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Diagnostic Utility of Array-Based Comparative Genomic Hybridization in a Clinical Setting

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Array-based comparative genomic hybridization is a recently introduced technique for the detection of submicroscopic genomic imbalances (deletions or duplications) across the entire genome. To assess the potential utility of a widely available array-based comparative genomic hybridization platform that targets specific, clinically relevant, loci across the genome for cytogenetic diagnosis in a clinical setting, we reviewed the medical records of all 373 patients at Children's Hospital Boston who had normal chromosomal analysis and were tested with this targeted array-based comparative genomic hybridization over a 1-year period from November 1, 2004 to October 31, 2005. These patients were tested because of a suspicion of chromosomal abnormalities based on their clinical presentation. Thirty-six patients (9.7%) had abnormal array-based comparative genomic hybridization results. Twenty patients (5.4%) had potentially pathogenetic genomic imbalances and 16 patients (4.3%) had copy number variations that are not believed to be pathogenetic. Thirteen of 234 patients (5.6%) with mental retardation/global developmental delay, 10/114 patients (8.8%) with facial dysmorphism, 5/58 patients (8.6%) with multiple congenital anomalies, and 4/35 patients (11.4%) with both facial dysmorphism and multiple congenital anomalies had potentially pathogenetic genomic imbalances. Targeted array-based comparative genomic hybridization is a clinically available test that is useful in the evaluation of patients suspected of having chromosomal disorders. However, it is best used as an adjunct to chromosomal analysis when a clear genetic diagnosis is unavailable. © 2007 Wiley-Liss, Inc.

Key words: chromosome aberrations; chromosome disorders; gene dosage; gene duplication; gene deletion; microarray comparative genomic hybridization; copy number variations

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INTRODUCTION

Chromosomal disorders are often suspected in patients who present with mental retardation, dysmorphic features, or multiple congenital anomalies. However, cryptic structural aberrations or submicroscopic segmental aneuploidies cannot be detected by routine chromosomal analysis. Array-based comparative genomic hybridization (aCGH) is a relatively new molecular cytogenetic technique that permits the detection of submicroscopic genomic imbalances, that is, deletions and duplications, in the human genome. In this technique, test and normal reference DNA samples are labeled with two different-colored fluorochromes and hybridized simultaneously onto bacterial artificial chromosome (BAC) clones of mapped sequences. The presence of copy number changes at a particular locus is suggested by a deviation from the expected 1:1 fluorescence intensity ratio between the test and normal samples at that chromosomal locus [Solinas-Toldo et al., 1997; Pinkel et al., 1998].

Clinical studies utilizing aCGH with BAC clones spaced at 1–1.4 Mb intervals across the genome have detected potentially pathogenetic copy number changes in 10–17% of patients with mental retardation and normal chromosomal analyses [Vissers et al., 2003; Shaw-Smith et al., 2004; Schoumans et al., 2005; Tyson et al., 2005; Menten et al., 2006; Miyake et al., 2006; Rosenberg et al., 2006]. Using an aCGH platform with complete coverage of the human

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genome, de Vries et al. [2005] detected potentially pathogenetic copy number changes in up to 15% of their patients with mental retardation.

Recently, "targeted" aCGH with BAC clones mapping to clinically significant loci, rather than at fixed intervals across the genome, have been developed for routine clinical diagnostic use [Bejjani et al., 2005; Cheung et al., 2005]. In this study, we aim to determine the diagnostic yield and the limitations of a commercially available targeted aCGH platform among patients clinically suspected of having chromosomal disorders.

MATERIALS AND METHODS

Patients

We conducted a comprehensive retrospective review of the medical records of all patients at Children's Hospital Boston who had a normal chromosomal analysis and were tested with a targeted aCGH between November 1, 2004 and October 31, 2005. For each patient, we determined the presence or absence of three specific clinical features—global developmental delay (GDD) or mental retardation (MR), facial dysmorphism, and multiple congenital anomalies (MCA). We grouped GDD and MR into a single category because there are no valid instruments for measuring intelligence in children younger than 5 years of age, and MR usually first presents as GDD in the younger child [Curry et al., 1997; Shevell et al., 2003; Shaffer, 2005]. Patients with significant delays in speech and either fine or gross motor skills, as documented by pediatric neurologists, developmental pediatricians, or clinical geneticists, were categorized as having GDD. Patients who had at least three facial dysmorphic features identified by a clinical geneticist were categorized as having "facial dysmorphism." Patients with congenital structural abnormalities in more than one organ system, resulting in significant morbidity or disability, were categorized as having MCA.

Exclusion criteria for this study were: (a) parents of the probands who were tested with aCGH, (b) prenatal patients because of our lack of ability to determine their developmental milestones and facial dysmorphism, and (c) children in whom the clinical diagnoses were suspected a priori based on the classical features of the syndromes.

This study was approved by the Committee on Clinical Investigation at Children's Hospital Boston.

