Copy number variations associated with autism spectrum disorders contribute to a spectrum of neurodevelopmental disorders

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Purpose: Autism spectrum disorders represent a range of neurodevelopmental disorders that have been shown to have a strong genetic etiological component. Microarray-based comparative genomic hybridization and other molecular cytogenetic techniques are discovering an increasing number of copy number variations in individuals with autism spectrum disorder. **Methods:** We examined the yield of abnormal microarray-based comparative genomic hybridization findings in our laboratory for individuals referred for testing for autism spectrum disorder. We also examined the presence of autistic features among 151 additional individuals who were referred for microarray-based comparative genomic hybridization testing for indications other than autism spectrum disorder but had genomic alterations overlapping those found in cases referred for autism spectrum disorder. **Results:** We identified 1461 individuals referred for testing for autism spectrum disorder, with likely significant abnormalities reported in approximately 11.6% of individuals analyzed with whole-genome arrays. These abnormalities include alterations that encompass novel candidate genes such as *SNTG2, SOX5*, *HFE,* and *TRIP38*. A minority of individuals with overlapping abnormalities (19%) had autistic features, and many of the copy number variations identified in our study are inherited (69% among those found in individuals with autism spectrum disorder). **Conclusions:** Our results suggest these copy number variations are one of multiple factors contributing to the development of an autism spectrum disorder phenotype. Additionally, the broad phenotypic spectrum of the patients with these copy number variations suggests that these copy number variations are not autism spectrum disorder-specific but likely more generally impair neurodevelopment. ***Genet Med*** **2010:12(11):694–702.**

Key Words: *autism, ASD, microarray, CNV, neurodevelopment*

Autism spectrum disorders (ASDs, OMIM 209850) describe a range of behaviors that involve varying degrees of impaired language development, socialization, and interests. Individuals with autism, or autistic disorder, at the severe end of the spectrum, have findings before 3 years of age in three categories: impaired reciprocal social interaction, impaired communication, and restricted, repetitive, or stereotyped behaviors. Individuals with Asperger syndrome have all characteristic impairments except for language deficits, manifesting before 3 years of age. For those with impairments in only two of the three categories or impairments manifesting after 3 years of age, a diagnosis of pervasive developmental delay (PDD) not otherwise specified is given.[1] ASD is estimated to have an incidence of approximately 0.6% in the general population, although a recent study suggests the prevalence of ASD may be increasing, nearing 1%.[2,3] ASD occurs more commonly in males, with a recent study demonstrating a 4.5:1 male-to-female ratio.[2,3]

The genetics of ASD are heterogeneous and not fully understood. Only 10–20% of individuals with ASD have a known etiology, which may include single-gene disorders and cytogenetic abnormalities.[4] However, the heritability of autism is high, with twin studies yielding estimates of 90% or higher for the narrow phenotype of autism.[5] This has prompted a search for susceptibility loci, with linkage studies and genome-wide screens yielding many candidates. Because of this large number of loci and the differences in monozygotic and dizygotic concordance rates, ASD is frequently considered to have a complex, multigenic etiology with environmental influence.[6]

A variety of cytogenetic abnormalities has been detected in patients with ASD, including rearrangements such as maternally derived duplications of the Prader-Willi/Angelman syndrome region on 15q11q13, estimated to be present in 1% of ASD cases[4,5,7]; terminal deletions of 2q and 22q[8]; and deletions of 7q31.[5] Smaller, novel deletions and duplications have been found in large-population studies of individuals with ASD using microarray-based comparative genomic hybridization (aCGH),[9–13] including some recurrent abnormalities, such as the microdeletion and microduplication of 16p11.2.[14–16] Detection rates for copy number variations (CNVs) in these large studies are 7.0–11.5% for detrimental abnormalities,[9,12] 7–10% for de novo abnormalities in sporadic autism,[14,17] and 11.6–12.5% for autism-specific abnormalities.[10,11] This detection rate increases when looking at syndromic ASD (ASD cooccurring with other symptoms suggestive of a genetic syndrome), with one study showing a detection rate of 27.5%.[13]

Because of the finding of genomic imbalances in individuals with ASD, aCGH has been recommended as a first-tier test in the evaluation of patients with ASD, with the potential of having the highest yield of any single, clinically available test.[9,18] In this report, we examine the yield of abnormal aCGH findings in our laboratory for individuals referred for testing for ASD. Additionally, we survey the presence of autistic features in individuals with deletions and duplications overlapping those found in individuals with ASD to elucidate the spectrum of neurodevelopmental abnormalities associated with these CNVs.

