Chromosomal microarray testing influences medical management

Michael E. Coulter, BA[1,2], David T. Miller, MD, PhD[1,2,3], David J. Harris, MD[1,2], Pamela Hawley, MS[1], Jonathan Picker, MD, PhD[1,2], Amy E. Roberts, MD[1,2,4], Magdi M. Sobeih, MD, PhD[2,5], and Mira Irons, MD[1,2]

Purpose: Chromosomal microarray (CMA) testing provides the highest diagnostic yield for clinical testing of patients with developmental delay (DD), intellectual disability (ID), multiple congenital anomalies (MCA), and autism spectrum disorders (ASD). Despite improved diagnostic yield and studies to support cost-effectiveness, concerns regarding the cost and reimbursement for CMA have been raised because it is perceived that CMA results do not influence medical management. **Methods:** We conducted a retrospective chart review of CMA testing performed during a 12-month period on patients with DD/ID, ASD, and congenital anomalies to determine the proportion of cases where abnormal CMA results impacted recommendations for clinical action. **Results:** Among 1792 patients, 13.1% had clinically relevant results, either abnormal (n = 131; 7.3%) or variants of possible significance (VPS; n = 104; 5.8%). Abnormal variants generated a higher rate of recommendation for clinical action (54%) compared with VPS (34%; Fisher exact test, P = 0.01). CMA results influenced medical care by precipitating medical referrals, diagnostic imaging, or specific laboratory testing. **Conclusions:** For all test indications, CMA results influenced medical management in a majority of patients with abnormal variants and a substantial proportion of those with VPS. These results support the use of CMA as a clinical diagnostic test that influences medical management for this patient population. ***Genet Med* 2011:13(9):770–776.**

Key Words: *chromosomal microarray, array comparative genomic hybridization, autism, intellectual disability, developmental delay, genetic testing*

Chromosome microarray (CMA) or array comparative genomic hybridization has revolutionized the ability of medical geneticists to detect clinically significant copy number variants (CNVs) across the entire human genome. Most current CMA is based on oligonucleotide platforms and reliably detects deletions and duplications as small as ~200 kb, a significant improvement in detection compared with conventional cytogenetics, among patients with intellectual disability or developmental delay (ID/DD), autism spectrum disorders (ASD), and/or multiple congenital anomalies (MCA).[1–3] Pathogenic CNVs have also been shown to be associated with several other conditions, including epilepsy and a spectrum of neuropsychiatric disorders.[4–6]

CMA with whole genome coverage has a much higher yield than G-banded karyotype followed by fluorescence in situ hybridization of subtelomeric regions in patients with ID/DD, ASD, and MCA.[7,8] Diagnostic yield of CMA ranges from approximately 12 to 19% for this patient population, when compared with <3% for G-banded karyotyping.[1–3] Decision analytic modeling has suggested that using CMA as a first-tier test before karyotype provides good value among patients with unexplained ID/DD, ASD, or MCA.[9] Recently updated practice guidelines of the American College of Medical Genetics endorse the use of CMA as the first-line diagnostic test in the following groups of patients: (1) multiple anomalies not specific to a well-delineated genetic syndrome, (2) apparently nonsyndromic DD/ID, and (3) ASD.[10]

Despite consensus among clinicians, laboratory geneticists, and genome scientists about the effectiveness of CMA in this patient population, some remain skeptical about the necessity for clinical CMA testing. Several health insurers have issued new reimbursement guidelines restricting the use of CMA or have stopped reimbursement for CMA in children with nonsyndromic ID/DD or ASD. The rationale behind these decisions is that CMA should not be reimbursed because it is only used to clarify a diagnosis but does not change clinical management of the patient.

On the basis of our clinical expertise, we hypothesized that CMA results cause new clinical action to be recommended in at least 30% of patients. We reviewed the results from 1 year of clinical CMA testing at a tertiary children's hospital and found that CMA results frequently initiate new clinical action.