Targeted Microarray CGH

A clinically available targeted aCGH (SignatureChip™ version 2, Signature Genomic Laboratories, LLC, Spokane, WA) was used to investigate all the patients in this study. This targeted aCGH contains 831 BAC clones covering 230 loci of known microdeletion and microduplication syndromes, subtelomeric and pericentromeric regions. Each locus is covered by at least three partially overlapping BAC clones; each interstitial locus is flanked by control contigs placed about 1 Mb on either side. The design and clinical validation of this aCGH by Signature Genomic Laboratories, LLC, has been published [Bejjani et al., 2005]. This validation included the analysis of 36 patients with known chromosomal abnormalities, as well as 50 phenotypically normal individuals (25 males and 25 females) drawn from the Baylor Polymorphism Resource (http://www.bcm.edu/blg/showned.cfm?01-106) representing four ethnic groups (12 African-American, 11 Asians, 16 Caucasians, and 11 Hispanics).

Fluorescence In Situ Hybridization (FISH) Confirmation

For all patients who had abnormal aCGH results using standard protocols, confirmatory FISH was performed by Signature Genomic Laboratories, LLC [Shaffer et al., 1994]. This helped to delineate the chromosomal rearrangements that resulted in the copy number changes detected by aCGH.

Evaluation of the Clinical Significance of Abnormal aCGH Results

Whenever possible, the parents of each patient with an abnormal aCGH result were tested to determine whether the observed abnormality was de novo or inherited. De novo findings were classified as potentially pathogenetic genomic imbalances. Inherited aberrations from phenotypically normal parents were considered to be normal copy number variations (CNV). In addition, a search of the Database of Genomic Variants (http://projects.tcag.ca/variation/) [Iafrate et al., 2004] and MEDLINE (http://www.pubmed.gov/) was performed to determine whether the observed finding had been previously reported to be a normal population variant.

Statistical Analysis

We compared the proportion of deletions and duplications in patients with potentially pathogenetic genomic imbalances to those with CNV. The two-tailed Fisher's exact test was used because of the relatively small number of patients in each category.

We also examined the proportion of patients with one or more of three specific clinical features (MR/GDD, facial dysmorphism, and MCA) who had potentially pathogenetic genomic imbalances.

RESULTS

A total of 373 patients with normal chromosomal analysis were tested by aCGH over the 12-month

period. Clinical geneticists ordered aCGH testing for 190 (51%) of these patients, and pediatric neurologists ordered aCGH testing for 168 (45%) of these patients. The clinical features in these 373 patients included GDD/MR in 234/352 (66%), facial dysmorphism in 114/286 (40%), and/or MCA in 58/372 (16%). Of these 373 patients, 36 (9.7%) had abnormal aCGH results, including 20 (5.4%) with potentially pathogenetic genomic imbalances and 16 (4.3%) with CNV (Fig. 1). Deletions were more common in patients with potentially pathogenetic genomic imbalances, while duplications were more common in patients with CNV; in addition we identified three patients with unbalanced translocations and two patients with mosaic chromosomal trisomies (Table I). Figure 2 shows representative samples of the aCGH plots for the patients with potentially pathogenetic genomic imbalances. FISH analysis confirmed the aCGH findings in all patients with abnormal aCGH results, except for six patients with duplications (Table III, Patients #iv, v, ix, xii, xiv, and xv). The inability of FISH to confirm these duplications could be due to the fact that it can be difficult to visualize small duplications on interphase FISH; however, it is believed that these duplications are not "artifactual," as supported by the observation that they were also observed in the parents of these patients, where the parents were available for testing (Table III, Patients #iv, v, and ix), using the same aCGH platform [LG Shaffer, personal communication]. The major clinical and cytogenetic findings in the patients with potentially pathogenetic genomic imbalances and normal CNV are summarized in Tables II and III respectively.

Either one or both parents were not available for analysis in 11 of the 20 patients with potentially pathogenetic genomic imbalances (Table II). Nonetheless, they were categorized as having potentially pathogenetic aCGH findings based on our knowledge of the chromosomal loci and genes involved in these microdeletions and microduplications. In seven of these patients, the deletions encompassed genes or loci known to result in abnormal clinical phenotypes. Patient #5 had a deletion on chromosome 2q37.3, which is now recognized as a microdeletion syndrome [Aldred et al., 2004; Wassink et al., 2005]; Patients #10 and #11 had a deletion in the *SNRPN/UBE3A* locus on chromosome 15q11.2q12 known to cause Angelman syndrome; patient #14 had a deletion on chromosome 22q11.2 involving the *HIRA* (*TUPLE1*)/*TBX1* locus known to contribute to velocardiofacial syndrome; Patient #16 had a deletion on chromosome Xp22.3 involving *STS* (steroid sulfatase) known to be responsible for X-linked icthyosis; Patient #17, who was autistic, had a deletion on chromosome Xp22.3 involving *NLGN4X*, a gene that has been implicated in some cases of autism [Laumonnier et al., 2004]. Patient #18 had a mosaic duplication of chromosome 1q44 and a non-mosaic deletion of chromosome 2q37.3; although up to 70% of the segment that was duplicated on chromosome 1q44 has previously been observed in normal individuals [Iafrate et al., 2004], the chromosome 2q37.3 deletion is known to be pathogenetic [Aldred et al., 2004; Wassink et al., 2005], and in retrospect, the patient's phenotype was thought to be consistent with that of monosomy 2q37.3.

