Not just one: the utility of whole genome sequencing for making a dual molecular diagnosis

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OBJECTIVES

Conventional phenotype-driven and stepwise genetic testing is time-consuming and may yield inconclusive results. Moreover, the genetic investigation paradigm often stops at the point of identifying a single diagnosis, potentially overlooking additional underlying genetic etiologies. Whole genome sequencing (WGS) offers a comprehensive solution for genetic investigation by capturing a wide range of genomic variations in a single test. The utility of WGS for making multiple molecular diagnosis in individuals with suspected genetic etiologies was investigated.

METHODS

WGS results from over 1,000 individuals who were referred for investigation of genetic conditions at Baylor Genetics were evaluated. Additional WGS results since the time of abstract submission have been retrieved, analyzed, and included in this cohort. The molecular findings and characteristics of the variants contributing to multiple diagnoses were examined.

RESULTS

Reportable multi-locus findings were reported in 78 cases; including 1 case with triple diagnoses, 24 cases with dual diagnoses, and 36 individuals with a definite diagnosis in conjunction with other reportable finding(s) (VUS or above) in another locus [2 variants in an autosomal recessive AR locus or 1 variant in an autosomal dominant (AD) locus](Figure 1). Variants in autosomal dominant loci contributed to 57.1% of the multiple diagnoses. Around half of the multiple diagnoses involved multiple variant types (Figures 2 & 3).

FIGURE 1

WGS and Rapid WGS yielded single and multiple reportable findings

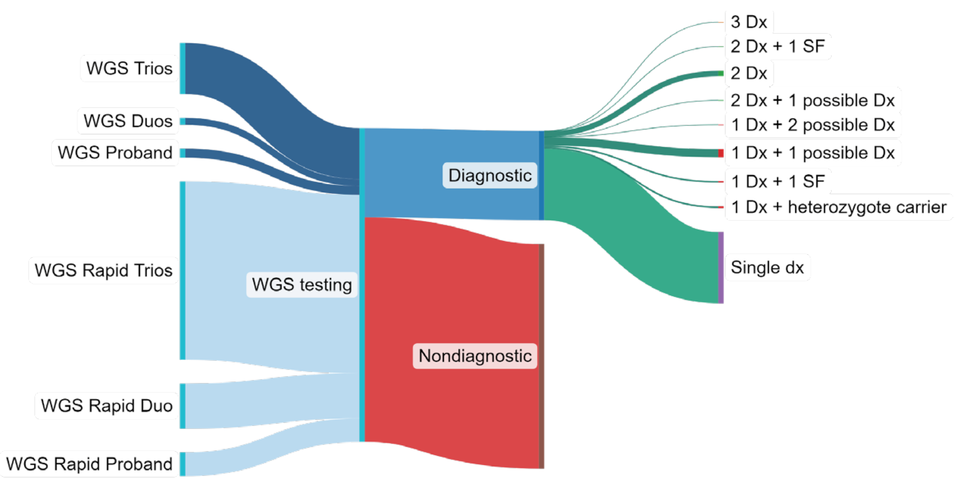
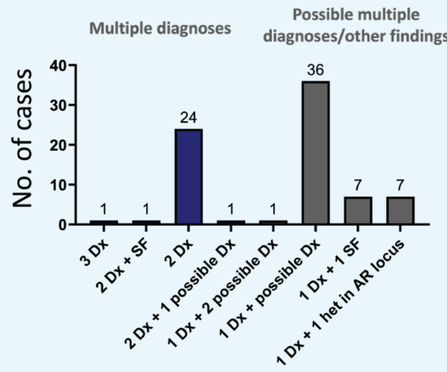


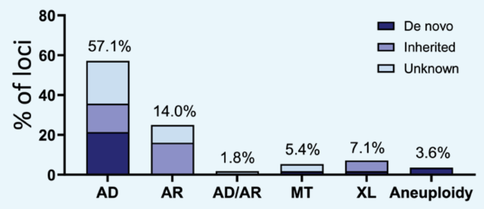
Figure 1: The proportion of WGS and Rapid WGS yielding single and multiple molecular diagnoses. Dx, diagnosis(es). SF, secondary findings based on the ACMG v3.2 gene list. A possible diagnosis is defined by one VUS in an autosomal dominant disease-causing locus or one pathogenic/likely pathogenic variant in addition to a VUS in an autosomal recessive disease-causing locus.

