Clinical Utility of Chromosomal Microarray Analysis

AUTHORS: Jay W. Ellison, MD, PhD,[a] J. Britt Ravnan, PhD,[a] Jill A. Rosenfeld, MS,[a] S. Annie Morton, MS,[a] Nicholas J. Neill, BS,[a] Marc S. Williams, MD,[b] Jodi Lewis, BS,[a] Beth S. Torchia, PhD,[a] Cathryn Walker, BS,[a] Ryan N. Traylor, BS,[a] Kimberly Moles, MS,[a] Elizabeth Miller, BS,[a] Jennifer Lantz, BS,[a] Caitlin Valentin, MS,[a] Sara L. Minier, MS,[a] Kimberly Leiser, MHPA,[a] Berkley R. Powell, MD,[c] Timothy M. Wilks, MD,[d] and Lisa G. Shaffer, PhD[a]

[a]Signature Genomic Laboratories, PerkinElmer, Inc, Spokane, Washington; [b]Intermountain Healthcare Clinical Genetics Institute, Salt Lake City, Utah; [c]Children's Hospital Central California, Madera, California; [d]Madigan Army Medical Center, Joint Base Lewis-McChord, Washington

KEY WORDS

microarray analysis, clinical utility, DNA copy number variants

ABBREVIATION

BAC—bacterial artificial chromosome

Drs Ellison, Ravnan, and Shaffer and Ms Morton made substantial contributions to conception and design; Drs Ellison, Ravnan, Torchia, Wilks, and Powell and Mr Neill, Ms Lewis, Ms Walker, Ms Traylor, Ms Moles, Ms Miller, Ms Lantz, Ms Valentin, Ms Minier, and Ms Leiser made substantial contributions to the acquisition of data; Drs Ellison, Ravnan, Torchia, and Williams and Ms Rosenfeld, Ms Morton, and Mr Neill analyzed and interpreted the data; Dr Ellison, Ms Rosenfeld, Ms Morton, and Mr Neill participated in drafting the article; and all authors critically revised the paper for important intellectual content and approved the final version to be published.

The views of expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Army, Department of the Navy, Department of Defense, or the US government.

Dr Williams' current affiliation is Genomic Medicine Institute, Geisinger Health System, Danville, PA.

www.pediatrics.org/cgi/doi/10.1542/peds.2012-0568

doi:10.1542/peds.2012-0568

Accepted for publication Jun 14, 2012

Address correspondence to Jay W. Ellison, MD, PhD, Signature Genomic Laboratories, 2820 N Astor St, Spokane, WA 99207. E-mail: jay.ellison@perkinelmer.com

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

FINANCIAL DISCLOSURE: *Drs Ellison, Ravnan, Torchia, and Shaffer, as well as Ms Rosenfeld, Ms Morton, Mr Neill, Ms Lewis, Ms Walker, Ms Traylor, Ms Moles, Ms Miller, Ms Lantz, Ms Valentin, Ms Minier, and Ms Leiser were employees of Signature Genomic Laboratories at the time of the study; Dr Wilks is active duty and an employee of the US government; and Drs Williams, Powell, and Wilks have indicated they have no financial relationships relevant to this article to disclose.*

FUNDING: This study was funded in part by Signature Genomic Laboratories, PerkinElmer, Inc.

WHAT'S KNOWN ON THIS SUBJECT: Chromosomal microarray analysis offers a superior diagnostic yield over karyotyping for the evaluation of individuals with developmental disabilities. Many third-party payers, however, do not reimburse for microarray testing, citing a lack of evidence that patients benefit from testing.

WHAT THIS STUDY ADDS: This study demonstrates that microarray testing frequently identifies conditions that include features requiring specific medical follow-up and that referring physicians respond to abnormal test results with appropriate clinical actions. Microarray testing, therefore, provides direct benefits to patients.

abstract

OBJECTIVE: To test the hypothesis that chromosomal microarray analysis frequently diagnoses conditions that require specific medical follow-up and that referring physicians respond appropriately to abnormal test results.

METHODS: A total of 46 298 postnatal patients were tested by chromosomal microarray analysis for a variety of indications, most commonly intellectual disability/developmental delay, congenital anomalies, dysmorphic features, and neurobehavioral problems. The frequency of detection of abnormalities associated with actionable clinical features was tallied, and the rate of physician response to a subset of abnormal tests results was monitored.

RESULTS: A total of 2088 diagnoses were made of more than 100 different disorders that have specific clinical features that warrant follow-up. The detection rate for these conditions using high-resolution whole-genome microarrays was 5.4%, which translates to 35% of all clinically significant abnormal test results identified in our laboratory. In a subset of cases monitored for physician response, appropriate clinical action was taken more than 90% of the time as a direct result of the microarray finding.

CONCLUSIONS: The disorders diagnosed by chromosomal microarray analysis frequently have clinical features that need medical attention, and physicians respond to the diagnoses with specific clinical actions, thus arguing that microarray testing provides clinical utility for a significant number of patients tested. *Pediatrics* 2012;130:e1085–e1095

In recent years, chromosomal microarray analysis has had a large impact on the genetic evaluation of patients with intellectual disability/developmental delay, multiple congenital anomalies, and/or autism spectrum disorder. For these clinical indications, microarray testing has a significantly higher diagnostic yield than conventional karyotype analysis.[1–3] This superior diagnostic utility has led to recommendations that genomic microarray analysis be the first-tier test over karyotyping for the genetic evaluation of patients with these indications.[1,4,5] Yet, whereas karyotyping is routinely reimbursed by third-party payers, often microarray testing is not.[6–9] A major reason given for denial of coverage is that microarray testing is not medically useful. Many payers have indicated that there is an inadequate amount of published evidence that microarray testing offers clinical utility,[6–10] defined as a positive effect on patient management and/or clinical outcomes.[11]

To assess the degree to which microarray testing provides medically useful information, we examined genomic copy number abnormalities detected in our laboratory to determine how often these abnormalities reveal diagnoses that warrant specific clinical follow-up. In a subset of cases, we tracked the clinical actions taken by referring physicians in response to the abnormal test result. Our findings indicate that disorders diagnosed by microarray testing often include clinical features that need to be directly addressed and that referring physicians frequently initiate specific and appropriate clinical actions.

METHODS

Our database of 46 298 postnatal patients tested by microarray analysis at Signature Genomic Laboratories from April 29, 2004, through October 21, 2011, was searched for abnormalities associated with specific clinical disorders, the diagnosis of which would likely lead to changes in patient management. The disorders comprised 3 categories: (1) established microdeletion and microduplication syndromes with clinical features that require specific medical follow-up, (2) conditions associated with increased cancer susceptibility, and (3) phenotypes for which obvious medical intervention is indicated and that are caused by copy number changes in individual dosage-sensitive genes. A few cases were listed in more than 1 category, but each was counted only once when performing the detection rate calculations.

For a subset of cases with abnormal results (n = 122), the referring physicians were queried as to whether they responded by taking specific clinical action(s) pertaining to the disorder identified by the microarray finding. Obvious and straightforward clinical actions for each diagnosis were the criteria used to select the disorders within this subset. Individuals in this study for whom additional clinical information was obtained provided written informed consent using an Institutional Review Board Spokane–approved consent form. For all other cases, clinical outcomes were not addressed, and no additional patient information was sought beyond the indication for testing noted on the test requisition form received by the laboratory. This form lists the following test indications: developmental delay, dysmorphic features, multiple congenital anomalies, seizure disorder, autism spectrum disorder, and "other" with details to be filled in by the physician.

The microarray platforms used to test samples evolved during the reporting period of 2004 to 2011. Initial platforms (SignatureChip versions 1–4, Signature Genomic Laboratories, Spokane, WA) used bacterial artificial chromosome (BAC) probes with targeted coverage of the genome.[12] Subsequent BAC-based arrays (SignatureChip WG versions 1–2, Signature Genomic Laboratories) featured whole-genome coverage and were used from 2007 to 2009.[13] Oligonucleotide-based whole-genome arrays (SignatureChip OS versions 1–3, Signature Genomic Laboratories)[14,15] have been offered since February 2008; the current version is a 135K array custom designed by Signature Genomic Laboratories and manufactured by Roche NimbleGen (Madison, WI).[15] Abnormalities detected on all array platforms were tallied for this report. For the measurement of detection rate, only cases tested on oligonucleotide platforms were included in the calculations because the lower-resolution BAC-based platforms did not assay all of the genes reported here.

