P1-94

Updated clinical experience with esoteric aneuploidies on a genome-wide cell-free DNA test

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I. Objectives

With the expansion of cell-free DNA (cfDNA) testing to include genome-wide aneuploidy and copy number variants, recent literature has begun to explore esoteric aneuploidies.[1,2] The clinical implications and complex biology of esoteric aneuploidies have stimulated many discussions on the counseling challenges of these pertinent aneuploidies. The outcomes of esoteric aneuploidies seen on a commercial genome-wide cfDNA test are described.

II. Methods

A retrospective analysis was performed on over 50,000 maternal blood samples submitted for genome-wide cfDNA analysis. Samples were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing.[3] Sequencing data were analyzed using a novel algorithm to detect trisomies and subchromosomal, genome-wide copy number variants 7Mb and larger.[4] Screen positive cases with a single esoteric aneuploidy were reviewed. Clinical outcomes were requested from ordering providers as part of routine follow-up of positive cases.

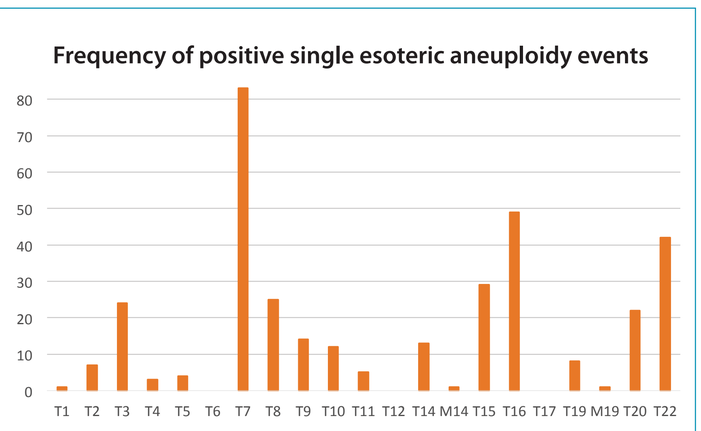
III. Results

There were 343 cases that screened positive for a single esoteric aneuploidy. The most common trisomies (excluding 21/18/13/X/Y), in descending order, were of chromosomes 7, 16, 22, 15, 8, 3, 20, 9, 14, and 10, having 10 or more cases each. Collectively, these most common aneuploidies account for 91.2% (n=313) of all of the single esoteric aneuploidies.

Figure 1: Frequency of positive single esoteric aneuploidy cases on a commercial genome-wide cfDNA test.

Note that there are no cases of trisomies 6, 12, or 17.

T=Trisomy, M=Monosomy



Only 161 cases (46.9%) had follow-up diagnostic testing reported to the laboratory. Considering only those cases with diagnostic testing, the retrospective observed PPV is approximately 26.7%. Confirmed fetal cases include full trisomy, mosaicism, or uniparental disomy of the implicated chromosome on diagnostic testing. Confirmed confined placental mosaicism (CPM) indicates a case in which the cfDNA result was confirmed on CVS or placental testing, but amniocentesis or neonatal testing returned a normal result. Four of the confirmed fetal cases had diagnostic testing results indicative of uniparental disomy (3 cases of T16 and 1 case of T15).

Reported adverse pregnancy outcomes were reviewed overall (**Figure 2**) and by each specific aneuploidy (**Table 1**).

For **Figure 2** and **Table 1**, the following definitions were used:

- **Adverse outcome:** a case was considered to have an adverse outcome if growth restriction, preterm labor/delivery, miscarriage/fetal demise, or structural ultrasound anomalies were reported. Cases that were confirmed by diagnostic testing in the fetus or placenta were combined with this group even if incomplete obstetric outcome was available.
- **Normal outcome:** A case was considered to have normal outcome if reasonably complete obstetric outcome information was available and diagnostic testing was performed and discordant with the cfDNA results. Also included are cases in which a phenotypically normal neonate was delivered at term and no additional genetic testing was pursued.
- **Unknown/incomplete:** Cases falling into the unknown/incomplete category were cases which did not report a normal outcome and did not report an adverse outcome/confirmed cfDNA result. This includes cases in which normal diagnostic testing was reported but no obstetric outcome information was obtained.

Figure 2: Overall distribution of outcomes in cases screening positive for an esoteric aneuploidy on a commercial genome-wide cfDNA test.

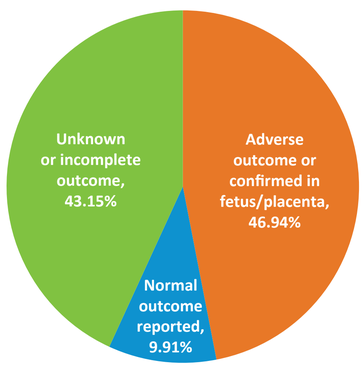


Table 1: Distribution of outcomes overall and by specific aneuploidy.