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MATERIALS AND METHODS

Bacterial artificial chromosome microarray analysis

Microarray analysis was performed on some individuals in this cohort (Table 1) with a bacterial artificial chromosome (BAC) microarray (the SignatureChip®; Signature Genomic Laboratories, Spokane, WA). The five versions of the microarray have increasing coverage of the genome, and the array version used was based on time of sample receipt. Version 1.0 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 the SignatureChip Whole Genome (SignatureChipWG) until December 2009. Array coverage was designed to target specific microdeletion/microduplication regions, subtelomeres, and pericentromeres. The more recent arrays have increasing inclusion of more microdeletion/microduplication regions and genes in important developmental pathways, with the WG array adding coverage to the spaces between targeted regions, with an average gap size between contigs of ~1.6 Mb. A comparison of the contents of versions 1.0-WG is available at: http://www.signaturegenomics.com/clone_list.html. Microarray analysis was performed as described.[19,20] Results were visualized using our laboratory-developed computer software program Genoglyphix (available at: http://www.signaturegenomics.com/genoglyphix.html).

Oligonucleotide aCGH

Oligonucleotide-based microarray analysis was performed on some of the individuals reported in this cohort (Table 1) using a 105K-feature whole-genome microarray (SignatureChip Oligo Solution™, custom designed by Signature Genomic Laboratories, made by Agilent Technologies, Santa Clara, CA) as described previously.[21] This oligonucleotide-based microarray was offered for clinical use beginning February 2008, and physicians have been given the option of using this array or the BAC-based microarray for their patients' samples since that time.

Fluorescence in situ hybridization

Abnormalities detected by aCGH were visualized by metaphase or interphase fluorescence in situ hybridization using one or more BAC clones determined to be abnormal by aCGH.[22,23]

Subject identification

We searched our database of more than 5,000 genomic abnormalities found in 22,680 samples submitted for clinical testing for CNVs in individuals with indications for study related to an ASD, including "autism," "autistic," "PDD," "pervasive" (developmental delay), and "Asperger" (syndrome). A genomic abnormality was considered potentially causative if it met at least one of the following criteria: (1) de novo in origin, (2) overlapping with another case in our database referred for ASD, (3) overlapping with autism-associated genes or loci reported in the literature, or (4) >1 Mb in size.

Evaluation of cases with abnormalities overlapping those detected in individuals with ASD

We pursued further analysis of some of the loci determined to potentially causative in the individuals with ASD, excluding abnormalities associated with a well-described syndrome and abnormalities of the sex chromosomes. All individuals with abnormalities of the same copy number state (i.e., gain or loss) overlapping these autosomal loci were identified in our database. Clinicians were asked to supply information about the presence or absence of ASD for all cases with overlapping abnormalities, with the exception of individuals younger than 15 months, who were too young to be evaluated for ASD. Information about how a diagnosis of ASD was made was not obtained.

RESULTS

Genomic abnormalities in individuals referred for ASD

Between March 2004 and July 2008, 22,680 samples were submitted for clinical testing. Of these, 1,461 had an ASD as an indication for study. Abnormalities were reported in 180 (12.3%) of these cases. The rate of abnormalities reported was higher with whole-genome arrays (16.8% for the BAC microarray and 23.5% for the oligonucleotide microarray) compared with 8.6% for Versions 1–4 of the targeted BAC microarray (Table 1). Based on the criteria stated earlier in the text, the abnormalities in 113 of these cases were determined to be potentially causative, yielding a detection rate of 7.7% among

