18p11.31 that overlapped *DLGAP1*.

We identified 1035 CNVs encompassing one of the six recurrent loci (Table 2). For all six recurrent schizophrenia susceptibility loci we analyzed, the spectrum of phenotypes remained diverse and heterogeneous and distinct from schizophrenia (Table 2). For at least two of these loci (16p11.2 and 22q11.2), there were substantially more cases with copy number losses than with copy number gains, reflecting a potentially benign outcome in some carriers of the duplication. Parental testing was limited except for 22q11.2 and 16p11.2 deletion cases.

Individuals with schizophrenia as IFS

Between March 2004 and February 2010, we analyzed 38,779 individuals by aCGH. Of these, six with a likely clinically relevant CNV were referred with an IFS of schizophrenia ($n = 5$) or family history of schizophrenia ($n = 1$) (Table 3). One abnormality, a 3.62 Mb duplication at 4q21.22q21.23, was present in a 33-year-old man with schizophrenia and his child with developmental delay. We also identified 37 individuals

Table 3 Summary of microarray results for six individuals referred for schizophrenia or family history of schizophrenia and 37 individuals with overlapping abnormalities

Patient	Age	Indication for study	CNV coordinates	Gain/ loss	Inheritance	Other CNVs	Parental features	TCAG-DGV and CHOP-CNV entries[a]
22q12.1q12.2 (chr22:21,583,587–33,269,269)								
GC2623	15 yr	Schizophrenia, known duplication 22q12.1q12.2	chr22:22,217,953–22,530,221	Gain	Unknown	None		21 gains; 781 losses
GC37754	13 yr	DD, hearing loss, learning disability	chr22:22,282,867–22,565,867	Gain	Mat	None		14 gains; 139 losses
GC42403	9 yr	DD, r/o Williams syndrome	chr22:22,369,475–22,581,617	Gain	Mat	None		2 gains; 9 losses
GC33668	3 mo	MCA	chr22:22,464,914–22,603,194	Gain	Unknown	None		1 gain; 14 losses
GC46253	5 yr	DD	chr22:22,476,593–22,603,194	Gain	Mat	None		1 gain; 14 losses
GC62502	23 mo	DD	chr22:22,468,728–22,600,357	Gain	Unknown	None		1 gain; 14 losses
13q13.3q14.11 (chr13:39,340,184–43,052,251)								
GC10217	9 yr	DD, family hx of schizophrenia	chr13:39,340,184–43,052,251	Gain	Unknown	None		257 gains; 104 losses
GC39030	20 yr	DD, short stature, autism	chr13:39,096,744–39,516,428	Loss	Pat	None		1 gain; 2 losses
GC55840	1 mo	Preterm infant, congenital hydrocephalus	chr13:40,101,000–40,406,145	Gain	Mat	None		2 gains; 0 losses
GC62258	7 yr	DD, DF, microcephaly	chr13:38,017,451–38,757,393	Loss	Unknown	None		5 gains; 309 losses
Xp22.31 (chrX:6,450,427–6,677,693)/*VCX, PNPLA4*								
GC18033	17 yr	Unspecified schizophrenia, post-traumatic stress disorder	chrX:7,633,882–8,039,507	Gain	Unknown	None		21 gains; 14 losses
GC10158	8 yr	DD, DF, strabismus	chrX:7,633,882–8,039,507	Gain	Unknown	None		21 gains; 14 losses
GC23724	8 yr	DD, seizure disorder, tuberous sclerosis	chrX:7,633,882–8,039,507	Gain	Mat	None		21 gains; 14 losses
GC17499	8 yr	DD, DF	chrX:7,633,882–8,039,507	Gain	Mat	None		21 gains; 14 losses
GC18547	5 mo	DF	chrX:7,633,882–8,039,507	Gain	Mat	None		21 gains; 14 losses
GC24565	11 yr	DD, hypotonia, MR	chrX:7,565,292–8,057,512	Gain	Unknown	None		21 gains; 14 losses
GC42155	2 mo	Congenital CMV	chrX:7,486,181–8,091,811	Gain	Pat	None		21 gains; 18 losses
GC19995	7 yr	DD, DF	chrX:7,466,397–8,039,507	Gain	Pat	None		21 gains; 18 losses
GC18598	0 mo	Pulmonary oligohydramnios	chrX:7,466,397–8,039,507	Gain	Unknown	None		21 gains; 18 losses
GC16863	7 yr	DD	chrX:7,466,397–7,884,342	Loss	Unknown	None		17 gains; 18 losses

