Reinterpretation of Chromosomal Microarrays with Detailed Medical History

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Objective To investigate the utility of a detailed medical history in the interpretation of chromosomal microarray results for pediatric patients with a constitutional disease.

Study design A retrospective review and reinterpretation of test results from chromosomal microarrays performed from 2011 to 2013. Previously reported genetic variants were reanalyzed after review of the patient's complete electronic medical record (cEMR). A 3-tier system was used for reclassification of variants: pathogenic or likely pathogenic (P/LP); variant of uncertain significance (VUS); or benign or likely benign (B/LB).

Results Over an 18-month period, 998 patients with chromosomal microarray results were identified. The most common reasons for chromosomal microarray testing were developmental delay (n = 336), autism spectrum disorder (n = 241), and seizures (n = 143). Chromosomal microarray testing identified 1 or more variants in 48% (482 of 998) of patients; 516 patients had a negative report. For the 482 patients with variants, the original interpretations were composed of 19.3% P/LP (93 of 482), 44.8% VUS (216 of 482), and 35.9% B/LB (173 of 482) variants. After review of the cEMR, 34% of patient results (164 of 482) were changed in interpretation. One case changed from B/LB to VUS, 7 VUS were upgraded to P/LP, and 156 VUS were downgraded to B/LB. No P/LP variants had a change in interpretation.

Conclusions Overall, 16.4% (164 of 998) of patients with chromosomal microarray testing had a change in interpretation. Access to the patient's cEMR improves the interpretation of chromosomal microarrays by decreasing the number of uncertain (VUS) interpretations. *(J Pediatr 2020;222:180-5)*.

Chromosomal microarray is recommended by the American College of Medical Genetics and Genomics (ACMG) as a first-tier clinical test to evaluate patients with developmental delay/intellectual disability, autism spectrum disorder, and multiple congenital anomalies.[1,2] Chromosomal microarray has a diagnostic yield of 15%-20% compared with approximately 3% for traditional karyotyping and fluorescence in situ hybridization.[2] Since their introduction, chromosomal microarrays have evolved to include single-nucleotide polymorphisms to identify regions with absence of heterozygosity (AOH) in addition to changes in copy number. These regions of AOH may be indicative of identity by descent or uniparental disomy.[3]

The interpretation of chromosomal microarrays is based on large databases containing the microarray results from patients with characterized phenotypes as well as control individuals. One study examining developmental delay compared the copy number variants (CNVs) in 15 767 pediatric patients with developmental delay with 8329 adult controls.[4] There was an increased burden of CNVs in patients (25.7%) compared with controls (11.5%). One of the largest resources correlating CNV changes to patient phenotypes is DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources).[5] The DECIPHER database is regularly updated and contains CNV changes in more than 35 000 patients. Although large catalogs of CNVs are important resources for interpreting microarray findings, the majority of microarray studies are uninformative, with negative findings or variants of uncertain significance (VUS). Interpretation of microarray CNVs requires an in-depth correlation between a patient's phenotype and the individual genes within a chromosomal region. A better understanding of a patient's phenotype and/or

ACMG	American College of Medical Genetics and Genomics
AOH	Absence of heterozygosity
B/LB	Benign or likely benign
cEMR	Complete electronic medical record
CNV	Copy number variant
DECIPHER	Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources
P/LP	Pathogenic or likely pathogenic
VUS	Variant of uncertain significance

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a better understanding of the genes contained within a chromosomal region will both improve the interpretation of microarray CNVs.

For our tertiary care pediatric institution, chromosomal microarray testing was performed by high-resolution array via a reference laboratory. Historically, chromosomal microarray ordering at our institution was available to multiple medical specialists and only limited clinical information was provided to the reference laboratory. The current study retrospectively assessed the importance of generating detailed phenotypic information for a patient by reviewing their complete electronic medical record (cEMR)—namely the history and physical; progress notes; specialist consult and follow-up notes; pathologic, laboratory, and radiologic data; growth charts; etc—in the interpretation of chromosomal microarray results.