Table 1 Frequency of abnormalities found by different microarray platforms

	Targeted BAC array version 1–4	Whole-genome BAC array	Whole-genome oligonucleotide	Total	*P* (targeted vs. whole genome)	*P* (whole-genome BAC vs. oligonucleotide)
Total cases analyzed	15,467	5,422	1,791	22,680		
Number of cases with ASD indication for study	881	482	98	1,461		
Number of ASD cases with abnormality reported	76	81	23	180	1.3×10^{-7}	0.15
Abnormality rate	8.6%	16.8%	23.5%	12.3%		
Number of ASD cases with potentially causative abnormality[a]	46	54	13	113	9.4×10^{-6}	0.60
Rate of potentially causative abnormalities[a]	5.2%	11.2%	13.3%	7.7%		

[a]See "Materials and Methods": subject identification for criteria used to classify abnormalities.
ASD, autism spectrum disorder; BAC, bacterial artificial chromosome.

all cases referred for ASD (Table 1). Detection rates of these abnormalities were not significantly different between the BAC and oligonucleotide whole-genome arrays ($P = 0.60$, two-tailed Fisher's exact test), but detection rates were significantly greater in the whole-genome arrays compared with earlier targeted BAC array Versions 1–4 ($P = 9.4 \times 10^{-6}$, one-tailed Fisher's exact test). The abnormalities found in the remaining 67 cases referred for ASD did not meet criteria for potentially causative abnormalities (Table, Supplemental Digital Content 1, http://links.lww.com/GIM/A122).

Of the 113 cases with potentially causative abnormalities (Table, Supplemental Digital Content 2, http://links.lww.com/GIM/A123), 34 abnormalities were associated with syndromes that have been well reported and associated with ASD, and these were excluded from the second part of the study in which autistic features were assessed in cases with overlapping abnormalities. Also excluded were the 16 cases with abnormalities considered to be potentially causative on the X or Y chromosome, leaving 63 cases with abnormalities in 41 distinct autosomal regions in which to investigate ASD features in cases with overlapping abnormalities.

ASD in individuals with genomic alterations in autosomal loci of interest

Information about ASD or autistic features was obtained for 151 individuals with overlapping abnormalities and without an ASD indication for study, 29 of whom (19%) were found to have autistic features or an ASD diagnosis (Table, Supplemental Digital Content 3, http://links.lww.com/GIM/A118). The altered genomic regions of all cases with autistic features were compared to determine the smallest region of overlap of their abnormalities, some of which overlapped with previous reports of CNVs found in ASD or included genes that have been associated with ASD (Table 2). The CNVs surveyed were mostly inherited (41/59, or 69%, among autistic individuals, and 42/68, or 62%, among nonautistic individuals).

DISCUSSION

Our survey of potentially causative CNVs detected in a series of 1461 individuals referred for aCGH testing because of an ASD or autistic features allows for a broader view of the contribution of these CNVs to neurodevelopmental disorders. For CNVs at most of these loci, autistic features were only present in a subset of individuals, indicating these genomic gains and losses are likely one of multiple factors contributing to features of ASD. This phenotypic variability may also be indicative of a more general contribution of the CNVs to a range of neurodevelopmental disturbances, and other factors likely influence the ultimate phenotypic penetrance and expressivity. These CNVs may affect biological pathways that generally impact neurodevelopment and manifest themselves phenotypically as developmental delays, mental retardation (MR), ASD, or other neuropsychiatric disorders. Our survey also identified CNVs not previously reported in association with ASD. Although the gene content of some of these novel CNVs is compelling, we have a limited number of cases in our study, so further research is required to determine their pathogenicity.

Our results demonstrate CNVs at loci associated with ASD do not always result in autistic features. These data also support previous studies of recurrent microdeletions and microduplications, such as those at 15q13.3[24–29] and at the distal region of 1q21.1,[30,31] in which autistic features are present in only a subset of cases. Although formal ASD evaluations were not performed for all patients in the study, we were able to ascertain that some patients' neurobehavioral profiles clearly did not raise clinical suspicion of an ASD. For example, patients found to have the 15q13.3 microdeletion who did not have autistic features instead had developmental delays, seizures, language difficulties, and other behavioral issues such as attention-deficit hyperactive disorder. In addition, some of these CNVs may be inherited from an apparently normal parent,[29,30,32,33] which further suggests these CNVs are not always associated with neurodevelopmental impairments; they demonstrate reduced penetrance and variable expressivity. Therefore, it is difficult to predict whether an individual with one of these CNVs will develop autistic features, another neurodevelopmental disorder, or neither, particularly in prenatal or asymptomatic cases.