RESULTS

Known Microdeletion and Microduplication Syndromes

On searching our database, we identified 1733 individuals who were found to have genomic copy number changes that encompassed regions corresponding to established microdeletion and microduplication syndromes that were selected for this study (Table 1). These disorders have complex phenotypes that typically include developmental and/or neurologic abnormalities, often accompanied by congenital malformations and other medical problems. Each of the 40 listed disorders shows a significant incidence of at least 1 clinical feature (eg, cardiac, renal, eye, or endocrine abnormalities) that requires specific medical follow-up. Note that these clinical features may not have been evident at the time of testing and, in fact, only rarely were they mentioned as an indication for testing.

The following case example illustrates the utility of microarray testing in directing the clinical care of a patient. A 6-year-old boy was tested because of

TABLE 1 Diagnosed Abnormalities That Include Critical Genomic Regions for Microdeletion and Microduplication Syndromes With Actionable Clinical Features

Chromosome Band(s) (Named Syndrome, OMIM No.)	Gene(s)[a]	Actionable Aspect of Phenotype[b]	Total Diagnoses
1p36 deletion (607872)		Cardiac abnormalities[31]	138
1p31–p32 deletion (613735)		Urinary tract, eye abnormalities[32]	5
1q21.1 distal deletion (612474)		Cardiac abnormalities, cataracts[33]	108
1q21.1 deletion with susceptibility to TAR (274000)	*RBM8A*	Thrombocytopenia[34]	43
2p15–p16.1 deletion (612513)		Optic atrophy, renal anomalies[35]	6
4p16.3 deletion (Wolf-Hirschhorn, 194190)		Cardiac, eye, renal abnormalities[36]	37
6p25.3 deletion (612582)	*FOXC1*	Eye and cardiac abnormalities, deafness[37]	12[c]
6q24–q25 deletion (612863)	*TAB2*	Cardiac abnormalities[38]	4
7q11.23 deletion (Williams, 194050)	*ELN*	Cardiac, serum calcium, other abnormalities[39]	117
7q11.23 duplication (609757)		Cardiac abnormalities[40]	48
8p23.1 deletion		Cardiac abnormalities[41]	25
8q12 duplication		Cardiac and eye abnormalities, deafness[42]	2
9q22.3 deletion		Seizures, multiple tumors[43]	6[d]
9q34 deletion (Kleefstra, 610253)	*EHMT1*	Cardiac abnormalities[44]	42
10p13–p14 deletion (DiGeorge 2, 601362)		Cardiac abnormalities[45]	11[e]
10q22–q23 deletion	*PTEN, BMPR1A*	Cardiac abnormalities, juvenile polyposis[46]	32[f]
11qter deletion (Jacobsen, 147791)	*FLI1*	Cardiac abnormalities, thrombocytopenia[47]	36[g]
12q14.1–q15 deletion		Cardiac abnormalities[48]	7
12q24 duplication		Cardiac abnormalities[49]	5[h]
14q22–q23 deletion		Eye abnormalities, pituitary hypoplasia[50]	11
15q11.2–q13 deletion (Prader-Willi; 176270; Angelman, 105830)	*SNORD116, UBE3A*	Obesity/seizures[51,52]	86
16p13.3 duplication (613458)	*CREBBP*	Cardiac abnormalities[53]	18
16p13.11 deletion		Seizures[54]	49
16p13.11 duplication		Cardiac abnormalities[55]	20
16p11.2–p12.2 deletion (613604)		Cardiac abnormalities[56]	5
16p12.1 deletion (136570)		Cardiac abnormalities[57]	60
16q11.2–q12.2 deletion		Hypothyroidism, seizures[14]	3
17p13.3 distal deletion	*YWHAE*	Eye abnormalities[58]	18
17p13.3 proximal deletion (Miller-Dieker, 247200)	*PAFAH1B1*	Seizures[59]	17
17p11.2 deletion (Smith-Magenis, 182290)	*RAI1*	Hearing loss; cardiac, eye, sleep abnormalitie[60]	49
17p11.2 duplication (Potocki-Lupski, 610883)		Cardiac, EEG abnormalities[61]	50
17q12 deletion (137920)		Renal cysts, diabetes risk[62]	29
17q21.31 deletion (610443)		Cardiac, urologic abnormalities[63]	43
17q23.1–q23.2 deletion (613355)		Cardiac, eye abnormalities[64]	4
22q11.1 tetrasomy (Cat-eye, 115470)		Cardiac, renal abnormalities[65]	18
22q11.2 deletion (DiGeorge 1, 188400; Velocardiofacial, 192430)	*TBX1*	Cardiac, immune system, other abnormalities[66]	262
22q11.21 duplication (608363)		Cardiac abnormalities[67]	166
22q11.2 distal deletion (611867)		Cardiac abnormalities[68]	51
22q13.3 deletion (Phelan-McDermid, 606232)		Hearing loss[69]	71
Xp11.22–p11.23 duplication (300801)		EEG abnormalities[70]	19

OMIM No., Online Mendelian Inheritance in Man reference number; TAR, thrombocytopenia-absent radius.

[a] Genes listed are those in the interval known to be associated with an actionable phenotype.

[b] References describe the actionable clinical features of the respective disorder.

[c] All of these patients have deletions of *FOXC1* and are also listed in Table 3.

[d] All of these patients have deletions of *PTCH1* and are also listed in Table 2.

[e] Nine of these patients have deletions of *GATA3* and are also listed in Table 3.

[f] Fourteen of these patients have a deletion of *BMPR1A*, and 1 of these 14 patients also has a deletion of *PTEN*. These cases are also listed in Table 2.

[g] Twenty-two of these patients have a deletion of *FLI1* and are also listed in Table 3.

[h] Two of these patients have mosaicism for trisomy 12.

developmental delay and dysmorphic features. Microarray analysis revealed a 1.3-megabase deletion of chromosome band 17q12 that includes the gene *HNF1B*, which is associated with a recurrent microdeletion syndrome called renal cysts and diabetes. Following the test result, he had a renal ultrasound that showed the presence of multiple cysts and was referred to a nephrologist for follow-up. He continues to be monitored periodically for elevated blood glucose levels.

Hereditary Cancer Predisposition

A search of our database found 189 patients who have copy number changes detected by microarray analysis of genes associated with hereditary cancer risk (Table 2). Based on the indication for testing, only 16 patients had a known or suspected tumor risk before testing; for the remaining 92% of patients, the indications for testing were not related to cancer predisposition. In all cases, the referring physician was informed of the association

TABLE 2 Detected Copy Number Changes of Cancer Susceptibility Genes in Postnatal Cases