(*Continued*)

Table 3 *Continued*

Patient	Age	Indication for study	CNV coordinates	Gain/ loss	Inheritance	Other CNVs	Parental features	TCAG-DGV and CHOP-CNV entries[a]
GC25423	8 yr	Learning disabilities	chrX:7,466,397–7,805,167	Gain	Unknown	None		17 gains; 17 losses
GC26540	14 mo	DD, DF	chrX:7,327,226–8,039,507	Gain	Unknown	None		19 gains; 20 losses
8p23.1 (chr8:10,291,322–13,026,502)/3′*GATA4, NEIL2, FDFT1*								
GC27235	20 yr	Schizophrenia, nonverbal learning disorder, peripheral neuropathy	chr8:11,538,850–11,778,975	Gain	Unknown	None		Larger gains; 39 losses
GC22726	15 yr	DD	chr8:11,538,850–11,778,975	Gain	Unknown	None		Larger gains; 39 losses
GC21874	8 yr	Autoimmune disorder	chr8:11,538,850–11,778,975	Gain	Mat	None		Larger gains; 39 losses
GC60769	2 yr	DD, DF	chr8:11,475,676–11,895,875	Gain	Unknown	None		9 gains; 131 losses
GC40731	8 yr	Metabolic acidosis	chr8:11,613,171–11,843,370	Gain	Unknown	None		4 gains; 39 losses
GC36743	13 mo	DD, hypotonia, gross motor delay	chr8:11,660,979–11,759,064	Loss	Mat	None		Larger gains; 35 losses
4q21.22q21.23 (chr4:83,648,321–87,269,792)								
GC10607	33 yr	Cleft palate, schizophrenia, DD, child with 4q21q22q21.23 duplication and DD	chr4:83,648,321–87,269,792	Gain	Unknown	None		19 gains; 670 losses
GC32594	5 yr	DD	chr4:83,648,321–87,269,792	Gain	Pat	None	Cleft palate, schizophrenia, DD	19 gains; 670 losses
GC66966	3 yr	DD, hypotonia	chr4:83,084,674–85,361,489	Loss	Unknown	None		3 gains; 214 losses
GC47800	17 yr	Encephalopathy	chr4:82,632,273–84,004,580	Loss	Unknown	None		1 gain; 135 losses
GC30526	18 mo	Lack of coordination, delayed milestones	chr4:83,661,721–84,056,444	Loss	Dn	None		0 gains; 152 losses
GC21877	5 yr	Seizures	chr4:83,776,912–84,056,444	Loss	Unknown	None		0 gains; 152 losses
Xq25q26.2 (chrX:129,594,577–130,607,672)/*ENOX2, IGSF1*								
GC35772	12 yr	Seizure disorder, MR, schizophrenia	chrX:129,594,577–130,607,672	Gain	Unknown	None		12 gains; 3 losses
GC47074	1 mo	Abnormal ultrasound, Dandy Walker variant, MCA	chrX:129,379,323–130,000,421	Gain	Mat	None		1 gain; 7 losses
GC43948	20 mo	Encephalopathy	chrX:130,131,441–130,775,694	Gain	Mat	None		10 gains; 3 losses
GC62183	4 yr	Microcephaly	chrX:129,597,964–130,221,615	Gain	Unknown	None		9 gains; 2 losses
GC60779	8 yr	DD, autism	chrX:129,928,410–130,416,452	Loss	Unknown	None		11 gains; 2 losses

(*Continued*)

Table 3 *Continued*

Patient	Age	Indication for study	CNV coordinates	Gain/ loss	Inheritance	Other CNVs	Parental features	TCAG-DGV and CHOP-CNV entries[a]
GC40249	16 yr	MCA	chrX:129,986,312–130,665,664	Gain	Mat	None		11 gains; 3 losses
GC43743	0 mo	DF, MCA	chrX:130,000,361–130,714,869	Gain	Mat	None		11 gains; 3 losses
GC42359	16 mo	Other conditions due to sex chromosome anomalies	chrX:130,221,555–130,585,032	Gain	Mat	None		3 gains; 1 loss
GC49270	2 yr	DD	chrX:130,221,555–130,714,869	Gain	Mat	None		3 gains; 1 loss