MATERIALS AND METHODS

Subjects and CMA testing

We identified all patients at Children's Hospital Boston (CHB) who had an "abnormal" or "variant of possible significance" result on CMA from July 1, 2009, to July 1, 2010. At CHB, interpretation of results are based on general principles for CNV interpretation as outlined elsewhere.[3,11] CMA variants were classified as follows:

1. "Abnormal" variants are those that encompass known microdeletion/microduplication syndromes, deletions of genes known to be associated with disease by loss of one copy (haploinsufficiency), and large deletions and/or duplications involving many genes.

From the [1]Division of Genetics, Children's Hospital Boston; [2]Harvard Medical School; and [3]Department of Laboratory Medicine, [4]Department of Cardiology, and [5]Neurolinguistics, Clinic, Behavioral Neurology Group, Department of Neurology, Children's Hospital Boston, Boston, Massachusetts.

Mira Irons, MD, Division of Genetics, Hunnewell-5, Children's Hospital Boston, 300 Longwood Avenue, Boston, MA 02115. E-mail: mira.irons@childrens.harvard.edu.

The first two authors contributed equally to this work.

Disclosure: David T. Miller is a consultant for Roche-Nimblegen regarding clinical issues for chromosomal microarray testing. The others authors have no potential conflicts of interest to declare.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.geneticsinmedicine.org).

Submitted for publication February 22, 2011.

Accepted for publication April 1, 2011.

Published online ahead of print June 28, 2011.

DOI: 10.1097/GIM.0b013e31821dd54a

2. "Variants of possible significance" (VPS) are those that include deletions or duplications that overlap with, but do not entirely match reported pathogenic deletions/duplications, deletions or duplications containing genes that are suspected, but not confirmed in disease pathogenesis, or changes that involve a gene for which loss of one copy through deletion would not cause a disease, but would imply that the person is a carrier for a recessive trait.
3. "Variants of unknown significance" are those that are not currently present in the literature or available databases and usually do not include genes known to be associated with disease. In addition, duplications involving disease genes are typically variants of unknown significance as it is unknown what effect a duplication might have on the disease state. However, deletions including a gene of unknown function are included as it is unknown whether a deletion of a gene could affect the patient.
4. "Reported copy number variants" are variants that have been previously reported as CNVs found in unaffected individuals.

Eight patients referred for CMA with a known or suspected diagnosis of Down syndrome were excluded because the clinical features of this syndrome are readily apparent and the diagnosis of Down syndrome itself is associated with changes in medical management. In addition, 31 of 104 (28%) patients with VPS who did not have completed parental studies at the time of analysis were excluded because CMA findings cannot be interpreted in these patients until parental studies are completed. There were 41 patients who had no documented follow-up after CMA testing was completed. In these cases, individual referring physicians were contacted. They were asked if undocumented follow-up did occur, and if not, asked what clinical action they would have recommended in follow-up. When the referring clinician did not respond or did not know what clinical action was warranted by the CMA result, two American Board of Medical Genetics board-certified clinical geneticists (D.T.M. and M.I.) reviewed the CMA finding and provided their professional opinion about recommended clinical action.

Patients with a previously known abnormal conventional cytogenetic result, such as partial or whole chromosome deletion or duplication, sometimes undergo CMA testing to more accurately delineate translocation breakpoints. These patients ($n = 8$) were retained for several reasons: (1) delineation of breakpoints can help with genotype-phenotype correlation and medical recommendations, (2) CMA is now recommended as first-tier testing, and we wanted to assess the potential impact of CMA if used as a first-tier test as recommended in current guidelines, and (3) CMA may reveal additional areas of imbalance not identified by previous testing. These patients were treated in the analysis as though their CMA was ordered first and identified their variant. This study was performed as a quality improvement initiative and so did not require formal institutional review board approval.

Linking clinical action to CMA result

A recommendation for clinical action was defined as a specialist referral, imaging study, diagnostic test, or medication prescription that was recommended by the patient's physician based on CMA results. An American Board of Medical Genetics board-certified clinical geneticist (D.T.M. or M.I.) reviewed the clinic notes from the ordering provider for all subjects with VPS or abnormal results. Only recommendations made because of the CMA results specifically were included. We did not include clinical recommendations that occurred chronologically after CMA testing but were not initiated by the CMA result. We also excluded recommendations that would have been indicated for the genomic disorder identified by CMA but had already been performed for another reason. Although many patients were recommended to have a follow-up fluorescence in situ hybridization study to determine whether the variant identified by CMA was the result of an insertional translocation, and many were recommended to have parental studies to determine the likelihood of recurrence in future pregnancies, these recommendations were not counted in our analysis because they should be a standard practice after all abnormal CMA results.