Disorder[a] (OMIM No.)	Gene(s)[b] (Chromosome Band)	No. of Cases[c,d]
Juvenile polyposis (174900)	*BMPR1A* (10q23.2)	15[e]
	SMAD4 (18q21.2)	2[f]
Beckwith-Wiedemann (130650)	*IGF2*[g] (11p15.5)	13
Familial adenomatous polyposis (175100)	*APC* (5q22.2)	13
Lynch (614337, 614350)	*PMS2* (7p22.1)	4
	MSH6 (2p16.3)	2
Neurofibromatosis 1 (162200)	*NF1* (17q11.2)	19
Paraganglioma, pheochromocytoma (168000, 115310, 171300)	*SDHB* (1p36.13)	8
	SDHD (11q23.1)	3
Von Hippel-Lindau (193300)	*VHL* (3p25.3)	10
Retinoblastoma (180200)	*RB1* (13q14.2)	12
Rubinstein-Taybi (180849, 613684)	*CREBBP* (16p13.3)	15
	EP300 (22q13.2)	1
Leiomyomatosis and renal cell cancer (150800)	*FH* (1q43)	7
Wilms tumor (194070)	*WT1* (11p13)	6
Monosomy 7 mosaicism (252270)	unknown	5
Basal cell nevus (109400)	*PTCH1* (9q22.32)	6[h]
Hereditary breast and ovarian cancer (612555)	*BRCA2* (13q13.1)	3
Peutz-Jeghers (175200)	*STK11* (19p13.3)	5
Tuberous sclerosis (613254)	*TSC2* (16p13.3)	4
Simpson-Golabi-Behmel (312870)	*GPC3* (Xq26.2)	2[f]
PTEN hamartoma (601728)	*PTEN* (10q23.31)	3[i,j]
Li-Fraumeni and Li-Fraumeni-like (151623, 609265)	*TP53* (17p13.1)	1
	CHEK2 (22q12.1)	1
Neurofibromatosis 2 (101000)	*NF2* (22q12.2)	1
Hereditary diffuse gastric cancer (137215)	*CDH1* (16q22.1)	1
Multiple exostoses (133700, 133701)	*EXT1* (8q24.11)	9
	EXT2 (11p11.2)	4
Hyperparathyroidism-jaw tumor (145001)	*CDC73* (1q31.2)	2
Acute myelogenous leukemia (601626)	*RUNX1* (21q22.12)	8
Melanoma (155601, 155755)	*CDKN2A* (9p21.3)	1
Rhabdoid predisposition (609322)/ Schwannomatosis (162091)	*SMARCB1* (22q11.23)	7

OMIM No., Online Mendelian Inheritance in Man reference number.

[a] Disorders taken from Lindor et al.[24]

[b] Other genes were queried, but no pathogenic abnormalities were found in our database. These genes are *MSH2*, *MLH1*, *SDHC*, *BRCA1*, *TSC1*, *PRKAR1A*, and *MEN1*.

[c] The *GPC3* and *SMAD4* cases are also included in Table 3.

[d] The 34 cases reported by Adams et al.[22] are included among these cases.

[e] Fourteen of these patients have a deletion of the 10q22–q23 microdeletion critical region, and one of these 14 patients also has a deletion of *PTEN*. These patients are also listed in Table 1.

[f] These cases are also presented in Table 3.

[g] Duplications, rather than deletions, are associated with tumor risk.

[h] These patients have the 9q22.3 deletion syndrome and are included in Table 1.

[i] One of these patients has the 10q22–q23 deletion syndrome and is included in Table 1.

[j] One of these patients also has a deletion of *BMPR1A*.

of the abnormality with cancer risk. It should also be noted that for 2 of the probands a parent was found to carry the same abnormality; therefore, risk was identified not just for the proband but also for other family members. Letters that specifically addressed the risk to these relatives were sent to referring physicians.

The patient reported by Heald et al.[16] provides a dramatic example of how microarray analysis can benefit patients. This patient was a 22-year-old woman who was tested in our laboratory as part of an evaluation for developmental delay and other features. She was found to have a 5q22.1–q22.2 deletion that included *APC*, the causative gene for familial adenomatous polyposis. Although she had not previously had suggestive symptoms, a diagnosis of familial adenomatous polyposis was confirmed when colonoscopy revealed hundreds of adenomatous polyps. A thyroid scan led to a finding of papillary thyroid cancer. She subsequently underwent the life-saving measures of ^{131}I therapy and a total colectomy.

Other Actionable Conditions Associated With Dosage-Sensitive Genes

We searched our database of abnormal microarray results for an additional set of 74 genes that are associated with a specific actionable phenotype when functional gene dosage is altered. Almost all of the phenotypes are associated with haploinsufficiency for the relevant gene, as evidenced by published reports of heterozygous whole-gene deletions or other null alleles in affected individuals. A total of 252 cases of copy number abnormalities representing these genes were detected in our laboratory. The conditions, corresponding genes, relevant clinical actions pertaining to the diagnosis, and numbers of cases diagnosed are listed in Table 3. We identified a number of patients who were at risk for more than one actionable phenotype as a result of a deletion that includes multiple dosage-sensitive genes. These cases included the following: (1) a patient with deletions of *EDNRB* and *RB1*, which put the patient at risk for hearing loss, Hirschsprung disease, and retinoblastoma; (2) 12 patients with deletions of *MNX1* and *KCNH2*, thus putting them at risk for urologic, spinal, and anal abnormalities, as well as cardiac arrhythmia; (3) 9 patients with deletions of *GATA3* and the DiGeorge 2 critical region, which put them at risk for hearing loss, renal anomalies, and cardiac abnormalities; (4) 4 patients with deletions of *LHX4* and *SERPINC1*, putting them at risk for pituitary insufficiency as well as a clotting predisposition; and (5) 14 patients whose deletions encompassed the 10q22–q23 microdeletion critical region, as well as the gene *BMPR1A*. The 10q deletion

TABLE 3 Conditions Caused by Dosage-Sensitive Genes for Which Specific Clinical Actions Are Indicated

Disorder/Phenotype (OMIM No.)	Gene[a,b] (Chromosome Band)	Relevant Clinical Actions	No. of Cases
Long QT (613688)	*KCNH2*[71] (7q36.1)	ECG, cardiology referral	14[c]
Brugada (601144)	*SCN5A*[72] (3p22.2)	ECG, cardiology referral	1
Waardenburg (various types; 193500, 193510, 277580)	*PAX3*[73] (2q36.1)	Audiology, ENT referral	4
	MITF[74] (3p14.1)	Audiology, ENT referral	2
	EDNRB[75] (13q22.3)	Audiology, ENT referral, gastroenterology referral	13[d]
Hyperparathyroidism, deafness, renal anomalies (146255)	*GATA3*[76] (10p14)	Audiology, renal ultrasound, specialist referral	12[e]
Seizures (300672, 604403, 612164, 613720, 613721)	*SCN1A*[77] (2q24.3)	EEG, neurology referral	12[f]
	SCN2A[78] (2q24.3)	EEG, neurology referral	11[f]
	KCNQ2[79] (20q13.33)	EEG, neurology referral	4
	STXBP1[80] (9q34.11)	EEG, neurology referral	3
	CDKL5[81] (Xp22.13)	EEG, neurology referral	3
Deafness (600101)	*KCNQ4*[82] (1p34.2)	Audiology, ENT referral	5
HHT (187300)	*ENG*[83] (9q34.11)	Vascular evaluation, head MRI, pulse oximetry	3
HHT plus juvenile polyposis (175050)	*SMAD4*[84] (18q21.2)	Above plus monitoring for GI bleeding, colonoscopy	2
Thrombocytopenia (188025)	*FLI1*[85] (11q24.3)	Check platelet count, monitor closely pre-op	22[g]
Pituitary hormone deficiency (300123)	*SOX3*[86] (Xq27.1)[h]	Endocrine referral	6
Simpson-Golabi-Behmel (312870)	*GPC3*[87] (Xq26.2)	Echocardiogram, tumor surveillance	2
Branchiootorenal (113650)	*EYA1*[88] (8q13.3)	Audiology, renal evaluation, specialist referral	4
Axenfeld-Rieger type 1 (180500)	*PITX2*[89] (4q25)	Ophthalmology referral	5
Axenfeld-Rieger type 3 (602482)	*FOXC1*[90] (6p25.3)	Ophthalmology referral	12[i]
Optic atrophy (165500)	*OPA1*[91] (3q29)	Ophthalmology referral	6
MODY (125850, 125851)	*HNF4A*[92] (20q13.12)	Glucose monitoring, endocrine referral	2
	GCK[93] (7p13)	Glucose monitoring, endocrine referral	7
Holt-Oram (142900)	*TBX5*[94] (12q24.21)	Cardiac evaluation	2
Stickler (108300, 604841)	*COL2A1*[95] (12q13.11)	Ophthalmology referral, audiology	1
	COL11A1[96] (1p21.1)	Audiology	3
Pseudohypoparathyroidism (103580, 603233, 612462)	*GNAS*[97] (20q13.32)	Endocrinology referral	1
Otodental (with coloboma; 166750)	*FGF3*[98] (11q13.3)	Ophthalmology referral, audiology	2
GLUT1 deficiency (606777, 612126)	*SLC2A1*[99] (1p34.2)	Neurology referral, ketogenic diet	3
Familial cavernous hemangioma (116860, 603284)	*KRIT1*[100] (7q21.2)	Brain MRI, avoidance of NSAIDs	4
	CCM2[101] (7p13)	Brain MRI, avoidance of NSAIDs	3
Polycystic kidney disease (173900, 613095)	*PKD1*[102] (16p13.3)	Nephrology referral	3
	PKD2[103] (4q22.1)	Nephrology referral	8
Pituitary insufficiency (262700)	*LHX4*[104] (1q25.2)	Endocrinology referral	9[j]
Adrenal hypoplasia congenital (300200)	*NR0B1*[105] (Xp21.2)	Endocrinology referral	5
Alport syndrome (301050)	*COL4A5*[106] (Xq22.3)	Nephrology referral	4
Heart malformations (108900)	*NKX2-5*[107] (5q35.2)	Cardiology referral	1
Choroideremia (303100)	*CHM*[108] (Xq21.2)	Ophthalmology referral	2
Currarino (176450)	*MNX1*[109] (7q36.3)	Urology, neurosurgery referral	27
Infantile spasms (606382)	*MAGI2*[110] (7q21.11)	Neurology referral, specific therapy	3
Kallmann (308700)	*KAL1*[111] (Xp22.31)	Endocrinology referral, renal ultrasound	1
Immune deficiency (308240)	*SH2D1A*[112] (Xq25)	Immunology referral	1
Ornithine transcarbamylase deficiency (311250)	*OTC*[113] (Xp11.4)	Specific medical and dietary therapy	6
Marfan (154700)	*FBN1*[114] (15q21.1)	Cardiology and ophthalmology referral	2
Protein S deficiency (612336)	*PROS1*[115] (3q11.2)	Hematology referral	1
Antithrombin III deficiency (613118)	*SERPINC1*[116] (1q25.1)	Hematology referral	6[k]
CHARGE syndrome (214800)	*CHD7*[117] (8q12.2)	Cardiology, ophthalmology, ENT referral	7
Diamond-Blackfan anemia (612528)	*RPL35A*[118] (3q29)	Hematology referral	2