[a]TCAG-DGV data from: http://projects.tcag.ca/variation/; CHOP-CNV data from: http://cnv.chop.edu/.
CMV, cytomegalovirus; DD, developmental delay; DF, dysmorphic features; dn, de novo; hx, history; ID, intellectual disability; mat, maternal; MCA, multiple congenital anomalies; MR, mental retardation; pat, paternal; r/o, rule out.

with an overlapping gain/loss of a CNV identified in an individual with schizophrenia. Table 3 lists inheritance, age at diagnosis, and copy number state of CNV for the six individuals with an IFS of schizophrenia and the 37 individuals with overlapping CNVs. Examination of the IFS for individuals with CNVs overlapping those identified in individuals referred for schizophrenia showed a spectrum of neurologic deficits, including developmental and speech delays, behavioral problems, autism/ASD, and seizure disorders. Although none of the individuals had an IFS of schizophrenia, medical records were not obtained to completely exclude the diagnosis. Of these individuals, the inheritance was de novo in one, maternal in 15, paternal in four, and unknown in 17 because parents were not tested. The age range for this group was from 1 month to 20 years; all but one individual was at or below 19 years of age. None of the CNVs identified in the individuals with an IFS of schizophrenia overlapped known or putative schizophrenia susceptibility loci.

DISCUSSION

Elucidating the genetic architecture of complex disorders is confounded by the large number, low frequency, and variable effect of predisposition loci. Genomic CNVs have been established as a major source of human genetic variation that underlie many neurologic and neurodevelopmental syndromes including schizophrenia. Although a number of large-scale studies have now revealed an increased load of copy number variation in patients with schizophrenia compared with the normal population,[21,22,24–28] no large-scale analysis has been performed to determine the phenotypic spectrum of the CNVs that have been implicated in schizophrenia. Although previous studies have characterized rare CNVs in large populations of individuals with schizophrenia, this study identified CNVs previously associated with schizophrenia in individuals referred for genetic testing for a broad spectrum of physical and developmental deficits. Our results further refine the clinical spectrum associated with schizophrenia susceptibility loci.

CNVs present in individuals with schizophrenia are associated with a diverse spectrum of neurodevelopmental deficits and are not unique to schizophrenia. For the nonrecurrent and recurrent loci, the phenotypic spectrum was diverse and included developmental delay, ID, autism spectrum, and multiple congenital anomalies. The 22q11.2 microdeletions are the most well-characterized example of heterogeneity; the deletion can be associated with multiple neuropsychiatric disorders including schizophrenia, attention deficit/hyperactivity disorder, bipolar disorder, and ASD.[10,39,40] We have now identified 280 cases of CNVs at 22q11.2 (deletions [$n = 186$] and duplications [$n = 94$]) in individuals with diverse neurologic deficits including ID, DD, and seizures (Table 2). Evidence from other disorders also suggests pleiotropic effects. CNVs of the 1q21.1 region, for example, are associated with a spectrum of neurodevelopmental deficits including autism[41] and schizophrenia.[23] Although phenotypic heterogeneity has been established for many of the recurrent CNVs, our results broaden the neurodevelopmental spectrum of sporadic CNVs that had previously been identified in a few individuals in large-population studies. For example, we identified 19 individuals with CNVs of 2p16.3 that encompass the *NRXN1* gene, which is a neurodevelopmentally important gene. The spectrum of neurologic deficits in our cases ranged from mild MR to seizure disorders and ASD.

The frequency in our patient and parent populations of some of the CNVs associated with neurodevelopmental conditions suggests that the CD-RV model may not sufficiently explain the role of these CNVs in schizophrenia. For all loci, we identified de novo, maternally and paternally inherited aberrations, although the origin of the CNV in many probands was unknown because one or both parents were unavailable for testing. In some cases, the CNV was inherited from a carrier parent with milder neurologic deficits than the proband. For example, the 177 kb microdeletion at 9p24.2 identified in patient GC15750 (Table 1) was inherited from his carrier father, who had a history of anxiety and depression. The inheritance of a CNV from a parent who displays a milder form of the IDs identified in the proband has been reported for some CNVs.[42] In addition, although recent evidence suggests CNVs of 15q11.2, which is within the larger Prader-Willi/Angelman syndrome deletion region between breakpoints I and II, may be enriched in patients with idiopathic generalized epilepsy, autism, and other neurocognitive phenotypes,[23,43] the 15q11.2 CNV is relatively common in the normal population and has rarely been reported to be de novo.[24,44] Although the CD-RV model hypothesizes that one or a few rare, highly penetrant CNVs contribute to schizophrenia, the presence of these CNVs in a high proportion of reportedly normal carrier parents suggests they may indeed not be highly penetrant. These results support other studies in which

