Clinical Genetic Testing for Patients With Autism Spectrum Disorders

Yiping Shen, PhD[a,b,c,d], **Kira A. Dies, ScM**[a,c], **Ingrid A. Holm, MD, MPH**[a,c,e,f], **Carolyn Bridgemohan, MD**[a,c,g], **Magdi M. Sobeih, MD, PhD**[a,c,h], **Elizabeth B. Caronna, MD**[a,i], **Karen J. Miller, MD**[a,j], **Jean A. Frazier, MD**[a,k,l], **Iris Silverstein, MD**[a,m], **Jonathan Picker, MBChB, PhD**[a,c,n], **Laura Weissman, MD**[a,c,g], **Peter Raffalli, MD**[a,c,h], **Shafali Jeste, MD**[a,c,h], **Laurie A. Demmer, MD**[a,j], **Heather K. Peters, MS**[a,e], **Stephanie J. Brewster, MS**[a,e], **Sara J. Kowalczyk, MA, MPH**[a,i], **Beth Rosen-Sheidley, MS**[a,j], **Caroline McGowan, MS**[a,n], **Andrew W. Duda III, MS**[a,m], **Sharyn A. Lincoln, MS**[a,n], **Kathryn R. Lowe, MS**[a,e], **Alison Schonwald, MD**[a,c,g], **Michael Robbins, MD**[a,c,h], **Fuki Hisama, MD**[a,c,n], **Robert Wolff, MD**[a,c,h], **Ronald Becker, MD**[a,c,g], **Ramzi Nasir, MD, MPH**[a,c,g], **David K. Urion, MD**[a,c,h], **Jeff M. Milunsky, MD**[a,i,o], **Leonard Rappaport, MD**[a,c,g], **James F. Gusella, PhD**[a,c,d], **Christopher A. Walsh, MD, PhD**[a,c,n], **Bai-Lin Wu, PhD, MMed**[a,b,c,p], and **David T. Miller, MD, PhD**[a,b,c,n] on behalf of the **Autism Consortium Clinical Genetics/DNA Diagnostics Collaboration**

[a]Autism Consortium, Boston, Massachusetts

[b]Department of Laboratory Medicine, Children's Hospital Boston, Boston, Massachusetts

[c]Harvard Medical School, Boston, Massachusetts

[d]Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts

[e]Program in Genomics, Children's Hospital Boston, Boston, Massachusetts

[f]Manton Center for Orphan Disease Research, Children's Hospital Boston, Boston, Massachusetts

[g]Developmental Medicine Center, Children's Hospital Boston, Boston, Massachusetts

[h]Department of Neurology, Children's Hospital Boston, Boston, Massachusetts

[i]Department of Pediatrics, Boston University School of Medicine, Massachusetts

[j]Floating Hospital for Children, Tufts Medical Center, Boston, Massachusetts

[k]University of Massachusetts Medical School, Worcester, Massachusetts

[l]UMass Memorial Medical Center, Worcester, Massachusetts

[m]Massachusetts General Hospital for Children LADDERS Clinic, Boston, Massachusetts

[n]Division of Genetics, Children's Hospital Boston, Boston, Massachusetts

Address correspondence to: Bai-Lin Wu, PhD, MMed, Children's Hospital Boston, 300 Longwood Ave, Farley 7, Boston, MA 02115. bai-lin.wu@childrens.harvard.edu.
Dr Shen is the first author for the DNA diagnostics team, Ms Dies is the first author for the Autism Consortium team, Dr Wu is the senior author for the DNA Diagnostics team, and Dr Miller is the senior author for the Autism Consortium team.

ºClinical Genetics, Boston University School of Medicine, Massachusetts

ᵖDepartments of Pediatrics and Pathology, Children's Hospital, Shanghai Medical College and Institutes of Biomedical Science, Fudan University, Shanghai, China

Abstract

BACKGROUND—Multiple lines of evidence indicate a strong genetic contribution to autism spectrum disorders (ASDs). Current guidelines for clinical genetic testing recommend a G-banded karyotype to detect chromosomal abnormalities and fragile X DNA testing, but guidelines for chromosomal microarray analysis have not been established.

PATIENTS AND METHODS—A cohort of 933 patients received clinical genetic testing for a diagnosis of ASD between January 2006 and December 2008. Clinical genetic testing included G-banded karyotype, fragile X testing, and chromosomal microarray (CMA) to test for submicroscopic genomic deletions and duplications. Diagnostic yield of clinically significant genetic changes was compared.

