Clinical Utility of Chromosomal Microarray Analysis

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

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,a Jodi Lewis, BS,a Beth S. Torchia, PhD,a Cathryn Walker, BS,a Ryan N. Taylor, 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,a Timothy M. Wilks, MD,a and Lisa G. Shaffer, PhDg

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 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 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).
Copyright © 2012 by the American Academy of Pediatrics

FINANCIAL DISCLOSURE: Drs Ellison, Ravnan, Torchia, and Shaffer, as well as Ms Rosenfeld, Ms Morton, Mr Neill, Ms Lewis, Ms Walker, 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

author: 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,a Jodi Lewis, BS,a Beth S. Torchia, PhD,a Cathryn Walker, BS,a Ryan N. Taylor, 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,a Timothy M. Wilks, MD,a and Lisa G. Shaffer, PhDg

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 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 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).
Copyright © 2012 by the American Academy of Pediatrics

FINANCIAL DISCLOSURE: Drs Ellison, Ravnan, Torchia, and Shaffer, as well as Ms Rosenfeld, Ms Morton, Mr Neill, Ms Lewis, Ms Walker, 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.
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. 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. Yet, whereas karyotyping is routinely reimbursed by third-party payers, often microarray testing is not. 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 defined as a positive effect on patient management and/or clinical outcomes.

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. Subsequent BAC-based arrays (SignatureChip WG versions 1–2, Signature Genomic Laboratories) featured whole-genome coverage and were used from 2007 to 2009. Oligonucleotide-based whole-genome arrays (SignatureChip OS versions 1–3, Signature Genomic Laboratories) 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). 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...
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 1: Diagnosed Abnormalities That Include Critical Genomic Regions for Microdeletion and Microduplication Syndromes With Actionable Clinical Features

