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Proband-only Diagnostic Exome Sequencing: Trends in Diagnostic Yield at One Commercial Company

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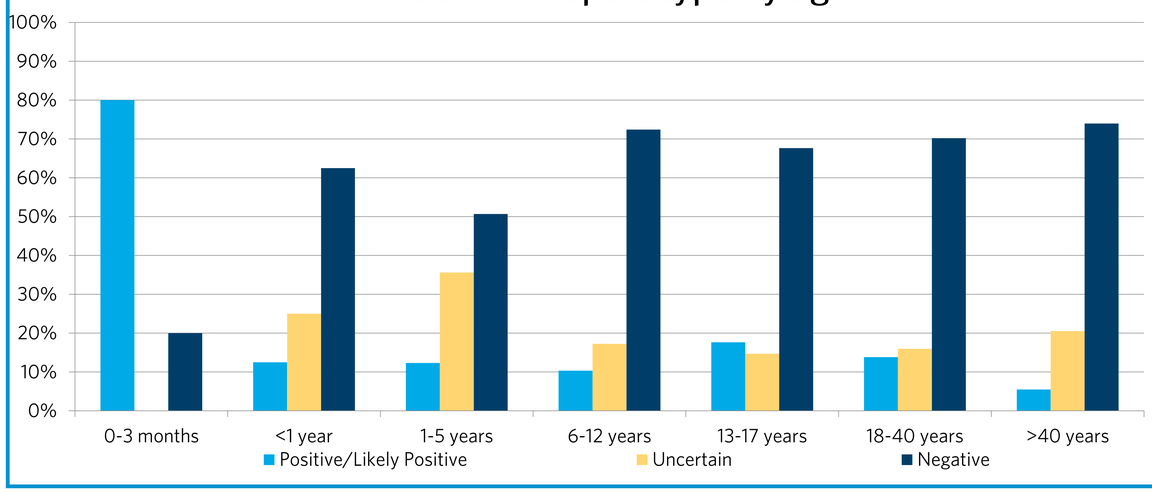
BACKGROUND

- Use of family samples for diagnostic exome sequencing (DES) has been shown to increase diagnostic yield compared to proband-only DES[1,2]
- Trio analysis can:
  - Optimize variant detection (joint variant call)
  - Determine phase of variants within the same gene
  - Highly prioritize *de novo* alterations
- Family samples provide information on variant co-segregation through:
  - Concurrent exome sequencing on family member(s)
  - Targeted Sanger analysis of relevant variants
- Family members may not be available for testing in cases of:
  - Adoption
  - Adult patients whose parents may be deceased
  - Complicated family dynamics