Some of the CNVs identified in this study overlap with those found in cohorts of patients with other neurodevelopmental disorders, further broadening the phenotypic spectra associated with these CNVs. For example, CNVs within *DPP6* and *CNTNAP2*, for which our study has one duplication and two deletions, respectively, in autistic individuals, have also been associated with schizophrenia and attention-deficit hyperactive disorder without being present in a control population.[34] CNVs in schizophrenia cohorts have included duplications of 7q36.1 and 9p24.2[35]; our study has one and two individuals, respectively, with these duplications and autistic features. Microdeletions of 15q13.3 have been significantly associated with schizophrenia,[36,37] and we have seven individuals in our study with this deletion and autistic features. Our laboratory has identified a 16p12.2 microdeletion in a patient referred for schizophrenia (Sahoo et al., in preparation) in addition to three in this study with autistic features. ASD is frequently comorbid with MR, and ASD is occasionally identified in individuals with genetic syndromes not typically associated with ASD, similar to three cases in this cohort referred for ASD who had the Williams syndrome microdeletion at 7q11.2. This MR-ASD comorbidity may be because individuals with reduced mental capacity cannot compensate for social impairments caused by other genetic or environmental factors[38] or because MR and the cognitive and social/behavioral impairments in ASD share common physiological pathways.[39,40] The phenotypic spectra associated with these various CNVs suggest that these genomic alterations likely contribute to abnormal neurodevelopment, but other factors, both genetic and environmental, may be needed to contribute to the development of an ASD or other specific phenotype. This overlap between causes of ASD and other neurodevelopmental disorders implicates an increasing number of potential genetic causes for ASD, and the causes likely differ from one case to the next, complicating the identification of any single factor.

The CNVs surveyed in our study include some with a novel association with ASD, although further study of these genes and replication in other cohorts will be necessary to determine whether this association with ASD is significant. The CNVs include a de novo deletion of *SOX5* (OMIM 604975), a transcription factor shown to play roles in chondroblast function and oligodendrocyte differentiation and migration; a de novo deletion of *HFE,* mutations of which are associated with the autosomal-recessive *HFE*-associated hereditary hemochromatosis (OMIM 235200), *TRIP38,* whose function is unknown, and multiple histone genes at 6p22.1; and a maternally inherited deletion of *SNTG2* (OMIM 608715), which encodes a scaffolding protein shown to interact with neuroligins implicated in autism.[41] This deletion involving part of *SNTG2* in a patient with autistic features was inherited from a mother with a mild personality disorder and was absent in a normal brother. As these are only single cases, further studies in other populations

Table 2 Summary of autistic features and inheritance of chromosome abnormalities involving candidate ASD loci