RESULTS—Karyotype yielded abnormal results in 19 of 852 patients (2.23% [95% confidence interval (CI): 1.73%–2.73%]), fragile X testing was abnormal in 4 of 861 (0.46% [95% CI: 0.36%–0.56%]), and CMA identified deletions or duplications in 154 of 848 patients (18.2% [95% CI: 14.76%–21.64%]). CMA results for 59 of 848 patients (7.0% [95% CI: 5.5%–8.5%]) were considered abnormal, which includes variants associated with known genomic disorders or variants of possible significance. CMA results were normal in 10 of 852 patients (1.2%) with abnormal karyotype due to balanced rearrangements or unidentified marker chromosome. CMA with whole-genome coverage and CMA with targeted genomic regions detected clinically relevant copy-number changesin7.3%(51 of 697) and 5.3%(8 of 151) of patients, respectively, both higher than karyotype. With the exception of recurrent deletion and duplication of chromosome 16p11.2 and 15q13.2q13.3, most copy-number changes were unique or identified in only a small subset of patients.

CONCLUSIONS—CMA had the highest detection rate among clinically available genetic tests for patients with ASD. Interpretation of microarray data is complicated by the presence of both novel and recurrent copy-number variants of unknown significance. Despite these limitations, CMA should be considered as part of the initial diagnostic evaluation of patients with ASD.

Keywords

Autism is a complex neurobehavioral disorder that includes impairments in social interaction, developmental language and communication deficits, and rigid, repetitive behaviors. The *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision* (DSM-IV-TR) category of pervasive developmental disorders includes autistic disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS), Asperger disorder, childhood disintegrative disorder, and Rett disorder. These diagnoses are also collectively known as autism spectrum disorders (ASDs). ASD occurs in all racial, ethnic,

and social groups. The prevalence of autistic disorder is ~1 per 1000, and the prevalence of ASD is ~6 per 1000, affecting many more males than females.[1]

Genetic factors increase the risk of developing ASD,[2] but the specific genetic cause for an individual patient can be elusive. Autism may be a component of genetic syndromes with distinct clinical features, as in tuberous sclerosis and Rett disorder. Other syndromes are not easily recognized in young children, as in fragile X syndrome, which accounts for ~2% of ASD cases.[3] Most children with ASD do not have dysmorphic features or other medical problems associated with a recognizable genetic syndrome, and genetic testing is crucial to identifying a cause for ASD in this population.

G-banded karyotyping for chromosomal abnormalities and fragile X testing are currently recommended as first-tier genetic tests, and are abnormal in up to 5% of patients.[3,4] Karyotyping will not detect submicroscopic genomic deletions and duplications or copy-number variants (CNVs) smaller than ~5 megabases (Mb). Subtelomeric fluorescence in situ hybridization (ST-FISH) can detect submicroscopic CNVs in patients with mental retardation (MR), but authors of the largest study of ST-FISH found pathogenic changes in only 2.6% of 11 688 unselected cases of MR,[5] and no changes were found by ST-FISH in 1 small study of patients with ASD.[6]

Array comparative genomic hybridization (array CGH) also called chromosomal microarray analysis (CMA), detects clinically significant CNVs in at least 10% of patients with a variety of developmental problems such as developmental delay, MR, and multiple congenital anomalies.[7–9] Research studies for patients with ASD suggest a similar detection rate of ~10% using CMA,[10–12] but the diagnostic yield in large clinical cohorts has not been well studied. We present here clinical genetic test results, including those from karyotype, fragile X testing, and CMA, and discuss the implications for clinical care for a large cohort of patients with ASD.

METHODS

We evaluated a combined cohort of 933 patients (755 males and 178 females [ratio: 4.24:1]) (Table 1). Autistic disorder ($n = 447$) and PDD-NOS ($n = 454$) were the predominant diagnoses. A total of 461 patients, aged 13 months to 15 years and clinically diagnosed with ASD, were recruited through the Autism Consortium (AC), a research and clinical collaboration that included 5 Boston-area medical centers (see "Acknowledgments"). Protocols and consent forms were approved by the institutional review boards of each center. ASD diagnosis for patients in the AC cohort was made by the patient's referring clinician (developmental-behavioral pediatrician, neurologist, pediatric psychologist, or psychiatrist) by using the criteria for a pervasive developmental disorder as outlined by the American Psychiatric Association's DSM-IV-TR. These 461 patients completed at least 1 of 3 genetic tests, with 433 individuals completing all 3 tests, and data were entered into the registry (see Supporting Information, which is published at www.pediatrics.org/content/full/125/4/e727).

Another 472 patients, aged 15 months to 22 years, were added through samples submitted for clinical genetic testing to the Children's Hospital Boston DNA Diagnostic laboratory. ASD diagnosis was based on clinical test requisition forms and medical record review to confirm that DSM-IV-TR criteria were used.

Among multiplex families, test results from only 1 affected family member were included. For cases in which only 1 sample per family was submitted for testing, we were not able to determine if the family was simplex or multiplex; thus, the overall proportion of cases from simplex versus multiplex families was not determined.

