

Yield of additional genetic testing after chromosomal microarray for diagnosis of neurodevelopmental disability and congenital anomalies: a clinical practice resource of the American College of Medical Genetics and Genomics (ACMG)

Darrel Waggoner, MD¹, Karen E. Wain, MS², Adrian M. Dubuc, PhD³, Laura Conlin, PhD⁴, Scott E. Hickey, MD⁵, Allen N. Lamb, PhD⁶, Christa Lese Martin, PhD², Cynthia C. Morton, PhD^{3,7,8}, Kristen Rasmussen, MS⁹, Jane L. Schuette, MS¹⁰, Stuart Schwartz, PhD¹¹ and David T. Miller, MD, PhD¹² on behalf of the ACMG Professional Practice and Guidelines Committee

Disclaimer: This practice resource is designed primarily as an educational resource for medical geneticists and other clinicians to help them provide quality medical services. Adherence to this practice resource is completely voluntary and does not necessarily assure a successful medical outcome. This practice resource should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinician should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen.

Clinicians are encouraged to document the reasons for the use of a particular procedure or test, whether or not it is in conformance with this practice resource. Clinicians also are advised to take notice of the date this practice resource was adopted, and to consider other medical and scientific information that becomes available after that date. It also would be prudent to consider whether intellectual property interests may restrict the performance of certain tests and other procedures.

Purpose: Chromosomal microarray (CMA) is recommended as the first-tier test in evaluation of individuals with neurodevelopmental disability and congenital anomalies. CMA may not detect balanced cytogenomic abnormalities or uniparental disomy (UPD), and deletion/duplications and regions of homozygosity may require additional testing to clarify the mechanism and inform accurate counseling. We conducted an evidence review to synthesize data regarding the benefit of additional testing after CMA to inform a genetic diagnosis.

Methods: The review was guided by key questions related to the detection of genomic events that may require additional testing. A PubMed search for original research articles, systematic reviews, and meta-analyses was evaluated from articles published between 1 January 1983 and 31 March 2017. Based on the key questions, articles were retrieved and data extracted in parallel with comparison of results and discussion to resolve discrepancies. Variables assessed included study design and outcomes.

Results: A narrative synthesis was created for each question to describe the occurrence of, and clinical significance of, additional diagnostic findings from subsequent testing performed after CMA.

Conclusion: These findings may be used to assist the laboratory and clinician when making recommendations about additional testing after CMA, as it impacts clinical care, counseling, and diagnosis.

Genetics in Medicine (2018) 20:1105–1113; <https://doi.org/10.1038/s41436-018-0040-6>

Keywords: Balanced rearrangement; Chromosomal microarray; genetic testing; mosaicism; UPD

¹Department of Human Genetics, University of Chicago, Chicago, Illinois, USA; ²Autism & Developmental Medicine Institute, Geisinger Health System, Danville, Pennsylvania, USA; ³Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA; ⁴Department of Pathology and Laboratory Medicine, Perelman School of Medicine, Philadelphia, Pennsylvania, USA; ⁵Department of Pediatrics, The Ohio State University College of Medicine, Columbus, Ohio, USA; ⁶Department of Pathology, ARUP Laboratories, University of Utah School of Medicine, Salt Lake City, Utah, USA; ⁷Department of Obstetrics and Gynecology and Reproductive Biology, Brigham and Women's Hospital, Broad Institute of MIT and Harvard, Harvard Medical School, Boston, Massachusetts, USA; ⁸Division of Evolution and Genomics Science, School of Biological Sciences, Manchester Academic Health Science Center, Manchester, UK; ⁹Department of Medical Genetics, Marshfield Clinic, Marshfield, Wisconsin, USA; ¹⁰Department of Human Genetics, University of Michigan School of Medicine, Ann Arbor, Michigan, USA; ¹¹Laboratory Corporation of America® Holdings, Burlington, North Carolina, USA; ¹²Division of Genetics and Genomics, Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts, USA. Correspondence:

Darrel Waggoner (dwaggon@genetics.uchicago.edu) or David T. Miller (david.miller2@childrens.harvard.edu)

The Board of Directors of the American College of Medical Genetics and Genomics approved this statement on 31 January 2018

Submitted 28 March 2018; accepted: 4 April 2018

Published online: 18 June 2018

INTRODUCTION

Chromosomal microarray (CMA) is a widely used clinical genetic diagnostic test, having been endorsed as a first-tier test in the postnatal evaluation of patients with unexplained developmental delay, intellectual disability, congenital anomalies, and autism spectrum disorder.^{1,2} The diagnostic yield of CMA for this patient population has been well documented, including a separate systematic evidence review (SER).³

In the clinic, CMA testing is ordered on a large number of patients, and by a diverse group of clinicians, and it is often ordered in place of the traditional G-banded karyotype. The American College of Medical Genetics and Genomics (ACMG) guideline states that ordering providers should be aware of cytogenomic aberrations not detectable by CMA, including those relevant to various microarray platforms (e.g., single-nucleotide polymorphism [SNP] versus oligonucleotide).² The rationale for this document is that additional testing is often done if the results are negative, or to determine if an abnormal finding is familial or *de novo*, or to identify the underlying mechanism of an abnormal finding, which could also have importance for family counseling. There is variability between laboratory practices with respect to follow-up testing (e.g., fluorescence in situ hybridization (FISH) or quantitative polymerase chain reaction (qPCR) to confirm variants or identify the mechanism of copy-number variants. Until now, there has been no SER to summarize how often specific cytogenomic aberrations are undetected by CMA alone or when additional testing is needed to clarify the mechanism of abnormal findings; this information is important for clinical decision-making regarding the need or benefit of additional testing following CMA.