Table 4 Estimates of frequency for recurrent CNVs associated with schizophrenia in our patient population and a previously published control population

CNV	Frequency[a]	Controls[b]	Significance[c]	Previously reported case/control comparison in schizophrenia populations	Previously reported case/control comparison in variable neurodevelopmental deficit[d] population[45]
1q21.1 microdeletion	107/23,250 (0.46%)	3/5674 (0.02%)	$P<0.0001$	0.2%/0.023%[22]	0.47%/0.0%
1q21.1 microduplication	113/23,250 (0.49%)	3/5674 (0.02%)	$P<0.0001$	NA	0.17%/0.02%
15q11.2 microdeletion	96/13,670 (0.70%)	4/5674 (0.07%)	$P<0.0001$	0.6%/0.2%[22]	NA
15q11.2 microduplication	83/13,670 (0.61%)	0/5674 (0.0%)	$P<0.0001$	NA	NA
15q13.3 microdeletion	69/23,250 (0.30%)	0/5674 (0.0%)	$P<0.0001$	0.2%/0.017%[22]	0.48%/0.02%
15q13.3 microduplication	44/23,250 (0.19%)	13/5674 (0.23%)	$P=0.6598$	NA	NA
16p11.2 microdeletion	98/23,250 (0.42%)	3/5674 (0.05%)	$P<0.0001$	0.03%/0.03%[25]	0.78%/0.02%[25]
16p11.2 microduplication	59/23,250 (0.25%)	1/5674 (0.02%)	$P=0.0008$	0.03%/0.03%[25]	0.46%/0.02%[25]
16p13.11 microdeletion	32/23,250 (0.14%)	0/5674 (0.0%)	$P=0.0101$	0.12%/0.04%[46]	0.48%/0.0%
16p13.11 microduplication	74/23,250 (0.32%)	0/5674 (0.0%)	$P<0.0001$	0.3%/0.09%[46]	0.48%/0.25%
22q11.2 microdeletion	115/23,250 (0.49%)	0/5674 (0.0%)	$P<0.0001$	0.2%/0.0%[22]	NA
22q11.2 microduplication	63/23,250 (0.27%)	0/5674 (0.0%)	$P<0.001$	NA	NA

[a]Based on period from November 2007 to February 2010. Frequency of 15q11.2 CNVs was determined based on cases analyzed on oligonucleotide-based arrays (n=13670).
[b]Based on meta-analysis of 5674 controls.[47]
[c]Based on χ^2 test with Yates correction.
[d]Includes individuals with autism and intellectual disability.

the penetrance of recurrent schizophrenia candidate loci is between 2 and 7.4%.[45]