Methods

This study was reviewed and approved by the UT Southwestern institutional review board. Chromosomal microarray test results performed on peripheral blood during an 18-month period (September 2011 to May 2013) were retrospectively examined to document all variants identified by the reference laboratory. The chromosomal microarray used by the laboratory had a combination of 400 000 comparative genomic hybridization oligonucleotide probes and 120 000 single-nucleotide polymorphism probes for the detection of genome-wide CNVs and the presence of AOH (each region of AOH counted as a variant). The same test order and general methodology was used throughout the study, but different versions of the array were introduced during the study period with an increase in number of probes targeting genes at the exon-level; the earliest arrays in the study targeted exon-level resolution of 1900 genes compared with later arrays which targeted 4500 genes with exon-level resolution. Throughout the study period, the reference laboratory used the same reporting standards and database for interpretation. Of note, the laboratory's reporting standards at the time predated and did not incorporate the 2011 ACMG standard classification system for variants.[6] The laboratory interpreted variants as "abnormal" and did not further classify pathogenicity.

As part of this study, the study team consisting of 4 pathologists, a genetics counselor, and a medical geneticist, translated the original descriptive reports into a 3-tier classification scheme of P/LP (pathogenic/likely pathogenic); VUS; or B/LB (benign/likely benign). The study team also assessed whether chromosomal microarray testing aided in making a diagnosis relevant to the patient's clinical presentation. If the reference laboratory provided evidence for disease association fitting with the patient's symptoms in the result report (disease-associated CNV or AOH), the original interpretation was classified as P/LP; if evidence for non-pathogenicity was provided, then the original interpretation was classified as B/LB. If a variant was mentioned to be of unclear significance by the reference laboratory, or if it was only listed in the report without any additional explanation, or it was a variant that did not explain the patient's symptoms, then we categorized the original interpretation as a VUS.

Next, all cases with reported variants were reinterpreted using the cEMR, which again included review of progress notes, history and physical notes, and any laboratory data available at the time of testing. The retrospective cEMR review was limited to the information available to the clinical team at the time the chromosomal microarray report was originally finalized. Based on cEMR, a consensus reinterpretation was performed for each variant according to the categories of P/LP, VUS, or B/LB per the 2011 ACMG CNV interpretation guidelines.[6] Follow-up parental studies were included in the analysis whenever available. Results of other genetic testing (eg, Fragile X analysis, Sanger sequencing, gene panels done by next-generation sequencing, etc) also were included in the analysis if they were available at the time the chromosomal microarray results were reported. To focus on the utility of detailed medical history rather than growth in medical knowledge over the passage of time, subsequent genetic test results were not included in the cEMR review. Of note, updated CNV interpretation guidelines were published in 2019 by the ACMG.[7] These updated guidelines are not reflected in the current study as they were released after the reinterpretation analysis had been completed.

The clinical significance of genes found within deletions or duplications identified were evaluated via search of the medical literature, DECIPHER, the Online Mendelian Inheritance in Man database, and the University of California Santa Cruz Genome Browser.[5,8,9] We limited our interpretation and literature review of genes and genomic regions to knowledge available at the time of chromosomal microarray testing. Publications of novel gene–disease associations or new microdeletion/duplication syndromes that occurred after chromosomal microarray testing was complete, were not included in the analysis. Diagnostic yield was defined as the percentage of patients with clinically significant CNVs found relative to all patients tested. Both diagnostic yield and changes in interpretation were counted at a per patient-level rather than at a per-variant level. When a patient had multiple CNVs, the most clinically relevant change, if any, was used for the patient's chromosomal microarray interpretation.

Results

A total of 998 patients (average age of 6 years; 650 male and 348 female) with chromosomal microarray test results were identified in the 18-month study period. There were 482 patients (48.3%) with a variant reported and 516 patients (51.7%) with no variant reported (**Figure**). The 482 patients had a total of 837 variants identified, which ranged from 1 to 24 variants identified per individual patient. Of