At the request of the ACMG Professional Practice and Guidelines Committee, we convened a working group in December 2014 to conduct an evidence review.

The working group devised the following key questions:

1. What types of genomic abnormalities (e.g., balanced rearrangement, mosaicism, and uniparental disomy) that may not be reliably detected by CMA are identified with other related tests such as karyotype or fluorescence in situ hybridization (FISH) in a child with unexplained developmental delay, intellectual disability, congenital anomalies, or autism spectrum disorder, and what are the frequencies of these genomic abnormalities when there are (a) normal CMA results or (b) abnormal CMA results?
2. Does management change as a result of additional testing that identifies a pathogenic abnormality not detected by CMA or that further clarifies the original CMA findings?

The evidence review group (ERG) expanded these key questions according to categories of genomic events. Questions were developed using the Agency for Healthcare Research and Quality (AHRQ) Effective Healthcare Program that includes the use of the PICOTS framework (Population, Intervention, Comparison, Outcomes, Timing, and Setting) of

the topic to be reviewed. Key questions were drafted by individuals within the ERG and then agreed upon by the entire group (Supplemental Table 1).

We considered diagnostic yield an important outcome because previous studies have documented the benefit of a genetic diagnosis with regard to avoiding additional testing and ensuring accurate genetic counseling and informed reproductive decisions.⁴⁻⁷

This evidence review has potential to impact providers' recommendations for children with unexplained developmental delay, intellectual disability, congenital anomalies, or autism spectrum disorder who are candidates for additional testing after CMA. In turn, knowledge obtained from this review may affect genetic counseling regarding diagnosis and reproductive risks, and laboratory practices for ancillary testing related to CMA.

MATERIALS AND METHODS

Evidence Review Group

A multidisciplinary group, including experts in cytogenetics, molecular genetics, CMA analysis (specifically including SNP array), genetic counseling, and clinical genetics, was assembled to define the key questions, determine inclusion and exclusion criteria, formulate literature search strategies, screen titles and abstracts, extract relevant data, grade the evidence, and synthesize the findings. ERG members were all volunteers, and there was no funding support for this effort. Volunteers were divided into four subgroups of three individuals per group; each subgroup consisted of one clinician (medical geneticist or genetic counselor) and two laboratory geneticists. This grouping was done to balance expertise among the groups and also to balance any perceived conflict of interest (COI) among individuals who receive compensation for working in a laboratory that performs CMA or other genetic testing.

Data sources and article selection

Each subgroup performed a search of PubMed for evidence relevant to the specific key questions assigned to their subgroup. Search terms are available as Supplemental Table 2.

Literature searches were focused on publications written in English and appearing between 1 January 1983 and 31 March 2017. Searches were done using keywords related to each key question (linked by the words "OR" or "AND"; Supplemental Table 2). The patient population for the evidence review was defined as postnatal patients with nonspecific intellectual disability (ID), developmental delay (DD), autism spectrum disorder (ASD), and/or multiple congenital anomalies (MCA) and is summarized as "PATIENTS" in Supplemental Table 1.

Inclusion/exclusion criteria for articles

Articles were selected for inclusion if they addressed our key questions and represented one of the following: meta-analysis of prior studies, systematic reviews of the literature, evidence-based guidelines, case series, randomized controlled trials, population studies, cohort studies, or case-control studies.

Articles were selected for exclusion if they represented animal studies, languages other than English, and case reports of fewer than three cases, or if the article was already represented in another review article that was included.

Data extraction

After conducting the literature searches, subgroups extracted data for their questions. Titles of articles were reviewed and then publications excepted based on exclusion criteria. Remaining articles were then reviewed in more detail by reading the abstract, or the entire publication, as needed. Articles were selected for inclusion based on the criteria above for the specific questions being addressed by each subgroup. Some subgroups ended up with papers overlapping with those pertinent to other subgroups. We included information about the population studied, type of genetic testing, types and frequencies of genomic events observed, and diagnostic yield of testing.

Grading strength of evidence

Each subgroup graded the quality of evidence for each included reference using a four-tiered grading scheme for diagnostic evidence (Supplemental Table 3). The entire ERG was reconvened as needed (April–December 2015) to discuss the grading of evidence.

Risk of bias

The outcomes of interest for this review related to diagnostic yield. As such, none of the studies included involved an intervention. Therefore, we considered our grading of the evidence as a means to assess risk of bias for individual studies.

Synthesis of evidence

After grading of the evidence, subgroups drafted a synthesis of evidence from the retained studies (Supplemental Table 4). The synthesis of evidence is organized around genomic events that may contribute to a diagnosis in the patient, including balanced rearrangements, chromosomal mosaicism, unbalanced insertions, multiple copy-number variants (CNVs), and regions of homozygosity and uniparental disomy.

RESULTS

The number of articles discovered through the PubMed search, and the number selected based on inclusion criteria, are available in Supplemental Table 2. We identified a total of 9841 publications and selected 25 to include in our review. Of those identified (and number included), 7561 addressed balanced rearrangements ($n = 10$), 403 insertions ($n = 4$), 297 UPD ($n = 7$), and 1580 mosaicism ($n = 6$). The majority were retrospective or mixed retrospective/prospective studies. Grading and summary of the studies included are available in Supplemental Table 4, grouped according to key questions evaluated.