<table>
<thead>
<tr>
<th>Chromosome Band(s) (Named Syndrome, OMIM No.)</th>
<th>Gene(s)</th>
<th>Actionable Aspect of Phenotype</th>
<th>Total Diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p36 deletion (607872)</td>
<td>RBM8A</td>
<td>Cardiac abnormalities*</td>
<td>138</td>
</tr>
<tr>
<td>1p31–p32 deletion (613735)</td>
<td></td>
<td>Urinary tract, eye abnormalities*</td>
<td>5</td>
</tr>
<tr>
<td>1q21.1 distal deletion (612474)</td>
<td></td>
<td>Cardiac abnormalities, cataracts*</td>
<td>108</td>
</tr>
<tr>
<td>1q21.1 deletion with susceptibility to TAR (274000)</td>
<td>FOXC1</td>
<td>Thrombocytopenia*</td>
<td>43</td>
</tr>
<tr>
<td>2p15–p16.1 deletion (612513)</td>
<td>TAB2</td>
<td>Optic atrophy, renal abnormalities*</td>
<td>6</td>
</tr>
<tr>
<td>4p16.3 deletion (Wolf-Hirschhorn, 194190)</td>
<td></td>
<td>Cardiac, eye, renal abnormalities*</td>
<td>37</td>
</tr>
<tr>
<td>6q25.3 deletion (612582)</td>
<td>FOXC1</td>
<td>Eye and cardiac abnormalities, deafness*</td>
<td>12</td>
</tr>
<tr>
<td>6q24–q25 deletion (612863)</td>
<td>PTEN, BMPR1A</td>
<td>Cardiac abnormalities*</td>
<td>4</td>
</tr>
<tr>
<td>7q11.25 deletion (Williams, 194050)</td>
<td>FLI1</td>
<td>Cardiac abnormalities, juvenile polyposis*</td>
<td>32</td>
</tr>
<tr>
<td>7q11.23 duplication (609757)</td>
<td>PTEN, BMPR1A</td>
<td>Cardiac abnormalities, thrombocytopenia*</td>
<td>36</td>
</tr>
<tr>
<td>8p23.1 deletion</td>
<td></td>
<td>Cardiac abnormalities*</td>
<td>7</td>
</tr>
<tr>
<td>8p12.2 deletion (613604)</td>
<td>YWHAE</td>
<td>Cardiac abnormalities*</td>
<td>5</td>
</tr>
<tr>
<td>8q23.2 deletion (613355)</td>
<td></td>
<td>Cardiac, eye abnormalities, deafness*</td>
<td>2</td>
</tr>
<tr>
<td>9q34 deletion (Kleefstra, 610253)</td>
<td>SNORD11E, UBE3A</td>
<td>Seizures, multiple tumors*</td>
<td>6</td>
</tr>
<tr>
<td>10p13–p14 deletion (DiGeorge 2, 601362)</td>
<td>CREBBP</td>
<td>Cardiac abnormalities*</td>
<td>42</td>
</tr>
<tr>
<td>10q22–q23 deletion</td>
<td></td>
<td>Cardiac abnormalities*</td>
<td>11</td>
</tr>
<tr>
<td>11qter deletion (Jacobsen, 147791)</td>
<td></td>
<td>Cardiac abnormalities, juvenile polyposis*</td>
<td>32</td>
</tr>
<tr>
<td>12q14.1–q15 deletion</td>
<td>FLT1</td>
<td>Cardiac abnormalities, thrombocytopenia*</td>
<td>36</td>
</tr>
<tr>
<td>12q24 duplication</td>
<td></td>
<td>Cardiac abnormalities*</td>
<td>7</td>
</tr>
<tr>
<td>14q22–q33 deletion</td>
<td>PTEN, BMPR1A</td>
<td>Cardiac abnormalities, juvenile polyposis*</td>
<td>32</td>
</tr>
<tr>
<td>15q11.2–q13 deletion (Prader-Willi; 176270; Angelman, 105830)</td>
<td>PTEN, BMPR1A</td>
<td>Cardiac abnormalities, juvenile polyposis*</td>
<td>32</td>
</tr>
<tr>
<td>16p13.3 duplication (613458)</td>
<td></td>
<td>Cardiac abnormalities*</td>
<td>18</td>
</tr>
<tr>
<td>16p13.11 deletion</td>
<td></td>
<td>Seizures*</td>
<td>49</td>
</tr>
<tr>
<td>16p13.11 duplication</td>
<td></td>
<td>Cardiac abnormalities*</td>
<td>20</td>
</tr>
<tr>
<td>16p12.2–p12.2 deletion (613504)</td>
<td></td>
<td>Cardiac abnormalities*</td>
<td>5</td>
</tr>
<tr>
<td>16p12.1 deletion (136570)</td>
<td></td>
<td>Cardiac abnormalities*</td>
<td>60</td>
</tr>
<tr>
<td>16q12.1–q12.2 deletion</td>
<td></td>
<td>Hypothyroidism, seizures*</td>
<td>3</td>
</tr>
<tr>
<td>17p13.1 deletion</td>
<td>YWHAE</td>
<td>Cardiac abnormalities*</td>
<td>18</td>
</tr>
<tr>
<td>17p13.3 proximal deletion (Miller-Dieker, 247200)</td>
<td>RA1</td>
<td>Seizures*</td>
<td>17</td>
</tr>
<tr>
<td>17p11.2 deletion (Smith-Magenis, 182290)</td>
<td></td>
<td>Hearing loss, cardiac, eye, sleep abnormalities*</td>
<td>49</td>
</tr>
<tr>
<td>17p11.2 duplication (Potocki-Lupski, 610883)</td>
<td></td>
<td>Cardiac, EG abnormalities*</td>
<td>50</td>
</tr>
<tr>
<td>17q12 deletion (137920)</td>
<td></td>
<td>Renal cysts, diabetes risk*</td>
<td>29</td>
</tr>
<tr>
<td>17q21.3 deletion (608443)</td>
<td></td>
<td>Cardiac, urologic abnormalities*</td>
<td>43</td>
</tr>
<tr>
<td>17q23.1–q23.2 deletion (613555)</td>
<td></td>
<td>Cardiac, eye abnormalities*</td>
<td>4</td>
</tr>
<tr>
<td>22q11.2 deletion (Velocardiofacial, 193240)</td>
<td></td>
<td>Cardiac, renal abnormalities*</td>
<td>18</td>
</tr>
<tr>
<td>22q11.21 duplication (608365)</td>
<td></td>
<td>Cardiac, immune system, other abnormalities*</td>
<td>262</td>
</tr>
<tr>
<td>22q11.2 distal deletion (611867)</td>
<td>TBX1</td>
<td>Cardiac abnormalities*</td>
<td>166</td>
</tr>
<tr>
<td>22q13.3 deletion (Phealan-McDermid, 606232)</td>
<td></td>
<td>Cardiac abnormalities*</td>
<td>51</td>
</tr>
<tr>
<td>Xp11.22–p11.23 duplication (500081)</td>
<td></td>
<td>Hearing loss*</td>
<td>71</td>
</tr>
</tbody>
</table>