Location	Copy state	Total ASD cases	Inheritance of ASD cases				SRO coordinates	Total non-ASD cases	Inheritance of non-ASD cases				Candidate genes and references
			De novo	Mat	Pat	Unknown			De novo	Mat	Pat	Unknown	
1p36.22	Loss	1	0	0	0	1	chr1:10,075,582-11,158,005	1	0	0	0	1	
1q21.1	Loss	4	0	1	2	1[a]	chr1:144,124,744-144,396,898	10	4	3	2	1	9
1q21.1	Gain	3	0	1	1	1[a]	chr1:145,119,362-145,761,156	4	2	2	0	0	12, 16, 30, 31, and 50
1q25.2	Loss	3	0	1	0	2	chr1:178,294,383-178,599,040[b]	3	0	0	0	3	
1q41 q42.12	Loss	1	1	0	0	0	chr1:221,260,860-224,709,317	6[c]	5	0	0	1	10
2p25.3	Loss	1	0	1	0	0	chr2:1,145,606-1,556,673	0	0	0	0	0	*SNTG2*[11]
2q13	Loss	3	0	2[d]	0	1	chr2:111,267,923-111,495,488	3	0	1	1	1	9
2q23.3	Loss	1	0	0	0	1	chr2:150,728,853-154,119,732	0	0	0	0	0	
2q33.3 q34	Loss	1	0	0	0	1	chr2:207,584,984-213,908,936	0	0	0	0	0	*MAP2*[51–55]
2q37.1	Gain	2	0	0	1[e]	1	chr2:233,043,888-233,227,429	2	0	0	1	1	
3p12.3 p12.2	Gain	1	0	1[f]	0	0	chr3:80,395,073-83,498,191	1	0	0	0	1	12 and 14
3q29	Loss	2	0	0	2	0	chr3:196,335,426-196,496,805[g]	1	0	1	0	0	
4q28.3	Loss	4	0	4	0	0	chr4:135,162,685-135,399,620	9	0	2	4	3	*PABPC4L*
4q33	Loss	2	1	0	1	0	chr4:170,841,800-171,547,755[h]	4	1	0	0	3	*GRIA2, GLRB, NPY1R,* and *NPY5R*[56]

(*Continued*)

Table 2 *Continued*

Location	Copy state	Total ASD cases	Inheritance of ASD cases				SRO coordinates	Total non-ASD cases	Inheritance of non-ASD cases				Candidate genes and references
			De novo	Mat	Pat	Unknown			De novo	Mat	Pat	Unknown	
4q35.2[i]	Gain	3	0	2	1	0	chr4:188,225,707-188,492,275	1	0	0	0	1	11 and 12
4q35.2[i]	Gain	2	0	1	1	0	chr4:189,051,560-189,277,198	3	0	0	1	2	12 and 14
5q35.3	Loss	1	1	0	0	0	chr5:179,175,186-179,769,465	3	0	1	0	2	
6p22.1	Loss	1	1	0	0	0	chr6:26,092,436-26,282,972	0	0	0	0	0	*HFE, TRIP38*
6q27	Loss	4	1	0	0	3	chr6:170,423,676-170,669,360	5	1	0	0	4	57–59
7p22.3	Gain	2	0	1[j]	0	1	chr7:124,996–292,633	0	0	0	0	0	11
7p21.1	Loss	1	1	0	0	0	chr7:16,222,136-19,648,978	0	0	0	0	0	
7q11.23	Gain	1	1	0	0	0	chr7:74,989,544-75,807,629	0	0	0	0	0	
7q31.33 q32.1	Loss	3	0	0	0	3	chr7:125,243,153-127,266,866[k]	1	0	1	0	0	*GRM8*[10,12,60]
7q35 q36.3	Loss	2	1	0	0	1	chr7:145,337,612-158,490,400	4	2	2[l]	0	0	*CNTNAP2, EN2*[14,61–63]
7q36.1 q36.2	Gain	1	0	0	0	1	chr7:150,219,239-152,619,553	0	0	0	0	0	
7q36.2	Gain	1	0	1	0	0	chr7:152,918,872-153,711,308	1	0	0	1	0	*DPP6*[14]
9p24.2	Gain	2	0	0	1	1	chr9:2,922,366-4,437,068	7	0	2	1	4	12
9q34.3	Gain	1	0	0	0	1	chr9:137,590,561-139,935,299	3	0	1	1	1	50
9q34.3	Loss	2	2	0	0	0	chr9:139,633,668-139,799,808	4	3	0	0	1	*EHMT1*[64]
10p15.3	Gain	5	0	0	2	3	chr10:2,650,802–2,839,892	8	0	1	1	6	No genes in SRO

(*Continued*)