BALANCED REARRANGEMENTS

CMA will not detect balanced rearrangements, such as balanced translocations, insertions, or balanced inversions,

which may be detected by karyotype or FISH. Ten articles were included to provide evidence for the frequencies of such events, and whether it is likely to be clinically significant with respect to establishing a genetic diagnosis. Approximately 1% or fewer of cases with a normal CMA will have a balanced rearrangement detected by karyotype; multiple studies revealed incidences of 0.8%, 1.3%, and 0.28%, respectively.^{8–10} A meta-analysis of 36,325 patients estimated an incidence of 0.78% for an apparently balanced rearrangement.¹¹

There are now several studies providing evidence that approximately 40% of patients with an abnormal phenotype and an apparently balanced cytogenetic rearrangement by G-banding have a cryptic imbalance at the site of the breakpoints potentially explaining the phenotype.^{12–16} However, when CMA is normal and a cryptic imbalance is not identified, it is difficult to estimate the likelihood that the balanced rearrangement detected by karyotype explains the abnormal phenotype without the use of sequencing technology that is not clinically available at this point. Parental studies can provide information as to whether a rearrangement is *de novo* versus inherited. While inheritance status does not always predict pathogenicity, it is likely to be more so in *de novo* cases. A limited number of publications have investigated the phenotypic consequences of balanced rearrangements, as it can be difficult to determine causation due to disruption and/or dysregulation of gene expression. These studies indicate that an apparently balanced rearrangement at the G-banding level may be associated with or causative of the phenotype in less than half of cases in the setting of a patient with congenital anomalies or intellectual disability.^{12–16} Further details are summarized in the Supplemental Material.

Notably, these studies and others investigating apparently balanced rearrangements found clinically relevant imbalances seemingly unrelated to the breakpoints of the primary rearrangements (e.g., distant to the breakpoint or on a different chromosome). If these cases had been studied by CMA first, the imbalance would have been detected but the unrelated balanced rearrangement would have been missed. This observation indicates that some patients with an abnormal phenotype and a pathogenic CNV could also have a concomitant balanced rearrangement not detected by CMA. In a case series of 33 subjects, 2 of 7 cases with a balanced rearrangement had CNVs distant from the balanced breakpoints.¹⁷ Additional research will be needed to estimate the frequency of this occurrence. It is unclear if the co-occurrence of a balanced rearrangement and an unrelated CNV has a combined effect on the phenotype, or the frequency with which this type of event occurs, but it may not be uncommon.

CHROMOSOMAL MOSAICISM

Six studies addressing incidence and detection of mosaicism were included. Chromosomal mosaicism (whole or segmental) detected by CMA analysis, typically from a peripheral blood specimen (sometimes from fibroblasts), was present in approximately 0.5–1.2% of individuals with unexplained DD, ID, ASD, and MCA.^{18–23} The ability to detect mosaicism

appeared to be increased with CMA due to the greater sensitivity of molecular testing technologies as compared with cytogenetic techniques. Mosaicism for whole-chromosome aneuploidy has been detected at levels as low as 20% and 5% for array comparative genomic hybridization (CGH)²⁰ and SNP arrays,²¹ respectively, and was comparable with levels determined by FISH and/or chromosome analysis (depending on the number of cells counted). Furthermore, current array platforms detected segmental mosaicism at levels beyond resolution of standard chromosome analysis (<4 Mb), including mosaicism for CNVs within a single gene.¹⁸

Although most instances of mosaicism are detected by either method, there are cases for which karyotype and CMA revealed mosaicism missed by the alternative. Results of five studies comparing detection of chromosomal mosaicism between routine chromosome analysis and CMA included cases in which CMA had increased detection. In 0.13% (5/3710 cases), CMA detected mosaicism not discovered by standard karyotyping.⁸ Conversely, mosaicism was found by karyotype alone in 6/3710 cases (0.16%). Further details are summarized in Supplemental Material.

UNBALANCED INSERTIONS

Four studies addressing unbalanced insertions were included. CMA results may show a copy-number gain that could represent either a tandem duplication or an unbalanced insertion. Distinguishing between these two scenarios requires additional methods and can have implications for pathogenicity as the phenotype could be complicated by a gene disruption from the insertion in addition to the copy-number gain. Moreover, apparently *de novo* pathogenic or likely pathogenic deletions or duplications in affected individuals can be a result of balanced insertions in parents with important implications for counseling about recurrence risk. The review here addresses the question of whether parental studies are indicated to help determine recurrence risk and do not address the concept of parental studies being utilized for interpreting CNVs of uncertain clinical significance by determining inheritance.

Unbalanced insertions appear to account for approximately 1:500 individuals referred for CMA analysis and were involved in roughly 2–2.5% of all abnormal CMA studies, including both deletions and duplications. Approximately 2% were due to balanced insertions in one of the parents.^{24–27}

In addition to the risk of triplosensitivity of genes contained in the duplicated interval or gene disruption, the potential for gene disruption, fusion genes, or more complex rearrangements to be causative of the phenotype was investigated by sequencing technologies. Interstitial duplications appeared to be tandem and in direct orientation in the majority of cases (83%) leaving one intact copy of the duplicated gene. The remaining 17% of apparent duplications represented triplications embedded within duplications (8.4%), adjacent duplications (4.2%), insertions (2.5%), and complex rearrangements (1.7%);²⁶ further details are summarized in the Supplemental Material.

