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Understanding prenatal chromosomal abnormalities: Importance of both chromosomal and microarray aberrations

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Introduction

As cytogenetic analysis continues to morph from the examination of microscopic aberrations, it becomes more important to understand the variety of these submicroscopic changes and their association with phenotypic outcomes. It is estimated that every year, ~6% of worldwide births (~7.9 million infants) are born with serious birth defects. Over a 10-year period (2012-2022), more than 25,000 patients with a major ultrasound (US) abnormality detected prenatally have been referred for single-nucleotide polymorphism (SNP) microarray testing. The presence of one major abnormality was associated with an increased detection of ~4.9% cytogenetic anomalies by array (when conventional cytogenetics was normal) and ~5.3% increased detection of cytogenetic abnormalities by array when there were two or more major US abnormalities. This study demonstrates an increased diagnostic yield by microarray and refines our estimates of cytogenomic anomalies (combined array and conventional cytogenetics) for four specific US defects (cystic hygroma, diaphragmatic hernia, omphalocele and holoprosencephaly).

Methods

In this study, standard cytogenetic and SNP microarray analyses were performed on patients ascertained with four specific types of US abnormality detected prenatally: 9,577 with a prenatally detected cystic hygroma; 1,283 with a diaphragmatic hernia; 2,417 with an omphalocele; and 1,097 with holoprosencephaly. The majority of these were isolated abnormalities, but 929 (9.7%) fetuses with cystic hygroma had additional abnormalities (therefore considered non-isolated), 161 (12.5%) fetuses with diaphragmatic hernia had additional abnormalities, 429 (17.7%) fetuses with an omphalocele had additional abnormalities, and 938 (9.7%) fetuses with holoprosencephaly had additional abnormalities (Tables 1-4).

Cytogenetic studies were done using standard analysis and analyzed at the 500-550 band level. SNP microarray studies were done utilizing the CytoScan™ HD array (ThermoFisher Scientific).

In this study, some patients received chromosome analysis only, some microarray analysis only, and others both chromosome and microarray analysis (CMA).

	CHROMOSOMES		MICROARRAY	
PROTOCOL	TOTAL NO.	% ABNORMAL	TOTAL NO.	% ABNORMAL
ISOLATED - TOTAL	1988	20.7%	896	1.79%
<13 WEEKS	602	30.3%	270	0.74%
>15 WEEKS	1386	16.6%	626	2.24%
NON-ISOLATED - TOTAL	429	48.5%	194	7.20%
<13 WEEKS	159	75.5%	59	5.10%
>15 WEEKS	270	32.6%	135	8.15%

Table 1. Chromosome and microarray abnormalities associated with an omphalocele. This table provides the frequency of chromosome and microarray abnormalities in both cases of isolated omphaloceles and where there are additional malformations (non-isolated). The frequencies are given for the total group as well those identified in the first trimester and those identified in fetuses 15 weeks or greater.

	CHROMOSOMES		MICROARRAY	
PROTOCOL	TOTAL NO.	% ABNORMAL	TOTAL NO.	% ABNORMAL
ISOLATED - TOTAL	8648	43.1%	2394	4.6%
<13 WEEKS	5975	46.9%	1672	4.8%
>15 WEEKS	2673	34.6%	722	3.9%
NON-ISOLATED - TOTAL	929	60.2%	218	12.8%
<13 WEEKS	469	65.0%	90	6.67%
>15 WEEKS	254	55.2%	128	17.1%

Table 2. Chromosome and microarray abnormalities associated with a cystic hygroma. This table provides the frequency of chromosome and microarray abnormalities in both cases of isolated cystic hygromas and where there are additional malformations (non-isolated). The frequencies are given for the total group as well those identified in the first trimester and those identified in fetuses 15 weeks or greater.

	CHROMOSOMES		MICROARRAY	
PROTOCOL	TOTAL NO.	% ABNORMAL	TOTAL NO.	% ABNORMAL
ISOLATED - TOTAL	1122	8.83%	637	5.65%
<13 WEEKS	20	15.0%	13	0%
>15 WEEKS	1113	8.7%	624	5.8%
NON-ISOLATED - TOTAL	161	22.4%	92	9.8%
<13 WEEKS	4	0%	4	0%
>15 WEEKS	157	22.9%	88	10.2%

Table 3. Chromosome and microarray abnormalities associated with a diaphragmatic hernia. This table provides the frequency of chromosome and microarray abnormalities in both cases of isolated diaphragmatic hernia and where there are additional malformations (non-isolated). The frequencies are given for the total group as well those identified in the first trimester and those identified in fetuses 15 weeks or greater.