In three of these 11 patients, the deletions involved multiple genes. Although it is uncertain whether these deletions contributed to the patients' phenotype, we classified these deletions as being potentially pathogenetic. Patient #4 (Table II), who was reported to have Fryns syndrome, had a deletion on chromosome 1q41q42 encompassing a region with multiple known and hypothetical genes [Kantarci et al., 2006]; Patient #13 had a deletion on chromosome 20p13 involving at least nine known and putative genes. Patient #6 had a mosaic deletion on chromosome 7p21.1 involving *HDAC9*, *TWIST1*, and *FERD3L*. The FISH confirmation, along with the aCGH plot, for this mosaicism has been reported [Ballif et al., 2006]. *HDAC9* is believed to be involved in hematopoiesis, *TWIST1* is responsible for Saethre–Chotzen syndrome, while the function of *FERD3L* is unclear but it may be involved in the regulation of transcription. Although this deletion was observed in only 5.7% of her peripheral blood lymphocytes and she did not have any clinical features suggestive of Saethre–Chotzen syndrome or anemia, we could not exclude the possibility that this deletion might be present in a non-mosaic state in other tissues of her body and contributed to her phenotype. Hence, her deletion was classified as being potentially pathogenetic.

The remaining patient (Table II, Patient #3) had a duplication on chromosome 1q21.1 involving a segment that is at least 285 kb and contains several ubiquitously expressed genes including *PIAS3* (regulating activity of transcription factors), *RBM8A* (regulating mRNA splicing), *POLR3C* (part of the RNA polymerase III complex), *HFE2* (regulating iron metabolism in liver, heart, and skeletal muscle), and *ITGA10*, the precursor of integrin α-10, which is involved in collagen binding in articular cartilage. Given that the child has mental retardation and bilateral club feet, we speculated that over-expression of *ITGA10* might be responsible for her phenotypic features [UCSC Genome Browser, 2006]. Hence we classified this duplication as being potentially pathogenetic.

Among the 16 patients with CNV (Table III), nine had inherited their deletions and duplications from phenotypically normal parents. Of the other seven patients, Patient #i had an approximately 153 kb deletion on chromosome 2p25.3 that involved no known genes and only 1 predicted gene of unknown function [Ensembl database, 2006]; Patient #vi had a