MULTIPLE CNVS IDENTIFIED

Ten articles that addressed the findings of multiple CNVs were included. Of note, this review did not focus on CMA results suggestive of an unbalanced translocation. A CMA result with a single terminal deletion and single terminal duplication is suggestive of an unbalanced translocation, which may be inherited or *de novo*, and is typically referred for parental testing to determine if segregated from a balanced translocation carrier. A positive CMA result may also show multiple copy gains/losses (two or more), and these imbalances could represent a complex apparently balanced reciprocal rearrangement that would be detectable by G-banded karyotype or metaphase FISH. There are few studies that allow for an accurate estimate of the frequency of this situation. One study showed that 88% (23/26) of cases with two or more copy-number imbalances in the same chromosome and 87% (33/38) with two or more copy-number imbalances in two different chromosomes had a complex karyotype.⁸ Two other studies reported two cases with multiple CNVs and a complex karyotype, and nine cases where a complex karyotype was discovered in the setting of a single CNV (seven with duplication and two with deletion).^{9,28} These studies suggest that cases with two or more imbalances should have a karyotype or metaphase FISH, depending on the size of the CNVs, to evaluate the possibility of a more complex structural rearrangement.

In addition, multiple studies now document cases with a single CNV for which a karyotype or FISH clarifies the mechanism. These cases include ring and marker chromosomes as well as other complex rearrangements.^{8,28,29} This additional testing to clarify the genetic mechanism associated with the CNVs is necessary for accurate recurrence risk counseling.

DETECTION OF REGIONS OF HOMOZYGOSITY AND UNIPARENTAL DISOMY

Seven studies that addressed ROH and UPD were included. In addition to copy-number variation, SNP arrays may detect copy-neutral regions of homozygosity (ROH) through the targeting of biallelic markers dispersed throughout the genome. Large segments of copy-neutral ROH may be clinically significant as an indicator of uniparental disomy (UPD) or may reflect inheritance of a genomic segment from a common ancestor—identity by descent (IBD). ROH reflecting IBD has been observed in many different epidemiologic studies of healthy members of the general population.³⁰ In some cases where an autosomal recessive mode of inheritance is suspected, identification of ROH may aid the diagnostic evaluation by uncovering a gene in which a homozygous pathogenic mutation resides.

Although SNP array can be suggestive of UPD, all cases of UPD may not be detected, and detection rates may vary between laboratories. Detection varies based on the specifics of the UPD (i.e., isodisomy versus heterodisomy, whole chromosome versus segmental). In one study, more than 95% of patients with Beckwith–Wiedemann syndrome from

paternal UPD were detected by SNP array, as many of these were due to segmental UPD.³¹ In contrast, Prader–Willi syndrome (PWS) was frequently due to heterodisomy, although meiotic recombination sometimes leads to a region of isodisomy with a corresponding lower detection rate by SNP array.³² Therefore, additional testing may be warranted to assess UPD depending on the clinical phenotype.

In general, reportable ROH are 3–10 megabases in size. In large population studies, ROH were found in 5–10% of cases.^{23,33,34} In two large cohort studies, 70–80% of individuals with ROH had involvement of at least two chromosomes, suggestive of IBD.^{33,34} Efforts to refine cases of ROH that were suspicious for UPD have been successful. Criteria were developed to define a “UPD signature” and prospectively test 46 cases with ROH suspicious for underlying UPD.³⁵ This study found that further testing confirmed UPD in as many as 29/46 (63%) cases. Criteria used for suspected UPD included ROH involving only a single chromosome (especially one that was known to be associated with a UPD phenotype) and telomeric ROH.³⁵ Another study identified 11 cases to be suspicious for UPD, either due to mapping to a chromosome with a known human imprinting disorder or due to involvement of very large chromosomal segments, and microsatellite analysis confirmed UPD in 5/11 cases.²³

It is difficult to estimate how often a confirmed UPD chromosome or chromosome segment is responsible for a patient’s phenotype. Several studies have reported whether the ROH mapped to chromosomes with known human imprinting disorders or imprinted genes. One study reported that in 12/19 cases involving a single chromosome, the ROH mapped to a chromosome with a known human imprinting disorder.³³ Another study found single-chromosome ROH mapping to chromosome 14 in seven cases, but two cases had confirmation testing that was negative for UPD.³⁴ Finally, one study reported that approximately 50% of their cases mapped to a chromosome with imprinted genes and/or had a recognizable imprinting disorder phenotype.³⁵

Under certain specific clinical circumstances, ROH that is indicative of IBD may be used to narrow the search for a causative gene wherein a homozygous variant causing an autosomal recessive (AR) disease is suspected. It appears that this occurs only rarely (~1% of cases with ROH) as reported in two large studies.^{23,33,36} Move reference 36 to this box. In the setting of parental consanguinity and/or results consistent with close parental relatedness, an AR single-gene disorder was diagnosed in four patients from two families for a yield of 4/59 (7%).³³

DISCUSSION

The goal of this evidence review is to synthesize the literature regarding diagnostic yield and, where possible, clinical significance of additional testing after either a normal or abnormal CMA. Additional testing is considered either to clarify further CMA results or to detect genomic events, which cannot be detected by CMA.

It is known that there are certain types of genomic abnormalities not detected by CMA (simple and complex apparently balanced rearrangements, etc.). CMA results do not determine the genetic mechanism associated with a CNV and, therefore, are limited in the ability to inform family counseling. SNP array findings may suggest certain abnormalities such as UPD and IBD, but cannot confirm their clinical significance. In these situations, additional testing is warranted to inform further clinical significance and family counseling.