Table 2 *Continued*

Location	Copy state	Total ASD cases	Inheritance of ASD cases				SRO coordinates	Total non-ASD cases	Inheritance of non-ASD cases				Candidate genes and references
			De novo	Mat	Pat	Unknown			De novo	Mat	Pat	Unknown	
10q21.2 q21.3	Loss	1	1	0	0	0	chr10:64,607,543-64,854,701	0	0	0	0	0	*JMJD1C* and *REEP3*[65]
10q24.32	Gain	1	0	0	0	1	chr10:103,169,248-103,624,892	0	0	0	0	0	12
11q12.1 q12.1	Gain	2	0	1	0	1	chr11:56,613,461-60,165,703	1	0	0	0	1	
11q24.3 q25	Loss	5	2	0	0	3	chr11:128,252,353-131,612,360	3	0	2	0	1	8 and 66
11q25	Gain	4	0	1	1[j]	2	chr11:132,626,207-132,808,152	4	0	0	1	3	*OPCML*[12]
12p12.1	Loss	1	1[f]	0	0	0	chr12:23,543,231-23,699,047	0	0	0	0	0	*SOX5*
12q24.33	Gain	1	0	1	0	0	chr12:129,602,302-129,962,509	1	0	0	0	1	12, 14, 66, and 67
15q13.3	Loss	7	1	1	2	3	chr15:29,207,889-29,603,362[m]	8	0	4	1	3	*CHRNA7*[9,24,26–28]
15q13.3	Gain	1	0	0	1	0	chr15:29,207,889-29,603,362[m]	1	0	0	0	1	*CHRNA7*[9,11,26]
15q24	Loss	1	0	0	0	1	chr15:70,794,484-73,322,523	1	1	0	0	0	14, 68
16p13.11	Gain	3	0	2	1	0	chr16:15,446,641-15,843,228	5	1	0	1	3	*NDE1, NTAN1*[32,33]
16p12.2	Gain	2	0	0	1	1	chr16:21,509,120-21,698,983	1	0	0	1	0	*METTL9, IGSF6, OTOA*[14,69,70]
16p12.2	Loss	3	0	0	2	1	chr16:21,509,120-21,641,889	2	1	0	1	0	*METTL9, IGSF6, OTOA*[14]
17p11.2	Gain	1	1	0	0	0	chr17:20,753,347-21,191,540	0	0	0	0	0	*MAP2K3*[71]
17q12	Gain	1	0	0	0	1	chr17:32,117,729-33,246,206	1	0	1	0	0	

(*Continued*)

Table 2 *Continued*

Location	Copy state	Total ASD cases	Inheritance of ASD cases				SRO coordinates	Total non-ASD cases	Inheritance of non-ASD cases				Candidate genes and references
			De novo	Mat	Pat	Unknown			De novo	Mat	Pat	Unknown	
18p11.32 p11.23	Loss	2	0	0	0	2	chr18:140,284-7,574,236	4	1	0	0	3	9, 12, and 50
18p11.32 p11.21	Gain (×4)	1	0	0	0	1	chr18:140,284-14,065,199	0	0	0	0	0	
22q13.33	Loss	4	2	0	0	2	chr22:49,493,686-49,549,855	9	4	0	0	5	*SHANK3*[14,17,45,72,73]
Total		98	18	21	20	42		124	26	24	18	56	