RESULTS

Patients

These patients were generally representative of the broader population of patients with ASD (Table 1), including a male/female ratio of 4.24:1 (755 males and 178 females), a roughly equal proportion of patients with autistic disorder ($n = 447$ [47.9%]) and PDD-NOS ($n = 454$ [48.7%]) and a minority of patients with Asperger disorder ($n = 31$ [3.3%]). Age at diagnosis ranged from 13 months to 22 years.

Genetic Testing Results

Karyotype testing identified abnormal results in 19 of 852 patients (2.23% [95% confidence interval (CI): 1.73%–2.73%]) (Table 2). CMA also detected the abnormality in 8 of 19 (42.1%) with an abnormal karyotype, but 10 of 19 (52.6%)had balanced rearrangements and appeared normal according to CMA. Patient ASD-09-009 had low-level mosaicism not detected by CMA. CMA results corrected or clarified ambiguous karyotype results by demonstrating that a 15q duplication was a clinically insignificant repetitive sequence (patient ASD-09-011) and by precisely defining cytogenetically ambiguous translocation break points (patient ASD-09-016). Fragile X testing results were abnormal for 4 patients (0.46% [95% CI: 0.36%–0.56%]) (Table 3), 2 of whom were premutation carriers.

CMA was performed on 848 of 933 patients (90.9%). Most patients were tested by CMA with whole-genome coverage (697 of 848 patients [82.2%]), either Agilent (Santa Clara, CA) 244k comparative genomic hybridization arrays (589 of 848 patients [69.5%]) or Affymetrix (Santa Clara, CA) 500k or v5.0 single-nucleotide polymorphism arrays (108 of 848 patients [12.7%]). CNVs were identified in 154 of 848 patients (18.2% [95% CI: 14.76%–21.64%]). Of these, 59 of 848 (7.0% [95% CI: 5.5%–8.5%]) had results considered "abnormal" or "possibly significant," and 95 (11.2%) had results considered variants of unknown significance (Table 4; see "Methods" for definitions). The detection rate for abnormal or possibly significant results by targeted array was 5.3% (8 of 151), and the rate for whole-genome array was 7.3% (51 of 697). Variants classified as variants of unknown significance (VUS) or benign CNVs are listed in Supporting Information.

Among abnormal variants, 50 of 60 (83%) were below the size range routinely detectable by karyotype (typically ~5 Mb). Many variants were relatively large compared with the range of typical CNVs. Previous surveys of copy-number variation suggested that more than 95% of CNVs are <500 kilo-bases (kb),[13] and more recent data with higher-resolution arrays

suggested that many more "small" CNVs exist but were previously undetectable because of technologic limitations.[14] Abnormal CNVs in this study had a mean size of 1896 kb and median of 546 kb (excluding 5 chromosomal aneuploidy cases), with 35 of 60 (~58%) larger than 500 kb. VUS (Supplemental Data 2) identified in this study had smaller size (mean size: 261 kb; median: 141 kb). It should be noted that 32 of 154 patients (~21%) had 2 abnormal CNVs or VUS, and 9 of 204 patients (4.4%) had 3 abnormal CNVs or VUS.

Secondary diagnoses were collected from physician referral notes for the AC cohort. In total, 54 of 461 individuals (12%) were noted to have MR, and of these, 12 of 54 (22%) had abnormalities detected by microarray, 2 of 54 (3.7%) by karyotype, and 3 of 54 (5.6%) by fragile X testing. In addition, 16 of 461 individuals (3.5%) were noted to have dysmorphic features, of which 10 of 16 (63%) had abnormalities detected by microarray and 2 of 16 (13%) by karyotype testing. Seizures were reported in 36 of 461 individuals (7.8%), and of these, 8 of 36 (22%) had abnormalities detected by microarray and 2 of 36 (5.6%) by karyotype testing. Those chromosomal abnormalities detected by karyotype testing were also detected by microarray analysis.

The male/female ratio in patients with abnormal CMA findings was 3.2:1 (45 males/14 females). Slightly more female patients with ASD had abnormal CMA results (14 of 157 [8.9%]) compared with male patients (45 of 691 [6.5%]). Slightly more abnormal CMA results were found among patients with autistic disorder (34 of 403 [8.4%]) than patients with PDD-NOS (25 of 414 [6.2%]). Females with autistic disorder had the highest abnormal CMA rate (8 of 82 [9.8%]). Males with autistic disorder and females with PDD-NOS had a similar abnormal CMA rate (both 8.1% [26 of 321 and 6 of 74, respectively]). Males with PDD-NOS diagnosis had the lowest abnormal CMA rate (5.5% [19 of 340]). No abnormal CMA results were reported among the small number of patients with Asperger disorder (*n* = 31).