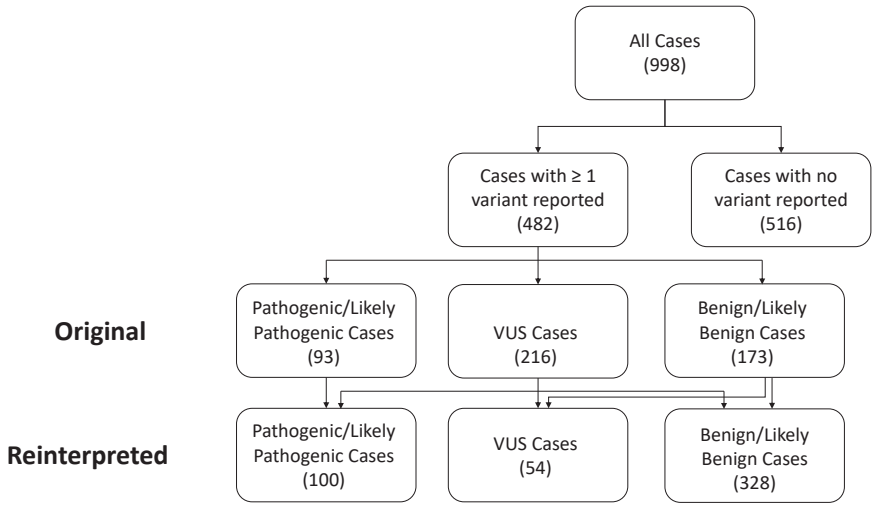

Figure. Study profile. A total of 998 patients had chromosomal microarray testing performed as part of their clinical evaluation. Among the 482 patients who had a variant, reports were classified as either P/LP (n = 93), VUS (n = 216), or B/LB (n = 173). After review of the cEMR, a consensus reinterpretation was performed and resulted in decrease in number of VUS, which were either upgraded to P/LP or downgraded to B/LB.

the types of variants identified, there were 162 patients with 1 or more deleted (loss) segment and 202 patients with 1 more duplicated (gain) segments, 27 patients showed AOH, and 7 had aneuploidy. The remaining patients (n = 84) had complex findings of both deletions and duplications or copy number variants combined with AOH. Findings were divided based on the primary indication for testing. The most common indication for chromosomal microarray testing was developmental delay (n = 336 cases, 34%), followed by a workup for autism spectrum disorder (n = 241 cases, 24%), and seizures (n = 143 cases, 14%) (Table I). Other clinical reasons included other neurologic issues, multiple congenital anomalies, urogenital anomalies, heart defects, and failure to thrive.

Of the 482 patients with reported variants, the original interpretations by the reference laboratory were composed of 19.3% (93 of 482) P/LP variants, 44.8% (216 of 482) VUS, and 35.9% (173 of 482) B/LB variants as enumerated by the study team. For the 93 patients with a P/LP variant, a subset of 39 had a CNV in a known deletion/duplication region (DECIPHER). The original overall diagnostic yield was 9.3% (93 P/LP cases of 998 patients tested). Based on the primary indication, the diagnostic yield ranged from 3% to 33%. The highest diagnostic yield was observed in patients with failure-to thrive (33%, 2 of 6); however, this may be skewed due to the low number of cases. The next highest diagnostic yields were for indications of multiple congenital anomalies (24.5%, 13 of 53) and heart defects (18%, 4 of 22).

Table I. Original classifications categorized by clinical indication for testing

Primary indications	Total cases	Cases with variant	Diagnostic yield*	P/LP	VUS	B/LB
Developmental delay	336	169	31/336 (9.2%)	31	85	53
Autism spectrum disorder	241	94	9/241 (3.3%)	9	43	42
Seizures	143	51	12/143 (8.4%)	12	21	18
Neurologic	84	60	13/84 (15.5%)	13	25	22
Multiple congenital anomalies	53	41	13/53 (24.5%)	13	17	11
Urogenital	47	23	2/47 (4.2%)	2	9	12
Heart defects	22	10	4/22 (18.2%)	4	3	3
Failure to thrive	6	4	2/6 (33.3%)	2	2	0
Other	66	30	7/66 (10.6%)	7	11	12

*Diagnostic yield is the percent of total cases with a pathogenic or likely pathogenic variant.