We have described the occurrence of additional genomic events in the presence of both normal and abnormal CMA results in a defined population. These results can be utilized to inform the likelihood of additional testing having clinical implications. The published literature mostly focuses on describing the incidence of the genetic abnormalities investigated in this review, and was not designed to assess clinical outcomes or cost–benefit analysis. There are studies documenting the benefit of a genetic diagnosis allowing accurate genetic counseling and informed reproductive decisions.^{4–7} Therefore, our working group was limited in its ability to make recommendations as to when to perform additional tests based on the lack of studies documenting clinical outcomes or cost–benefit analysis. The evidence is summarized below according to negative or positive CMA results and genomic event type. When appropriate, the expert opinion of the group is reported, and the types of tests to consider and clinical implications are documented, but the decision to pursue additional testing should be made on an individual case basis.

The findings of the review are summarized in Table 1.

BALANCED REARRANGEMENTS

A negative or normal CMA may miss a balanced rearrangement or possible mosaicism. Approximately 0.8% of cases with negative CMA results will be expected to have a balanced rearrangement (e.g., translocation, inversion, insertion) that would be detected by a karyotype.⁸ We believe this could be an underestimate because karyotype can additionally detect other structurally abnormal chromosomes, such as rings and markers, which were not included in the search of the literature when examining this specific question. This review focused specifically on a postnatal population with a defined phenotype and does not account for every clinical scenario that may be encountered. There are other circumstances that may be clinically suggestive of the need for a karyotype after normal CMA, including prenatal findings of clinical abnormalities or a family history suggestive of a balanced rearrangement.

A balanced rearrangement may interrupt a gene or dysregulate gene expression and hence be the pathogenic cause of the patient’s phenotype. However, given an incidence of 0.78–1.3% for missed balanced events,^{8–10} overall it is estimated that such an event could be the cause of an abnormal phenotype in about 0.56–0.91% of all cases with normal CMA.

Table 1 Summary of focused evidence review of the literature of CMA findings in patients with unexplained DD, ID, MCA, or AD

CMA finding	Genetic event	Clinical importance/counseling issues	Additional diagnostic yield	Additional tests considered
Normal or negative	Balanced rearrangement (e.g., translocation, inversion, insertion)	A balanced rearrangement may interrupt a gene or disrupt regulation of gene expression; recurrence risk counseling is indicated for offspring of parent with a balanced rearrangement	An estimated additional diagnostic yield of 0.78–1.3% after a negative CMA	G-banded karyotype
	Mosaicism for segmental or whole-chromosome aneuploidy	There is very limited evidence indicating whether the presence of mosaicism missed by CMA is associated with an abnormal phenotype	It is not clear what percent of cases is the result of a mosaic chromosomal abnormality that is not detected by either method	G-banded karyotype, FISH
Pathogenic or likely pathogenic nonrecurrent single CNV	Copy-number gains due to insertion (versus tandem duplication)	CMA results may show a copy-number gain that could represent either tandem duplication or an unbalanced insertion. When inherited from parent with balanced insertion, there are implications for recurrence risk counseling	Approximately 2% of deletion and duplications are due to balanced insertion in one of the parents	Karyotype or metaphase FISH on the proband and/or parents (depending on the size of the rearrangement)
Multiple CNVs (≥2)	Insufficient evidence to determine incidence	Associated with a complex karyotype in 88% of cases in one study	Additional studies may be considered as appropriate on an individual case basis	G-banded karyotype and/or metaphase FISH
Copy-neutral ROH on a single chromosome	UPD	Accurate diagnosis of UPD is only possible with additional molecular testing: follow-up testing should be considered to confirm UPD whenever ROH involves only one chromosome and raises the clinical suspicion for an imprinting disorder or the phenotype is suggestive of an imprinting syndrome	63% of cases when (1) large ROH, (2) telomeric, (3) affecting chromosome with known imprinting disorder	Microsatellite markers and/or methylation studies
Copy-neutral ROH on multiple chromosomes (≥2)	Identity by descent (IBD)	May assist in identifying an autosomal recessive disorder caused by a gene in the homozygous region	Results in diagnosis in up to 7% of cases (in a setting of known parental relatedness/consanguinity)	Follow-up sequencing and/or targeted copy-number detection

CNV copy-number variation, DD developmental delay, ID intellectual disability, MCA multiple congenital anomalies, AD UPD uniparental disomy, ROH regions of homozygosity, FISH fluorescence in situ hybridization, CMA chromosomal microarray

Sequencing has tremendous potential to determine the nature and effect of the breakpoints on specific genes. A recently published large cohort study suggests that balanced rearrangements are often associated with genomic imbalances <10 kb, direct gene disruption/truncation, or modulation of long-range regulatory interactions; the expression and known function of genes involved in these disruptions are suggestive of their role in causing a phenotype.³⁷ A prenatal study similarly showed disruption of regulatory domains and the associated genes can be predictive of the phenotype.³⁸ We believe these studies provide further evidence of the importance of incorporating sequencing technology into the investigation and interpretation of cytogenomic abnormalities. The clinical consequence of these disruptions is related to more than just the gene(s) involved in the translocation, deletion, or duplication, but is also related to the change in gene expression from disrupted regulatory domains.

