The Genetics Corner: Congenital Microcephaly and a Region of Homozygosity on Chromosome 1 that was not Reported on the Prenatal Chromosome Microarray

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Case History:

A genetics consultation was requested for a 6-day old male with prenatally diagnosed IUGR, microcephaly, and bilateral talipes equinovarus. A fetal MRI demonstrated agenesis of the corpus callosum, severe lateral and third ventriculomegaly, and extensive parenchymal thinning. The mother had an amniocentesis with normal oligo-SNP chromosome microarray (CMA) results: arr(1-22) x2,(XY)x1, and negative PCR for CMV and Toxoplasma gondii. She was a 27-year old G3P1011, who denied teratogenic exposures or travel outside of the country. Parents, who denied consanguinity, reported that they were both from the same small town in Mexico.

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The infant was born at 36w5d gestation, after spontaneous rupture of membranes with clear amniotic fluid, by vaginal delivery in the vertex presentation. APGAR scores were 81 and 95. Birth weight 2041 g (2nd percentile), birth length 40.6 cm (<1st percentile), head circumference 27 cm (Z score -4 SD). A repeat CMA, which was sent to the same cytogenetic laboratory as the prenatal test, identified a long contiguous region of homozygosity (ROH) on chromosome 1, of approximately 21.4 megabases (Mb). Upon further request, the laboratory gave a verbal report of additional regions of homozygosity on chromosomes 2, 4, 11, and 16, indicating that the parents likely had a distant common ancestor. A trio whole-exome sequencing test identified a homozygous (likely causative) variant, c.929T>G/p.Leu310Arg, in MFSD2A. This gene, which is located within the ROH on chromosome 1, is responsible for an autosomal recessive form of primary microcephaly (MCPH15, MIM 616486) that matches this baby's phenotype.

Assessment and Counseling:

CMA is the first-tier diagnostic test for patients with multiple congenital anomalies. It can be performed prenatally or postnatally. A single nucleotide polymorphism (SNP) CMA uses SNPs to detect DNA copy number gains or losses at high resolution. It can also detect regions of homozygosity (ROH, or absence of heterozygosity, AOH) when the copy number is normal. ROH are stretches of identical DNA shared by the maternally-derived and paternally-derived copies of a chromosome pair. ROH are not necessarily associated with a genetic disease, but they may pose an increased risk for autosomal recessive disorders when a common ancestor carried an autosomal recessive trait that was then passed on by both parents to an affected child. ROH occur when parents are closely related (consanguineous), or more distantly related as members of a reproductively isolated group, as in this case. ROH can also be caused by uniparental disomy (UPD), which occurs when only one parent contributes both copies of a chromosome pair (in whole or in part). When UPD involves an imprinted chromosome region that is differentially expressed based on the parent of origin, it can cause a significant genetic disorder (e.g., Prader-Willi syndrome).

The laboratory that performed both the prenatal and postnatal CMAs in our patient clarified the discrepancy in their reports when we called asking for more details. This lab uses different reporting standards for prenatal and postnatal microarray studies. Their prenatal microarray report is designed to limit information of uncertain significance. They report terminal ROH >5 Mb or interstitial ROH >10 Mb when they occur on chromosomes that cause imprinting disorders consistent with UPD (e.g., chromosomes 6, 7, 11, 14, 15). Multiple ROH that make up greater than 10% of the genome are also reported prenatally, as this indicates close consanguinity, a first or second-degree relationship between the parents. In postnatal microarrays, ROH is reported when it makes up >2% of the genome or when a terminal ROH is >5 Mb, or an interstitial ROH is >10 Mb on an individual chromosome regardless of its imprinting status. Because the largest ROH in our patient was on chromosome 1, which is not imprinted, and was therefore of uncertain significance, it was not reported on the prenatal microarray. When we called the laboratory, they released five additional ROH that were not included in the postnatal CMA report as these did not meet those postnatal reporting criteria.