[a]This individual with ASD had a deletion of the proximal, TAR-associated region of 1q21.1 and a duplication of the distal region of 1q21.1.
[b]Three cases with ASD had this region of overlap, but two of the cases had 16p abnormalities (including a 16p11.2 deletion) that are associated with autism. The only case with ASD without another abnormality had a deletion at chr1:169,650,629-178,599,040.
[c]These nonautistic patients have been previously reported.[74]
[d]Twin brothers with ASD were found to have this deletion.
[e]The father is reportedly normal, and the proband had congenital anomalies that are likely not explained by this deletion.
[f]This patient had a 3p12 duplication from his mother and a 12p12.1 de novo deletion.
[g]Two cases had ASD, one at chr3:194,057,893-196,496,805 and the other going into the typically deleted region for the 3q29 microdeletion syndrome, which is associated with autism,[75] chr3:196,335,426-197,719,622. Causes of ASD may be independent.
[h]The case with ASD and the deletion of the SRO alone also had the 4q28.3 deletion. The larger case with ASD had a deletion of chr4:154,119,050-172,932,223.
[i]Four cases with ASD had a duplication within 4q35.2, two of which were small and did not overlap each other but overlapped at least one other ASD case.
[j]This individual with ASD had a maternally inherited duplication of 7p22.3 and a paternally inherited duplication of 11q25.
[k]One case with ASD has a 15.9 Mb deletion that includes *FOXP2*, which is associated with speech delays,[76] and other genes with possible autism associations such as *ST7*,[14,77,78] *WNT2*,[79] *MET*,[80] and *CADPS2*.[81] Removing this case makes the SRO chr7:125,243,153-128,988,832. One of the cases with ASD has a deletion that includes candidate gene *UBE2H*,[82] whereas the other does not include this gene.
[l]These are siblings with an unbalanced translocation inherited from a balanced carrier mother.
[m]Alteration was measured by BAC array; it may be larger and span BP4-BP5 as described in the 15q13.3 microdeletion syndrome.[28]
ASD, autism spectrum disorder; Mat, maternally inherited; Pat, paternally inherited; SRO, smallest region of overlap; coordinates are according to UCSC hg18 build.

may confirm this association with autism, show a more general association with neurodevelopmental disorders, as we have shown with other CNVs in this survey, or may show the CNVs to be likely benign.

We also identified a recurrent microdeletion and microduplication at 16p12.2, although population data suggest these recurrent CNVs may not be clinically significant. This microdeletion has been identified in controls (20/6712),[83] and the frequency is not significantly different than either the frequency among cases referred to our laboratory for clinical aCGH testing (33/16,773) or among the subset of these cases referred for an ASD (2/580). This microduplication had a frequency of 14/6,712 in the same control population,[83] which is not significantly different than the frequency in cases referred for aCGH testing (22/16,773) or the subset of cases being tested for ASD (1/580). This example illustrates a potential limitation to this study, as truly benign variants may be identified and in the absence of control data may be difficult to implicate or reject as candidates. Conversely, some of the CNVs that are not categorized as potentially causative may be rare loci that predispose to ASD, as the criteria used in this study are a screening tool and may eliminate some smaller or inherited abnormalities that have a legitimate connection with ASD. Larger, independent studies are required to identify and replicate the association between any specific CNV and ASD.

Despite the studies that suggest high heritability for autism and, therefore, a strong genetic contribution, the interaction between genetic factors and ASD is not fully understood. Because of the absence of common genetic factors in individuals with ASD, and as a result of an analysis of family history data, a multigenic model is the commonly accepted model for ASD.[42] If ASD is truly multigenic then a CNV could be one of several genetic factors required to lead to ASD. These CNVs could be in normal individuals in the population and, therefore, may be unrecognized in studies of de novo abnormalities or "autism-specific" changes. It is possible that some of the small, recurrent, inherited abnormalities in this cohort, such as the 4q28.3 microdeletion or the 16p12.2 microdeletion and microduplication, could represent one of these common CNVs that can contribute to ASD when other unknown factors are present. The paucity of obvious results in all these searches for common CNVs in ASD suggests that the contributory factors are varied, so it may be rare to find multiple families with the same abnormalities.

An increasing number of microdeletions and microduplications are being reported in association with ASD, and the nature of the association between the CNV and ASD, whether it is causative, contributory, or potentially benign, is slowly being elucidated. Although some genomic disorders have been associated with a specific neurobehavioral profile,[43–45] and the relative contribution that other CNVs, such as 16p11.2 microdeletions and microduplications, to ASD is being described,[11,14,16,46,47] the roles, if any, that most CNVs play in ASD etiology have not been defined. Some of these CNVs are implicated in disease, even when inherited from a normal parent,[24,30] a paradigmatic shift from traditional cytogenetic orthodoxy.[32,48,49] Furthermore, these CNVs may be present in individuals with a spectrum of neurodevelopmental impairments, which suggests they play a general role in altering brain development. In any individual with ASD, multiple, varied factors, one of which may be a CNV, likely contribute to the phenotype.

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