Overall, we find that the data support balanced rearrangements associated with or causative of the phenotype in a meaningful proportion of cases in the setting of a patient with congenital anomalies or intellectual disability. At the level of an individual patient, however, the presence of a balanced rearrangement by karyotype is not predictive of a phenotype. To correlate the balanced event with the phenotype, knowledge of the specific gene(s) involved in the breakpoint is required. Due to low overall incidence and current implementation of clinical genome sequencing to determine if balanced rearrangements interrupt/dysregulate a gene, it may not be cost effective to follow up all negative CMA test results with karyotype analysis. Further technological development is anticipated to address this area (e.g., low pass genome sequencing).

CHROMOSOMAL MOSAICISM

Consideration of mosaicism is complicated by the level of mosaicism in the sample and the likelihood that the abnormality contributes to pathogenicity. Most studies document that detection of whole-chromosome mosaicism is similar for both karyotype and CMA, at levels of ~5% with SNP arrays.²¹ SNP arrays have greater sensitivity for detection of segmental mosaicism. Although several cases⁸ document mosaicism detected by karyotype or FISH and missed by CMA, this group's expert opinion is that the unknown overall incidence of these events and absent data documenting pathogenicity preclude routine recommendation of follow-up studies.

CMA may still have high sensitivity when there are clinical features suspicious for chromosomal mosaicism, such as phenotypic findings consistent with X chromosome mosaicism, pigmentary abnormalities, or growth asymmetry associated with intellectual disability. However, ancillary testing should be considered, including a karyotype plus FISH, as well as sampling more than one tissue type.

UNBALANCED INSERTIONS

A copy-number gain may be due to interstitial duplication (83%)²⁴⁻²⁷ or to an insertion. An insertion may be inherited

from a parent with a balanced insertion (~2%),²⁴⁻²⁷ which would have implications for recurrence risk counseling. Follow-up testing of the proband to differentiate the type of copy gain and of the parents to assess for a balanced insertion requires karyotype or FISH, depending on the size of the gain. An insertion that disrupts a gene at the site of insertion or dysregulates a gene(s) due to rewiring of a chromatin domain may also have implications for the phenotype. Genomic sequencing to detect these types of events is not widely clinically available at this time. A single CNV deletion may also be inherited from a parent who has a balanced insertion, and detection requires parental karyotyping and/or FISH studies.

Follow-up testing of the proband and parents, for the purposes of identifying insertional events, only applies to unique, nonrecurrent CNVs as recurrent events are due to low copy repeat (segmental duplication) mediated rearrangements. We do not recommend routine parental follow-up studies by metaphase FISH for duplications or deletions that are classified as a variant of uncertain significance (VUS) for the purposes of identifying parental balanced insertions. This recommendation does not apply to the utilization of parental studies for the purpose of VUS interpretation.

MULTIPLE CNVS IDENTIFIED

A positive CMA detecting more than two CNVs is associated, in one study, with a complex chromosome rearrangement in nearly 90%⁸ of cases, although these rearrangements may appear as balanced rearrangements by chromosome analysis. Following from this review, we believe G-banded karyotype and/or metaphase FISH may be considered on an individual basis when there is a positive CMA test. Detection of a complex karyotype can also occur in cases with a single CNV and occurs more frequently with duplications than deletions.⁸

DETECTION OF REGIONS OF HOMOZYGOSITY AND UNIPARENTAL DISOMY

SNP array may reveal ROH on one chromosome suggestive of possible whole-chromosome or segmental UPD, or of multiple chromosomes suggestive of IBD. Additional studies including microsatellite markers and/or methylation studies are needed to confirm UPD. Determining UPD may have importance for establishing a diagnosis and for recurrence risk counseling. SNP array may not always detect heterodisomy UPD, so this group's expert opinion is that a clinical phenotype suggestive of an imprinting disorder should determine the need for additional testing. When ROH involves multiple chromosomes, IBD would be considered and may indicate an autosomal recessive disease. A careful search of the homozygous regions may reveal specific genes identified in autosomal recessive diseases that match the patient's phenotype. We suggest consideration of follow-up sequencing and/or targeted copy-number detection to help establish a diagnosis when multiple regions of ROH are detected.

Conclusion

The studies that are available report the incidence of varying cytogenomic abnormalities and allow an estimation of the likelihood that a specific abnormality may be missed after CMA has been completed. Our group found it difficult to make specific recommendations for or against additional testing because the studies do not document the clinical impact and outcomes of the diagnosis of each genomic event. In addition, there are no cost-based analyses of additional testing. An additional limitation to this review is the lack of evidence documenting the medical outcomes and benefits of establishing a genetic diagnosis.

The benefit of establishing a diagnosis and providing accurate recurrence risk counseling is widely accepted as critical to informing reproductive options, yet there appears to be a dearth of research published investigating these important outcomes or health outcomes. Future studies that investigate clinical outcomes may enable more specific recommendations. Currently, there is insufficient evidence for recommendations to develop guidelines/guidance for next steps in diagnosis, and as such, an expert consensus process could be a next step for guideline development. Methods for this could include an expert consensus process based on the Delphi method.^{39,40}

We acknowledge certain limitations to this review. We relied on only one database for the ascertainment of published evidence, which might limit the scope of publications available. However, deficiencies in identifying key literature was not found by the diverse expertise of the working group members. This study did not meet the full requirement of a systematic evidence review, and thus, there is a possibility of bias in the results presented and in the opinions formulated. Specifically, we did not develop methods to assess risk of bias for individual studies, we did not summarize the strength of evidence for each main outcome of interest, nor did we describe the limitations at the study and outcome level. However, because our main outcomes related to diagnostic yield, we chose to use the grading of evidence to assess individual study bias. Because of the limited research available, we also chose to summarize the strength of the evidence and limitation in aggregate.