FIGURE 2

A Number of patients with multi-locus findings



B Mode of inheritance of disease-causing loci contributing to multiple diagnoses



C Characteristics of the variants contributing to multiple diagnoses

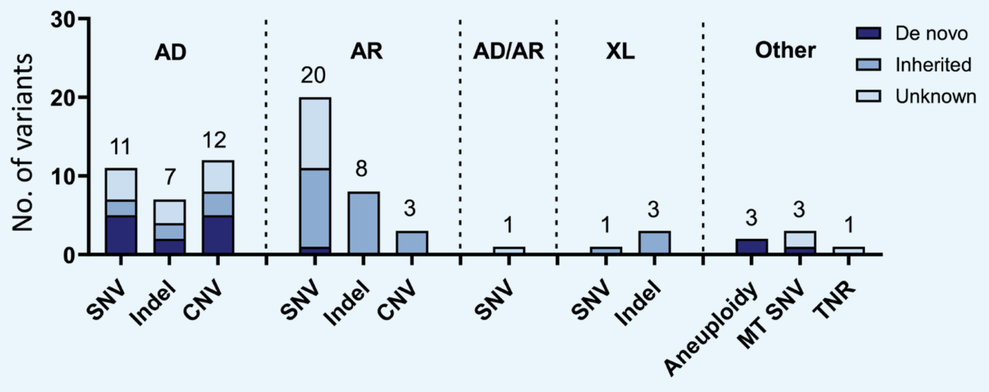


Figure 2: A. The number of individuals with multi-locus reportable findings.
B & C. The mode of inheritance of the loci and variant types contributing to multiple molecular diagnoses in 27 cases. Dx, diagnosis(es); SF, secondary finding; het, heterozygous; AD, autosomal dominant; AR, autosomal recessive; MT, mitochondrial; SNV, single nucleotide variant; Indel, small insertions and deletions; CNV, copy-number variant; TNR, triplet repeat expansion.

FIGURE 3

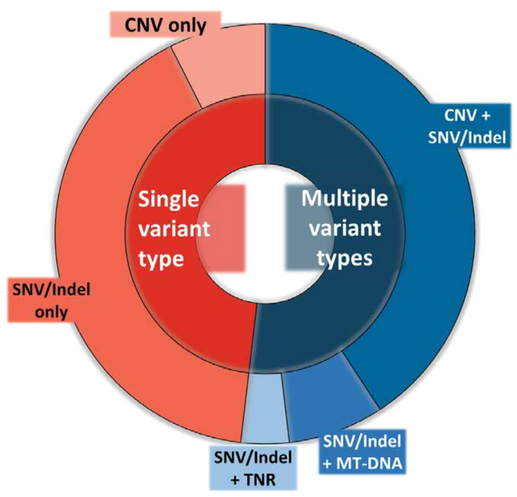


Figure 3: Approximately half of the multiple diagnoses involved a combination of different variant types. WGS or multiple lines of testing would be required to reach these multiple diagnoses. TNR, triplet repeat expansion.

CONCLUSIONS

WGS demonstrated utility in unraveling multiple molecular diagnoses. This finding is significant not only in ending diagnostic odysseys but also in demonstrating the need to account for heterogenous variant types and multiple molecular etiologies associated with complex or overlapping phenotypes. Multiple molecular diagnoses will also improve the understanding about the clinical effects of multiple variants at more than one locus.

Prenatal CMA using exon-level coverage for 'disease gene' CNV interrogations in the era of non-invasive prenatal testing

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BACKGROUND

Non-invasive prenatal testing (NIPT) with cell-free DNA is widely accepted as standard of care to screen for common aneuploidies, and some platforms also include screening for microdeletions/duplications. This shift in clinical practice affects the use of chromosomal microarray analysis (CMA) in the prenatal setting. In addition, microarrays with exon-by-exon coverage for disease genes enable detection of intragenic copy number variants (CNVs) leading to increased sensitivity to postnatal diagnosis but such arrays are rarely used in prenatal CMA.

This study investigated the role of prenatal CMA in the era of NIPT based on a single laboratory's experience.

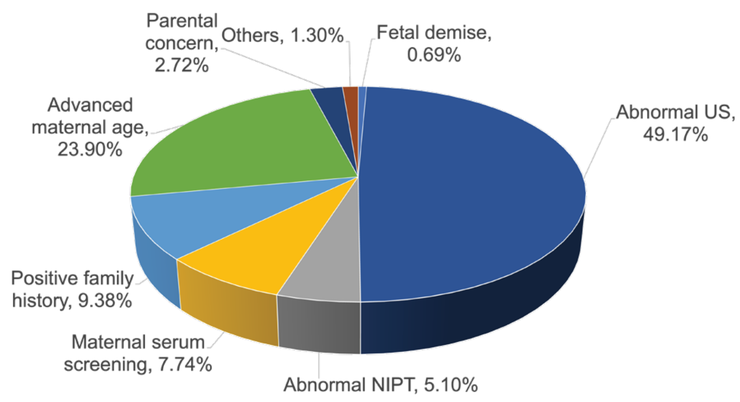
METHODS

- We retrospectively reviewed the results of all amniotic fluid and chorionic villus samples that were analyzed by CMA using custom-designed Agilent arrays during the last 11 years at Baylor Genetics.
- For 80% of samples, CMA was performed using arrays that include exon-by-exon coverage for >1,700 genes.
- Parental samples were received concurrently to evaluate maternal cell contamination and to facilitate data interpretation for most cases.