For all patients with reported variants, retrospective review of the cEMR was performed via the study team to determine whether additional medical information or genetics expertise would change the original chromosomal microarray interpretation. Based on cEMR review, 34.0% (164 of 482) of abnormal patient results were reinterpreted (Figure). Almost all of the reinterpreted variants were from the original VUS category; 7 VUS were upgraded to P/LP and 156 VUS were downgraded to B/LB; one variant was changed from B/LB to VUS. No variant reinterpretations occurred for original interpretations of P/LP. Of the 7 additional P/LP variants, one CNV occurred in a known deletion/duplication region (DECIPHER). After reinterpretation, 20.7% (100 of 482) of variants were P/LP, 11.2% (54 of 482) VUS, and 68.0% (328 of 482) B/LB variants (Table II). The cEMR review with genetics expertise resulted in a slight increase in overall diagnostic yield by 0.7%, from 9.3% to 10.0%.

VUS reclassification occurred across all clinical indications (Table III). Overall, 75% (163 of 216) of VUS were reclassified. The patients with a testing indication of developmental delay had 71.8% (61 of 85) of VUS reclassified. Patients with a testing indication of autism spectrum disorder group had 72.1% (31 of 43) of VUS

Table II. Classifications after reinterpretation

Classifications	Cases* (%)	Cases after reinterpretation (%)	Percentage change
P/LP	93 (19.3)	100 (20.7)	+1.4
VUS	216 (44.8)	54 (11.2)	−33.6
B/LB	173 (35.9)	328 (68.0)	+32.1

*Cases refer to the number of individuals with at least 1 variant identified.

reclassified. Patients with other neurological indications has 76% (19 of 25) of VUS reclassified.

Seven cases had an upgraded clinical significance from VUS to P/LP (**Table IV**; available at www.jpeds.com). These cases show both deletions and duplications, which the study team determined were best interpreted as pathogenic or likely pathogenic. One example of a case where a variant was reinterpreted from a VUS to P/LP involved a deletion of part of chromosome 9. The only information available to the reference laboratory was that the patient was a one-year-old male with a seizure disorder. The original report identified a de novo loss of approximately 7.5 Mb from chromosomal region 9p13.3-p13.1. This variant was simply listed in the original report, and no interpretation of the variant or evidence supporting its pathogenic or benign nature was given. Review of the cEMR found that, in addition to seizures, the patient had developmental delay and white matter changes on magnetic resonance imaging of the brain. At the time of chromosomal microarray report, interstitial 9p13 deletions had been reported in the literature in a few patients with variable symptoms including developmental delay, myoclonic jerks, intention tremor, partial absence of the corpus callosum, dysmorphic features, feeding difficulties, craniosynostosis, precocious puberty, and short stature.[10-12] Considering these data, this variant was reinterpreted as P/LP.

A case example of a variant that was reinterpreted from a VUS to B/LB involved a 0.016-Mb duplication of chromosomal region 6p21.1. There was no clinical indication provided to the reference laboratory, which listed this copy number variant as a gain of "unclear clinical relevance." This region included 2 genes: *GNMT* and *PEX6*. Pathogenic changes to the *PEX6* gene are associated with an autosomal-recessive peroxisome biogenesis disorder associated with the following features: hypotonia, developmental delay, hepatic dysfunction, hearing loss, retinal dystrophy, and visual impairment.[13,14] Mutations in the *GNMT* gene are associated with a rare autosomal-recessive condition called glycine N-methyltransferase deficiency. Clinical features include persistent hypermethioninemia, hepatomegaly, and chronic elevation of serum transaminases.[15] A review of the cEMR revealed that the patient was a 5-year-old girl with a history of truncus arteriosus, coloboma, cleft lip and palate, and left lid ptosis. With cEMR review, it was deemed that the duplication did not contribute to the patient's clinical features.

Table III. Reclassified VUS by indication for testing

Primary indications	Original VUS	VUS reclassified (%)*	New P/LP	New B/LB	Remaining VUS
Developmental delay	85	61 (71.8)	2	59	24
Autism spectrum disorder	43	31 (72.1)	1	30	12
Seizures	21	15 (71.4)	2	13	6
Neurologic	25	19 (76.0)	0	19	6
Multiple congenital anomalies	17	15 (88.2)	2	13	2
Urogenital	9	8 (90.9)	0	8	1
Heart defects	3	3 (100.0)	0	3	0
Failure to thrive	2	1 (50.0)	0	1	1
Other	11	10 (90.9)	0	10	1
Total cases	216	163	7	156	53

*Percentage of total number of cases with reported variants that had a VUS that was reclassified.