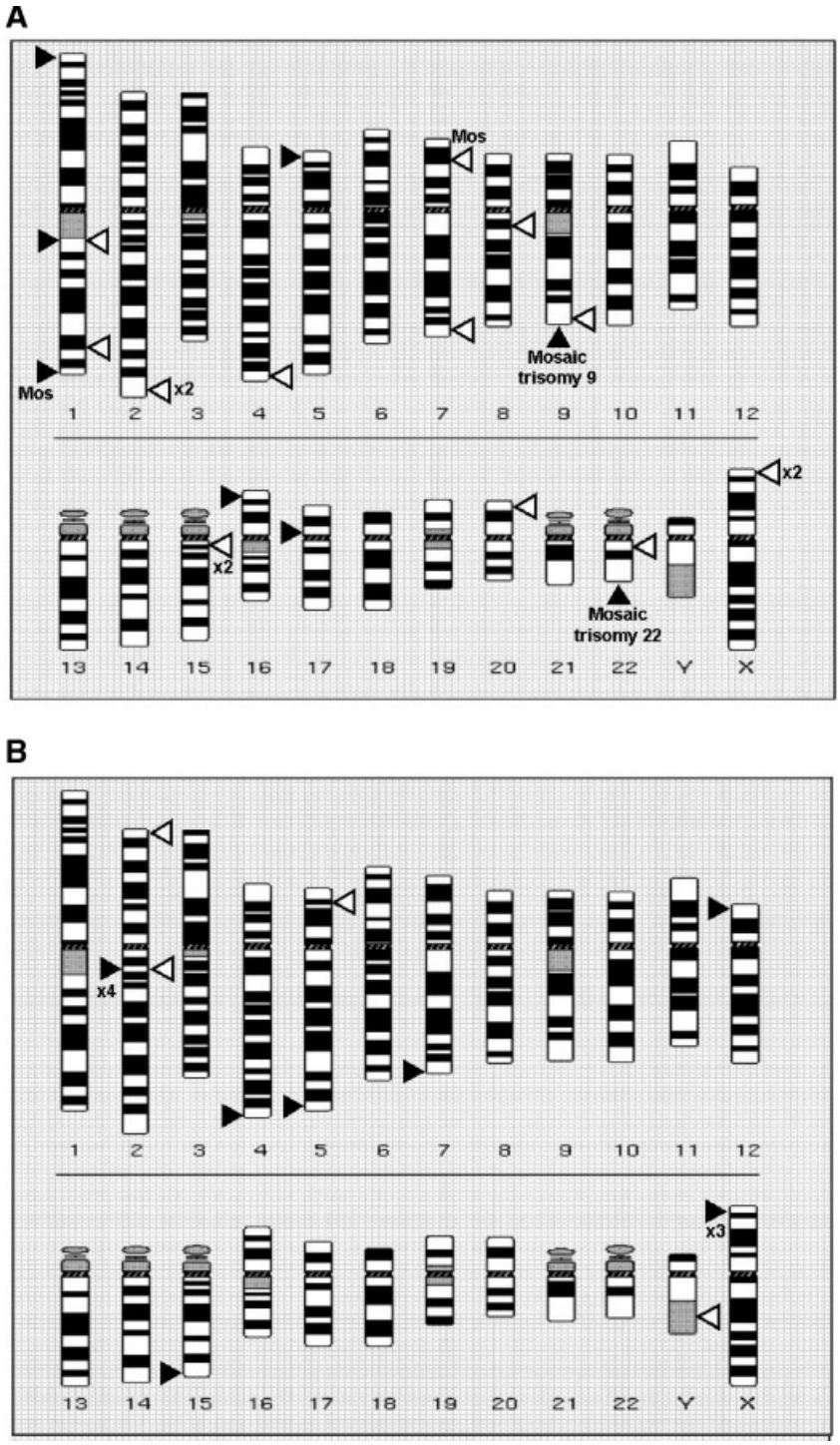

FIG. 1. Human idiograms with the distribution of duplications and deletions identified by aCGH. Black arrowheads—genomic positions of segmental duplications and trisomies; White arrowheads—genomic positions of segmental deletions; Mos, mosaic; Numbers adjacent to arrows indicate the number of patients with the same change at the given genomic position. [The idiograms are modified from those copyrighted by David Adler and are used with permission] **a**: Distribution of duplications and deletions in 20 patients with potentially pathogenetic genomic imbalances. **b**: Distribution of duplications and deletions in 16 patients with CNV.

heterozygous deletion of *NPHP1* (chromosome 2q13), which is not known to be deleterious; Patient# (xii) had a duplication on chromosome 15q26.3 that has been reported as a normal variant [Shaffer et al., 2006]; three patients (Patients #xiii–xv) had duplication of *STS* (chromosome Xp22.3), which is considered a normal variant [Shaw-Smith et al., 2004]; and one patient (Patient #xvi) had a deletion on Yq12 that has also been reported to be a normal variant [Shaffer et al., 2006].

TABLE I. Types of Chromosomal Aberrations in Patients With Potentially Pathogenetic Genomic Imbalances and CNV

Aberration	Potentially pathogenetic	CNV	*P* value (Fisher's exact test)
Deletions	12/20 (60%)	4/16 (25%)	0.049
Duplications	3/20 (15%)	12/16 (75%)	<0.001
Unbalanced translocations	3/20 (15%)	0/16	
Chromosomal mosaic trisomies	2/20 (10%)	0/16	

CNV, copy number variations.

One patient with CNV (Table III, Patient #viii) had a 384 kb deletion on chromosome 5p15.2 between the BACs RP11-107O20 and RP11-553D6, which does not overlap with the Cri-du-chat critical region [Zhang et al., 2005]. Although it overlaps with a region that is thought to be responsible for mild MR [Zhang et al., 2005], all the patients in that study had cytogenetically visible deletions, unlike our patient who had a 384 kb deletion, which is too small to be resolved on routine chromosomal analysis. More importantly, her mother, who carried the same deletion, was phenotypically normal. This deletion was therefore classified as a CNV.

Among our patients, 13/234 (5.6%) with MR/GDD, 10/114 (8.8%) with facial dysmorphism, 5/58 (8.6%) with MCA, and 4/35 (11.4%) with both facial dysmorphism and MCA had potentially pathogenetic genomic imbalances. In addition, 3/52 (5.8%) of patients with none of these three features (Table II, Patients #2, #14, #17) had potentially pathogenetic genomic imbalances, suggesting that aCGH should be considered in patients referred for evaluation with behavioral, learning, or other developmental problems that are not associated with significant mental retardation and/or facial dysmorphism.

DISCUSSION

We have conducted a large study of 373 patients to assess the clinical utility of targeted aCGH testing