RESULTS

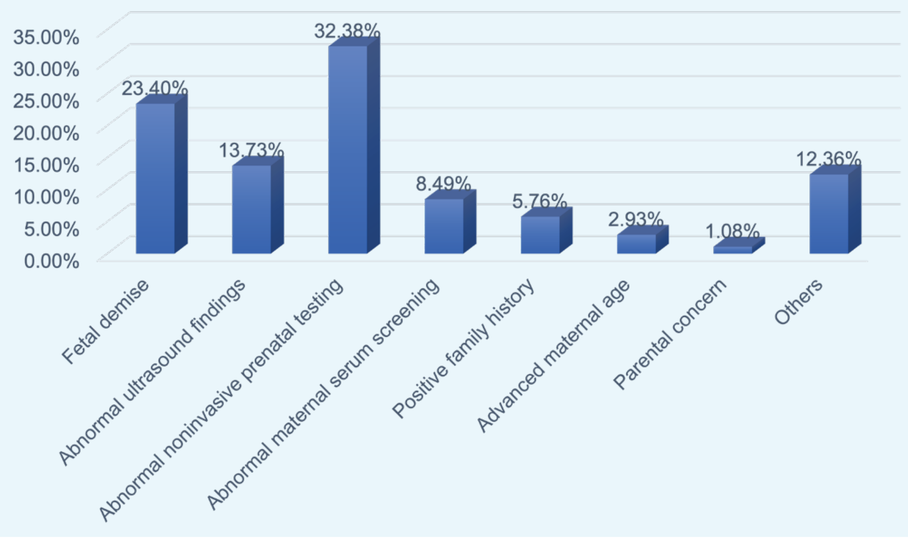
- CMA cases were grouped according to the primary indication. If there were multiple indications, the case was classified based on the primary indication with the highest priority in the following order: fetal demise, abnormal ultrasound findings, abnormal NIPT, positive maternal serum screening, positive family history, advanced maternal age (AMA), parental concern, and others.
- The most common primary indications were abnormal prenatal ultrasound findings (US) and AMA. This is consistent with previous literature (Breman et al. 2012). Abnormal or atypical NIPT was observed in 5.1% of cases, and another 1.0% cases had both an abnormal ultrasound and abnormal NIPT indication (Figure 1).

Figure 1 Distribution of CMA cases by primary indication



- The overall CMA detection rate of clinically significant findings was 10.7% and the diagnostic rate for pregnancies with abnormal ultrasound findings was 13.7%.
- The diagnostic rate was highest (32.4%) for cases with abnormal or atypical NIPT results as the primary indication. Among those, the most frequent NIPT findings were increased risk or positive result for autosomal aneuploidy, followed by increased risk or positive result for sex chromosome aneuploidy, microdeletion/duplication, and inconclusive or nonreportable findings.

Figure 2 Diagnostic rate by primary indication



Cases with increased risk for microdeletion/duplication by NIPT

- Of the 70 cases with increased risk for microdeletion/duplication, CMA confirmed the CNV in 13 (18.6%) of cases (Table 1).
- Of the 32 cases with increased risk of 22q11.2 deletion, the typical 22q11.2 deletion was detected by CMA in four cases, while the other 28 cases showed no copy number changes in this region.
- For the other 9 cases with CMA confirmed CNVs other than 22q deletion, all the CNVs are >10 Mb in size except for one case with a 1.4 Mb maternally inherited duplication in 21q22.2.
- Notably, in one case, while NIPT reported increased risk for 1p36 deletion, CMA did not detect copy number variants in chromosome 1, instead, but CMA showed a terminal deletion in chromosome 3 that is the typical deletion for 3q29 microdeletion syndrome.

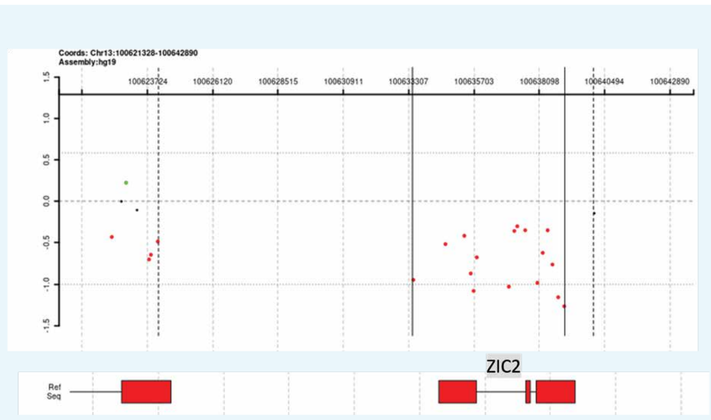
Table 1 Comparison of the CMA results with the NIPT results for the cases with increased risk for deletions/duplications

NIPT showing increased risk	# of cases	# of cases with concordant CMA results	Confirmation rate
22q11.2 deletion	32	4	12.5%
1p36 deletion	8	0	0.0%
15q PWS/AS deletion	8	0	0.0%
5p deletion	6	0	0.0%
18p duplication	3	2	66.7%
Others	13	7	53.8%
Total	70	13	18.6%

Single gene deletions/duplications detected by prenatal CMA

- Prenatal CMA findings include aneuploidy, triploidy, and deletions/duplications not involving an entire chromosome (N=321 cases).
- Due to the increased probe coverage in genes for established rare disease traits, CMA detected clinically significant CNVs affecting single protein coding genes in 18 cases. The genes affected in these cases include *DMD* (N=9 cases), *NRXN1* (N=2 cases), and seven genes in each of one case (*ATP7A, KAL1, MED13L, PAFAH1B1, RPL11, STS,* and *ZIC2*).
- The smallest finding is a de novo ~5.6 Kb deletion of the *ZIC2* gene in a fetus with holoprosencephaly detected by ultrasound (Figure 2). Haploinsufficiency of *ZIC2* causes holoprosencephaly 5 (OMIM # 609637). Holoprosencephaly is a complex brain malformation resulting from incomplete cleavage of the prosencephalon, affecting both the forebrain and the face.