The abnormal CNVs detected in this cohort are quite diverse in terms of chromosome distribution and size (Table 4). The only recurrent CNVs identified were a 1.8-Mb region of chromosome 15q13.2q13.3 (chr15:28.7Mb-30.5Mb; hg18; 2 deletions and 2 duplications) and a 600-kb region of chromosome 16p11.2(chr16:29.5Mb-30.1Mb;hg18;4 deletions and 2 duplications), together accounting for 17% (10 of 59) of all abnormal CMA findings. No other recurrent CNVs were identified. Overall, CMA had a higher yield than karyotype or fragile X testing for clinical genetic testing in this large cohort of patients with ASD (Table 5).

DISCUSSION

Our findings indicate that CMA with whole-genome coverage detects more abnormalities than G-banded karyotype and fragile X DNA testing in patients with ASD, and suggest that CMA should be a first-tier test in this patient population. CMA could not entirely replace a G-banded karyotype in this patient population because of the inability of CMA to detect balanced rearrangements, but these are a small proportion of abnormal results. We identified 10 patients with a balanced rearrangement representing 1.2% of all patients tested (*n* = 852).

If these patients had only been tested by CMA, then it is possible that a pathogenic change would be missed.

Although CMA does not detect balanced rearrangements, a significant proportion of balanced rearrangements are probably not clinically significant. The balanced pericentric inversions on chromosome 2 (patient ASD-09-002) and chromosome 9 (patients ASD-09-017 and ASD-09-018) could also occur in healthy individuals and likely are not related to ASD. In fact, the chromosome 2 inversion was maternally inherited. Chromosome 9 inversions are known polymorphisms, and also likely inherited, but parental samples were not available for testing.

We found 6 cases of balanced translocations, but they are also not necessarily pathogenic. They may be inherited from an unaffected parent, making the child a balanced carrier like the parent. Among 6 balanced translocations in our cohort, 1 patient (ASD-09-004) had the identical result as the parent, 2 cases had no parent data, and 3 cases were de novo. The de novo balanced translocations are not necessarily pathogenic, either. Balanced rearrangements are known to occur in healthy individuals, even when they interrupt a known gene. In a recent study of balanced rearrangements that interrupt a gene, approximately half (16 of 31) were found in healthy individuals.[15]

Pathogenic balanced rearrangements are likely to account for only a small number of ASD cases. Studies of cytogenetically balanced rearrangements in large cohorts of patients with ASD are not available, but such studies have been done for patients with MR and should be comparable. Balanced rearrangements make up only ~10% of cytogenetically visible abnormalities in patients with developmental disabilities such as MR, meaning that only ~0.3% of patients would have such changes.[16–18] Although traditional karyotyping could detect these events, they represent a similarly small proportion of cases in our cohort and may or may not be related to ASD.

Patient ASD-09-009 had mosaicism for a marker chromosome that is probably of little clinical significance because (1) the level of mosaicism is low, and (2) small marker chromosomes typically contain gene-poor repetitive DNA. CMA does not contain probes from these repetitive DNA regions, and the failure of whole-genome CMA to detect this anomaly is actually evidence that the marker is repetitive DNA. Karyotype testing and CMA can detect mosaicism at the level of ~5% to 10% abnormal cells and 30% abnormal cells, respectively. We only found 1 such example of low-level mosaicism, demonstrating that these events also occurred at low frequency in our ASD cohort.

The proportion of patients with positive results for any of the 3 tests in this study was similar to other studies on ASD, some of which were performed on research samples.[3,10,11,19] Our yield for CMA was <10%, perhaps for several reasons. Whole-genome scans for copy-number variation have identified large de novo CNVs in 7% to 10% of simplex ASD families (1 child affected), 2% to 3% of multiplex families, and only 1% of control families.[10,19] Our patients were added through clinical care and were not selected on the basis of simplex versus multiplex families and are, therefore, not enriched for simplex cases. Diagnostic yield of CMA may have been limited by technical factors. Some tests (~17%)

were performed on platforms that have coverage below the ability to detect all 500-kb copy-number changes. However, most of our samples (83%) were tested by Agilent 244k or Affymetrix 500k and v5.0 whole-genome arrays. The trend toward higher yield with whole-genome arrays as compared with targeted arrays has been reported by authors of other studies.[7]

We might have expected to find higher numbers of definite abnormal results for CMA on the basis of yields for patients with generalized MR, which are 10%.[20,21] Our yield was lower, but our cohort of patients with ASD almost certainly contains more high-functioning individuals than a cohort of patients with MR, including 31 individuals with Asperger disorder in whom no clinically significant CNVs were identified. This suggests that yield from CMA may be lower in patients with high functioning autism, and this is consistent with other reports.[22] Our cohort had a relatively low proportion of patients with secondary diagnoses known to have a high rate of abnormalities on CMA. Only 54 of 461 patients (11.7%) in the AC cohort were diagnosed with MR by medical record review. Similarly, only 16 of 461 patients (3.5%) in the AC cohort had a secondary diagnosis of multiple congenital anomalies, which was reported to have CMA abnormalities in 19.9% of patients.[8] Our yield of abnormal results for fragile X testing was also lower than expected but may represent a selection bias against patients with fragile X syndrome, as has been suggested in similar studies.[23] Two of these patients with fragile X syndrome were premutation carriers, but their results were included as abnormal because recent studies revealed that there may be a higher incidence of neuropsychiatric conditions, including autism, among fragile X pre-mutation carriers.[24]