The ultimate goal for this evidence review was to document the evidence for observed frequency of different types of genomic events, and how well they can be detected with available clinical testing. The broader perspective is that an ever-increasing proportion of future diagnostic genetic testing will be based on massively parallel sequencing, and the potential for more sensitively identifying and accurately describing complex genomic events by sequencing makes this an important time to reflect on how it may impact clinical diagnostic testing.

There is evidence to suggest that sequence-based data will complement the information obtained by CMA and/or traditional cytogenetics when it becomes more widely available in a clinical setting. Research studies have demonstrated that structural variants not involving known disease

genes can cause clinical symptoms through disruption of higher-order genomic organization such as interference with normal regulation of gene expression.^{15,37} How often this occurs in individuals with apparent genomic syndromes remains to be determined.

We hope current and future efforts to incorporate sequence-based methods for detection of chromosomal aberrations will be designed to provide two goals. One, more precise detection of the chromosomal aberrations summarized here, including apparently balanced rearrangements, imbalances representing complex rearrangements, mosaicism, and isodisomy. Second, to add additional clinically relevant information through characterization of complex rearrangements in either apparently balanced or unbalanced genomes. Considering next-generation sequencing (NGS)-based methods in the context of diagnostic yield for the types of genomic changes discussed in this evidence review will help to determine whether NGS-based methods can be employed in place of CMA or conventional cytogenetic methods based on the clinical situation. As a next step, it will be important to assess the role of NGS approaches in diagnosing the cause of neurodevelopmental disabilities and congenital anomalies, as the ability to detect copy-number variation and complex rearrangements by NGS improves.

This review documents the incidence of cytogenomic abnormalities that may be missed or need further testing for clarification after CMA has been performed for patients with neurodevelopmental issues and birth defects. These issues are relevant to healthcare providers, patients, policy makers, and payers. The decision to perform additional testing requires consideration of the specific clinical indication, the cytogenomic abnormality being considered, and the availability of clinical testing. Careful review by a geneticist or healthcare professional familiar with the limitations of CMA would inform these determinations.

ELECTRONIC SUPPLEMENTARY MATERIAL

The online version of this article (<https://doi.org/10.1038/s41436-018-0040-6>) contains supplementary material, which is available to authorized users.

ACKNOWLEDGEMENTS

Support is recognized by the National Institutes of Health (GM061354 to C.C.M.)

DISCLOSURE

Allen Lamb is an employee of ARUP. Christa Lese Martin is an employee of Geisinger, has received grants from the National Institutes of Health (NIH) and Simons Foundation, serves on ACMG Board of Directors, and is a consultant for the Jackson Laboratory. David Miller is a clinical consultant to Claritas Genomics (nonequity professional services agreement; ended 1 August 2017). Stuart Schwartz is an employee of Laboratory Corporation of America® Holdings and serves on the Cancer Genomics Consortium Board of Directors. Karen Wain is an

employee of Geisinger. The remaining authors declare no conflict of interest.