Figure 3 A ~5.6 kb deletion affecting only the *ZIC2* gene was detected



CONCLUSION

- Prenatal CMA remains essential for the detection of microdeletions/duplications because NIPT screening coverage of submicroscopic copy number changes is limited.
- We also show that increased probe coverage of disease genes on prenatal microarrays enables detection of single gene copy number changes.
- Finally, this confirmation data demonstrates the importance of a diagnostic test such as CMA in follow up to NIPT results that are positive or increased risk for microdeletions/duplications.

REFERENCES

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Advancing Precision Diagnosis through Simultaneous Detection of Diverse Variant Types: Insights from Clinical Whole Genome Sequencing

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INTRODUCTION

Genetic disorders arise from diverse types of molecular variants. Historically, clinical genetic testing assays could only assess for specific types of variants (primarily sequencing-based). This resulted in many patients with complex phenotypes remaining undiagnosed or required to submit to multiple rounds of testing with alternate assays to reach a diagnosis. Whole genome sequencing (WGS) now offers healthcare providers the ability to test for many types of variants with one assay, providing a powerful tool to pinpoint an etiology accurately and more rapidly for these patients, thus avoiding the diagnostic odyssey. Here, we summarize our experience performing clinical WGS at Baylor Genetics to highlight its utility as a first-tier diagnostic test capable of detecting a spectrum of variant types.

METHODS

This is a retrospective evaluation of WGS results at Baylor Genetics. For each patient, the type of variant(s) reported as well as demographic data, clinical history, and diagnostic findings were investigated.

RESULTS

Single nucleotide variations (SNVs) and small indels were the most common variant types in 233 cases with significant findings related to reported phenotype.

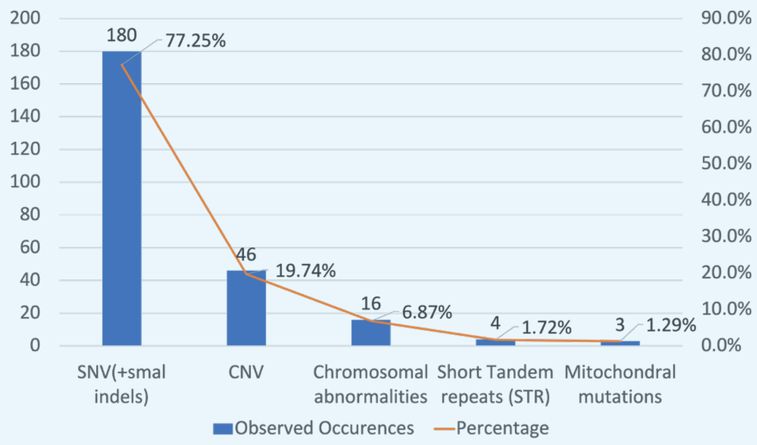

Figure 1: Reported occurrences and percentage of different variant types

Nearly 30% of cases with WGS findings related to phenotypes would not have been captured by assays only focusing on SNV/indels.

Case Type	Numbers	Percentage
Cases with SNV(+small indels) only	164	70.4%
Cases involved other variant types	69	29.6%

Examples of cases solved by variants other than SNV

Variant Type	Major Phenotypes	Variants Detected	Diagnosis
CNV	cleft lip/palate, PDA, ASD, respiratory failure	*De novo* deletion of exons 3-4 in *SPECC1L*	Teebi hypertelorism syndrome-1
Chromosomal	IUGR, dysmorphic features, thrombocytopenia	deletion of 11q23.3q25 and duplication of 16p13.3	Jacobsen syndrome & 16p13.3 microduplication
STR	congenital hypotonia	~2450 CTG repeats in *DMPK*	Congenital myotonic dystrophy-1
Mitochondrial	failure to thrive, chronic muscle weakness	*De novo* Heteroplasmic (~39%) m.14453G>A	*MT-ND6*-related disorders

An example of complex WGS findings: a mosaic monosomy X plus Xq21.31q28 deletion detected by the comprehensive ability of WGS analysis (Figure 2-5)