FIGURE 1: Report type by age



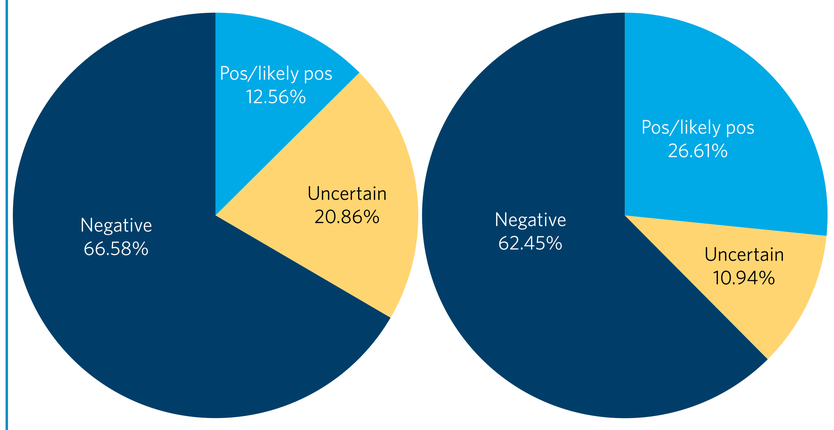
METHODS

- Assessed all proband-only DES (no other family members available for co-segregation analysis) ordered through a commercial lab over a 2 ½ year period.
- Defined diagnostic yield as resulting in a positive or likely positive overall report
  - Uncertain reports issued if a variant of interest has either uncertain pathogenicity or clinical overlap
- Evaluated trends in diagnostic yield based on:
  - Age at testing
  - Age of disease onset
  - Clinical features
  - Previously reported testing
- Analyzed trends of uncertain reports due to:
  - Unclear clinical overlap
  - Inconsistent zygosity -- one mutant allele identified in autosomal recessive (AR) disorder
  - Identification of variant of unknown significance (VUS)

DIAGNOSTIC YIELD

- 374 proband-only DES cases were tested at Ambry Genetics over a 2 ½ year period
- Diagnostic yield by age (Figure 1)
  - Highest among neonate age group (80.0%; 4/5)
  - Lowest among individuals over 40 (5.48%; 4/73)
  - Rate of uncertain results consistent across age groups excluding neonates
- Adults with childhood-onset disease had higher diagnostic yield compared to those with adult-onset disorders (14.9% vs. 7.0%)
- Compared to parent-proband trios*, (Figure 2) proband-only DES had:
  - Half the diagnostic rate (12.56% vs. 26.61%)
  - Twice the uncertain results (20.86% vs. 10.94%)
  - Similar rates of negative reports
- Retrospective data (2012-2014) of proband-only DES with family member samples for co-seg analysis showed higher diagnostic yield (not pictured)
  - Demonstrates the importance of familial samples for determining zygosity/inheritance for variant interpretation
  - Earlier cohort is likely enriched for Mendelian disorders given these were ran before DES became widely adopted

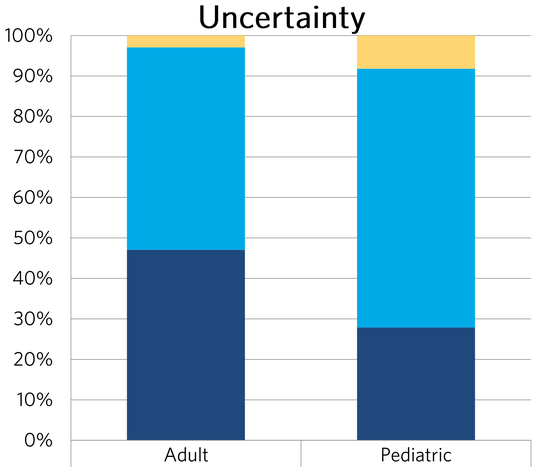
FIGURE 2: Proband-only vs. Trio diagnostic yield



CLINICAL vs. VARIANT UNCERTAINTY

- In total, 78 uncertain reports were issued containing 96 genes.
  - 34 genes on 30 reports for adults
  - 62 genes on 48 reports for children
- 1 case was uncertain due to inconsistent zygosity (heterozygous mutation in AR gene).
- Remaining cases were due to VUS and/or uncertain clinical overlap.
  - Across all ages, an uncertain report was more likely due to a VUS (Figure 3)
  - However, in adults there was greater certainty when it comes to clinical overlap
- 47.06% of adult cases had good clinical overlap, but were issued an uncertain report based on the VUS variant classification.
- Only 27.42% of pediatric cases were uncertain due to VUS classification alone.
- 1 adult case (2.94%) was classified as uncertain for clinical overlap alone.