RESULTS

A total of 1792 patients had CMA testing from July 1, 2009, to July 1, 2010, at CHB. Two hundred thirty-five of these patients were found to have an abnormal variant (131 patients, 7.3% of total tested) or variant of possible significance (104 patients, 5.8% of total tested). One hundred and ninety-four of these patients were included in our analysis; 121 patients with abnormal CMA variants and 73 patients with VPS (Table, Supplemental Digital Content 1, http://links.lww.com/GIM/A186). Abnormal variants were significantly larger than VPS. The mean size of an abnormal variant was 5.1 Mb, and mean size of a VPS was 655 kb (one-tailed t-test, $P = 6.8 \times 10^{-10}$) (Note: seven abnormal variants that were duplications of the entire X or Y chromosome were not included in mean size calculation.) Patients with abnormal variants and patients with VPS were considered separately in our analysis because the rate of recommended clinical action after CMA was significantly different between the two groups. The rate of recommendation for clinical action in patients with abnormal variants was 54%, and the rate in patients with VPS was 34% (Fisher exact test, $P = 0.01$).

General demographic features of these two groups are shown in Table 1. Of note, there were more males than females who had abnormal variants (61% male and 39% female) or VPS (56% male and 44% female). Among the cases reviewed, CMA testing was ordered most frequently by neurologists, followed closely by geneticists. ASD was the indication for testing in 21% of patients with an abnormal variant and 32% of patients with a VPS. DD/ID was the indication for testing in 44% of patients with an abnormal variant and 29% of patients with a VPS. Congenital anomalies were the indication for testing in 16% of patients with an abnormal variant and 16% of patients with a VPS; 7% of patients with an abnormal variant were referred for CMA to confirm a previously identified genetic abnormality (Table 2).

The majority of patients referred for CMA underwent other testing in an attempt to identify a genetic or neurometabolic cause for their disease. The most common tests were karyotype, Fragile X CGG repeat analysis, Prader-Willi/Angelman Syndrome methylation analysis, plasma amino acids, and urine organic acids. One hundred ten patients with an abnormal variant (90%) underwent a total of 210 other tests before or at the same time as CMA testing. Sixty-one patients with a VPS (84%) underwent a total of 146 other tests before or at the same time as CMA testing. These tests were all negative except for the following: two patients positive for Prader-Willi/Angelman Syndrome methylation analysis diagnosed concurrently by CMA and 23 patients with karyotype abnormalities at the band identified by CMA. Importantly, in these 23 patients, the boundaries of their variants were identified at much higher resolution by CMA than by karyotype. In total, 356 other tests were performed on the 194 patients in this study that served the same

Table 1 Demographic and clinical features of patients with abnormal and VPS CMA results

	Patients with abnormal variant (%)	Patients with VPS (%)
Sex		
Male	73 (61)	41 (56)
Female	48 (39)	32 (44)
Age (yr)		
<2	36 (30)	11 (15)
2–5	27 (22)	27 (37)
5–10	37 (31)	14 (19)
>10	21 (17)	21 (29)
Inclusion		
Total	131 (7.3)	104 (5.8)
Excluded	10	31
Included	121	73
Clinical features[a]		
ASD	26 (21)	23 (32)
DD/ID	53 (44)	21 (29)
Congenital anomalies	19 (16)	12 (16)
Dysmorphic features	66 (55)	31 (42)
Seizures	19 (16)	17 (23)
Hypotonia	41 (34)	24 (33)

[a]Some patients had more than one clinical feature, so percentages add to greater than 100%.