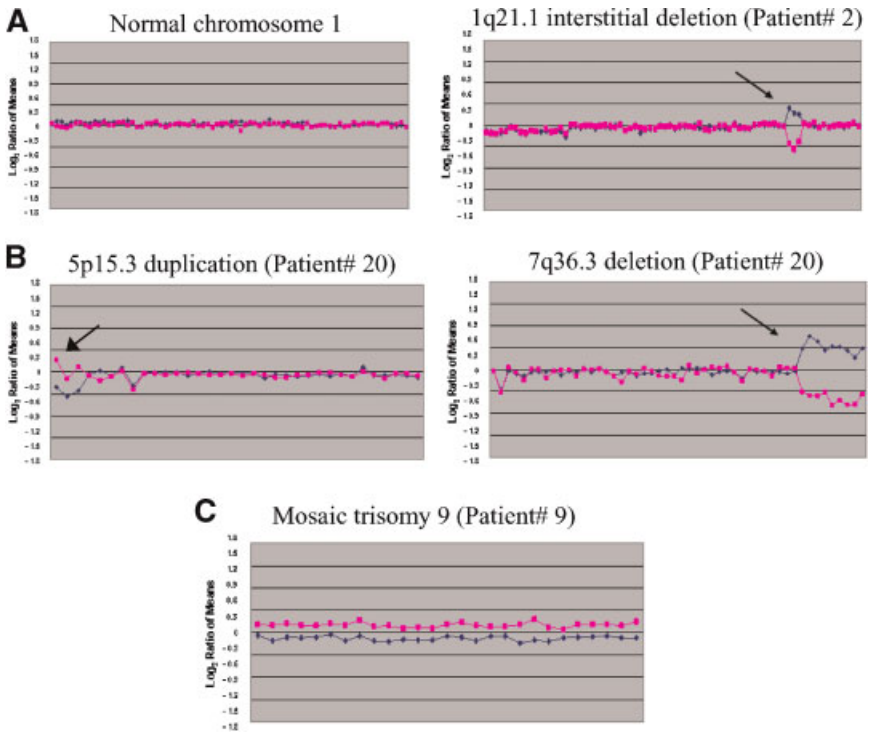

FIG. 2. Profiles for chromosomal aberrations detected with the SignatureChip® targeted aCGH. Each clone on the plot is arranged along the X-axis according to its location on the chromosome with the most distal telomeric short arm clones on the left and the most distal/telomeric long-arm clones on the right. The dark blue line represents the control:patient fluorescence intensity ratios for each clone, whereas the pink (light) line represents the fluorescence intensity ratios obtained from a second hybridization in which the dyes have been reversed (patient:control). All the following plots are from patients with potentially pathogenetic genomic imbalances (Table II), and the abnormalities identified by aCGH were confirmed by conventional FISH. **a**: (Patient #2): Detection of DNA copy loss at chromosome 1q21.1. The left plot shows a normal chromosome 1, with a ratio of 0 on a $\log_2$ scale for all clones. The right plot shows DNA copy loss at chromosome 1q21.1, indicating an interstitial deletion at this locus, for Patient #2. **b**: (Patient #20): Detection of an unbalanced terminal translocation between chromosomes 5 and 7. The left plot shows DNA copy gain at 5p15.3, and the right plot shows DNA copy loss at 7q36.3. **c**: (Patient #9): Detection of DNA copy gain across all of chromosome 9. The plot shows a significant deviation from 0 for all chromosome 9, indicating mosaic trisomy 9. The presence and level of mosaicism was investigated further with FISH. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II. Patients With Potentially Pathogenetic Genomic Imbalances

Patient	aCGH findings [genes involved]	BAC clones involved	Confirmed by FISH?	Inherited?	MR/GDD	Dysmorph	MCA	Other major clinical findings
1	1p36.3 duplication and 1p36.3 triplication [multiple genes]	(RP11-361M21, RP4-740C4, RP4-713A8)×3, (RP3-484O5, RP11-547D24, RP1-140A9)×4	Yes	De novo	Y	N	N	Macrocephaly, seizures, left ptosis, inverted nipples
2	1q21.1 deletion [multiple genes]	RP11-315I20 → RP11-1112C1	Yes	De novo	N	N	N	Overgrowth (height, weight and head circumference >97th centile), mood disorder, ADD
3	1q21.1 duplication [multiple genes]	RP11-315I20, RP11-841D19, RP11-1112C1	Yes	Unknown	Y	?	N	Bilateral club feet
4	1q41q42 deletion [multiple genes]	RP11-139E20, RP11-1031M6, RP11-61M2	Yes	Unknown	?	Y	Y	Many clinical features of Fryns syndrome including coarse facies, cleft soft palate, VSD, diaphragmatic hernia; died at 1 day of life
5	2q37.3 deletion [multiple genes]	RP11-875C22, RP11-367H1, RP13-925E23	Yes	Unknown	Y	?	N	Seizure, PDD, stereotyped self-stimulatory behavior
6	7p21.1 mosaic (5.7%) deletion [*HDAC9*, *TWIST1*, *FERD3L*]	RP11-10E7, RP11-5G13	Yes (3/53 metaphase cells, D7Z1 probe as control)	Unknown	Y	Y	N	No clinical features of Saethre–Chotzen syndrome. No ptosis. Congenital pendular nystagmus. Overgrowth (length, weight and head circumference >97th centile), congenital pendular nystagmus, aqueductal stenosis. No clinical features of Saethre-Chotzen syndrome. No ptosis.
7	8q12.2 deletion [*RAB2*, *CHD7*]	RP11-414L17, RP11-668C3, RP11-33I11	Yes	De novo	?	Y	Y	Absent septum pellucidum, dysplastic corpus callosum, panhypopituitarism, transposition of great arteries, bilateral hearing loss, bilateral vesico-ureteric reflux, microphallus
8	9q34.3 interstitial deletion [multiple genes]	RP11-48C7, RP13-467E5	Yes	De novo	?	Y	Y	Not tracking visually at 3 months, ASD, hypotonia, choreoathetotic movements, hemosiderin in cerebellum
9	Mosaic (10%) trisomy 9 [multiple genes]	RP13-354N4 → RP11-31M4	Yes (2/7 metaphase, and 10/100 interphase cells, D9Z3 probe as control)	Not applicable	Y	Y	N	Triangular face, bulbous nasal tip with narrow bridge, smooth philtrum, high-arched palate; swirling areas of hyperpigmentation bilatarally; bilateral sensorineural hearing loss; Left vertical talus; Left 4th finger camptodactyly; Delayed myelination, mildly malformed pons
10	15q11.2q12 deletion [*SNRPN*, *UBE3A*]	RP11-125E1 → RP11-466L14	Yes	Unknown	Y	N	N	Microcephaly, infantile spasm, VSD
11	15q11.2q12 deletion [*SNRPN*, *UBE3A*]	RP11-125E1 → RP11-466L14	Yes	Unknown	Y	?	N	Had a few words, but lost them after 1 year old—now non-verbal. No seizures but brain electrical activity mapping (BEAM) suggests seizure tendency [follow-up studies → deletion on maternal chromosome]
12	17p11.2 duplication [multiple genes]	RP11-138I1, RP11-404D6, RP11-384N5, RP11-524F11, RP11-1149K20, RP11-958E14	Yes	De novo	Y	Y	N	Mild hypotonia, Wide-based gait
13	20p13 interstitial deletion [multiple genes]	RP5-1103G7, RP11-112D6	Yes	Mother—normal; Father—died: colon cancer	N	Y	N	Relative macrocephaly, speech and fine motor difficulties, hypothyroidism