To determine the relative frequencies of the recurrent CNVs among our study population, we compared the identification rates of each recurrent CNV from November 2007 to February 2010, during which time our laboratory performed testing using whole-genome platforms that had dense coverage for all six recurrent CNV regions. This comparison allowed us to retain consistency in size and breakpoints for the recurrent deletions and duplications. During this interval, we tested 23,250 individuals. The frequency of the recurrent CNVs ranged from 0.14% for 16p13.11 microdeletions to 0.70% for 15q11.2 microdeletions (Table 4). The incidence of these CNVs in the general population can be estimated by comparing their detection rate in our laboratory with that of a genomic disorder with a well-established incidence, such as Smith-Magenis syndrome (SMS), which has a frequency in the general population of approximately 1/15,000.[48] During this same period, we identified 27 SMS deletions (0.12% of patient population). By comparison, 1q21.1 microduplications were identified in 113 individuals (0.49% of our study population). Thus, because they were identified more than four times more frequently in our study populations than SMS deletions, the incidence of 1q21.1 microduplications in the general population may be inferred to be >1/3700. These may be overestimates of frequency because some cases of SMS are diagnosed by other methods, and therefore, not all individuals with these syndromes will have aCGH, whereas the abnormalities associated with neurodevelopmental disorders do not have clearly recognizable constellations of clinical features and would not be expected to be diagnosed by other methods. Comprehensive compilation of data from our clinical collection and that from focused study populations provide a comparison of the frequencies between the groups. Taken in concert with the frequency of these recurrent CNVs in apparently normal carrier parents, our data indicate that some of the recurrent CNVs associated with schizophrenia may not fit the CD-RV model. Rather, such CNVs may lie on a spectrum between rare highly penetrant mutations and common low-risk CNVs that, in aggregate, contribute to complex disorders. These CNVs may act as modifiers or vulnerability factors for neurologic deficits rather than directly influencing the specific disease outcome.[42,45] Additionally, incomplete penetrance and variable expressivity may be valid explanations for assessing the outcome of many of these inherited CNVs. Most of these recurrent CNVs—with the notable exception of the 15q13.3 microduplication—seem to be enriched in our study population compared with a previously published population of normal control individuals (Table 4); the statistical significance of the frequency of these 11 recurrent CNVs compared with their frequency in the control population (χ^2 with Yates' correction) revealed significant P values (<0.0001) for microdeletions at 1q21.1, 15q11.2, 15q13.3, 16p11.2, and 22q11.2 and microduplications at 1q21.1 and 16p13.11, respectively. The significance for the remaining loci was less profound (Table 4). These data further suggest that the odds of most of these abnormalities to be associated with a variable phenotype are significant or approach significance, with the exception of duplication 15q13.3. Further studies of the frequencies of these CNVs in normal control populations are necessary to establish their degree of enrichment in individuals with neurodevelopmental impairments.

For some of the rare rearrangements that have been previously reported in only one case, our data suggest that these abnormalities are indeed clinically relevant and not rare benign polymorphisms. For example, we identified eight CNVs (seven deletions and one duplication) encompassing an approximately 440 kb interval on 9p24.2 that includes the *RFX3* gene, deletion

of which was identified in one individual with schizophrenia in the cohort described by Walsh et al.[20] The deletion sizes in these individuals ranged from 177 kb to 3.5 Mb, and the clinical features in these individuals included autism, behavioral problems, developmental delay, and ID. The deletion was de novo in two individuals, paternally inherited in two, maternally inherited in one, and of unknown origin in three. Thus, although these CNVs may not be unique to individuals with schizophrenia, our results suggest rare, nonrecurrent CNVs that have only been identified in single cases in previous large-population studies[6,20–22,24] may be clinically significant.

Because the symptoms of schizophrenia typically do not appear until early adulthood (with the exception of the more severe childhood-onset schizophrenia), we cannot rule out the future manifestation of schizophrenia in the young patients in our study. However, because the prevalence of schizophrenia in individuals with IDs and developmental delay is reportedly three times that of the normal population,[49] we would expect that schizophrenia would be enriched in patients older than 16 years identified by our laboratory. Of the 1035 individuals with recurrent CNVs, 123 were older than 16 years at diagnosis. However, none of these individuals had an IFS of schizophrenia, and many had an IFS of a distinct neurologic deficit (e.g., ASD). It has been suggested that based on structural studies of the brains of individuals with comorbid learning disability and schizophrenia and those with only schizophrenia that the presence of schizophrenia (whether diagnosed or destined to develop) predisposes some individuals to develop IDs.[50,51] This raises the possibility that schizophrenia may be at one end of a phenotypic continuum, starting with early childhood-onset developmental/ID that eventually culminates in schizophrenia in adulthood in a fraction of cases. Thus, one might conclude that the cooccurrence of similar genetic aberrations in individuals with MR/ID and individuals with schizophrenia is a result of comorbidity between schizophrenia and MR. However, the presence of heterogeneous neurocognitive deficits in our study, including some quite distinct from schizophrenia—such as attention deficit/hyperactivity disorder—and the absence of schizophrenia in any of our patients with MR suggest not that schizophrenia predisposes to MR/DD but that a common genetic insult predisposes to a myriad of neurologic problems. Long-term follow-up of our patients to determine and document any evolving phenotype will be required to address these issues. The other, more acceptable explanation may be that the genetic defects are truly pleiotropic and the outcome in an individual harboring a CNV can be very different and potentially influenced by environmental factors and modifier effects of other loci. These data also imply the existence of shared biologic pathways among multiple neurodevelopmental conditions, and the suggestion that any specific locus is unequivocally associated with only schizophrenia or any other specific neurodevelopmental conditions should be made with caution.

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