Table 2 Ordering physicians and indication for CMA

	Patients with abnormal variant (%)	Patients with VPS (%)
Ordering physician		
Genetics	52 (43)	25 (34)
Neurology	57 (47)	33 (45)
Developmental medicine	7 (6)	12 (16)
Other	5 (4)	3 (4)
Indication for CMA		
ASD	26 (21)	23 (32)
DD/ID	55 (45)	21 (29)
Congenital anomalies	19 (16)	12 (16)
Previous genetic diagnosis	8 (7)	0 (0)
Other	13 (11)	17 (23)

purpose as CMA to identify a genetic, metabolic, or neurologic cause for these patients' disease (Table 3).

The rate of recommended clinical action after CMA testing is presented in Tables 4 and 5. Patients with an abnormal variant had a significantly higher rate of recommended clinical action (54%) than patients with a VPS (34%; Fisher exact test, $P = 0.01$), although a recommendation for clinical action occurred in over a third of patients with a VPS. The rate of recommended clinical action for patients with ASD was similar in both groups (abnormal, 27%; VPS, 30%), as was the rate of recommended clinical action for patients with DD/ID (abnormal, 62%; VPS, 62%). Specific recommendations were divided into three categories: specialist referral, imaging, and laboratory test. In 65 patients with abnormal variants for whom clinical action was recommended, 67 specialist referrals, 25 imaging studies, and 20 laboratory tests were recommended (Table 4). In 25 patients with VPS for whom clinical action was recommended, 11 specialist referrals, 9 imaging studies, and 18 laboratory tests were recommended (Table 5).

Table 3 Previous and concurrent other testing in patients with CMA testing

	Count (%)	No. tests
Patients with abnormal variant		
ASD (n = 26)	23 (88)	63
DD/ID (n = 55)	54 (98)	98
Congenital anomalies (n = 19)	14 (74)	17
Previous genetic diagnosis (n = 8)	8 (100)	9
Other (n = 13)	11 (85)	23
Total (n = 121)	110 (90)	210
Patients with VPS		
ASD (n = 23)	21 (91)	51
DD/ID (n = 21)	15 (71)	38
Congenital anomalies (n = 12)	9 (75)	22
Previous genetic diagnosis (n = 0)	NA	NA
Other (n = 17)	16 (94)	35
Total (n = 73)	61 (84)	146

Tests included karyotype, Fragile X, Prader-Willi methylation study, plasma amino acids, urine organic acids, various single gene tests, and various other biochemical tests.

There were a few notable specific CMA results in this group of patients. Twelve patients had a duplication or deletion over chromosome 16p11.2, a region previously found to be associated with ASD and DD/ID.[12–15] Two cases of Angelman Syndrome and one case of Prader-Willi syndrome were newly identified, as were seven cases of sex chromosome aneuploidy (XXX [2], XXY [3], and XYY [2]).

Case examples

A few examples illustrate the importance and often unexpected nature of new, clinically significant information revealed by CMA testing.

Case 1: A 15-year-old girl with learning difficulties and behavioral problems

A 15-year-old girl was evaluated for history of learning difficulties and behavioral problems. CMA was ordered to determine if there was a genetic basis for her problems, and it revealed a 3.8-Mb deletion on 2q that included the *PROC* gene,

Table 4 Rate of recommendation for clinical action and specific recommendations for patients with abnormal CMA results

	Indication for CMA					
	ASD	DD/ID	Congenital anomalies	Previous genetic diagnosis	Other	Total
Clinical action recommended	7 (27%)	34 (62%)	13 (68%)	5 (63%)	6 (46%)	65 (54%)
No action recommended	19 (73%)	21 (38%)	6 (32%)	3 (37%)	7 (54%)	56 (46%)
Recommended action						
Referral[a]	5	36	15	4	7	67 (60%)
Endocrine	2	7	1	2	1	13
Ophthalmology	0	6	4	0	1	11
Cardiology	0	8	4	1	2	16
Imaging[b]	5	14	5	0	1	25 (22%)
Renal ultrasound	1	6	3	0	1	12
Spine x-ray	2	6	2	0	0	10
MRI	2	1	0	0	0	4
Laboratory test[c]	3	12	3	1	1	20 (18%)
Hearing test	0	1	2	0	0	3
EEG	0	2	0	1	0	3