CHARGE, coloboma, heart defect, atresia choanae, retarded growth and development, genital hypoplasia, ear anomalies/deafness; ECG, electrocardiogram; ENT, ear, nose, and throat; GI, gastrointestinal; HHT, hereditary hemorrhagic telangiectasia; MODY, maturity-onset diabetes of the young; NSAID, nonsteroidal anti-inflammatory drug; OMIM No., Online Mendelian Inheritance in Man reference number.

[a] Other dosage-sensitive genes were queried, but no pathogenic abnormalities were found in our database. These genes are *SOX10*, *PCDH19*, *ACVRL1*, *HNF1A*, *PRPF31*, *CYP11A*, *MYBPC3*, *LMNA*, *PAX2*, *AR*, *F8*, *F9*, *AVPR2*, *HPRT1*, *OCRL*, *ATP7A*, *NDP*, *CYBB*, *RPS10*, *RPS17*, *RPS19*, *RPS24*, *RPS26*, *RPL5*, *RPL11*, and *RP2*.

[b] Listed references provide evidence for functional dosage sensitivity of the phenotype for the corresponding gene.

[c] Twelve of these patients also have Currarino syndrome owing to deletion of *MNX1*.

[d] Six of these patients are at risk for retinoblastoma due to deletion of *RB1*.

[e] Nine of these patients also have deletions of the DiGeorge 2 critical region and are included in Table 1.

[f] Both *SCN1A* and *SCN2A* are deleted in 8 of these cases.

[g] These patients represent a subset of patients with Jacobsen syndrome (Table 1).

[h] Duplications, rather than deletions, result in the phenotype.

[i] These patients all have the 6p25.3 deletion syndrome (Table 1).

[j] Four of these patients also have deletions of *SERPINC1*.

[k] Four of these patients also have deletions of *LHX4*.

predisposes these patients to cardiac abnormalities, whereas deletion of *BMPR1A* puts them at risk for juvenile polyposis; 1 of these 14 patients also had a deletion of *PTEN*, therefore, greatly increasing the risk of developing numerous tumor types, including thyroid, breast, and endometrial malignancies. These examples clearly illustrate the nature of disorders caused by copy number abnormalities: they often have multiple clinical features resulting from altered doses of multiple genes.

Another example of a patient who benefited from the information given by microarray analysis was the case of a patient who was referred for microarray testing because of developmental delay, dysmorphic features, and multiple congenital anomalies. This 3-month-old infant was one of the patients noted previously with a deletion of 7q36 that included the *MNX1* and *KCNH2* genes. These findings not only provided a diagnosis of Currarino syndrome as a result of the *MNX1* deletion (with its predisposition to urologic, spinal, and anal anomalies), but also susceptibility to long QT syndrome (owing to deletion of *KCNH2*). Following the array result, the patient had an electrocardiogram that showed an elongated QT interval, and prophylactic medical therapy was subsequently instituted.

Physician Responses to Microarray Results

Our data clearly show that microarray testing can identify individuals at risk for specific medical problems that warrant follow-up care. To determine whether these risks are in fact being addressed, for a subset of cases we queried referring physicians as to whether they took specific actions pertinent to the particular diagnosis made by microarray testing. Of the 122 inquiries made, we received 81 responses (from 46 different clinicians), which are tallied by gene in Table 4. In 76 (94%) of the 81 cases, at least 1 of the appropriate clinical actions was taken by the referring physician after the receipt of the microarray result. Examples of these actions included an electrocardiogram and cardiology referral for those at risk for long QT syndrome; glucose monitoring and endocrine referral for those at increased risk of diabetes; renal ultrasound for those at risk for renal pathology; and platelet count monitoring for those at risk for thrombocytopenia.

Detection Rate of Clinically Actionable Abnormalities

A total of 46 298 microarray analyses were performed during the reporting period on postnatal proband samples, with nearly equal numbers tested on BAC and oligonucleotide array platforms (23 142 and 23 156, respectively). Of the 151 clinically actionable disorders reviewed, we detected pathogenic abnormalities for 118, resulting in a total of 2088 diagnoses. Of these cases, 1968 (94%) involved DNA segments that were shorter than 10 megabases and so would likely be missed by routine karyotyping. Our initial BAC arrays were targeted and did not probe the entire genome, and later BAC platforms did not provide the resolution necessary to detect all small copy number alterations that we currently address. Therefore, to obtain a more accurate estimate of our current detection rate of actionable conditions, we separately tallied cases tested on higher-resolution oligonucleotide arrays. The total number of diagnoses made using these arrays was 1259, giving a detection rate of 5.4%. We previously determined that our rate of detection of clinically significant alterations on oligonucleotide arrays is 15.4%.[17] Therefore, 35% of all pathogenic copy number changes found in our laboratory identify conditions for which specific clinical actions are warranted.

DISCUSSION

Karyotype analysis has long been used for the genetic evaluation of individuals with developmental abnormalities. The

TABLE 4 Responses to Abnormal Microarray Results for Specific Genes

Disorder/Phenotype	Gene (Chromosome Band)	Cases Queried	Responses Received	Appropriate Action Taken
17q12 deletion/renal cysts and diabetes	*HNF1B* (17q12)	28	16	15
Long QT	*KCNH2* (7q36.1)	13	8	8
Waardenburg	*PAX3* (2q36.1)	2	2	2
	MITF (3p14.1)	2	2	1
Hyperparathyroidism, deafness, renal anomalies	*GATA3* (10p14)	6	5	5
Miller-Dieker/lissencephaly, seizures	*PAFAH1B1* (17p13.3)	1	1	1
Epilepsy	*SCN1A/2A*[a] (2q24.3)	3	1	0
	SCN2A (2q24.3)	1	1	1
	STXBP1 (9q34.11)	1	0	–
HHT	*ENG* (9q34.11)	3	2	2
HHT plus juvenile polyposis	*SMAD4* (18q21.2)	2	2	2
11qter deletion/ thrombocytopenia	*FLI1* (11q24.3)	9	9	8
Pituitary hormone deficiency	*SOX3* (Xq27.1)	3	2	1
Cardiomyopathy, tumor risk	*GPC3* (Xq26.2)	2	1	1
Williams	*ELN* (7q11.23) and others	20	10	10
DiGeorge/velocardiofacial	*TBX1* (22q11.21) and others	26	19	19
	Totals	122	81	76

HHT, hereditary hemorrhagic telangiectasia.