Discussion

Based on cEMR review, we changed the interpretation of 34% (164 of 482) of previous chromosomal microarray patient reports. Almost all reinterpreted variants (n = 163) were from the original VUS category, and the majority of reinterpreted VUS were downgrades to B/LB (n = 155). Determination that a variant is benign is helpful because it supports the continued investigation for alternative genetic etiologies. Although this is a retrospective study utilizing cEMR review, the potential implication is that additional clinical information improves the interpretation of chromosomal microarray testing.

In 1 case, review of the history did not change the variant classification (initially a VUS and remained so after reinterpretation) but led to a diagnosis that directly affected treatment. A 3-month-old female patient with a history of failure to thrive and clinical features suggestive of an autosomal recessive skeletal dysplasia; these features included profound hypomineralization, a low alkaline phosphatase level, and a family history of distant consanguinity. The original chromosomal microarray report had identified three regions of AOH, but these were only listed in the report without further enumeration of any potentially relevant genes in those regions. The only clinical feature available to the reference laboratory for this patient was failure to thrive. Review of cEMR revealed the aforementioned additional clinical features, and review of the AOH regions found that one included the *ALPL* gene; this gene is associated with hypophosphatasia, a condition characterized by defective mineralization of the bones and/or teeth with a low alkaline phosphatase level.[16,17] This chromosomal microarray finding was important, as it guided subsequent sequencing of the *ALPL* gene to identify a pathogenic variant, identifying the cause of the patient's symptoms, and leading to a Food and Drug Administration–approved therapeutic treatment (asfotase alfa).

This study demonstrates that detailed clinical description of a patient improves the interpretation of

chromosomal microarray tests. In addition to detailed clinical information, there are other variables that have been demonstrated to affect the interpretation and diagnostic yield of genomic tests. For example, recent studies have identified the passage of time as a factor in reinterpreting previous clinical test results.[18-20] For pediatric epilepsy gene panels, 36.2% (67 of 185) of previously reported variants had a change in variant interpretation.[18] In the case of hereditary cancer genetic testing, 6.4% (2868 of 44 779) of all unique variants were reclassified; however, when considering variants identified in more than one individual, 25.4% (46 890 of 184 327) of VUS were reclassified.[19] Finally, for chromosomal microarray testing, 11.9% of cases (8 of 67) were reassessed as having a potentially pathogenic array result after reanalysis was done 2 years following the initial study.[20] Changes in variant interpretation over time may result from a combination of new medical literature, and evolving signs and symptoms in a patient. Other factors that may impact the interpretation and diagnostic yield of a genomic test include advances in technology, the quality of a laboratory, and the experience and expertise of the interpreter of the test result. The present study is unique in that time, technology, patient age, and laboratory factors were controlled variables; therefore, the importance of descriptive clinical information in microarray interpretation is highlighted.

The practice implication of this study is that laboratories providing chromosomal microarray testing can improve their interpretation with access to the patient's EMR or by requiring in-depth histories at the time the test is performed. Physicians should understand that chromosomal microarray test interpretations can have diagnostic variability depending on the depth of clinical information available to the chromosomal microarray testing laboratory. The depth of clinical information provided could also be increased by patient referral to specialists (eg, examination of a patient for dysmorphism by a geneticist, for example). Our study would urge that all physicians ordering chromosomal microarray testing should list all of the patient's clinical features prior to analysis, and that they should contact the laboratory with new clinical findings or to discuss specific findings which may be of significance. This practice, together with standardization of clinical features (ie, use of Human Phenotype Ontology terms) allows for more precise and patient-specific variant interpretation and a more phenotypic-driven analysis.[21,22] This is particularly important for the general pediatrician, who may see patients with a VUS and relies on the laboratory's interpretation. The findings of this study indicate that detailed clinical information may reduce the number of VUS and improve the certainty of chromosomal microarray test results. Alternatively, referral to a geneticist should be considered if there is uncertainty over the significance of a genetic variant. Chromosomal microarray and other genomic tests are complex and have improved interpretations when integrated with the patient's complete medical history. ■

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50 Years Ago in *The Journal of Pediatrics*

Hand-Foot-Genital Syndrome and Its Multiple Genetic Mechanisms

Stern M, Hall JC, Perry BL, Stimson CW, Weitkamp LR, Poznanski AK. The hand-foot-uterus syndrome: a new hereditary disorder characterized by hand and foot dysplasia, dermatoglyphic abnormalities, and partial duplication of the female genital tract. J Pediatr 1970;77:109-16.