The most commonly recommended clinical actions are shown; others are listed below.
[a]Other referrals: neuromuscular (1), neurology (4), renal (3), immunology (2), developmental medicine (2), physical/occupational therapy (1), velocardiofacial (1), psychiatry (2), dermatology (3), gastroenterology (4), oncology (1), autism evaluation (1), ENT (1), dentistry (1), and sleep medicine (1).
[b]Other imaging: other x-ray (1).
[c]Other laboratory tests: creatine kinase (1), *DMD* sequencing (1), CBC (1), serum Ca++ (1), sulfatase (1), *CLN6* sequencing (1), EKG (1), prescription of l-carnitine (1), mucopolysacharrides (1), sulfatide (1), plasma proline (1), protein C antigen (1), liver function tests (1), and *RAI1* sequencing (1).

Table 5 Rate of recommendation for clinical action and specific recommendations for patients with VPS CMA results

	Indication for CMA				
	ASD	DD/ID	Congenital anomalies	Other	Total
Clinical action recommended	7 (30%)	13 (62%)	2 (17%)	3 (18%)	25 (34%)
No action recommended	16 (70%)	8 (38%)	10 (83%)	14 (82%)	48 (66%)
Recommended action					
Referral[a]	4	5	0	2	11 (29%)
Endocrine	0	1	0	1	2
Ophthalmology	3	1	0	0	4
Neurology	0	2	0	0	2
Cardiology	1	1	0	0	2
Imaging[b]	1	7	1	0	9 (24%)
MRI	1	5	0	0	4
Laboratory test[c]	6	9	1	2	18 (47%)
EEG	1	1	0	0	2
Urine organic acids	0	1	1	0	2
Urinalysis	0	1	0	1	2

The most commonly recommended clinical actions are shown, others listed below.
[a]Other referrals: metabolism (1).
[b]Other imaging: renal ultrasound (1), hand/forearm x-ray (1), and MRS (1).
[c]Other laboratory tests: transferrin glycoslyation (1), bone tests (1), *PARK2* sequencing (1), *GBA* sequencing (1) beta-glucosidase activity (1), plasma amino acids (1), serum proline (1), *NIP1A* sequencing (1), *COH1* sequencing (1), CBC (1), drug dosing (1), and thyroid function tests (1).

a gene associated with hereditary thrombophilia caused by protein C deficiency (MIM ID #176860). The patient's geneticist ordered a protein C antigen test to determine if the patient should avoid procoagulant medications, such as oral contraceptives, in the future. Indeed, protein C testing showed that the patient had a low level of functional protein C. The incidental finding on CMA and confirmation of the corresponding biochemical deficit provided important information that will help the patient's future care providers avoid procoagulant medications that are more likely to cause her to clot.

Case 2: A 9-year-old girl with a complicated medical history

A 9-year-old girl with a complicated medical history was referred to genetics by her cardiologist for a genetic evaluation to identify an underlying cause of her multiple problems. She had a history of bicuspid aortic valve, atrial septal defect, anteriorly placed anus, polysplenia, vesicoureteral reflux, hearing loss, sacral dimple, and myopia and strabismus. Previously, she had normal karyotype, 500K single nucleotide polymorphism array, connexin 26 gene test, and sweat test. A CMA was ordered because the current array has more uniform coverage than the 500K array. CMA testing showed a 244-kb deletion at 22q12.1; the deleted region contained the *CHEK2* gene that when mutated causes greatly increased risk of several types of cancer (Li-Fraumeni Syndrome 2, LFS2, MIM ID #609265). Because of this finding, the patient was referred to an oncologist who specializes in genetics for further evaluation and counseling. An incidental finding on CMA generated important medical information that initiated a change in clinical management.