[a] *SCN1A* and *SCN2A* genes were both deleted in the cases.

diagnostic yield of this testing for patients with developmental delay/mental retardation varies in different studies, but the average is 4% to 5%.[18,19] Karyotyping has been consistently reimbursed by third-party payers, but current concerns about health care costs are leading to higher expectations for the usefulness of laboratory tests, beyond simply providing a diagnosis.[20] It is increasingly expected that testing provide clinical utility, in the form of changes in patient management and improved clinical outcomes.

The first reported examples of the clinical utility of microarray testing were descriptions of deletions of tumor suppressor genes, which put the patients at a high risk of developing hereditary cancer syndromes.[16,21,22] Such patients benefit from awareness of tumor risk and appropriate clinical surveillance.[23–26] Other studies showed that clinical actions were taken after abnormal microarray results,[27–30] although these studies were limited in scope and/or did not tie specific actions to diagnoses. These reports have not provided sufficient evidence to universally convince third-party payers that microarray testing is worthy of reimbursement.

The goal of our study was to examine the evidence for the clinical utility of chromosomal microarray analysis, which has already been demonstrated to have a superior diagnostic yield over karyotyping for similar clinical indications.[1,4,5] Our approach was to identify specific diagnoses made by microarray testing, which are expected to lead to specific clinical actions and improved patient care. We identified more than 100 such disorders, ranging from complex syndromes involving multiple organ systems, to disorders with discrete problems that need obvious and specific medical follow-up. Admittedly, some of the patients may have displayed such problems before testing, but many of the disorders diagnosed have variable features that frequently are not evident or suspected. The test result thus serves to alert physicians to the possibility of these treatable problems. We showed that these actionable diagnoses constitute a significant proportion (35%) of all pathogenic abnormalities detected by microarray analysis and that the detection rate of these disorders is greater than the overall detection rate of karyotype analysis for similar testing indications. We can expect the frequency of actionable diagnoses to increase in the future as we learn more about the clinical consequences of copy number abnormalities. Finally, we showed that physicians respond to abnormal microarray results with specific and appropriate clinical actions and noted several illustrative cases where the clinical outcome was optimized. Our findings, therefore, argue strongly that chromosome microarray analysis provides clinical utility for a significant number of tested patients.

CONCLUSIONS

Our data show that the diagnoses made by chromosomal microarray analysis frequently involve specific clinical features that may have been present but not apparent or were not yet manifest at the time of testing. Alerting physicians and families to these potential problems leads to optimal health management of patients, as demonstrated in the cases in which we queried the referring physicians. It is expected that anticipatory medical care of children and adults with developmental disabilities will lead to improved outcomes in terms of both general health and fulfillment of their developmental potential. Long-term follow-up studies could be performed to confirm this assumption, but in the meantime, our data show that microarray testing provides immediate clinical utility for patients and such testing should be considered worthy of reimbursement by insurers.

ACKNOWLEDGMENTS

The authors thank Erin Dodge, MFA, MA (Signature Genomic Laboratories), and A. Michelle Caldwell, BS (Signature Genomic Laboratories), for preparation and editing of the manuscript, and the many physicians and genetic counselors who responded to our queries regarding follow-up clinical actions.

REFERENCES

1. Miller DT, Adam MP, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet*. 2010;86(5):749–764
2. Sagoo GS, Butterworth AS, Sanderson S, Shaw-Smith C, Higgins JP, Burton H. Array CGH in patients with learning disability (mental retardation) and congenital anomalies: updated systematic review and meta-analysis of 19 studies and 13,926 subjects. *Genet Med*. 2009;11(3):139–146
3. Hochstenbach R, van Binsbergen E, Engelen J, et al. Array analysis and karyotyping: workflow consequences based on a retrospective study of 36,325 patients with idiopathic developmental delay in the Netherlands. *Eur J Med Genet*. 2009;52(4):161–169
4. Manning M, Hudgins L; Professional Practice and Guidelines Committee. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. *Genet Med*. 2010;12(11):742–745
5. Shen Y, Dies KA, Holm IA, et al; Autism Consortium Clinical Genetics/DNA Diagnostics Collaboration. Clinical genetic testing for patients with autism spectrum

disorders. *Pediatrics*. 2010;125(4). Available at: www.pediatrics.org/cgi/content/full/125/4/e727