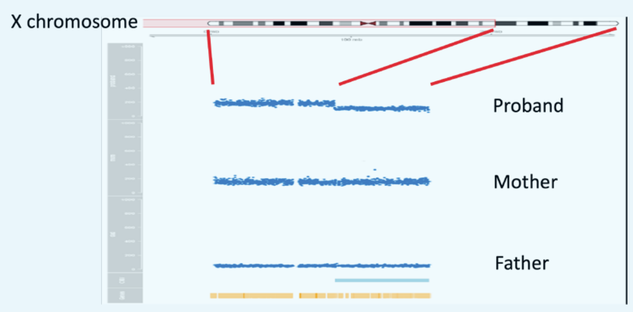

Figure 2: CNV analysis detected a *de novo* Xq terminal deletion in the proband

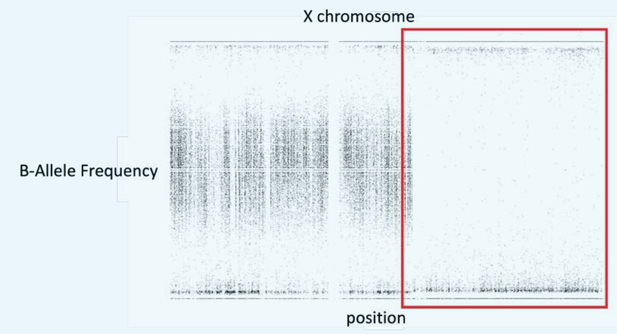

Figure 3: Terminal deletion in chromosome X consistent with the regions of homozygosity (ROH, indicated by red box) detected by WGS on the X chromosome.

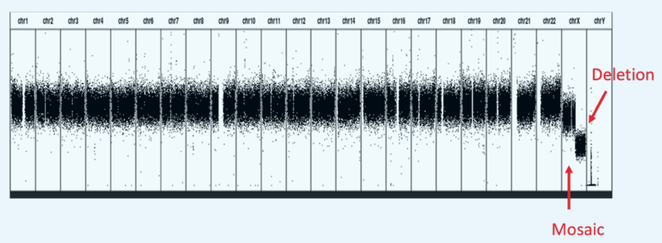

Figure 4: Genome wide review of read depth presentation however revealed a more complex scenario, suggestive of mosaicism for monosomy X in addition to the Xq terminal deletion.

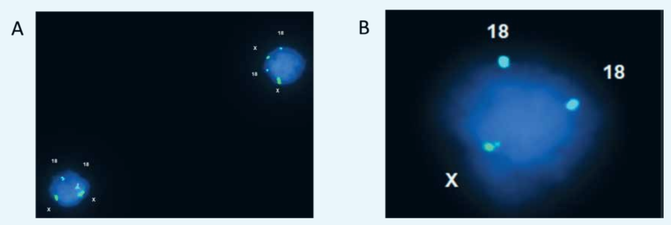

Figure 5: Further confirmatory rapid FISH test with X centromere probes detected 64% cells with two X chromosome (represented in picture A), while 36% cells with monosomy X (represented in picture B). Probe set for simultaneously detecting centromere of chromosome 18 and X were deployed.

CONCLUSIONS

These data affirm the utility of WGS as a comprehensive test for detecting an assortment of molecular variants to make a diagnosis. Many patients with hard-to-diagnose phenotypes are critically ill, and this data supports WGS as an expedient tool which can improve patient outcomes and avoid diagnostic odysseys. The availability of rapid WGS (~5 calendar day TAT) further supports the clinical utility of WGS.

Products of conception analysis by chromosomal microarray: a single center experience.

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INTRODUCTION

Chromosome aberrations detectable by conventional cytogenetics are a common cause of missed abortions and intrauterine fetal demise. However, chromosome analysis of products of conception (POC) may be unsuccessful or uninformative due to maternal cell contamination (MCC) and reduced viability of fetal cells in culture. Chromosomal microarray (CMA) may circumvent such issues, as it does not require culturing; in addition, MCC studies may be performed on extracted DNA prior to running the array. If those data indicate significant MCC, testing may be cancelled to avoid incurring the expense for a likely false negative result. A retrospective review of data from 484 cases submitted to Baylor Genetics for targeted chromosomal microarray analysis (CMA) following a stillbirth or a spontaneous loss of pregnancy was undertaken to assess clinical utility and determine best practices.

METHODOLOGY

Samples submitted following pregnancy loss were triaged for amount and type of tissue as well as for test orders. The most common tissue types received were placental tissue including chorionic villi and cord; on rare occasions, skin was submitted. If CMA was the only ordered test on fresh tissue, a direct sample was sent for DNA extraction, and if available, a piece of tissue was frozen for additional extraction if needed. When chromosome analysis was also ordered, every effort was made to send a direct sample to extraction before initiating cultures. FFPE samples were also accepted for testing.

Prior to DNA extraction, the sample was incubated overnight at 37°C in Puregene Cell Lysis Buffer combined with 3 µl of Proteinase K (20mg/mL) and 1.5 µl RNaseA (100.0mg/mL). DNA extraction was performed using a modified Qiagen method as described previously (Breman et al 2012).

CMA was performed on a custom designed 180k array chip from Agilent containing 60,000 SNP probes for detection of AOH and triploidy in addition to >100,000 oligonucleotide probes targeting virtually all the known microdeletion or microduplication syndromes as well as the pericentromeric and subtelomeric regions with an average probe density of 10–20 kb/probe in the targeted regions. The average probe density over the entire genome (between disease regions) is 30 kb/probe.