	Number of cases	Adverse outcome or confirmed in fetus	Normal outcome reported	Unknown or incomplete outcome
Total number of cases	343	46.9%	9.91%	43.2%
T7	83	28.9%	19.3%	51.8%
T16	49	65.3%	2.0%	32.7%
T22	42	66.7%	9.5%	23.8%
T15	29	75.9%	10.3%	13.8%
T8	25	32.0%	12.0%	56.0%
T3	24	37.5%	20.8%	41.7%
T20	22	27.7%	4.6%	68.2%
T9	14	71.4%	14.3%	14.3%
T14	13	38.5%	7.7%	53.9%
T10	12	25.0%	0.0%	75.0%
T19	8	25.0%	0.0%	75.0%
T2	7	57.1%	14.3%	28.6%
T11	5	60.0%	0.0%	40.0%
T5	4	75.0%	25.0%	0.0%
T4	3	33.3%	33.3%	33.3%
T1	1	100.0%	0.0%	0.0%
M19	1	0.0%	0.0%	100.0%
M14	1	0.0%	0.0%	100.0%

The most frequent adverse outcome reported is fetal demise or miscarriage (14.29%) followed closely by growth restriction (12.83%) (**Figure 3**). Individual aneuploidies had varying degrees and types of adverse outcomes (**Table 2**). Many cases had incomplete follow-up information, as indicated by the last column in **Table 2**. A case was considered to have "complete" follow-up information if both diagnostic testing results and obstetric outcome (pregnancy course and delivery, pregnancy loss, etc.) was available. Most cases had either diagnostic testing information (but not obstetric outcome information) or obstetric outcome information but never had diagnostic testing, especially in the cases of pregnancy loss. **Table 4** reviews the outcomes of the ten most frequent esoteric aneuploidies, as they collectively account for more than 91% of the cases.

Table 3 reviews the genetic testing outcomes of the ten most common esoteric aneuploidies. Notably, a co-twin demise was reported in 5.83% of all positive cases.

Figure 3: Frequency of specific adverse outcomes in cases with results suggestive of an esoteric aneuploidy.

Outcomes are not mutually exclusive; i.e. a single case may have had both growth restriction and preterm labor.

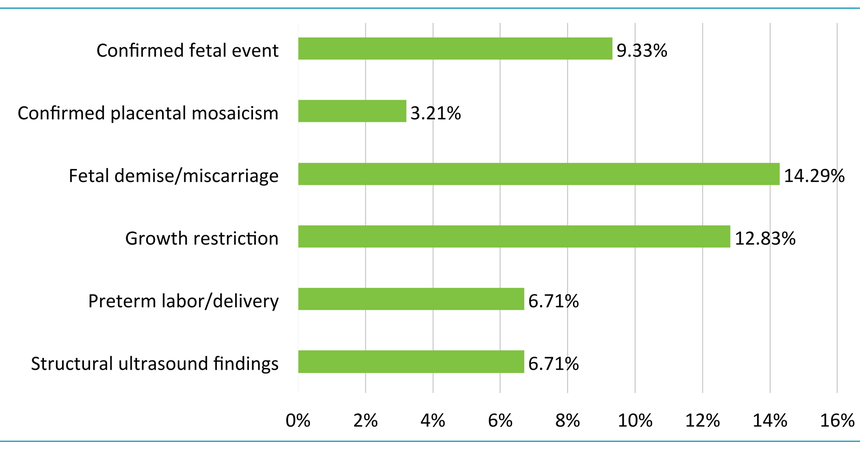


Table 2:
Complete outcome information includes both complete obstetric outcome and diagnostic genetic testing results. Partial outcome information indicates some outcome information was available, but it was incomplete. Lost to follow-up denotes cases with no outcome information.

Trisomy (# of cases)	Total number of cases	Complete outcome info (A)	Partial outcome information (B)			Lost to follow-up (C)
			Diagnostic results only	Obstetric outcome only	Diagnostic results with partial obstetric outcome	
T3	24	9		**9**		6
			4	5	0	
T7	83	16		**43**		24
			21	16	6	
T8	25	3		**14**		8
			8	4	2	
T9	14	3		**10**		1
			3	4	3	
T10	12	0		**7**		5
			3	2	2	
T14	13	2		**8**		3
			4	4	0	
T15	29	4		**21**		4
			5	13	3	
T16	49	8		**28**		13
			4	18	6	
T20	22	2		**9**		11
			5	1	3	
T22	42	5		**28**		9
			5	16	7	

Table 3:
Diagnostic genetic testing outcomes

Trisomy (total # of cases)	Cases with diagnostic testing results	Confirmed in fetus or placenta	Co-twin demise reported
T3 (24)	12	1	2
T7 (83)	43	4	2
T8 (25)	13	3	1
T9 (14)	9	6	0
T10 (12)	5	0	0
T14 (13)	6	0	2
T15 (29)	12	5	2
T16 (49)	18	9	6
T20 (22)	10	2	1
T22 (42)	17	10	3