6. Cigna Medical Coverage Policy: comparative genomic hybridization testing (chromosomal microarray analysis) for autism spectrum disorders, developmental delay, mental retardation and multiple or unspecified congenital anomalies. 2011. Available at: www.cigna.com/assets/docs/health-care-professionals/coverage_positions/mm_0493_coveragepositioncriteria_array_comparative_genomic_hybridization.pdf. Accessed November 1, 2011
7. Aetna Clinical Policy Bulletin. Comparative genomic hybridization (CGH). 2011. Available at: www.aetna.com/cpb/medical/data/700_799/0787.html. Accessed November 1, 2011
8. Wellmark Blue Cross and Blue Shield medical policies and authorizations: comparative genomic hybridization. 2011. Available at: www.wellmark.com/Provider/MedPoliciesAndAuthorizations/MedicalPolicies/policies/Comparative_Genomic_Hybridization.aspx. Accessed November 1, 2011
9. Blue Cross and Blue Shield of Montana medical policy/codes: chromosomal microarray (CMA) analysis for the genetic evaluation of patients with developmental delay/intellectual disability or autism spectrum disorder 2012. Available at: https://www.bcbsmt.com/MedReview/Policies/ChromosomalMicroarray/v102.aspx. Accessed July 18, 2012
10. Special Report: aCGH for the Genetic Evaluation of Patients with Developmental Delay/Mental Retardation or Autism Spectrum Disorder. Chicago: Blue Cross and Blue Shield Association, Technology Evaluation Center; April 2009. Available at: www.bcbs.com/blueresources/tec/vols/23/aCHG_genetic_evaluation_patients.pdf. Accessed October 31, 2011
11. Grosse SD, Khoury MJ. What is the clinical utility of genetic testing? *Genet Med*. 2006;8(7):448–450
12. Bejjani BA, Saleki R, Ballif BC, et al. Use of targeted array-based CGH for the clinical diagnosis of chromosomal imbalance: is less more? *Am J Med Genet A*. 2005;134(3):259–267
13. Ballif BC, Theisen A, Coppinger J, et al. Expanding the clinical phenotype of the 3q29 microdeletion syndrome and characterization of the reciprocal microduplication. *Mol Cytogenet*. 2008;1(1):8
14. Ballif BC, Theisen A, McDonald-McGinn DM, et al. Identification of a previously unrecognized microdeletion syndrome of 16q11.2q12.2. *Clin Genet*. 2008;74(5):469–475
15. Duker AL, Ballif BC, Bawle EV, et al. Paternally inherited microdeletion at 15q11.2 confirms a significant role for the SNORD116 C/D box snoRNA cluster in Prader-Willi syndrome. *Eur J Hum Genet*. 2010;18(11):1196–1201
16. Heald B, Moran R, Milas M, Burke C, Eng C. Familial adenomatous polyposis in a patient with unexplained mental retardation. *Nat Clin Pract Neurol*. 2007;3(12):694–700
17. Neill NJ, Torchia BS, Bejjani BA, Shaffer LG, Ballif BC. Comparative analysis of copy number detection by whole-genome BAC and oligonucleotide array CGH. *Mol Cytogenet*. 2010;3:11
18. Shevell M, Ashwal S, Donley D, et al; Quality Standards Subcommittee of the American Academy of Neurology; Practice Committee of the Child Neurology Society. Practice parameter: evaluation of the child with global developmental delay: report of the Quality Standards Subcommittee of the American Academy of Neurology and The Practice Committee of the Child Neurology Society. *Neurology*. 2003;60(3):367–380
19. Michelson DJ, Shevell MI, Sherr EH, Moeschler JB, Gropman AL, Ashwal S. Evidence report: genetic and metabolic testing on children with global developmental delay: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology*. 2011;77(17):1629–1635
20. Trevathan E. So what? Does the test lead to improved health outcomes? *Neurology*. 2011;77(17):1586–1587
21. Adam MP, Justice AN, Schelley S, Kwan A, Hudgins L, Martin CL. Clinical utility of array comparative genomic hybridization: uncovering tumor susceptibility in individuals with developmental delay. *J Pediatr*. 2009;154(1):143–146
22. Adams SA, Coppinger J, Saitta SC, et al. Impact of genotype-first diagnosis: the detection of microdeletion and microduplication syndromes with cancer predisposition by aCGH. *Genet Med*. 2009;11(5):314–322
23. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med*. 2009;11(1):35–41
24. Lindor NM, McMaster ML, Lindor CJ, Greene MH. Concise handbook of familial cancer susceptibility syndromes - second edition. *J Natl Cancer Inst Monogr*. 2008;38:3–93
25. Screening for breast cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med*. 2009;151(10):716–726, W-236
26. Gala M, Chung DC. Hereditary colon cancer syndromes. *Semin Oncol*. 2011;38(4):490–499
27. Runke C, Thorland E, Hodge J, Babovic-Vuksanovic D, Al-Owain M, Ellison J. Currarino syndrome and associated findings in three patients with distal 7q deletions. Paper presented at: 2011 ACMG Annual Clinical Genetics Meeting; March 18, 2011; Vancouver, BC, Canada
28. Oundjian N, Parisotto S, Chung W, et al. Complex chromosomal findings, including deletion of the SCN5A gene by array comparative genomic hybridization in a patient with developmental delay and Brugada syndrome. Paper presented at: 2011 ACMG Annual Clinical Genetics Meeting; March 18, 2011; Vancouver, BC, Canada
29. Saam J, Gudgeon J, Aston E, Brothman AR. How physicians use array comparative genomic hybridization results to guide patient management in children with developmental delay. *Genet Med*. 2008;10(3):181–186
30. Coulter ME, Miller DT, Harris DJ, et al. Chromosomal microarray testing influences medical management. *Genet Med*. 2011;13(9):770–776
31. Shapira SK, McCaskill C, Northrup H, et al. Chromosome 1p36 deletions: the clinical phenotype and molecular characterization of a common newly delineated syndrome. *Am J Hum Genet*. 1997;61(3):642–650
32. Lu W, Quintero-Rivera F, Fan Y, et al. NFIA haploinsufficiency is associated with a CNS malformation syndrome and urinary tract defects. *PLoS Genet*. 2007;3(5):e80
33. Mefford HC, Sharp AJ, Baker C, et al. Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. *N Engl J Med*. 2008;359(16):1685–1699
34. Klopocki E, Schulze H, Strauss G, et al. Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thrombocytopenia-absent radius syndrome. *Am J Hum Genet*. 2007;80(2):232–240
35. Rajcan-Separovic E, Harvard C, Liu X, et al. Clinical and molecular cytogenetic characterisation of a newly recognised microdeletion syndrome involving 2p15-16.1. *J Med Genet*. 2007;44(4):269–276
36. Battaglia A, Filippi T, Carey JC. Update on the clinical features and natural history of Wolf-Hirschhorn (4p-) syndrome: experience

with 87 patients and recommendations for routine health supervision. *Am J Med Genet C Semin Med Genet*. 2008;148C(4):246–251