Data was analyzed with proprietary in house software (Figure 1) until January, 2023 at which time, the use of NxClinical software was initiated. Reporting criteria have changed and continue to evolve; thus copy number variants have been reported for some later samples.

Receipt of a maternal blood sample allowed the degree of maternal cell contamination (MCC) to be evaluated by comparative analysis of maternal and fetal DNA using multiple unlinked polymorphic markers. CMA was not performed on samples showing ≥75% MCC. Figure 1B illustrates a diagnosis made in the face of ~50% MCC.

RESULTS

484 samples submitted for targeted CMA

- 84 cancellations issued by the laboratory
  - 62 showed 75-100% MCC
  - 22 cases yielded insufficient quantity/quality of DNA after multiple extractions
- 400 CMA results issued
  - 152 normal male samples
  - 130 reported as normal female
    - 77 were negative for maternal cell contamination
    - 12 with <5% MCC
    - 7 with 6-10% MCC
    - 3 with 10-20% MCC
    - 5 with 20-50% MCC
    - 2 with 100% MCC (maternal sample submitted after a normal female report)
    - 24 cases had no maternal sample submitted
  - 118 reported with an abnormal or variant result
    - Table 1 shows breakdown by type of abnormality and gestational age
      - As expected, the majority of aneuploidies occur in the 1st and 2nd trimesters
      - Trisomy 21 is equally represented across all gestational ages
    - Table 2 shows specific aneuploidies and triploidies by gestational age
    - Table 3 shows clinically significant CNVs unlikely to be the etiology of the loss

TABLE 1

Trimester					
Gestational Age	1st	2nd	3rd	Unknown	Total
Total # of Cases	124	182	75	19	400
# Aneuploid Cases	49	24	8	4	85
Triploidy	7	1	0	0	8
# Structural Abn. Cases	1	2	0	0	3
# CNVs	3	11	8	0	21

Table 1: Breakdown of results by type of abnormality and gestational age. The gestational age was not provided in 19 cases. Data does not include samples that were unable to undergo CMA analysis but for which chromosome analysis was performed.

FIGURE 1: CMA PLOTS

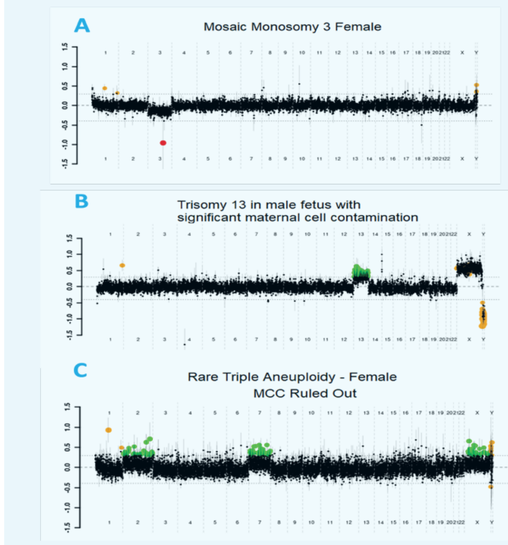

TABLE 2

Aneuploidy	Trimester		
	1st	2nd	3rd
Monosomy X	14*	4	0
Trisomy 22	9	0	0
Triploidy	7	1	0
Trisomy 15	5	0	0
Trisomy 21	5	6**	5
Trisomy 13	4	3	0
Trisomy 9	3**	2	0
Double Aneuploidy	2	1	0
Trisomy 8	1	0	0
Trisomy 4	1	0	0
Trisomy 14	1	0	0
Trisomy 16	1	1	0
Trisomy 18	1	4	2
Trisomy 20	1	0	0
Triple Aneuploidy	1	0	0
Monosomy 3	0	1**	0

Table 2: Specific aneuploidies in descending order of frequency in first trimester losses as compared to 2nd and 3rd losses

* 5 mosaic for XX cell line/** 1 mosaic for normal cell line

RESULTS (CONTINUED)

Chromosome analysis ordered on 186 samples

- 52 did not yield sufficient metaphase cells for analysis
- 26 yielded a normal male karyotype
- 8 samples showed both XX and XY cells (3 with abnormal male results also included in 22 abnormals)
- 81 yielded a normal female karyotype
  - MCC studies and concordant CMA results showed 27% of the 81 female results were truly normal female.
    - Data consistent with well known problem of maternal cell overgrowth of fetal cells in culture.
- 22 yielded abnormal results
  - 9 gave information not obtained from the array results
    - Table 4 shows 5 cases for which cytogenetics provided the diagnosis or additional context to the CMA findings.
    - For 5 cases identified as Trisomy 21 or 13 by CMA, karyotypes ruled out Robertsonian translocations.