Our study has potential limitations. Our patients were diagnosed by clinical evaluation using DSM-IV-TR criteria. The gold standard for research studies of ASD would include the Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview-Revised (ADI-R) in addition to meeting criteria for a pervasive developmental disorder as defined by the DSM-IV-TR. Some of the patients included in this study may not have met full research criteria for an ASD diagnosis if tested with the ADOS and ADI-R. Removing some patients from our sample on the basis of failure to meet criteria for an ASD diagnosis because of ADI-R/ADOS may actually increase the proportion of patients with an abnormality by removing patients with a milder phenotype. We cannot exclude the possibility of bias based on ascertainment of patients through tertiary care centers. These patients may be more likely to have abnormal genetic test results because they were referred because of other complicating factors such as specific family history or dysmorphic features. We did not observe a high rate of such issues, but we cannot rule out underreporting of complex features at the time of ascertainment.

The causal relationships between many of the abnormal CNVs identified in these patients with ASD and the clinical symptoms will require further study. Our conclusions about pathogenicity are based on the best current knowledge but could evolve over time. In general, sporadic cases of autism may be more likely caused by de novo mutations.[25] Inherited CNVs may also contribute to autism or autistic symptoms but may have more mild effects that could vary among family members. It is ironic that many apparently common recurrent pathogenic copy-number changes may not be de novo but exhibit decreased

penetrance and variable expressivity, such as 16p11.2, 15q13.2q13.3 and 1q21.[26–29] This has important implications for recurrence risk counseling. Identifying rare de novo copy-number changes is equally important for genetic counseling.

The increased yield of CMA, especially in comparison with G-banded karyotype testing, has important clinical impact. Genetic testing can be expensive, and payers may not be willing to reimburse for 2 tests that provide similar information. In such cases, CMA would be an appropriate choice despite a small number of balanced rearrangements that would be undetectable. Although we identified slight differences in the rate of abnormal CMA results based on gender and specific ASD category, these should not influence clinical decisions about offering CMA given the small magnitude of differences and also the potential variability of diagnosis over time, particularly in young children.[30–32] Also, other genetic testing may be indicated in select populations of patients with ASD (eg, testing for *MECP2* mutations among girls with ASD and microcephaly or testing for *PTEN* mutations among boys or girls with ASD and macrocephaly).[33,34]

Establishing a clear diagnosis may lead to earlier initiation of services and consequently improve outcome.[35–38] In most cases of ASD, some clinical symptoms are apparent before the age of 3 years, but in many cases children may not be diagnosed until they are much older.[39] ASD will remain a clinical diagnosis, but identifying a clear genetic etiology is advantageous in several ways. A clear genetic diagnosis can affect patient management decisions, availability of developmental services, and accuracy of genetic counseling about recurrence risks, which may range from <5% to as high as 50% depending on the cause. A clear genetic diagnosis also spares the patient and family a diagnostic odyssey involving multiple rounds of diagnostic testing.

Specific clinical recommendations for including CMA as a first-tier test in the evaluation of patients with ASD have not kept pace with this rapidly evolving technology. Considerations for including CMA in the evaluation of children with ASD have been outlined elsewhere[4,40,41] but have stopped short of recommending that CMA be offered as a first-tier genetic diagnostic test for ASD. On the basis of our results, genetic diagnosis will be missed in at least 5% of ASD cases without CMA, and our results suggest that CMA with whole-genome coverage should be adopted as a national standard of care for genetic testing among patients with ASDs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

ABBREVIATIONS

DSM-IV-TR	*Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision*
PDD-NOS	pervasive developmental disorder-not otherwise specified
ASD	autism spectrum disorder
CGH	comparative genomic hybridization
CNV	copy-number variant
ST-FISH	subtelomeric fluorescence in situ hybridization
CMA	chromosomal microarray analysis
MR	mental retardation
AC	Autism Consortium
CI	confidence interval
VUS	variants of unknown significance
ADOS	Autism Diagnostic Observation Schedule
ADI-R	Autism Diagnostic Interview-Revised

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WHAT'S KNOWN ON THIS SUBJECT

Multiple lines of evidence indicate a strong genetic contribution to ASD. Current guidelines for clinical genetic testing recommend a G-banded karyotype to detect chromosomal abnormalities and fragile X DNA testing, but guidelines for CMA have not been established.