Case 3: A 9-year-old boy with sensory-seeking behavior

A 9-year-old boy presented to the neurology service for concerns about sensory-seeking behaviors, speech delay, and toe walking. After his initial evaluation, the neurologist concluded the boy had Asperger syndrome and developmental coordination disorder. The neurologist ordered a CMA to identify any underlying genetic cause, which showed a deletion at Xp21.1 that included exons 45–48 of the *DMD* gene. The deletion showed that the boy had a form of Becker Muscular Dystrophy; he was referred to the neuromuscular and cardiology services and his creatine kinase was measured. His creatine kinase was highly elevated at 19,530. Reflecting on the CMA results, the neurologist noted that on thorough physical examination, the patient had calf pseudohypertrophy and a modified Gower sign. The CMA result not only explained the boy's history of clumsiness, lack of coordination, and toe walking but also directed his physicians to monitor him for serious complications of muscular dystrophy such as cardiac problems. In addition, this result led to carrier testing and surveillance of the patient's mother.

DISCUSSION

Our findings indicate that CMA results lead to recommendations for clinical action in more than half of patients with abnormal variants and over one third of patients with VPS. Our analysis clearly indicates that positive (abnormal or VPS) CMA results change medical management in a substantial proportion of patients. We identified some trends in the effect on clinical management.

Throughout our analysis, we separated patients with abnormal variants from those with VPS, even though positive results on CMA refer to the combination of these groups. We analyzed the groups separately because there were differences in clinical characteristics between the groups and a significant difference in rate of recommended clinical action.

There were significantly more patients younger than 2 years who had abnormal variants compared with VPS (Fisher exact test, $P = 0.02$). It might be assumed that patients younger than 2 years are enriched for neonates with MCA, a test indication documented to result in a substantial rate of abnormal CMA results.[16] Indeed, patients younger than 2 years referred for CMA in this study were significantly more likely to have congenital anomalies as an indication for testing compared with all other age groups (Fisher exact test, $P = 0.001$). There were significantly more patients with DD/ID compared with ASD who had abnormal variants (Fisher exact test, $P = 0.02$). There was a trend toward significance that more patients with ASD had VPS compared with patients with DD/ID (Fisher exact test, $P = 0.12$). There was also a higher rate of dysmorphic features, which would suggest the presence of other anomalies, in patients with abnormal variants compared with patients with VPS (55% vs. 42%; Fisher exact test, $P = 0.14$). The mean size of an abnormal variant was 5.1 Mb, whereas the mean size of a VPS was 655 kb (one tailed t-test, $P = 6.8 \times 10^{-10}$). Several factors could explain why patients with abnormal results had an increased rate of recommended clinical action compared with patients with VPS, including (1) younger age at time of testing, (2) more DD/ID compared with ASD, (3) more patients with dysmorphic features, (4) larger mean variant size resulting in increased number of genes cumulatively identified, and (5) more recognized genomic disorders where medical recommendations are more clear based on prior experience.

Our analysis also showed that 88% of patients with positive CMA results (abnormal variants and VPS) underwent other testing to identify a genetic, metabolic, or neurologic cause for their disease. A total of 356 other tests were ordered for these 194 patients to address the same presenting complaint(s) as CMA testing. There were only 25 cases in which other testing identified a cause for these patients' medical issue(s), and in no case was this cause different from that identified by CMA. We did not perform a formal analysis of cost-benefit or determine if the cost of CMA testing is offset by the savings in other laboratory testing that might be avoided. Several issues make it difficult to perform this type of analysis, including the heterogeneity of genomic conditions identified by CMA, and the difficulty of comparing relative benefits of the various diagnostic tests and interventions. These results suggest that many additional diagnostic tests could be avoided in patients with positive CMA results, and this could represent tangible savings in healthcare resource expenditures as a result of CMA testing.

Previous studies on the clinical use of CMA testing in patients with DD/ID, ASD, and congenital anomalies have primarily focused on the yield of CMA in these patient populations. Positive results (abnormal variants and VPS) were found in 13.1% of CHB patients referred for CMA over the year we studied, similar to previous studies.[1–3] Beyond the rate of positive results after CMA testing, we were interested in the impact of these results on patients' clinical management. CMA can detect numerous genetic disorders, and it is therefore not possible to anticipate the effect on clinical management until the test is performed. For example, the incidental discovery of deleted or duplicated cancer genes in patients referred for other reasons would influence clinical management in an entirely unexpected way. One study found deletions or duplications of regions known to cause hereditary cancer syndromes in 34 of 18,437 individuals tested with CMA (0.18%).[17] Importantly,

they observed that in 24 of these patients (70.6%) the indication for CMA was not a suspected cancer syndrome. Rather, indications among these patients were development delay or dysmorphic features. Their work highlights the presence of unexpected results from CMA that impact a patient's medical management. Similar to their results for hereditary cancer syndromes, we found a CNV that included a cancer causing gene in three patients tested by CMA (1.5% of patients with positive CMA results).