37. DeScipio C. The 6p subtelomere deletion syndrome. *Am J Med Genet C Semin Med Genet*. 2007;145C(4):377–382
38. Nowaczyk MJ, Carter MT, Xu J, et al. Paternal deletion 6q24.3: a new congenital anomaly syndrome associated with intrauterine growth failure, early developmental delay and characteristic facial appearance. *Am J Med Genet A*. 2008;146(3):354–360
39. Burn J. Williams syndrome. *J Med Genet*. 1986;23(5):389–395
40. Van der Aa N, Rooms L, Vandeweyer G, et al. Fourteen new cases contribute to the characterization of the 7q11.23 microduplication syndrome. *Eur J Med Genet*. 2009;52(2-3):94–100
41. Ballarati L, Cereda A, Caselli R, et al. Genotype-phenotype correlations in a new case of 8p23.1 deletion and review of the literature. *Eur J Med Genet*. 2011;54(1):55–59
42. Lehman AM, Friedman JM, Chai D, et al. A characteristic syndrome associated with microduplication of 8q12, inclusive of CHD7. *Eur J Med Genet*. 2009;52(6):436–439
43. Muller E, Hudgins L. 9q22.3 Microdeletion. In: Pagon R, Bird T, Dolan C, Stephens K, eds. *GeneReviews [Internet]*. August 18, 2011 ed. Seattle: University of Washington, Seattle; 1993. Available at: www.ncbi.nlm.nih.gov/books/NBK61984/. Accessed October 15, 2011
44. Kleefstra T, van Zelst-Stams WA, Nillesen WM, et al. Further clinical and molecular delineation of the 9q subtelomeric deletion syndrome supports a major contribution of EHMT1 haploinsufficiency to the core phenotype. *J Med Genet*. 2009;46(9):598–606
45. Schuffenhauer S, Lichtner P, Peykar-Derakhshandeh P, et al. Deletion mapping on chromosome 10p and definition of a critical region for the second DiGeorge syndrome locus (DGS2). *Eur J Hum Genet*. 1998;6(3):213–225
46. van Bon BW, Balciuniene J, Fruhman G, et al. The phenotype of recurrent 10q22q23 deletions and duplications. *Eur J Hum Genet*. 2011;19(4):400–408
47. Mattina T, Perrotta CS, Grossfeld P. Jacobsen syndrome. *Orphanet J Rare Dis*. 2009;4:9
48. Lynch SA, Foulds N, Thuresson AC, et al. The 12q14 microdeletion syndrome: six new cases confirming the role of HMGA2 in growth. *Eur J Hum Genet*. 2011;19(5):534–539
49. Doco-Fenzy M, Mauran P, Lebrun JM, et al. Pure direct duplication (12)(q24.1—> q24.2) in a child with Marcus Gunn phenomenon and multiple congenital anomalies. *Am J Med Genet A*. 2006;140(3):212–221
50. Nolen LD, Amor D, Haywood A, et al. Deletion at 14q22-23 indicates a contiguous gene syndrome comprising anophthalmia, pituitary hypoplasia, and ear anomalies. *Am J Med Genet A*. 2006;140(16):1711–1718
51. Carrel AL, Myers SE, Whitman BY, Allen DB. Growth hormone improves body composition, fat utilization, physical strength and agility, and growth in Prader-Willi syndrome: A controlled study. *J Pediatr*. 1999;134(2):215–221
52. Robb SA, Pohl KR, Baraitser M, Wilson J, Brett EM. The 'happy puppet' syndrome of Angelman: review of the clinical features. *Arch Dis Child*. 1989;64(1):83–86
53. Thienpont B, Béna F, Breckpot J, et al. Duplications of the critical Rubinstein-Taybi deletion region on chromosome 16p13.3 cause a novel recognisable syndrome. *J Med Genet*. 2010;47(3):155–161
54. Heinzen EL, Radtke RA, Urban TJ, et al. Rare deletions at 16p13.11 predispose to a diverse spectrum of sporadic epilepsy syndromes. *Am J Hum Genet*. 2010;86(5):707–718
55. Nagamani SC, Erez A, Bader P, et al. Phenotypic manifestations of copy number variation in chromosome 16p13.11. *Eur J Hum Genet*. 2011;19(3):280–286
56. Ballif BC, Hornor SA, Jenkins E, et al. Discovery of a previously unrecognized microdeletion syndrome of 16p11.2-p12.2. *Nat Genet*. 2007;39(9):1071–1073
57. Girirajan S, Rosenfeld JA, Cooper GM, et al. A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. *Nat Genet*. 2010;42(3):203–209
58. Schiff M, Delahaye A, Andrieux J, et al. Further delineation of the 17p13.3 microdeletion involving YWHAE but distal to PAFAH1B1: four additional patients. *Eur J Med Genet*. 2010;53(5):303–308
59. Dobyns WB, Das S. LIS1-associated lissencephaly/subcortical band heterotopia. In: Pagon R, Bird T, Dolan C, Stephens K, eds. *GeneReviews [Internet]*. March 3, 2009 ed. Seattle: University of Washington, Seattle; 1993. Available at: www.ncbi.nlm.nih.gov/books/NBK5189/. Accessed September 28, 2011
60. Smith AC, McGavran L, Robinson J, et al. Interstitial deletion of (17)(p11.2p11.2) in nine patients. *Am J Med Genet*. 1986;24(3):393–414
61. Potocki L, Bi W, Treadwell-Deering D, et al. Characterization of Potocki-Lupski syndrome (dup(17)(p11.2p11.2)) and delineation of a dosage-sensitive critical interval that can convey an autism phenotype. *Am J Hum Genet*. 2007;80(4):633–649
62. Mefford HC, Clauin S, Sharp AJ, et al. Recurrent reciprocal genomic rearrangements of 17q12 are associated with renal disease, diabetes, and epilepsy. *Am J Hum Genet*. 2007;81(5):1057–1069
63. Koolen DA, Sharp AJ, Hurst JA, et al. Clinical and molecular delineation of the 17q21.31 microdeletion syndrome. *J Med Genet*. 2008;45(11):710–720
64. Ballif BC, Theisen A, Rosenfeld JA, et al. Identification of a recurrent microdeletion at 17q23.1q23.2 flanked by segmental duplications associated with heart defects and limb abnormalities. *Am J Hum Genet*. 2010;86(3):454–461
65. Schinzel A, Schmid W, Fraccaro M, et al. The "cat eye syndrome": dicentric small marker chromosome probably derived from a no.22 (tetrasomy 22pter to q11) associated with a characteristic phenotype. Report of 11 patients and delineation of the clinical picture. *Hum Genet*. 1981;57(2):148–158
66. McDonald-McGinn DM, Emanuel BS, Zackai EH. 22q11.2 Deletion Syndrome. 1999 Sep 23 [Updated 2005 Dec 16]. In: Pagon RA, Bird TD, Dolan CR, et al., editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993-. Available at: www.ncbi.nlm.nih.gov/books/NBK1523/. Accessed October 15, 2011
67. Ensenauer RE, Adeyinka A, Flynn HC, et al. Microduplication 22q11.2, an emerging syndrome: clinical, cytogenetic, and molecular analysis of thirteen patients. *Am J Hum Genet*. 2003;73(5):1027–1040
68. Rauch A, Zink S, Zweier C, et al. Systematic assessment of atypical deletions reveals genotype-phenotype correlation in 22q11.2. *J Med Genet*. 2005;42(11):871–876
69. Phelan K, Rogers C. *Phelan-McDermid syndrome*. In: Pagon R, Bird T, Dolan C, Stephens K, eds. *GeneReviews [Internet]*. May 11, 2005 ed. Seattle: University of Washington, Seattle; 1993. Available at: www.ncbi.nlm.nih.gov/books/NBK1198/. Accessed October 18, 2011
70. Giorda R, Bonaglia MC, Beri S, et al. Complex segmental duplications mediate a recurrent dup(X)(p11.22-p11.23) associated with mental retardation, speech delay, and EEG anomalies in males and females. *Am J Hum Genet*. 2009;85(3):394–400
71. Splawski I, Shen J, Timothy KW, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A,