WHAT THIS STUDY ADDS

We present here clinical genetic test results, including karyotype, fragile X testing, and CMA, and discuss the implications for clinical care for a large cohort of patients with ASD.

TABLE 1

Characteristics of Patients in the AC and CHB Cohorts

	AC	CHB	Combined
Patients, *n*	461	472	933
Age range, y/mo (at visit)	1/7 to 21/10	1/3 to 22/0	–
Gender, *n*			
Male	369	386	755
Female	92	86	178
Diagnosis, *n*			
Autistic disorder	211	236	447
PDD-NOS	227	227	454
Asperger disorder	22	9	31
CDD	1	0	1
Secondary diagnosis, *n*			
MR	54	NA	NA
Seizures	36	NA	NA
Multiple congenital anomalies	16	NA	NA

CHB indicates Children's Hospital Boston; NA, not available; CDD, childhood disintegrative disorder.

TABLE 2

Karyotype Results

Study ID	Age at Diagnosis, y/mo	Gender	Karyotype	Parental Karyotype	CMA
ASD-09-001	2/5	M	46,XY,t(5;16)(p13.2:p13.2)	ND	Normal
ASD-09-002	3/0	F	46,XX,inv(2)(p11;2q13)	Maternal	Normal
ASD-09-003	2/5	M	46,XY,t(5;17)(q33:p13)	ND	Normal
ASD-09-004	2/6	M	46,XY,t(3;6)(q26.2:q16.2)	Paternal	Normal
ASD-09-005	11/2	M	46,XY,t(3;5)(q26.2;q22)	De novo	Normal
ASD-09-006	1/7	M	46,XX,t(6;7)(q13;q11.2)	De novo	Normal
ASD-09-007	2/11	M	46,XY,t(6;9)(q16.2;q13)	De novo	Normal
ASD-09-008	5/0	M	Duplication (13)(q14.1q21.3)	ND	ND
ASD-09-009	4/0	F	47,XX, +mar.ish der(13) or der(21) (D13Z1/D21Z1+) [4]/46,XX [17]	ND	Normal
ASD-09-010	3	M	46,XY,del (6)(q16.1q21)	ND	16.4 Mb del 6q16.1-q21
ASD-09-011	2/6	M	46,XY,dup(15)(q11q13)	ND	730 kb dup 16q22.1
ASD-09-012	3/8	M	46,XY,del(10)(q26.3).ish del(10) (q telomere)(D10S2490–)	De novo	4.1 Mb del 10q23
ASD-09-013	2/11	M	47,XY, + idic(15)(q13)	ND	11.9 Mb dup 15q11q13.3
ASD-09-014	2/8	F	47,XX, + 21	De novo	47,XX, + 21
ASD-09-015	5/4	M	47,XY, + 21	De novo	47,XY, + 21
ASD-09-016	10	M	46,XY,?ins(6)(?p23?q13?q21)	De novo	3.3 Mb del 18p11.31-p11.23; 313 kb del 6q16.3
ASD-09-017	3	M	46,XY,inv(9)(p11q13)	Unknown	Normal
ASD-09-018	3/4	M	46,XY,inv(9)(p11q13)	Unknown	Normal
ASD-09-019	2/10	M	47,XXY	De novo	X Chromosome duplication

M indicates male; t, translocation (involved chromosomes in parentheses); F, female; inv, inversion of chromosome region; ND, not done; de novo, not observed in blood sample from either parent; mar, marker chromosome; ish, in situ hybridization; del, deletion of chromosomal material; dup, duplication of chromosomal material; idic, isodicentric chromosome; ins, insertion of chromosome material.

TABLE 3

Fragile X Testing Results

Study ID	FMR1 Test Result	Age, y/mo	Gender	CGG Repeat No.	Methylation	Parent of Origin	Karyotype Result	CMA Result
ASD-09-020	Female premutation	2/6	F	69; 32	Normal	Paternal	XX,46	1.6 Mb maternal duplication at Xp22.31
ASD-09-021	Female premutation	2/0	F	56; 46	Normal	Unknown	XX,46	Normal
ASD-09-022	Male full mutation	2/6	M	200	Abnormal	Maternal	XY,46	Normal
ASD-09-023	Female mosaic for full mutation and premutation	5/1	F	>200; 59	Abnormal	Unknown	XX,46	Normal

Normal alleles: ~5 to 40 repeats; intermediate alleles (also termed "gray zone"): ~41 to 58 repeats; premutation alleles: ~59 to 200 repeats; full mutation alleles: >200 repeats; methylation of the *FMR1* promoter region typically occurs in full mutation alleles, resulting in silencing of gene expression.