TABLE 3

Copy Number Variation	Size (Mb)	Association	Classification
arr 7q11.23(72772522-74133319)x1	1.361	Williams Syndrome	Pathogenic
arr 17p12(14128550-15551647)x3	1.423	CMT1A	Pathogenic
arr 17q12(34522398-36136177)x3	1.614	17q12 duplication syndrome	Pathogenic
arr 22q11.23(23747662-24991856)x3	1.244	Variable phenotype	Pathogenic
arr 7q11.23(72772522-74128940)x3	1.356	WBS Duplication	Pathogenic
arr 17q12(34522398-36214026)x3 mat	1.692	17q12 duplication syndrome	Pathogenic
arr[GRCh37] Xq22.2(103031457_103172394)x2 mat	0.141	*PLP1* gene Pelizaeus-Merzbacher	Pathogenic

Table 3: Selected cases with clinically significant CNVs that are unlikely to result in pregnancy loss but are of significant clinical utility

TABLE 4

Cytogenetic Result	CMA Result	Indication	Discussion
92,XXYY/46,XY	Normal Male	Severe growth restriction	79% of cells tetraploid; CMA does not detect tetraploidy
92,XXYY,t(7;13)(p15;q14)x2[16]/46,XX[4]	Normal male	Blighted ovum	Tetraploid cells in two cultures; possible paternal translocation if XX cells are maternal; CMA showed <5% MCC
46,XY,t(2;22)(q12;q12)	Normal Male	IUFD	Possible parental translocation
47,XY,+14[20]	Normal Male	Missed AB	Different pieces of tissue used for each method
47,XY,+18[26]/46,XY[5]	Normal male	Missed AB	Different pieces of tissue used for each method

Table 4: Cases in which chromosome analysis provided diagnostic information after a normal CMA

DISCUSSION

- Chromosomal microarray analysis of DNA from direct extractions provides rapid, accurate diagnostic information on the etiology of pregnancy losses in any trimester without the classic problems incurred in cell culture
  - MCC studies allow assessment of the origin of normal female results, i.e., fetal or maternal
  - CMA may also provide information on pathogenic CNVs that may be of clinical utility to the family
- G-banded chromosome analysis, perhaps as reflex test, provides diagnostic information in some cases

Advancing Tay Sachs Disease Carrier Screening: Insights from Combined Enzyme and Molecular Approaches

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INTRODUCTION

Tay-Sachs (TS) disease (MIM 272800) is an autosomal recessive neurodegenerative disorder caused by α subunit deficiency of β-hexosaminidase (Hexo A). Population based carrier screening for individuals of Ashkenazi Jewish ancestry by enzyme analysis successfully reduced the incidence of TS in US and Canada.

In a diverse, pan-ethnic population, Tay-Sachs carrier screening is endorsed by the American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics and Genomics (ACMG). It typically employs both the Hexo enzyme assay and *HEXA* gene sequencing. Molecular testing can range from targeted variant to full-exon sequencing. The β-hexosaminidase enzyme assay, which measures lysosomal Hexo A and B enzymatic activities in leukocytes, has long been a cornerstone in Tay-Sachs screening.

In recent years, molecular screening panels have gained popularity, harnessing next-generation sequencing technology to cover a wide spectrum of diseases. Both enzyme analysis and molecular testing present challenges: Enzyme testing traditionally employs an artificial substrate and pseudodeficiency alleles result in carrier-range enzyme results despite not truly being a carrier of a pathogenic allele; DNA testing, particularly in diverse populations with low carrier frequency can identify variants of uncertain significance (VUS). Because of this VUS challenge, screening panels often report only selected variants, omitting potentially pathogenic novel variants.

To assess the utility of enzymatic carrier testing, we conducted a retrospective analysis of carrier sequencing results from our laboratory database. NGS carrier panel results were cross-referenced with leukocyte enzyme results. Any inconsistencies prompted retrieval of full *HEXA* sequence results for further variant curation. Results of total 44 patient results reported here support an integrated approach employing both molecular and enzymatic testing in Tay Sachs carrier screening to improve carrier detection.

METHODS

- The internal lab database was searched retrospectively for positive cases of Tay Sachs carrier Hexo enzyme screening by leukocytes. These enzyme results were further corroborated with available NGS carrier panel results. The sequencing data of cases with inconsistent enzyme and NGS sequencing results were subsequently reviewed for non-reported variants. All uncovered variants were curated.
- Tay Sachs enzyme assay was performed in leukocytes with heat inactivation to determine hexosaminidase A and B activities. Hexo A% range for carriers is 30-49%.
- NGS carrier panel is a reproductive carrier screening testing. Over 410 disorders may be screened in the customized NGS panel. Only (likely) pathogenetic variants are reported.
- This study was conducted according to Baylor College of Medicine (BCM) Institutional Review Board (IRB) approved protocols.