Table 4:
Outcomes reported for the 10 most common esoteric aneuploidies

Trisomy (total # of cases)	Cases with outcome information available*	Cases with adverse outcome or confirmed result	IUFD/SAB** (A)	IUGR** (B)	PTL/PTD** (C)	USF** (D)	Discordant testing, no obstetric outcome available	Normal, term, live-born infant reported***
T3 (24)	18	9 (50.0%)	2	4	2	6	4 (22.2%)	5 (27.7%)
T7 (83)	59	24 (40.1%)	1	6	4	18	19 (32.2%)	16 (27.1%)
T8 (25)	17	8 (47.1%)	1	1	0	4	7 (42.2%)	2 (11.7%)
T9 (14)	13	9 (69.2%)	5	1	0	5	2 (15.4%)	2 (15.4%)
T10 (12)	7	3 (42.9%)	1	1	0	2	3 (42.9%)	1 (14.2%)
T14 (13)	10	5 (50.0%)	4	0	0	1	4 (40.0%)	1 (10.0%)
T15 (29)	25	22 (88.0%)	14	0	0	5	2 (8.0%)	1 (4.0%)
T16 (49)	36	32 (88.9%)	8	15	8	20	3 (8.3%)	1 (2.8%)
T20 (22)	11	6 (54.5%)	2	1	0	2	5 (45.4%)	0 (0.0%)
T22 (42)	33	28 (84.8%)	11	4	5	11	2 (6.06%)	3 (9.1%)

* Derived from columns A+B in **Table 2**

** Outcomes in columns A through D are not mutually exclusive; i.e., a single case may have had both growth restriction and preterm labor.

***Normal, term, live-born infant reported includes cases in which genetic testing was not pursued due to lack of phenotype.

IV. Conclusions

Approximately 47% of all cases reported positive by cfDNA with an esoteric aneuploidy had an adverse outcome. When considering cases in which outcome information is available and stratifying by specific aneuploidies, some chromosomes confer a significantly higher risk of adverse outcome. These notable differences in adverse outcomes by chromosome are consistent with previous literature and likely related to the meiotic or mitotic origin of the aneuploidy.[5,6]

Of the cases with known diagnostic testing, the retrospective observed PPV is approximately 26.7%. Many cases only have amniocentesis and no additional placental testing, which limits assessment of diagnostic testing concordance, especially since many of these events would likely be confined to the placenta. The rates of adverse outcomes support the concept that many of these events exist in the placenta even if unconfirmed in the fetus. Previous literature has treated cases with signs of placental insufficiency (such as might be expected with CPM) as evidence of a likely true event in the fetus.[1] If we considered those cases with signs of CPM as true positive, the PPV would be significantly higher, as 46.9% of all cases were reported with either diagnostic confirmation or adverse obstetric outcomes.

Additionally, some cases only had karyotype as follow-up testing, which would not provide clarity on the presence of UPD. In cases with cfDNA result for an aneuploidy involving a known imprinted chromosome, this risk is communicated to the provider on the report for consideration in appropriate follow-up testing. However, even in non-imprinted chromosomes, UPD may carry residual risk for an autosomal recessive condition in the fetus and can help direct follow-up testing based on fetal phenotype.

A limitation of this dataset is the number of cases with incomplete or unknown outcome information, despite significant effort to collect it. Additional outcome information would allow for more in depth analysis of the differences between individual aneuploidies and rates of adverse outcomes. As a clinical laboratory, ongoing feedback from ordering providers is instrumental in collecting these outcomes. Ordering providers may have a difficult time obtaining follow-up if the patient was referred from another provider (such as an obstetrician referring to maternal fetal medicine specialists); it is also not uncommon for a patient with a confirmed, true positive to be lost to follow-up if they pursue a termination of pregnancy or have a spontaneous pregnancy loss and do not return for care.

Notably, over 14% of cases reported to have an esoteric aneuploidy ended in stillbirth or fetal demise. While confirmatory testing is always recommended after a screen positive cfDNA result, in some of these cases the loss occurred before diagnostic testing could be completed and testing on products of conception could not be facilitated. While cfDNA will not provide information about the structure or arrangement of a trisomy, the information about the likely chance of an esoteric aneuploidy as the explanation for the pregnancy loss may prove valuable to the patient and provider. Additionally, another 5.8% of cases had a known co-twin demise and families may find value in knowing the likely explanation for the loss of the co-twin.

Ultimately, this data demonstrates the clinical relevance of esoteric aneuploidies, even in the absence of diagnostic confirmation, and provides useful counseling insight on the implications of events by chromosome.

V. References

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