TABLE 4

Abnormal Chromosomal Microarray Results

Study ID	Chromosome Location	Deletion/ Duplication	Size, kb	Chromosome Coordinates	Parent of Origin	Diagnosis	Gender	Karyotype	Fragile X
ASD-09-024	1p36.13	Duplication	16 386	762978–17148920	ND	Autistic disorder	M	Normal	Normal
ASD-09-025	1q21.1	Deletion	400	147290000–147700000	De novo	PDD-NOS	M	Normal	Normal
ASD-09-026	1q21.1	Deletion	298	1441540012–144451954	Paternal	Autistic disorder	M	Normal	Normal
ASD-09-027	1q43q44	Duplication	3000	239338812–242339608	Maternal	PDD-NOS	F	Normal	Normal
ASD-09-028	2p16.3	Deletion	109	51236317–51344921	De novo	Autistic disorder	M	Normal	Normal
ASD-09-029	2p16.3	Deletion	139	50714297–50853329	ND	PDD-NOS	M	Normal	Normal
ASD-09-030	2p16.3	Deletion	122	51090504–51212385	Paternal	Autistic disorder	M	Normal	Normal
ASD-09-031	2p21	Deletion	112	47460399–47572748	ND	Autistic disorder	F	ND	ND
ASD-09-032	2q13	Deletion	1700	111108666–112819065	ND	Autistic disorder	M	ND	ND
ASD-09-033	2q13	Deletion	298	110050724–110348639	ND	PDD-NOS	M	Normal	Normal
ASD-09-034	2q33.1	Deletion	542	198603505–199146109	De novo	Autistic disorder	M	Normal	Normal
ASD-09-035	3p22.1	Deletion	4317	43443229–47760421	De novo	Autistic disorder	F	Normal	Normal
ASD-09-036	3q23	Deletion	352	143605199–143957178	ND	Autistic disorder	F	Normal	Normal
ASD-09-037	3q29	Duplication	453	198370256–198823726	ND	Autistic disorder	M	ND	Normal
ASD-09-038	4q23	Deletion	1348	100611813–101959551	De novo	Autistic disorder	M	Normal	Normal
ASD-09-039	4q35.2	Deletion	1120	185535833–186654005	ND	Autistic disorder	M	Normal	Normal
ASD-09-040	6p21.32	Duplication	550	31802268–32352051	De novo	Autistic disorder	M	Normal	Normal
ASD-09-041	6q16.1q21	Deletion	16 417	94102643–110520288	De novo	Autistic disorder	M	46,XY,del (6) (q16.1q21)	Normal
ASD-09-042	6q16.3	Deletion	312	1018122515–102124648	De novo	Autistic disorder	M	46,XY,?ins(6) (?p23?q13?q21)	ND
ASD-09-043	7q11.22	Deletion	43	69475350–69519211	ND	PDD-NOS	M	Normal	Normal
ASD-09-044	7q11.23	Duplication	1817	71 949 830–73 767 523	De novo	Autistic disorder	F	Normal	ND
ASD-09-045	8pq	Mosaic duplication	Entire chr 8	Entire chr 8	De novo	PDD-NOS	M	Abnormal	Normal
ASD-09-046	8q23.3	Deletion	229	114185479–114414476	ND	PDD-NOS	M	Normal	ND
ASD-09-047	8q24.22q24.3	Deletion	5000	136429381–141456935	Maternal	Autistic disorder	F	Normal	Normal
ASD-09-048	9q34.2	Duplication	285	136013220–136298049	De novo	PDD-NOS	M	Normal	Normal
ASD-09-049	10q11.21q11.23	Duplication	5950	45520815–41468963	Maternal	PDD-NOS	M	Normal	Normal
ASD-09-050	10q26.3	Deletion	4100	131300000–135400000	De novo	PDD-NOS	M	46,XY,del(10)(q26.3). ish del(10)(q telomere) (D10S2490-)	Normal
ASD-09-051	12p11.22	Deletion	211	28364520–28575366	De novo	PDD-NOS	F	Normal	Normal