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TABLE II. (*Continued*)

Patient	aCGH findings [genes involved]	BAC clones involved	Confirmed by FISH?	Inherited?	MR/GDD	Dysmorph	MCA	Other major clinical findings
14	22q11.2 deletion [multiple genes]	RP11-195I8 → RP11-1107H2	Yes	Unknown	N	N	N	Learning difficulties (but full-scale IQ on WISC-III: 83), Slightly high-arched palate (no cleft), hyperextensible and tapering fingers, Tetralogy of Fallot
15	Mosaic (77%) trisomy 22 on skin biopsy [multiple genes]	RP11-701M12 → RP11-825H3	Yes (23/30 metaphase cells, RP11-676E13 probe as control)	Not applicable	Y	N	Y	ASD, VSD, left pulmonary artery hypoplasia, left lung hypoplasia, left pelvic kidney; right hemi-hypertrophy; variegate pattern of hyper- and hypopigmentation; fusion of C7, T1 ± C6 vertebrae
16	Xp22.3 deletion [*STS*]	GS1-221L7, CTD-2052I13, RP13-436H11	Yes	Unknown	Y	N	N	Icthyosis from day 3 of life; Family history of icthyosis in maternal male relatives; hypertonia, visual delay, early tooth eruption (3 months)
17	Xp22.3 deletion [*NLGN4X*] (*STS* not deleted)	RP11-910C18, RP11-323G19, RP11-109P4	Yes	Unknown	N	N	N	Autism; self-stimulatory behavior; Small skull-base encephalocele
18	1q44 mosaic (34%) duplication [multiple olfactory receptor genes]	RP11-280A15, RP11-438F14, RP11-68F13	Yes (4/60 metaphase and 34/100 interphase cells, D1Z1 probe as control)	Mother—normal; Father—not available	Y	Y	N	Features consistent with del(2)(q37.3) syndrome; truncus arteriosus, interrupted aortic arch, VSD; eczema; short 4th and 5th metacarpals
	2q37.3 deletion [multiple genes]	RP11-875C22, RP11-367H1, RP13-925E23	Yes					
19	4q35.2 deletion [2 predicted genes—*HSP90AA4P* and a gene of unknown function]	RP11-347P3, RP11-706F1, RP11-45F23	Yes—der(4)t(4;16)(q35;p13.3)	De novo	Y	Y	N	VSD, seizures, feeding difficulties, partial agenesis of corpus callosum, periventricular gray matter heterotopia
	16p13.3 duplication [multiple genes]	RP11-252I11, RP11-97H22						
20	5p15.3 duplication [*AHRR*, *EXOC3*, and 1 provisional gene—*SLC9A3*]	RP11-1006P13	Yes—der(7)t(5;7)(p15.3;q36)	Mother has balanced translocation—46,XX,t(5;7)(p15.3;q36)	Y	Y	Y	Hypotonia, ASD, feeding difficulties, cortical blindness, sensorineural hearing loss, arthritis, contractures of knees and elbows, vesicouretric reflux, prolonged QTc interval on electrocardiogram
	7q36.3 deletion [*SHH*, and 2 provisional genes—*PRR8*, *RBM33*]	RP11-69O3						

Genes are listed if fewer than 4 genes involved.
MR, mental retardation; GDD, global developmental delay; Dysmorph, facial dysmorphic features; MCA, multiple congenital anomalies; ASD, atrial septal defect; ADD, attention deficit disorder; PDD, pervasive developmental disorder; Y, yes; N, no; ?, no information available.