KCNE1, and KCNE2. *Circulation*. 2000;102 (10):1178–1185

72. Kapplinger JD, Tester DJ, Salisbury BA, et al. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test. *Heart Rhythm*. 2009;6(9):1297–1303
73. Tassabehji M, Newton VE, Leverton K, et al. PAX3 gene structure and mutations: close analogies between Waardenburg syndrome and the Splotch mouse. *Hum Mol Genet*. 1994;3(7):1069–1074
74. Nobukuni Y, Watanabe A, Takeda K, Skarka H, Tachibana M. Analyses of loss-of-function mutations of the MITF gene suggest that haploinsufficiency is a cause of Waardenburg syndrome type 2A. *Am J Hum Genet*. 1996;59(1):76–83
75. Tüysüz B, Collin A, Arapoğlu M, Suyugül N. Clinical variability of Waardenburg-Shah syndrome in patients with proximal 13q deletion syndrome including the endothelin-B receptor locus. *Am J Med Genet A*. 2009; 149A(10):2290–2295
76. Van Esch H, Groenen P, Nesbit MA, et al. GATA3 haplo-insufficiency causes human HDR syndrome. *Nature*. 2000;406(6794): 419–422
77. Madia F, Striano P, Gennaro E, et al. Cryptic chromosome deletions involving SCN1A in severe myoclonic epilepsy of infancy. *Neurology*. 2006;67(7):1230–1235
78. Boone PM, Bacino CA, Shaw CA, et al. Detection of clinically relevant exonic copy-number changes by array CGH. *Hum Mutat*. 2010;31(12):1326–1342
79. Heron SE, Cox K, Grinton BE, et al. Deletions or duplications in KCNQ2 can cause benign familial neonatal seizures. *J Med Genet*. 2007;44(12):791–796
80. Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet*. 2008; 40(6):782–788
81. Mei D, Marini C, Novara F, et al. Xp22.3 genomic deletions involving the CDKL5 gene in girls with early onset epileptic encephalopathy. *Epilepsia*. 2010;51(4):647–654
82. Kamada F, Kure S, Kudo T, et al. A novel KCNQ4 one-base deletion in a large pedigree with hearing loss: implication for the genotype-phenotype correlation. *J Hum Genet*. 2006;51(5):455–460
83. Shovlin CL, Hughes JM, Scott J, Seidman CE, Seidman JG. Characterization of endoglin and identification of novel mutations in hereditary hemorrhagic telangiectasia. *Am J Hum Genet*. 1997;61(1): 68–79
84. Howe JR, Sayed MG, Ahmed AF, et al. The prevalence of MADH4 and BMPR1A mutations in juvenile polyposis and absence of BMPR2, BMPR1B, and ACVR1 mutations. *J Med Genet*. 2004;41(7):484–491
85. Raslova H, Komura E, Le Couédic JP, et al. FLI1 monoallelic expression combined with its hemizygous loss underlies Paris-Trousseau/Jacobsen thrombopenia. *J Clin Invest*. 2004;114(1):77–84
86. Woods KS, Cundall M, Turton J, et al. Over- and underdosage of SOX3 is associated with infundibular hypoplasia and hypopituitarism. *Am J Hum Genet*. 2005;76(5): 833–849
87. Hughes-Benzie RM, Pilia G, Xuan JY, et al. Simpson-Golabi-Behmel syndrome: genotype/phenotype analysis of 18 affected males from 7 unrelated families. *Am J Med Genet*. 1996;66(2):227–234
88. Abdelhak S, Kalatzis V, Heilig R, et al. Clustering of mutations responsible for branchio-oto-renal (BOR) syndrome in the eyes absent homologous region (eyaHR) of EYA1. *Hum Mol Genet*. 1997;6(13):2247–2255
89. Lines MA, Kozlowski K, Kulak SC, et al. Characterization and prevalence of PITX2 microdeletions and mutations in Axenfeld-Rieger malformations. *Invest Ophthalmol Vis Sci*. 2004;45(3):828–833
90. Chanda B, Asai-Coakwell M, Ye M, et al. A novel mechanistic spectrum underlies glaucoma-associated chromosome 6p25 copy number variation. *Hum Mol Genet*. 2008;17(22):3446–3458
91. Marchbank NJ, Craig JE, Leek JP, et al. Deletion of the OPA1 gene in a dominant optic atrophy family: evidence that haploinsufficiency is the cause of disease. *J Med Genet*. 2002;39(8):e47
92. Carette C, Dubois-Laforgue D, Saint-Martin C, et al. Familial young-onset forms of diabetes related to HNF4A and rare HNF1A molecular aetiologies. *Diabet Med*. 2010; 27(12):1454–1458
93. Osbak KK, Colclough K, Saint-Martin C, et al. Update on mutations in glucokinase (GCK), which cause maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemic hypoglycemia. *Hum Mutat*. 2009;30(11):1512–1526
94. Akrami SM, Winter RM, Brook JD, Armour JA. Detection of a large TBX5 deletion in a family with Holt-Oram syndrome. *J Med Genet*. 2001;38(12):E44
95. Van Der Hout AH, Verlind E, Beemer FA, Buys CH, Hofstra RM, Scheffer H. Occurrence of deletion of a COL2A1 allele as the mutation in Stickler syndrome shows that a collagen type II dosage effect underlies this syndrome. *Hum Mutat*. 2002;20(3):236
96. Tompson SW, Bacino CA, Safina NP, et al. Fibrochondrogenesis results from mutations in the COL11A1 type XI collagen gene. *Am J Hum Genet*. 2010;87(5):708–712
97. Germain-Lee EL, Groman J, Crane JL, Jan de Beur SM, Levine MA. Growth hormone deficiency in pseudohypoparathyroidism type 1a: another manifestation of multihormone resistance. *J Clin Endocrinol Metab*. 2003;88(9):4059–4069
98. Gregory-Evans CY, Moosajee M, Hodges MD, et al. SNP genome scanning localizes oto-dental syndrome to chromosome 11q13 and microdeletions at this locus implicate FGF3 in dental and inner-ear disease and FADD in ocular coloboma. *Hum Mol Genet*. 2007;16(20):2482–2493
99. Seidner G, Alvarez MG, Yeh JI, et al. GLUT-1 deficiency syndrome caused by haploinsufficiency of the blood-brain barrier hexose carrier. *Nat Genet*. 1998;18(2):188–191
100. Gaetzner S, Stahl S, Sürücü O, et al. CCM1 gene deletion identified by MLPA in cerebral cavernous malformation. *Neurosurg Rev*. 2007;30(2):155–159, discussion 159–160
101. Denier C, Goutagny S, Labauge P, et al; Société Française de Neurochirurgie. Mutations within the MGC4607 gene cause cerebral cavernous malformations. *Am J Hum Genet*. 2004;74(2):326–337
102. Ariyurek Y, Lantinga-van Leeuwen I, Spruit L, Ravine D, Breuning MH, Peters DJ. Large deletions in the polycystic kidney disease 1 (PKD1) gene. *Hum Mutat*. 2004;23(1):99
103. Torra R, Badenas C, San Millán JL, Pérez-Oller L, Estivill X, Darnell A. A loss-of-function model for cystogenesis in human autosomal dominant polycystic kidney disease type 2. *Am J Hum Genet*. 1999;65(2): 345–352
104. Dateki S, Fukami M, Uematsu A, et al. Mutation and gene copy number analyses of six pituitary transcription factor genes in 71 patients with combined pituitary hormone deficiency: identification of a single patient with LHX4 deletion. *J Clin Endocrinol Metab*. 2010;95(8):4043–4047
105. Guo W, Mason JS, Stone CG Jr et al. Diagnosis of X-linked adrenal hypoplasia congenita by mutation analysis of the DAX1 gene. *JAMA*. 1995;274(4):324–330
106. Thielen BK, Barker DF, Nelson RD, Zhou J, Kren SM, Segal Y. Deletion mapping in Alport syndrome and Alport syndrome-diffuse leiomyomatosis reveals potential mechanisms of visceral smooth muscle overgrowth. *Hum Mutat*. 2003;22(5):419

107. Pabst S, Wollnik B, Rohmann E, et al. A novel stop mutation truncating critical regions of the cardiac transcription factor NKX2-5 in a large family with autosomal-dominant inherited congenital heart disease. *Clin Res Cardiol*. 2008;97(1):39–42
108. Ponjavic V, Abrahamson M, Andréasson S, et al. Phenotype variations within a choroideremia family lacking the entire CHM gene. *Ophthalmic Genet*. 1995;16(4):143–150
109. Hagan DM, Ross AJ, Strachan T, et al. Mutation analysis and embryonic expression of the HLXB9 Currarino syndrome gene. *Am J Hum Genet*. 2000;66(5):1504–1515
110. Marshall CR, Young EJ, Pani AM, et al. Infantile spasms is associated with deletion of the MAGI2 gene on chromosome 7q11.23-q21.11. *Am J Hum Genet*. 2008;83(1):106–111
111. Hardelin JP, Levilliers J, del Castillo I, et al. X chromosome-linked Kallmann syndrome: stop mutations validate the candidate gene. *Proc Natl Acad Sci U S A*. 1992;89(17):8190–8194
112. Bolino A, Yin L, Seri M, et al. A new candidate region for the positional cloning of the XLP gene. *Eur J Hum Genet*. 1998;6(5):509–517
113. Suess PJ, Tsai MY, Holzknecht RA, Horowitz M, Tuchman M. Screening for gene deletions and known mutations in 13 patients with ornithine transcarbamylase deficiency. *Biochem Med Metab Biol*. 1992;47(3):250–259
114. Hilhorst-Hofstee Y, Hamel BC, Verheij JB, et al. The clinical spectrum of complete FBN1 allele deletions. *Eur J Hum Genet*. 2011;19(3):247–252
115. Pintao MC, Garcia AA, Borgel D, et al. Gross deletions/duplications in PROS1 are relatively common in point mutation-negative hereditary protein S deficiency. *Hum Genet*. 2009;126(3):449–456
116. Bock SC, Prochownik EV. Molecular genetic survey of 16 kindreds with hereditary antithrombin III deficiency. *Blood*. 1987;70(5):1273–1278
117. Aramaki M, Udaka T, Kosaki R, et al. Phenotypic spectrum of CHARGE syndrome with CHD7 mutations. *J Pediatr*. 2006;148(3):410–414
118. Farrar JE, Nater M, Caywood E, et al. Abnormalities of the large ribosomal subunit protein, Rpl35a, in Diamond-Blackfan anemia. *Blood*. 2008;112(5):1582–1592

Clinical Utility of Chromosomal Microarray Analysis

Jay W. Ellison, J. Britt Ravnan, Jill A. Rosenfeld, S. Annie Morton, Nicholas J. Neill, Marc S. Williams, Jodi Lewis, Beth S. Torchia, Cathryn Walker, Ryan N. Traylor, Kimberly Moles, Elizabeth Miller, Jennifer Lantz, Caitlin Valentin, Sara L. Minier, Kimberly Leiser, Berkley R. Powell, Timothy M. Wilks and Lisa G. Shaffer

Pediatrics 2012;130;e1085; originally published online October 15, 2012;
DOI: 10.1542/peds.2012-0568

PEDIATRICS®
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Clinical Utility of Chromosomal Microarray Analysis

Jay W. Ellison, J. Britt Ravnan, Jill A. Rosenfeld, S. Annie Morton, Nicholas J. Neill, Marc S. Williams, Jodi Lewis, Beth S. Torchia, Cathryn Walker, Ryan N. Traylor, Kimberly Moles, Elizabeth Miller, Jennifer Lantz, Caitlin Valentin, Sara L. Minier, Kimberly Leiser, Berkley R. Powell, Timothy M. Wilks and Lisa G. Shaffer

Pediatrics 2012;130;e1085; originally published online October 15, 2012;
DOI: 10.1542/peds.2012-0568