Study ID	Chromosome Location	Deletion/Duplication	Size, kb	Chromosome Coordinates	Parent of Origin	Diagnosis	Gender	Karyotype	Fragile X
ASD-09-052	12p13.33	Deletion	31	1821094–1852794	ND	PDD-NOS	F	ND	ND
ASD-09-053	12q14.2	Duplication	993	61814661–62807656	Paternal	Autistic disorder	M	Normal	Normal
ASD-09-054	13q12.11	Deletion	304	19698883–20002569	ND	Autistic disorder	M	Normal	Normal
ASD-09-055	13q12.11	Deletion	311	19691189–19860032	ND	Autistic disorder	F	Normal	Normal
ASD-09-056	15q11.1	Duplication	11 870	18362555–30232544	ND	Autistic disorder	M	47,XY +idic(15)(q13) unknown parental	Normal
ASD-09-057	15q11.2	Deletion	222	20412298–20634262	Paternal	Autistic disorder	M	Normal	Normal
ASD-09-058	15q11.2	Duplication	277	20428073–20704897	De novo	PDD-NOS	M	Normal	Normal
ASD-09-059	15q11.2q13.1	Duplication	4900	21219452–26214052	Maternal	Autistic disorder	M	ND	Normal
ASD-09-060	15q13.2q13.3	Deletion	1687	28719136–30405675	Maternal	PDD-NOS	M	Normal	Normal
ASD-09-061	15q13.2q13.3	Duplication	1982	28719136–30701432	De novo	Autistic disorder	M	Normal	Normal
ASD-09-062	15q13.2q13.3	Duplication	1982	28719136–30701432	De novo	Autistic disorder	M	Normal	Normal
ASD-09-063	15q13.2q13.3	Deletion	1500	28719136–30298155	ND	PDD-NOS	F	Normal	Normal
ASD-09-064	15q14	Deletion	152	31861894–32014683	ND	Autistic disorder	M	ND	Normal
ASD-09-065	16p11.2	Deletion	220	28732295–28952277	De novo	Autistic disorder	M	Normal	Normal
ASD-09-066	16p11.2	Deletion	546	29560500–30106852	De novo	Autistic disorder	M	Normal	Normal
ASD-09-067	16p11.2	Deletion	546	29560550–30106852	De novo	PDD-NOS	F	Normal	Normal
ASD-09-068	16p11.2	Deletion	546	29560500–30106101	De novo	PDD-NOS	M	Normal	Normal
ASD-09-069	16p11.2	Deletion	546	29560500–30106101	De novo	PDD-NOS	M	Normal	Normal
ASD-09-070	16p11.2	Duplication	679	29560500–30240082	Maternal	PDD-NOS	M	ND	ND
ASD-09-071	16p13.2	Duplication	368	6694662–7062616	ND	PDD-NOS	M	Normal	Normal
ASD-09-072	16q23.3	Deletion	166	81412569–81578850	Maternal	Autistic disorder	M	Normal	Normal
ASD-09-073	7q12	Deletion	1400	31889297–33323031	De novo	PDD-NOS	M	Normal	Normal
ASD-09-042	18p11.31p11.23	Deletion	3300	3905938–7234642	De novo	Autistic disorder	M	46,XY,?ins(6) (?p23?q13?q21)	ND
ASD-09-073	119p13.13	Duplication	168	13378448–13546189	De novo	PDD-NOS	M	Normal	Normal
ASD-09-075	21q	Duplication	Entire chr 21	Entire chr 21	De novo	Autistic disorder	F	47,XX, +21	
ASD-09-076	21q	Duplication	Entire chr 21	Entire chr 21	De novo	Autistic disorder	M	47,XY, +21	Normal
ASD-09-077	Xp22.31	Deletion	1628	6463313–8091810	Maternal	Autistic disorder	M	Normal	Normal
ASD-09-078	Xp22.31	Duplication	1624	6492092–8116174	ND	PDD-NOS	F	Normal	ABNORMAL_FEMALE paternal 69 premutation, maternal 32
ASD-09-079	Xq12	Deletion	24	65729442–65753605	Maternal	Autistic disorder	M	Normal	Normal
ASD-09-080	Xq27.1	Deletion	706	138429944–139136376	ND	PDD-NOS	M	Normal	Normal
ASD-09-081	XXY	Duplication	Entire chr X	Entire chr X	De novo	PDD-NOS	M	47,XXY	Normal

Study ID	Chromosome Location	Deletion/ Duplication	Size, kb	Chromosome Coordinates	Parent of Origin	Diagnosis	Gender	Karyotype	Fragile X
ASD-09-082	XYY	Duplication	Entire chr Y	Entire chr Y	De novo	Autistic disorder	M	ND	Normal

Chr indicates chromosome (coordinates reflect human genome build 18 from March 2006); ND, not done; M, male; de novo, not observed in blood sample from either parent; F, female; del, deletion of chromosomal material; ins, insertion of chromosome material; ish, in situ hybridization; idic, isodicentric chromosome.

TABLE 5

Summary of Genetic Testing in ASD

Test	Abnormal Results
Fragile X DNA, *n/N* (%)	2/852 (0.23)
G-banded karyotype, *n/N* (%)	19/852 (2.2)
Chromosomal microarray, *n/N* (%)	
Variant (s) identified	204/848 (24.1)
Clinically significant	59/848 (7.0)
Deletions, *n/N* (% of abnormal results)	37/59 (62.7)
De novo, *n* (% of deletions)	16 (43.2)
Maternally inherited	5 (13.5)
Paternally inherited	2 (5.4)
Unknown	15 (40.5)
Duplications, *n/N* (% of abnormal results)	22/59 (37.3)
De novo, *n* (% of abnormal duplications)	12 (54.5)
Maternally inherited	4 (18.1)
Paternally inherited	1 (4.5)
Unknown	5 (22.7)