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TABLE III. Summary of Patients With Probable Copy Number Variations (CNV)

Patient	aCGH findings [genes involved]	BAC clones involved	Confirmed by FISH?	Inherited?	MR/GDD	Dysmorph	MCA	Other major clinical findings
i	2p25.3 deletion [1 predicted gene only]	RP11-97B21	Yes	Unknown—but only 1 predicted gene	Y	N	N	Microcephaly, seizures, blonde hair with blue eyes; Brain MRI—left choroidal fissure cyst, small thalamus
ii	2q13 duplication [*NPHP1*, *MALL*, 1 predicted gene]	RP11-335A19, RP11-528G9, RP11-264O8	Yes	Yes—Father	Y	Y	N	GDD but speech and language more severely affected
iii	2q13 duplication [*NPHP1*, *MALL*, 1 predicted gene]	RP11-335A19, RP11-528G9, RP11-264O8	Yes	Yes—Father	N	?	N	Little/no expressive language
iv	2q13 duplication [*NPHP1*, *MALL*, 1 predicted gene]	RP11-335A19, RP11-528G9, RP11-264O8	No	Yes—Father	Y	N	N	Autism, vesico-ureteric reflux
v	2q13 duplication [*NPHP1*, *MALL*, 1 predicted gene]	RP11-335A19, RP11-528G9, RP11-264O8	No	Yes—Mother	N	N	N	Growth failure (height and weight only—normal head circumference)
vi	2q13 deletion [*NPHP1*, *MALL*, 1 predicted gene]	RP11-335A19, RP11-528G9, RP11-264O8	Yes	Unknown—but heterozygotes usually asymptomatic	Y	Y	Y	Hypoplastic corpus callosum, bilateral perisylvian polymicrogyria, right congenital glaucoma, possible hearing loss, laryngomalacia
vii	4q35.2 duplication [no genes known to be affected]	RP11-347P3	Yes	Yes—mother	Y	N	N	ASD, dilated coronary artery, Mild bilateral hydronephrosis
viii	5p15.2 deletion [only 1 predicted snRNA pseudogene]	RP11-107O20, RP11-141G2, RP11-553D6	Yes	Yes—mother	N	N	N	Normal cry; subependymal nodular heterotopia, hypogenesis of corpus callosum, cerebellar dysgenesis; mother has normal brain MRI
ix	5q35.3 duplication [multiple genes]	RP11-754A4, RP11-2593A12, RP11-437D1, RP11-69N15	No	Yes—mother	Y	N	N	Seizure, hypertonia, depigmented streak on left thigh and pelvis
x	7q36.3 duplication [*VIPR2*, 1 predicted gene]	RP11-93F2, RP11-789H4, RP11-1112M14	Yes—der(16)t(7;16)(q36;q24)	Yes—father	Y	?	N	Autism
xi	12p13.3 duplication [multiple genes]	RP5-1154L15, RP11-283I3, RP11-350L7	Yes	Yes—mother	N	?	N	Language delay, possible Landau-Kleffner variant
xii	15q26.3 duplication [*PCSK6* (partial), *TM2D3*, *TARSL2*]	RP11-14C10, RP11-89K11	No	Mother—normal; Father—not tested	Y	N	N	Seizure, Tantrums and aggressive behavior, history of pyloric stenosis
xiii	Xp22.3 duplication [*STS*]	GS1-221L7, CTD-2052I13,RP13-436H11	Yes	Unknown	Y	N	N	EEG—sharp and spike waves bilaterally but no clinical seizures
xiv	Xp22.3 duplication [*STS*]	GS1-221L7, CTD-2052I13,RP13-436H11	No	Unknown	Y	Y	N	Possible developmental regression, seizures, mesial temporal sclerosis, hypoplastic corpus callosum, Klippel-Feil (fusion of C1-C4 vertebrae)
xv	Xp22.3 duplication [*STS*]	GS1-221L7, CTD-2052I13,RP13-436H11	No	Unknown	Y	?	N	Cleft palate (no cleft lip)
xvi	Yq12 (pseudoautosomal region) deletion [*SPRY3*, *SYBL1*]	RP11-179B5,RP11-479B17,RP11-1137B3	Yes	Unknown	Y	?	N	Spasticity, genito-urinary reflux, hearing impairment

Genes are listed if fewer than 4 genes involved.
MR, mental retardation; GDD, global developmental delay; Dysmorph, facial dysmorphic features; MCA, multiple congenital anomalies; ASD, atrial septal defect; MRI, magnetic resonance imaging; EEG, electroencephalogram; Y, yes; N, no; ?, no information available.

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in a pediatric tertiary care hospital, and we believe that these findings can be generalized to other similar pediatric tertiary care settings. Our study demonstrates that targeted aCGH is able to identify previously undetectable cytogenetic abnormalities in 5.4% of all patients suspected of having chromosomal disorders with previously normal karyotypes, and in 11.4% of our patients who had both facial dysmorphism and MCA.

