The Genetics Corner: Frequently Asked Questions, Part I: About Copy number variants (CNV), Variants of Uncertain Significance (VUS) in Chromosome Microarrays (CMA)

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Chromosome microarray (CMA) has become a first-tier test in the evaluation of newborns with congenital anomalies, who do not have a clear diagnosis. It is used by neonatologists and geneticists alike. Microarrays have proven their effectiveness by improving the diagnostic yield and changing the clinical management of newborns who have congenital anomalies. This two-part series of frequently asked questions will help the neonatologist interpret microarrays with more confidence and efficacy. Next month's FAQs focus on CNVs that are associated with congenital heart defects, both the isolated and the syndromic varieties.

What are CNVs?

CNVs or copy number variants refer to variants in the normal amount of chromosomal material. These are losses (microdeletions) or gains (microduplications) of chromosomal material that are usually longer than 500 bases in size but much smaller CNVs may be reported if they involve critical regions.

How common are CNVs in the general population?

CNVs are common and universal. CNVs account for most of the genetic diversity in human populations. Each of us has on average >1000 CNVs of >450 base pairs compared to a reference population. A chromosome microarray test detects reportable CNVs in 2-4% of the general population. CNVs may be familial (inherited) or de novo (sporadic), common or rare, recurrent and well described in the medical reports or unique ("novel"), without other examples in the published literature.

How are CNVs reported and classified?
Here is an example: arr[hg19] 16p13.1 1p12.3(15,324,775-18,242,713)x1

In this example, a 2.9 Mb deletion in the short arm of chromosome 16 starts at 16p13.11 (proximal breakpoint) and ends at 16p12.3 (distal breakpoint). The result designates the human genome map that was used to map these coordinates: arr[hg19]. This is followed by the chromosome number (1-22, X, Y), arm (p or q), band and sub-bands that is affected. Next, within the set of parentheses, 2 long strings of numbers are marked off, in sets of 3s, by commas, and separated by a dash. These two numbers represent the nucleic acid coordinates for the starting and stopping points of the variant region. Subtract the smaller number from the larger number and you get the number of bases involved in the CNV, which describes its size (18.2 million -15.3 million = 2.9 million). The copy number follows: "x1" for a deletion, "x3" for a duplication. The genomic size of a CNV is expressed as Kb or Kilobases (1Kb=1000 base pairs) or Mg or Megabases (1Mb=1000 Kilobases or a million base pairs).

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Following the guidelines established by the American College of Medical Genetics and Genomics, genetic laboratories classify CNVs using 5 options: pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign and benign. Unfortunately, not all laboratories use the same standards to "call" variants and discrepancies in how labs classify CNVs are not uncommon. CNVs can be reclassified as more data becomes available.

What can be done to interpret a VUS on a microarray?

VUSs are commonly reported on microarray tests. These are usually either novel variants that have not been seen in a reference population or they are regions of variation with incomplete data, making interpretation difficult and limited. VUSs cannot be considered disease-causing but neither can they be ignored. The lab may offer parental testing for the VUS (sometimes, at no charge). A de novo VUS, or one that is inherited from a similarly affected parent, is more likely to be significant. A VUS that is inherited from an unaffected parent, is less likely to be significant. You can ask the lab to reinterpret a VUS after more family data has been collected or after a suitable period (usually a year or more) has elapsed in case new data has been added to the reference databases.

Can a familial CNV be pathogenic if the carrier parent is unaffected?

Some familial CNVs are disease-relevant but incompletely penetrant: meaning that some individuals in the family with the CNV express an abnormal phenotype while others with the same CNV do not, possibly because of the effects of other modifying environmental or genetic factors. Such CNVs may be considered VUSs

because of the lack of consistent correlation with a phenotype or defect but they are in fact important risk factors. The increased prevalence of a VUS in the disease population compared to the unaffected or general population is a measure of the relative risk it may confer. In some cases, a deletion can "unmask" a heterozygous pathogenic variant on the intact homolog. This can cause expression of an autosomal recessive phenotype in the patient who has both a deletion and a genetic variant that affect both copies of the gene in question. Recognizing how these CNVs contribute to risk can improve the interpretation of VUSs in patients with congenital anomalies in general, and congenital heart defects (CHDs) in particular.

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Are some CNVs better tolerated than others?

In general, microduplications are better tolerated and are less likely to be disease-causing than microdeletions. A microdeletion that causes haploinsufficiency of dosage-sensitive genes is more likely to cause disease.

Smaller CNVs and microdeletions or duplications that are not gene-rich are less likely to be disease-causing. Conversely, as the size and number of CNVs increase, so do their chance of clinical relevance. Large CNVs, 3 Mb or greater, are almost always pathogenic.

What resources offer more information on the significance of a CNV?

A clinical geneticist is your best resource but a timely consultation may not be available for every patient who needs one. It is a good idea to be familiar with a few useful resources that are readily available. Most microarray reports include a list of disease-related genes involved in the CNV. The clinician can review this list for the presence of genes that are relevant to the patient's phenotype. Here are a few resources:

The online database, OMIM, Online Mendelian Inheritance in Man, is provides information about disease-associated genes: https://www.omim.org/

DECIPHER is a database of patients with CNVs that includes their phenotypic features: https://decipher.sanger.ac.uk/

UNIQUE is a resource for patient educational materials and informative booklets (some in languages other than English) on rare chromosome variants: https://www.rarechromo.org/

Practical applications:

1. Use chromosome analysis as the preferred first test when aneuploidy or a structural chromosome anomaly is suspected. Conventional chromosome analysis is appropriate when:
   a. Down syndrome, another autosomal trisomy or Turner syndrome is suspected.
   b. Multiple miscarriages or infertility is present in the family history.

2. Microarray testing is the most appropriate first line test for all other children with congenital anomalies, including heart defects.
   a. The background rate of CNVs in the general population is 2-4%.
   b. Patients with apparently isolated, nonsyndromic CHDs have an increased rate of both de novo and familial CNVs of 4-10%.
   c. The diagnostic yield is higher in CHD patients with extracardiac anomalies, 15-20%.

3. Only CNVs that are classified as pathogenic or likely pathogenic in the microarray report are considered to be responsible for the CHD.
   a. VUSs (variants of uncertain significance) should not be

considered causative until more information is available to change their classification.

b. Request parental studies to further characterize a VUS as de novo or familial.
c. Request laboratory reinterpretation of a VUS classification to resolve its status over time (usually one year or more after test report).

References:

1. *Miller DT, Adam MP, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet. 2010 May14;86(5):749-64. PMID: 20466091*
2. *Richards S, Aziz N, Bale S, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24. PMID: 25741868*

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