REFERENCES

- Miller DT, Adam MP, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic testing for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet.* 2010;86:749–64.
- Manning M, Hudgins L. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. *Genet Med.* 2010;12:742–5.
- Michelson DJ, Shevell MI, Sherr EH, et al. 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;77:1629–35.
- Turner G, Boyle J, Partington MW, et al. Restoring reproductive confidence in families with X-linked mental retardation by finding the causal mutation. *Clin Genet.* 2008;73:188–90.
- Saam J, Gudgeon J, Aston E, et al. How physicians use array comparative genomic hybridization results to guide patient management in children with developmental delay. *Genet Med.* 2008;10:181–6.
- Hoffmann TJ, Windham GC, Anderson M, et al. Evidence of reproductive stoppage in families with autism spectrum disorder: a large, population-based cohort study. *JAMA Psychiatry.* 2014;71:943–51.
- Wood CL, Warnell F, Johnson M, et al. Evidence for ASD recurrence rates and reproductive stoppage from large UK ASD research family databases. *Autism Res.* 2015;8:73–81.
- Bi W, Borgan C, Pursley AN, et al. Comparison of chromosome analysis and chromosomal microarray analysis: what is the value of chromosome analysis in today's genomic array era? *Genet Med.* 2013;15:450–7.
- Gekas J, Vallee M, Castonguay L, et al. Clinical validity of karyotyping for the diagnosis of chromosomal imbalance following array comparative genomic hybridization. *J Med Genet.* 2011;48:851–5.
- Pickering DL, Eudy JD, Olney AH, et al. Array-based comparative genomic hybridization analysis of 1176 consecutive clinical genetics investigations. *Genet Med.* 2008;10:262–6.
- 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;52:161–9.
- De Gregori M, Ciccone R, Magini P, et al. Cryptic deletions are a common finding in "balanced" reciprocal and complex chromosome rearrangements: a study of 59 patients. *J Med Genet.* 2007;44:750–62.
- Higgins AW, Alkuraya FS, Bosco AF, et al. Characterization of apparently balanced chromosomal rearrangements from the developmental genome anatomy project. *Am J Hum Genet.* 2008;82:712–22.
- Schluth-Bolard C, Labalme A, Cordier MP, et al. Breakpoint mapping by next generation sequencing reveals causative gene disruption in patients carrying apparently balanced chromosome rearrangements with intellectual deficiency and/or congenital malformations. *J Med Genet.* 2013;50:144–50.
- Lupianez DG, Kraft K, Heinrich V, et al. Disruption of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. *Cell.* 2015;161:1012–25.
- Baptista J, Mercer C, Prigmore E, et al. Breakpoint mapping and array CGH in translocations: comparison of a phenotypically normal and an abnormal cohort. *Am J Hum Genet.* 2008;82:927–36.
- Talkowski ME, Rosenfeld JA, Blumenthal I, et al. Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell.* 2012;149:525–37.
- Pham J, Shaw C, Pursley A, et al. Somatic mosaicism detected by exon-targeted, high-resolution aCGH in 10,362 consecutive cases. *Eur J Hum Genet.* 2014;22:969–78.
- Hoang S, Ahn J, Mann K, et al. Detection of mosaicism for genome imbalance in a cohort of 3,042 clinical cases using an oligonucleotide array CGH platform. *Eur J Med Genet.* 2011;54:121–9.
- Ballif BC, Rorem EA, Sundin K, et al. Detection of low-level mosaicism by array CGH in routine diagnostic specimens. *Am J Med Genet A.* 2006;140:2757–67.
- Conlin LK, Thiel BD, Bonnemann CG, et al. Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. *Hum Mol Genet.* 2010;19:1263–75.
- Cheung SW, Shaw CA, Scott DA, et al. Microarray-based CGH detects chromosomal mosaicism not revealed by conventional cytogenetics. *Am J Med Genet A.* 2007;143A:1679–86.
- Bruno DL, White SM, Ganesamoorthy D, et al. Pathogenic aberrations revealed exclusively by single nucleotide polymorphism (SNP) genotyping data in 5000 samples tested by molecular karyotyping. *J Med Genet.* 2011;48:831–9.
- Neill NJ, Ballif BC, Lamb AN, et al. Recurrence, submicroscopic complexity, and potential clinical relevance of copy gains detected by array CGH that are shown to be unbalanced insertions by FISH. *Genome Res.* 2011;21:535–44.
- Nowakowska BA, de Leeuw N, Ruivenkamp CA, et al. Parental insertional balanced translocations are an important cause of apparently de novo CNVs in patients with developmental anomalies. *Eur J Hum Genet.* 2012;20:166–70.
- Newman S, Hermetz KE, Weckselblatt B, Rudd MK. Next-generation sequencing of duplication CNVs reveals that most are tandem and some create fusion genes at breakpoints. *Am J Hum Genet.* 2015;96:208–20.
- Kang SH, Shaw C, Ou Z, et al. Insertional translocation detected using FISH confirmation of array-comparative genomic hybridization (aCGH) results. *Am J Med Genet A.* 2010;152A:1111–26.
- Caramschi E, Stanghellini I, Magini P, et al. Predictive diagnostic value for the clinical features accompanying intellectual disability in children with pathogenic copy number variations: a multivariate analysis. *Ital J Pediatr.* 2014;40:39.
- Sanmann JN, Pickering DL, Golden DM, et al. Assessing the utility of confirmatory studies following identification of large-scale genomic imbalances by microarray. *Genet Med.* 2015;17:875–9.
- McQuillan R, Leutenegger AL, Abdel-Rahman R, et al. Runs of homozygosity in European populations. *Am J Hum Genet.* 2008;83:359–72.
- Keren B, Chantot-Bastarud S, Brioude F, et al. SNP arrays in Beckwith-Wiedemann syndrome: an improved diagnostic strategy. *Eur J Med Genet.* 2013;56:546–50.
- Tucker T, Schlade-Bartusiak K, Eydoux P, et al. Uniparental disomy: can SNP array data be used for diagnosis? *Genet Med.* 2012;14:753–6.
- Wang JC, Ross L, Mahon LW, et al. Regions of homozygosity identified by oligonucleotide SNP arrays: evaluating the incidence and clinical utility. *Eur J Hum Genet.* 2015;23:663–71.
- Wisniewska J, Bi W, Shaw C, et al. Combined array CGH plus SNP genome analyses in a single assay for optimized clinical testing. *Eur J Hum Genet.* 2014;22:79–87.
- Papenhausen P, Schwartz S, Risheg H, et al. UPD detection using homozygosity profiling with a SNP genotypic microarray. *Am J Med Genet Av.* 2011;155A:757–68.
- Sund KL, Zimmerman SL, Thomas C, et al. Regions of homozygosity identified by SNP microarray analysis aid in the diagnosis of autosomal recessive disease and incidentally detect parental blood relationships. *Genet Med.* 2013;15:70–78.
- Redin C, Brand H, Collins RL, et al. The genomic landscape of balanced cytogenetic abnormalities associated with human congenital anomalies. *Nat Genet.* 2017;49:36–45.
- Ordulu Z, Kammin T, Brand H, et al. Structural chromosomal rearrangements require nucleotide-level resolution: lessons from next-generation sequencing in prenatal diagnosis. *Am J Med Genet.* 2016;99:1015–33.
- Brook RH, Chassin MR, Fink A, Solomon DH, Kosecoff J, Park RE. A method for the detailed assessment of the appropriateness of medical technologies. *Int J Technol Assess Health Care.* 1986;2:53–63.
- Fink A, Kosecoff J, Chassin M, Brook RH. Consensus methods: characteristics and guidelines for use. *Am J Public Health.* 1984;74:979–83.