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

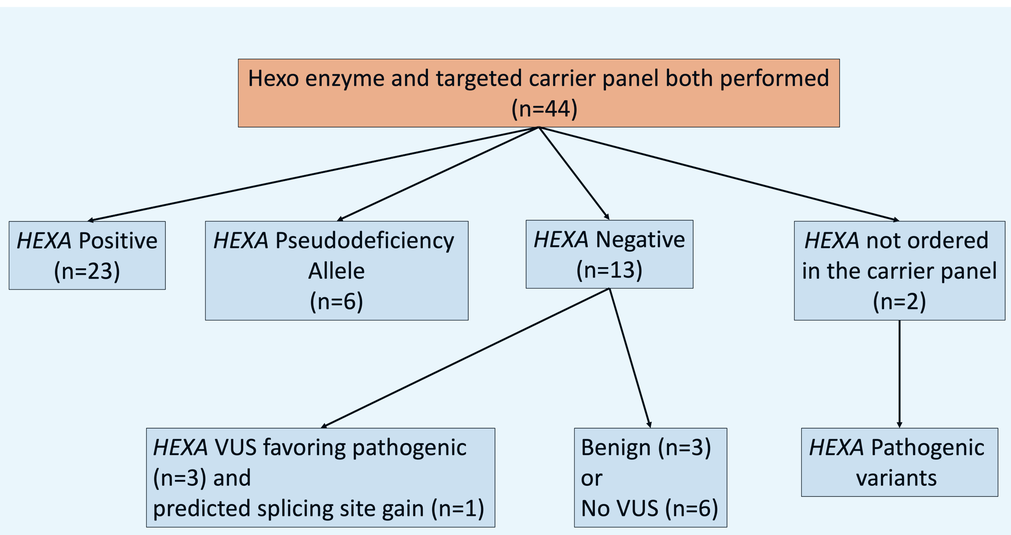

Figure 1: Follow-up molecular results of 44 Hexo A enzyme positive cases. *HEXA* results in NGS carrier panel results are corroborated. If *HEXA* is not reported in the initial customized panel, sequencing information are further retrieved and curated.

RESULTS

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

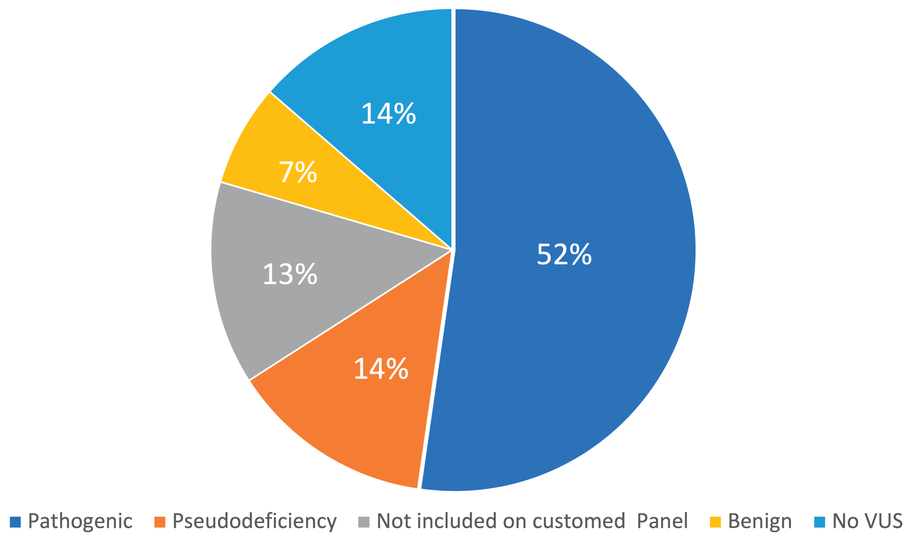

Table 1. Details of *HEXA* variant curations of 6 enzyme positive cases missed in targeted panel analysis.

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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