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This case raised several concerns and made us question when we should repeat a microarray that had been normal prenatally and when we should contact the laboratory seeking more information. Ultimately, the gene of interest in our patient was located in the initially unreported ROH on chromosome 1p34.2. Had the mother's obstetricians had access to this information prenatally, it is possible that a definitive prenatal diagnosis might have been possible using autozygosity mapping – which means looking at the genes in the region of homozygosity for candidates that match the phenotype in question. It is unclear how this information might have been used by the parents, but the question is moot because a definitive diagnosis was not possible until after delivery. The conservative reporting standards of the laboratory, in this case, serve the ideal of "doing no harm" by not offering information prior to delivery that might be misconstrued as pathogenic but at the cost of reducing options for the family, paternalistically withholding data and delaying the diagnostic process. The lab's lack of transparency conflicts with the family's right to autonomy, their clinicians' right to data, and the expectation by all parties of full disclosure. The laboratory's policy may not give sufficient weight to the fact that the amniocentesis was performed at a cost, both financial and in terms of risk to the pregnancy, which reflects the high importance that the parents placed on making a prenatal diagnosis. There was also no way for the neonatologists to know that there was more information to be had by repeating the microarray after delivery. In fact, up until this point, it had not been routine for us to repeat a microarray after delivery when the prenatal microarray was normal. We should admit here that the fact that the microarray was repeated postnatally was considered (at least by RDC) to be an error, unnecessary and even wasteful testing. Now that opinion seems less defensible.

The cost of withholding the information about the ROH may not be readily apparent, because we made a likely diagnosis with a genomic test (exome). This might argue that the lack of timely information about the ROH on chromosome 1 was not critical to the diagnostic process. However, the exome results took about four months and were very costly. We likely would have succeeded at a lower cost, and with a quicker turn-around-time had we been able to narrow our focus to candidate genes within the ROH and had we had that information in good time. The consequences of the lab's prenatal microarray reporting policy must include the stress of delayed diagnosis on the family, the cost to the patient of delays in offering meaningful treatment or surveillance, and the cost to the hospital of a redundant microarray and overly broad and expensive exome testing.

There are no professional standards or guidelines for reporting CNVs or ROH on microarrays, either prenatally or postnatally. Each laboratory determines its own reporting protocols. To document the variability in reporting standards that exist for prenatal and postnatal microarrays, we performed an informal telephone survey on a convenience sample of 10 cytogenetic laboratories. The results are summarized in Table 1. Among these ten labs, none had the same reporting criteria, even though many were similar. Furthermore, 8 of the 10 had different reporting standards for prenatal and postnatal microarrays, usually reporting less information prenatally. Knowing this, a clinician could reasonably expect that a "normal" prenatal microarray might not meet the criteria for the same "normal" interpretation when reported postnatally

Loss-of-function variants in MFSD2A are associated with autosomal recessive primary microcephaly, type 15 (MIM# 616486). The mechanism seems to be disruption of transport of the necessary omega-3 fat, docosahexaenoic acid, DHA, across the blood-brain barrier. This condition is characterized by a spectrum of severe microcephaly, structural brain anomalies, a paucity of white matter, hypotonia, spasticity, and intellectual disability with absent speech. Although the homozygous missense variant in MFSD2A in this child has been classified as of uncertain significance (VUS), c.929T>G/p.Leu310Arg, we considered it to be likely causative. This homozygous variant has not been reported in the literature, disease/mutation databases, or in allele frequency databases (gnomAD). The amino acid position is highly conserved through evolution, and in silico analyses predict this alteration has a deleterious effect on protein function. This variant is located at the 5' border of transmembrane domain 6; it has been hypothesized that variants in these transmembrane domains may interfere with substrate binding. The four other pathogenic variants in this gene reported in association with this phenotype are novel missense variants found in highly conserved residues in or at the border of the aforementioned transmembrane domains. At least 3 of these variants have had functional studies that show reduced or absent transporter activity. These findings suggest that this variant has a pathogenic consequence Taken together. We are in the process of investigating the CNS phenotype with more imaging studies.

Wang et al. (2015) demonstrate that ROH occur frequently and have clinical utility by reflecting parental relatedness, ascertaining autosomal recessive diseases, and unraveling UPD. In their study of over 14,500 consecutive oligo-SNP chromosome microarrays, these authors found that 6% of oligo-SNP microarrays harbored one or more ROH >10 Mb, of which 78% involved multiple regions, indicating identity by descent (consanguinity). Of the ROH involving single chromosomes, about 10% demonstrated UPD. Autosomal recessive disorders were confirmed in seven of nine cases from eight families because of the finding of a suspected gene within an ROH.