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

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

* References describe the actionable clinical features of the respective disorder.

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

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

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

* 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.

* Twenty-two of these patients have a deletion of FLI1 and are also listed in Table 5.

* Two of these patients have mosaicism for trisomy 12.
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 131I 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 MNT1 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 SERPING1, 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 BMPRIA. The 10q deletion

---

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

<table>
<thead>
<tr>
<th>Disorder a (OMIM No.)</th>
<th>Gene(s)b (Chromosome Band)</th>
<th>No. of Cases c,d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile polyposis (174900)</td>
<td>BMPRIA (10q23.2)</td>
<td>15</td>
</tr>
<tr>
<td>Beckwith-Wiedemann (130650)</td>
<td>IGF2 (11p15.5)</td>
<td>13</td>
</tr>
<tr>
<td>Familial adenomatous polyposis (175100)</td>
<td>APC (5q22.2)</td>
<td>13</td>
</tr>
<tr>
<td>Lynch (614357, 614350)</td>
<td>MSH6 (2p16.3)</td>
<td>2</td>
</tr>
<tr>
<td>Neurofibromatosis 1 (162200)</td>
<td>NF1 (17q11.2)</td>
<td>19</td>
</tr>
<tr>
<td>Paraganglioma, pheochromocytoma</td>
<td>SDHB (1p36.13)</td>
<td>8</td>
</tr>
<tr>
<td>Hereditary breast and ovarian cancer (137215)</td>
<td>BRCA1 (17q21.3)</td>
<td>3</td>
</tr>
<tr>
<td>Multiple exostoses (133700, 133701)</td>
<td>EXT1 (11q13.2)</td>
<td>4</td>
</tr>
<tr>
<td>Familial adenomatous polyposis</td>
<td>SMAD4 (3p25.3)</td>
<td>12</td>
</tr>
<tr>
<td>von Hippel-Lindau (193300)</td>
<td>VHL (3p25.3)</td>
<td>10</td>
</tr>
<tr>
<td>Retinoblastoma (180200)</td>
<td>RB1 (13q14.2)</td>
<td>12</td>
</tr>
<tr>
<td>Rubinstein-Taybi (180849, 613684)</td>
<td>CREBBP (18p13.3)</td>
<td>15</td>
</tr>
<tr>
<td>Melanoma (155601, 155755)</td>
<td>CDKN2A (9p21.3)</td>
<td>1</td>
</tr>
<tr>
<td>Schwannomatosis (168000, 115310, 171300)</td>
<td>G3P3 (10q23.2)</td>
<td>15</td>
</tr>
</tbody>
</table>

**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 MNT1 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 SERPING1, 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 BMPRIA. The 10q deletion

---

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 MEX1.

*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 Four 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 BMPRIA.
Four of these patients also have deletions of 6p25.3.

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

Six of these patients are at risk for retinoblastoma due to deletion of 13q14.1.

Twelve of these patients also have Currarino syndrome owing to deletion of 9q34.11.

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

Flanking regions provide evidence for functional dosage sensitivity of the phenotype for the corresponding gene.

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.