A potential limitation of this study is the lack of an appropriate control group. Certainly, other patients with developmental disabilities receive medical interventions such as specialist referrals, imaging studies, and other lab tests. Our retrospective study design limits our ability to prove that such interventions would not have happened in these patients if they had not received an positive result on CMA. We have the following comments on this issue: (1) Our review of the clinic notes indicates that a specific diagnosis was not suspected in most of these patients prior to CMA, and therefore it seems unlikely that the same pattern of actions would have occurred in the absence of CMA results; (2) An American Board of Medical Genetics board-certified clinical geneticist reviewed the clinic notes from the ordering provider for all subjects with abnormal (M.I.) or VPS (D.T.M.) results; only those clinical actions that were documented to result from the new CMA result were included in this study; (3) It would be difficult to obtain an appropriate control group due to the heterogeneity of conditions identified by CMA, where very few recurrent events are identified.

Another potential limitation of this study is the relatively small number of patients included in our analysis, and that they were only collected from one institution. Our findings can be validated through accumulation of future data from CHB and other institutions. We think that our institution is representative of the types of patients seen in similar settings, and therefore the results should be generalizable. Our hospital is, however, uniquely well suited to study the clinical impact of CMA testing because we have a large patient volume and essentially all patients tested at CHB are followed by physicians at this hospital. As a result, we are one of the largest CMA testing centers with access to patients' complete medical records.

A third potential limitation of this study is that many different physicians care for patients included in the analysis. This made it difficult to separate patients into well-defined clinical groups. As a result, we were only able to sort patients into a few broadly defined groups for study. In addition, because about half of the patients in this study were referred for CMA testing by physicians outside genetics (primarily neurologists), the rate of abnormal physical findings, particularly dysmorphic features, may have been underreported. In fact, geneticists reported dysmorphic features in significantly more patients than neurologists (43% vs. 25%; Fisher exact test, $P = 0.003$). Another possible explanation for this discrepancy is that patients with dysmorphic features may be more likely to be referred to genetics rather than neurology.

We have identified two areas where clinicians can focus to improve the quality of CMA clinical testing in the future. First, we found variability in completion of recommended parental studies. Parental studies are recommended free of charge at our institution for patients with VPS results to determine if the variant is de novo or inherited. Even so, only 63% of patients with VPS had parental studies completed for at least one parent. The completion rate may be improved by better pretest counseling about the potential need for parental studies.

Second, for CMA results to improve medical care, clinicians must be familiar with appropriate medical management for specific CNVs and especially for recurrent genomic disorders. The fact that clinicians from many specialty areas now order CMA revealed variability in clinical management among patients with the same genetic diagnosis. We found that several patients with a CNV known to be associated with medical problems had no clinical action recommended, even though it should have been. With recurrent disorders, it is possible to notice differences in management among patients when compared with more rare CNVs. For example, 16p11.2 microdeletion/microduplication (MIM ID #611913) is one of the most common genomic disorders identified on CMA, occurring in approximately 0.5% of all cases tested in clinical laboratories.[15,18] In a review of 16 patients with 16p11.2 microdeletion, 40% had seizures and 30% had congenital anomalies.[19] However, only two of the nine patients in our study with a deletion or duplication of 16p11.2 who had follow-up visits to discuss their CMA results received specific recommendations for any clinical action. Our results highlight the fact that our collective understanding of genomic disorders is constantly evolving, and clinicians must carefully perform periodic reviews of published information about the CNVs they find in their patients so that they can provide updated recommendations for appropriate clinical management.