RESULTS

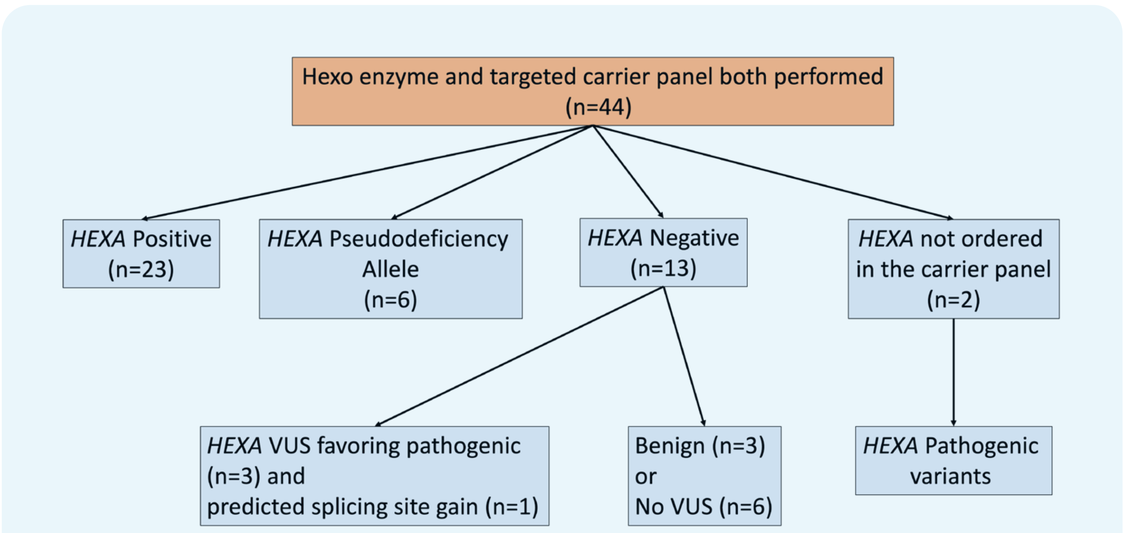

Figure 1: Follow-up molecular results of 44 Hexo A enzyme positive cases. *HEXA* results in NGS carrier panel results are corroborated. If *HEXA* is not reported in the initial customized panel, sequencing information are further retrieved and curated.

RESULTS

Figure 2. Distribution of molecular results in 44 Hexo A enzyme positive cases.

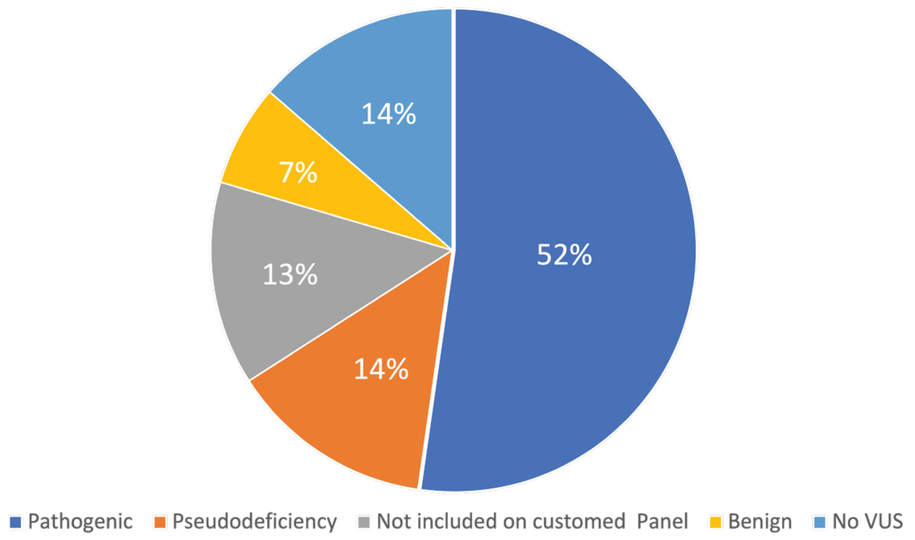

Table 1. Details of *HEXA* variant curations of 6 enzyme positive cases missed in targeted panel analysis.

No.	Gender	Ethnicity	Hexo A%	*HEXA* variant	Curation
1	F	Northern European Caucasian	37.5	c.1444G>A (p.E482K)	Pathogenic, *HEXA* not ordered
2	M	Hispanic American	47.3	c.409C>T (p.R137*)	Pathogenic, *HEXA* not ordered
3	F	NA	47.8	c.1061_1063del (p.F354del)	VUS favoring pathogenic, reported once in TSD patient
4	F	NA	40.3	c.590A>C (p.K197T)	VUS favoring pathogenic, reported once in TSD patient
5	F	NA	43.9	c.1288G>A (p.D430N)	VUS favoring pathogenic, CADD score 32, no disproving evidences
6	F	African American	44.7	c.673-13T>C	Likely benign, but has moderate splicing predictions for an acceptor gain

CONCLUSION

- In this Hexo A enzyme positive cohort
  - 52% enzyme positive patients were confirmed by targeted NGS panel testing.
  - Two patients were found to carry known pathogenic variants, yet no *HEXA* molecular screening was requested. One of these patients is of North European Caucasian descent.
  - Three pathogenic favoring variants were uncovered through additional sequence curation.
  - One novel intronic variant (possible splicing site gain) was found.
- Increased potential carrier detection rate by 13% when combining full sequence analysis and enzyme assay together for Tay Sachs carrier screening.
- Hexo enzyme assay in leukocytes remains essential in pan-ethnic Tay Sachs disease carrier screening.

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