Other dosage-sensitive genes were queried, but no pathogenic abnormalities were found in our database. These genes are SOX10, PCDH19, ADIAR1, HNF1A, PRPFS1, CYP11A, MYBPC3, LMNA, MRZ, AR, FGFR8, PRNP, NUPR1, Dicer, APTA, NOP, CYBR, RPS10, RPS17, RPS19, RPS24, RPS26, RPL5, RPL11, and RP2.

Twelve of these patients also have Currarino syndrome owing to deletion of MKNK1.

Six of these patients are at risk for retinoblastoma due to deletion of RB1.

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

Both SCN1A and SCN2A are deleted in 8 of these cases.

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

Twelve of these patients also have deletions of SERPINC1.

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

Four of these patients also have deletions of LRH1.
predisposes these patients to cardiac abnormalities, whereas deletion of BMPR1A puts them at risk for juvenile polypsis; 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 Curranino 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%. 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>
<thead>
<tr>
<th>Disorder/Phenotype</th>
<th>Gene (Chromosome Band)</th>
<th>Cases Queried</th>
<th>Responses Received</th>
<th>Appropriate Action Taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>17q12 deletion/renal cysts and diabetes</td>
<td>HNF1B (17q12)</td>
<td>28</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Long QT</td>
<td>KCNH2 (7q36.1)</td>
<td>13</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Waardenburg</td>
<td>PAK3 (2q36.1)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mitf (5p14.1)</td>
<td>MITF (5p14.1)</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hyperparathyroidism, deafness, renal anomalies</td>
<td>GATA3 (10p14)</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Miller-Dieker/lissencephaly, seizures</td>
<td>PAFAH1B1 (17p13.3)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>SCN1A (2q24.3)</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SCN2A (2q24.3)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>STXBP1 (9q34.11)</td>
<td>1</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>HHT</td>
<td>ENG (9q34.11)</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>HHT plus juvenile polyposis</td>
<td>SMAD4 (18q21.2)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>11pter deletion/thrombocytopenia</td>
<td>FLJ11 (11q24.3)</td>
<td>9</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Pituitary hormone deficiency</td>
<td>SOX3 (Xq27.1)</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Cardiomyopathy, tumor risk</td>
<td>GPC3 (Xq26.2)</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Williams</td>
<td>ELN (7q11.23)</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>and others</td>
<td>and others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DiGeorge/velocardiofacial</td>
<td>TBX1 (22q11.21)</td>
<td>26</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>and others</td>
<td>and others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Totals</td>
<td>122</td>
<td>81</td>
<td>76</td>
</tr>
</tbody>
</table>

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%.\textsuperscript{16,18,19} 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.\textsuperscript{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.\textsuperscript{16,21,22} Such patients benefit from awareness of tumor risk and appropriate clinical surveillance.\textsuperscript{23–26} Other studies showed that clinical actions were taken after abnormal microarray results,\textsuperscript{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.\textsuperscript{14,5} 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

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


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


47. Mattina T, Perrotta CS, Grossfeld P. Jacobsen syndrome. Orphanet J Rare Dis. 2009;4:9


61. Splawski I, Shen J, Timothy KW, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A,


Clinical Utility of Chromosomal Microarray Analysis
Pediatrics 2012;130;e1085; originally published online October 15, 2012;
DOI: 10.1542/peds.2012-0568

Updated Information & Services
including high resolution figures, can be found at:
/content/130/5/e1085.full.html

References
This article cites 105 articles, 20 of which can be accessed free at:
/content/130/5/e1085.full.html#ref-list-1

Subspecialty Collections
This article, along with others on similar topics, appears in the following collection(s):
Genetics
/cgi/collection/genetics_sub

Permissions & Licensing
Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
/site/misc/Permissions.xhtml

Reprints
Information about ordering reprints can be found online:
/site/misc/reprints.xhtml

PEDIATRICS is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since 1948. PEDIATRICS is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2012 by the American Academy of Pediatrics. All rights reserved. Print ISSN: 0031-4005. Online ISSN: 1098-4275.

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN™
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

The online version of this article, along with updated information and services, is
located on the World Wide Web at:
/content/130/5/e1085.full.html