FIGURE 3: Variant and Clinical Uncertainty



	Adult	Pediatric
Uncertain clinical overlap	2.94%	8.20%
Both VUS and uncertain clinical overlap	50.00%	63.93%
VUS	47.06%	27.87%

PREVIOUS TESTING

- One hypothesis for lower diagnostic yield in adults is that they already had extensive genetic testing
- In total, 612 molecular tests were reported for all patients (range 0 – 12 tests per patient)
- 97 patients had previous panel testing
  - Not a predictor for overall DES report type
- 87.4% of pediatric cases reported previous testing
  - Average of 2.1 tests per patient (range 0-12)
- Only 43.7% of adult cases reported previous testing
  - Average of 1.05 tests per patient (range 0-8)
  - No significant difference between childhood-onset and adult-onset disease cohorts (average of 1.19 vs. 1.01 tests)
- Patients with 4 or more previous tests were less likely to have a positive result than those with fewer tests (Table 1)

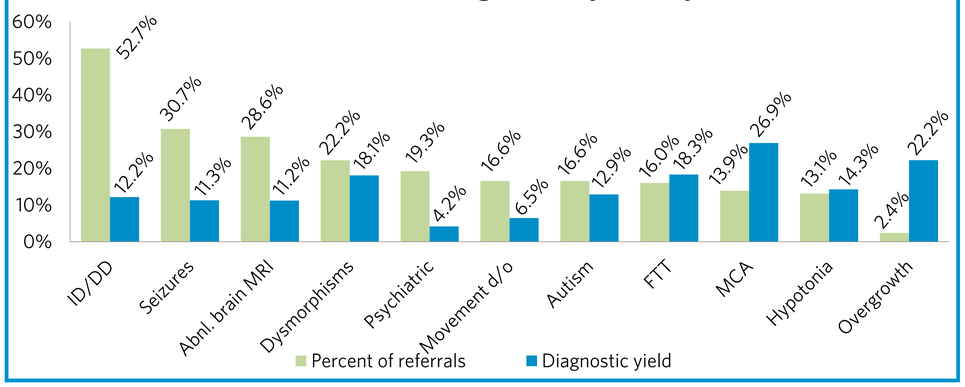
TABLE 1: Number of previous tests by report type

	Pos/likely pos	Uncertain	Negative
0 tests	12.1% (12/99)	18.2% (18/99)	69.7% (69/99)
1-3 tests	14.1% (33/234)	22.7% (53/234)	63.2% (148/234)
4+ tests	**4.9% (2/41)**	17.1% (7/41)	78.0% (32/41)

CLINICAL CATEGORIES

- Developmental delay/ intellectual disability, seizures, abnormal brain MRI, and dysmorphic features were most commonly reported features (Figure 4)
  - This is consistent with samples received for DES testing as a whole[1]
- Neurological, musculoskeletal and ophthalmological system involvement were the most common in both adult and pediatric groups
- Cases with multiple congenital anomalies had the highest diagnostic yield
- Individuals with psychiatric disorders had the lowest diagnostic yield

FIGURE 4: Referral rates & diagnostic yield by clinical feature



TAKE-HOME POINTS

- The diagnostic yield of proband-only DES with no familial samples for co-segregation analysis is nearly half that of DES performed on trios.
- Ability to add inheritance data to variant interpretation likely accounts for much of the diagnostic yield differences .
  - Cases with family member samples for co-segregation analysis show higher diagnostic rates and are comparable to trio analysis
- Pediatric proband-only DES had a higher overall diagnostic yield.
  - Neonates had highest yield, reinforcing utility of DES in newborns[3]
  - Adults over the age of 40 had the lowest diagnostic yield
- Despite lower diagnostic yield, there was a greater level of confidence for clinical overlap in adults compared to pediatric cases.
- Lower diagnostic yield in adults was not linked to previous extensive testing; however individuals of any age with ≥4 previous molecular tests had a decreased rates of positive or likely positive reports.
- In the absence of available family, proband-only DES is a useful diagnostic tool.

REFERENCES

1. Farwell KD, *et al.* (2015) *Genet Med* **17**(7):578-86. PMID:25356970
2. Lee H, *et al.* (2014) *JAMA* **312**(18):1880-7. PMID:25326637
3. Powis Z, *et al.* (2018) *Genet Med* doi:10.1038/gim.8018.11 [Epub ahead of print] PMID:29554516

*See poster 613 for additional data on parent-trio rates

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