Although our overall diagnostic yield (5.4%) appears lower than that reported by previous authors using other aCGH platforms [Shaw-Smith et al., 2004; de Vries et al., 2005; Schoumans et al., 2005; Tyson et al., 2005; Menten et al., 2006; Miyake et al., 2006; Rosenberg et al., 2006], this study differs from previous studies in several ways. We used a targeted aCGH that is readily available for routine clinical diagnosis. In addition, the patient populations in previous studies were relatively homogeneous as all those patients had MR/GDD with or without other accompanying features. However, because one of our aims was to determine the yield of aCGH in a clinical setting, we did not select for patients with specific clinical phenotypes. Many patients with clinical presentations suggestive of specific chromosomal conditions (e.g., velocardiofacial syndrome or Williams syndrome) would have had targeted testing for those conditions and were not part of this study population. We believe that this would provide a more realistic expectation of the diagnostic yield of aCGH when its use becomes widespread in the medical community.

Moreover, by having BACs at loci of known syndromes and at the subtelomeric regions, this targeted aCGH platform is able to diagnose patients with atypical presentations of typical diseases [Cheung et al., 2005]. For example, the patient with chromosome 8q12.2 deletion (Table II, Patient #7) had presented as a newborn with congenital heart disease, bilateral vesico-ureteric reflux, microphallus, and absent septum pellucidum with a dysplastic corpus callosum. However, CHARGE syndrome was not initially suspected in him because abnormalities in the septum pellucidum were not known to be associated with CHARGE syndrome, and his hearing had not been tested at the time of his presentation, so he did not meet the minimum diagnostic criteria for CHARGE syndrome. It was only after the microdeletion was identified that he was found to have profound hearing loss. Of note, he did not have choanal atresia nor coloboma, which are among the more common and specific clinical features of this syndrome. Thus, the use of aCGH in this patient facilitated the diagnosis of a well-described syndrome presenting with unusual features, and it enabled more appropriate management of the patient.

Routine chromosomal analysis is usually unable to detect chromosomal mosaicism below 14% [Hook, 1977]. Our study has demonstrated that this targeted aCGH is able to detect lower levels of chromosomal mosaicism, as observed in the patient with 7p21.1 deletion in 6% of her peripheral blood lymphocytes (Table II, Patient #6). This patient had routine chromosomal analysis at the 575-band resolution with 20 metaphases counted and 5 metaphases analyzed that was normal. Another patient who previously had normal chromosomal analysis in blood (550-band resolution; 20 metaphases counted, seven metaphases analyzed), and skin fibroblasts (425-band resolution; 20 metaphases counted, six metaphases analyzed) was found to have trisomy 9 in 10% of her peripheral blood lymphocytes by aCGH and follow-up FISH (Table II, Patient# 9). Following the detection of trisomy 9 by aCGH, the chromosomal analysis in peripheral blood was repeated at the 525-band resolution with 101 metaphases counted and 6 metaphases analyzed, and trisomy 9 was observed in three out of 101 cells.

It is now well-established that CNV are common in the human genome [Iafrate et al., 2004; Feuk et al., 2006]. This poses an enormous challenge to the interpretation of aCGH results since many of these CNV are not well studied. Although CNV have been considered to be benign, it has been postulated that they may be pathogenetic in some individuals even if they were inherited from phenotypically normal parents due to epigenetic effects, position effects, gene dosage effects or the unmasking of recessive mutations [Feuk et al., 2006; Menten et al., 2006]. Moreover, it has recently been shown that variations in the copy number of *FCGR3* could affect the risk of glomerulonephritis in patients with systemic lupus erythematosus [Aitman et al., 2006]. The classification of CNV as "benign" findings on aCGH may therefore need to be re-evaluated as our understanding of CNV improves.

Our findings that significantly more deletions were detected among the patients with potentially pathogenetic genomic imbalances, and significantly more duplications were detected among the patients with CNV suggests that segmental duplications of the human genome are better tolerated than segmental deletions. Hence, deletions identified by aCGH are more likely to be potentially pathogenetic as opposed to duplications, which are more likely to be CNV.

The increasing use of aCGH in routine clinical settings will lead to a better understanding of submicroscopic chromosomal aberrations and their contribution to clinical disease. However, at present, aCGH cannot detect balanced translocations and inversions because there is no genomic imbalance in those rearrangements. Moreover, deletions and duplications in regions not covered by aCGH will not be detected as well. It is therefore important to ensure that routine chromosomal analysis is normal prior to aCGH testing. This study has demonstrated

that aCGH is a useful diagnostic tool that is worth incorporating into the routine evaluation of patients with mental retardation/global developmental delay, facial dysmorphism or multiple congenital abnormalities, when they have had a normal karyotypic analysis. The identification of potentially pathogenetic but previously undetectable submicroscopic chromosomal aberrations will ultimately facilitate the care of these patients by enabling more appropriate genetic counseling, and by helping clinical researchers understand the pathogenesis and natural history of these segmental duplications and deletions.

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