Our survey found a variety of reporting criteria among ten commercial and academic cytogenetic laboratories and different stan-

Laboratory	Prenatal Microarray Reporting Criteria	Postnatal Microarray Reporting Criteria
Commercial Lab A	**Del >1 Mb, Dup >2 Mb** **Total percentage of ROH is reported when >5% of the genome** Single terminal ROH >3 Mb or single interstitial ROH >10-20 Mb are generally reported, dependent upon chromosomal location	**Del >50 kb, Dup >400kb** **ROH >3% of the genome** Single terminal ROH >3 Mb or single interstitial ROH >10-20 Mb are generally reported, dependent upon chromosomal location
Commercial Lab B	**At least 1 gene is included in CNV and Del >1 Mb, Dup >2 Mb** **AOH of unknown significance when greater than 5 Mb (terminal) and 10 Mb (interstitial) on imprinted (UPD-associated) chromosomes** **Total percentage of ROH is reported when >10% of the genome**	**Del >200 kb, Dup >500 kb** **ROH >2% of the genome**
Commercial Lab C	**Del >1 Mb, Dup 2 MB** ROH >8 MB multiple chromosomes or consanguinity	**Del >200 kb, Dup >500 kb** **Or >50 kb when CNV is in a clinically significant region** ROH >8 MB when multiple chromosomes are involved or consanguinity
Commercial Lab D	**CNV that is likely benign is not reported** AOH >5 Mb	**CNV that is likely benign may be reported** AOH >5 Mb
Academic Lab A	**CNVs: Del ≥1 Mb, Dup ≥2 Mb,** or smaller CNV in clinically significant regions ROH ≥5 Mb, reporting threshold ~10 Mb Total percentage of ROH is reported when ≥5% of the genome	CNVs: **Del ≥200 kb, Dup ≥400 kb** or smaller when CNV is in a clinically significant region ROH ≥5 Mb, reporting threshold ~10 Mb ROH ≥5% of the genome
Academic Lab B	CNV >50 kb AOH >5 Mb	Same as prenatal criteria
Academic Lab C	CNV > 400kb if VUS or >25 kb if pathogenic >400 kb VUS clinician determined AOH >3 Mb on a single chromosome or >1.5% of the genome	Same as prenatal criteria

Table 1. Prenatal and postnatal microarray reporting criteria

***Boldface** is used for reporting standards that are different for prenatal and postnatal microarrays*
AOH absence of heterozygosity (AOH=ROH), CNV copy number variant, Del deletion, Dup duplication, kb kilobase, Mb megabase, ROH region of homozygosity (AOH = ROH), VUS variant of uncertain significance

dards for prenatal and postnatal microarrays in 80% of those labs. When the prenatal and postnatal reporting standards differed, the tendency was to limit the reporting of CNVs and ROH prenatally. The challenges associated with the lack of uniform standards for reporting ROH among laboratories have been described by others (Grote et al., 2012). Our case reinforces the need for prenatal disclosure of ROH when an autosomal recessive disorder is suspected. Meanwhile, we are considering how best to unlock any hidden data that might exist in "normal" prenatal microarray reports. It will likely include more frequent communication with the cytogenetics lab and more repeat postnatal microarrays. We are also exploring more productive ways of partnering with the lab going forward. For instance, we asked whether the lab would be willing to report all ROH prenatally for autozygosity mapping when there is a high index of suspicion for an autosomal recessive disorder, which could be indicated on the test requisition form.

Practical Applications:

1. Understand that cytogenetic reporting criteria for prenatal and postnatal microarrays have not been standardized.
    1. Reporting algorithms vary between laboratories.
        1. Many cytogenetic laboratories also have different reporting criteria for prenatal and postnatal chromosome microarrays.
        2. Know your cytogenetic laboratory's microarray reporting protocols
2. After a normal prenatal microarray, consider repeating the test postnatally, especially when the infant has unexplained congenital anomalies, low birth weight, or poor feeding.
    1. Another option: contact the cytogenetic laboratory that reported the normal prenatal microarray results and ask for a reinterpretation using postnatal reporting standards.
3. Review regions of homozygosity (ROH) reported on the chromosome microarray, to identify candidates genes when an autosomal recessive disorder is suspected

References:

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