	CHROMOSOMES		MICROARRAY	
PROTOCOL	TOTAL NO.	% ABNORMAL	TOTAL NO.	% ABNORMAL
ISOLATED - TOTAL	644	26.4%	343	6.7%
<13 WEEKS	100	54.0%	36	16.7%
>15 WEEKS	544	21.3%	307	5.53%
NON-ISOLATED - TOTAL	294	38.0%	112	9.8%
<13 WEEKS	40	67.3%	9	0%
>15 WEEKS	254	33.5%	103	10.7%

Table 4. Chromosome and microarray abnormalities associated with holoprosencephaly. This table provides the frequency of chromosome and microarray abnormalities in both cases of isolated holoprosencephaly and where there are additional malformations (non-isolated). The frequencies are given for the total group as well those identified in the first trimester and those identified in fetuses 15 weeks or greater.

Results and discussion

In this study, we examined the frequency of chromosome abnormalities and additional microarray abnormalities in four major abnormalities detected by US (omphalocele, cystic hygroma, diaphragmatic hernia and holoprosencephaly) (Tables 1-4). Overall, this analysis revealed a number of interesting findings:

- The frequency of chromosome anomalies detected by karyotype for these US abnormalities, when isolated, ranged from 8.8% (diaphragmatic hernia) to 43.1% (cystic hygroma).
- The frequency of chromosome anomalies was higher when there were additional US abnormalities - ranging from 22.4% (diaphragmatic hernia) to 60.2% (cystic hygroma). Except for diaphragmatic hernia, these frequencies were all elevated in the patients ascertained in the first trimester.
- Rates of additional findings from microarray detected, for isolated abnormalities varied between 1.8% (omphalocele) and 6.7% (holoprosencephaly).
- Rates of additional findings from microarray analysis were all increased when there were additional US anomalies between 7.2% (omphalocele) to 12.8% (cystic hygroma).
- For three of these US abnormalities (diaphragmatic hernia, omphalocele and holoprosencephaly), the addition of a major heart defect resulted in the highest frequency of chromosome anomalies.
- Patients ascertained with diaphragmatic hernia, omphalocele and holoprosencephaly occurred mostly after 20 weeks. In contrast, we observed more cystic hygroma in the 1st trimester with differing rates of chromosome anomalies (46.9% for 1st trimester vs. 34.6% for >15 weeks for isolated abnormalities).
- The highest percent of structural chromosome/array anomalies (not aneuploidy) were detected in patients with holoprosencephaly. Overall, 77.7% of the structural abnormalities involved genes specifically associated with holoprosencephaly; 22.1% of these aberrations could only be detected by array.
- Additionally, 91.6% of all the chromosome and array anomalies detected in patients with holoprosencephaly could be related to the phenotypic findings; either by loss of specific genes detected by the array analysis or the presence of specific chromosome abnormalities (e.g., trisomy 13).
- Specific chromosome findings were associated with the different US findings: diaphragmatic hernias - GATA4 deletions and iso(12p); cystic hygroma - trisomy 21 and 45,X ; omphalocele - trisomy 18; holoprosencephaly - the deletion of multiple different genes (SIX2, ZIC2, SHH, TGIF and DLL1); and trisomy 13.
- During this study, 499 cases with cfDNA studies were identified that were submitted for chromosome/array analysis. For these cases, the positive predictive value (PPV) was 98.8%. Looking specifically at trisomy 18, trisomy 13 and 45,X, there were 306 cases and their respective PPVs were 98.9%, 98.2% and 98.6%. Literature values for these are much lower (84.6%, 43.9% and 14.5 - 40%, respectively), demonstrating how effective cfDNA studies can be with the presence of a significant US abnormality.

Conclusions

This is the most comprehensive known study to date utilizing CMA with specific congenital defects. It affirms that microarray provides added value over conventional cytogenetics and our findings carry significant implications for accurate genetic counseling for these families. The results show that:

- Patient care could be improved through greater use of microarray and less use of karyotyping because of the higher sensitivity with CMA.
- There is a wide difference in the frequency of abnormalities based on a number of factors including type of defects, complexity of defects and gestational age.

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