In the future, more studies will be needed to determine the effectiveness of the resulting clinical action (referrals, imaging, and tests) that were prompted by positive CMA results. This analysis is beyond the scope of this article and will be addressed in future investigations. Determining the effectiveness of clinical action after a positive CMA result will help better define the appropriate management for such patients and will provide valuable data for cost-benefit analysis.

Beyond the well-documented superiority of CMA over classical cytogenetic methods in terms of diagnostic yield in this patient population, our results support the position that CMA testing should be reimbursed by payers. Not only is CMA superior to G-banded karyotype, which is routinely reimbursed, but we have shown that positive CMA results frequently impact clinical management in this patient population. Furthermore, this impact was not limited to patients with dysmorphic features or congenital anomalies as a coding diagnosis but also included patients with ASD and DD/ID, suggesting that attempts to limit reimbursement to specific diagnostic categories is not valid.

ACKNOWLEDGMENTS

We thank Sharyn Lincoln, MS, CGC, and Brenda Barry, MS, CGC, for help with project planning; Ann Poduri, MD, MPH, who gathered follow-up information for patients referred for CMA by the Department of Neurology; and Kimberly Dunn, MSN, PNP, and Ramzi H. Nasir, MD, from the Developmental Medicine Center. We thank the DNA Diagnostic Laboratory where CMA testing is performed, including Bai-Lin Wu, PhD (Laboratory Director), Yiping Shen, PhD (Director of Research and Development), Va Lip, Xiaoming Shen, and Hong Fang. We would like to thank Mary-Ellen Cortizas, JD, from the Children's Hospital Boston, Department of Laboratory Medicine, for her encouragement and assistance with this QI study.

REFERENCES

1. 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;11:139–146.
2. 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;52:161–169.

3. 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;86:749–764.
4. Cook EH Jr, Scherer SW. Copy-number variations associated with neuropsychiatric conditions. *Nature* 2008;455:919–923.
5. St Clair D. Copy number variation and schizophrenia. *Schizophr Bull* 2009;35:9–12.
6. Mefford HC, Muhle H, Ostertag P, et al. Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet* 2010;6:e1000962.
7. Ravnan JB, Tepperberg JH, Papenhausen P, et al. Subtelomere fluorescence in situ hybridization analysis of 11 688 cases: an evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities. *J Med Genet* 2006;43:478–489.
8. Ballif BC, Sulpizio SG, Lloyd RM, et al. The clinical utility of enhanced subtelomeric coverage in array CGH. *Am J Med Genet A* 2007;143A:1850–1857.
9. Regier DA, Friedman JM, Marra CA. Value for money? Array genomic hybridization for diagnostic testing for genetic causes of intellectual disability. *Am J Hum Genet* 2010;86:765–772.
10. Manning M, Hudgins L. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. *Genet Med* 2010;12:742–745.
11. Lee C, Iafrate AJ, Brothman AR. Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. *Nat Genet* 2007;39(7 Suppl):S48–S54.
12. Kumar RA, KaraMohamed S, Sudi J, et al. Recurrent 16p11.2 microdeletions in autism. *Hum Mol Genet* 2008;17:628–638.
13. Weiss LA, Shen Y, Korn JM, et al. Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med* 2008;358:667–675.
14. Marshall CR, Noor A, Vincent JB, et al. Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 2008;82:477–488.
15. Hanson E, Nasir RH, Fong A, et al. Cognitive and behavioral characterization of 16p11.2 deletion syndrome. *J Dev Behav Pediatr* 2010;31:649–657.
16. Lu XY, Phung MT, Shaw CA, et al. Genomic imbalances in neonates with birth defects: high detection rates by using chromosomal microarray analysis. *Pediatrics* 2008;122:1310–1318.
17. 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;11:314–322.
18. Rosenfeld JA, Coppinger J, Bejjani BA, et al. Speech delays and behavioral problems are the predominant features in individuals with developmental delays and 16p11.2 microdeletions and microduplications. *J Neurodevelop Disord* 2010;2:26–38.
19. Shinawi M, Liu P, Kang SH, et al. Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. *J Med Genet* 2010;47:332–341.