A unique autosomal dominant syndrome was described in 1970 by Stern et al in which affected members in a large multigeneration family displayed phenotypic variability and had malformed thumbs, a hypoplastic thenar eminence, and clinodactyly of the fifth digit. There was shortening of the first metacarpal and metatarsal on radiograph examination. In addition to the previously described skeletal features, 4 females in 3 consecutive generations had duplication anomalies of the uterus, including a bifid uterus with single cervix, a double uterus and double cervix with a subseptate vagina, and a double uterus and septate vagina. Affected individuals had similar dermatoglyphic findings. The expanded phenotypic spectrum includes hypospadias in males and urinary tract abnormalities, such as vesicoureteral reflux, ectopic ureteric orifice, and ureteropelvic junction obstruction, resulting in renaming of the condition to "hand-foot-genital" syndrome (HFGS). Although the skeletal features display complete penetrance, the genital features are characterized by incomplete penetrance with phenotypic variability.

HFGS is caused by mutations in the homeobox gene *HOXA13*, a DNA-binding transcription factor involved with morphogenesis involving distal limb and lower urinary tract development. *HOXA13* is localized to the *HOXA* gene cluster on chromosome 7 and the second *HOX* gene reported to be associated with a human malformation syndrome. Mutation mechanisms in *HOXA13* causing HFGS include protein truncation, polyalanine tract expansion, and missense resulting in amino acid substitution and 7p15.2 microdeletions.[1] Protein truncating mutations are thought to function as null alleles. There are 5 polyalanine tracts in exon 1 with 15-18 amino acid residues and expanded alleles containing 7-15 meiotically stable extra alanine residues. The mechanism(s) associated with *HOXA13* polyalanine tract expansion include gain of function and protein inactivation causing a dominant negative effect. Missense mutations may alter *HOXA13* target DNA binding. Deletions in 7p15.2 containing the *HOXA* gene cluster resulting in hapoinsufficiency for *HOXA13* have been reported.[2] The presence of distal limb malformations in a child should prompt an investigation for urogenital anomalies.

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Table IV. Cases reclassified from VUS to P/LP

Microarray results	Indication for testing provided to laboratory	Clinical features identified in the complete medical record
4q13.1q13.2 deletion	Multiple congenital anomalies, digital abnormalities, brain malformation, cysts, aplasia cutis congenita	Multiple congenital anomalies, digital abnormalities, brain malformation, cysts, aplasia cutis congenita
7q35 deletion	No indication provided	Complex febrile seizures, gross motor delay, speech delay, amblyopia
9p13.3p13.1 deletion	Status epilepticus	Seizures, developmental delay, white matter changes on MRI of the brain
16p13.3 duplication	Autism spectrum disorder, developmental delay, speech impairment, obesity	Autism spectrum disorder, developmental delay, speech impairment, obesity, and a concern for Fragile-X syndrome
16p13.11 duplication	Developmental delay	Developmental delay, asthma, and arm shaking episodes
16q11.2 duplication; 17q21.31 deletion*	No indication provided	Prune belly syndrome, patent ductus arteriosus, vesicoureteral reflux, bilateral undescended testes
Xq27.3 duplication	Seizure disorders	Developmental delay and epilepsy

MRI, magnetic resonance imaging.

*The patient with both 16q11.2 duplication and 17q21.31 deletion had testing submitted without a clinical indication. 17q21.31 contains a portion of the *BRCA1* gene; this deletion is not likely associated with the patient's clinical presentation, but it is a significant incidental finding with implications for the patient and their family. The 16q11.2 duplication does not have a clear clinical association.