Use of an Enhanced Exome with Genome-Wide Structural Variant Detection for the Diagnosis of Mendelian Disease

Poster 124

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Introduction

Despite the theoretical power of exome sequencing, the realized diagnostic yield of clinical exome testing remains relatively low, ~25%. False negatives, which may account for a significant proportion of unsolved cases, stem from both technical and analytical challenges- including inadequate sequencing coverage, undetected variation (e.g. structural variation), non-optimized prioritization of variants, and limited understanding of the impact of genetic variation on phenotype. The ACE (Accuracy and Content Enhanced) Exome addresses these challenges and aims to increase diagnostic yield through the use of ACE sequencing, annotation, and a suite of phenotype-based analytics.

Materials and Methods

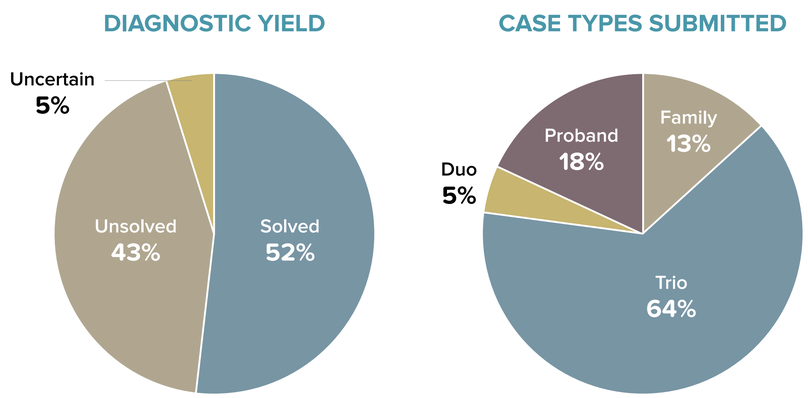
We used the ACE Exome and/or ACE analytics for whole genome/exome data (Personalis, Inc.) to sequence 83 cases of undiagnosed, presumed Mendelian, disease. 47% of cases had undergone array CGH prior to exome/genome sequencing. All research participants provided written informed consent as part of their enrollment in existing research studies with Institutional Review Board approval. Personalis, Inc. also employed an external Institutional Review Board to approve our involvement in the data analysis, where applicable. The remainder of the samples were submitted for clinical testing in our CLIA/CAP approved laboratory. Personalis ACE Exome sequencing was performed in-house, variants were called and annotated with the Personalis ACE pipeline, and the Personalis Annotation and Ranking Engine (PARE) was used to identify candidate variants. Ten samples were subjected to both ACE Exome and whole genome sequencing with ACE pipeline and analytics - bringing the total number of analyses to 93.

The ACE Exome constitutes an exome sequencing platform in which coverage is enhanced for >7800 biomedically relevant genes. The platform also incorporates genome-wide structural variant detection.

Results

Variants of interest were reported in 47 (57%) cases, with the majority having causative/likely causative variants in genes known to cause a phenotype consistent with the described presentation (**FIGURE 1**). A few cases had variants in genes partially explanatory of phenotype (2 cases), variants in candidate genes for phenotype (2 cases), or a single variant identified in a gene known to cause recessive disease (4 cases). Two causative SVs were identified - a large deletion and a previously reported single exon deletion.

FIGURE 1: Diagnostic Yield Overall and by Case Type



Results continued...

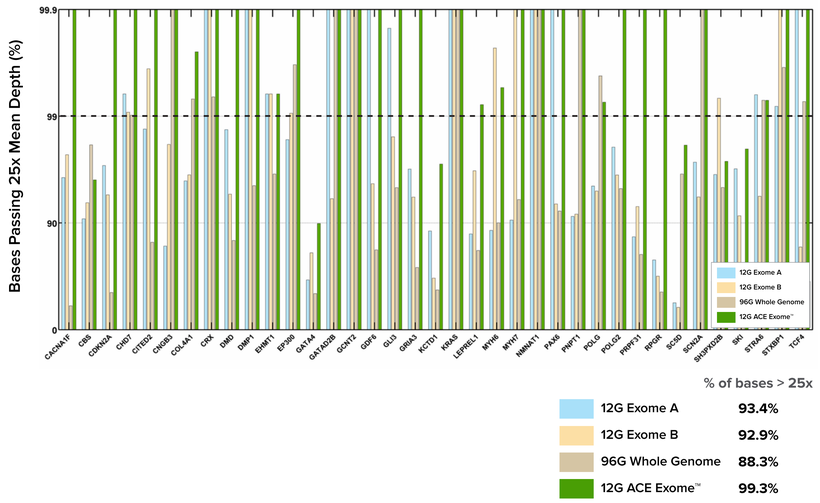
We find the overall diagnostic yield of the ACE Exome to be comparable to the diagnostic yield achieved with whole genome (**TABLE 1**). For ten of the trios described, both ACE Exome and whole genome sequencing was requested and performed. Three of these cases had causative variants reported by ACE Exome. Whole genome sequencing confirmed the three positive findings but did not find any additional likely causative variants in either positive or negative cases.

TABLE 1: Diagnostic Yield by Sequencing Technology

Sequencing Type	n=	Diagnostic Yield
ACE Exome™	**62**	**56%**
Whole Genome	**30**	**52%**

Average performance over the genes reported (genes not embargoed for publication presented here) was compared between two standard exome platforms, 96G whole genome, and the ACE Exome platform. Of the genes for which we reported variation, the majority showed deficits in sequencing coverage on standard exome platforms compared to supplementation with ACE Exome sequencing. ACE Exome sequencing reached target coverage thresholds (99% of bases covered at a minimum average 25x coverage) in 31/37 reported genes. By comparison, only 14/37 and 13/37 genes reached target threshold in two standardly utilized exome platforms. A 96G whole genome achieved coverage threshold on 15/37 genes.

FIGURE 2: Enhanced Gene Coverage with ACE Exome



% of bases > 25x

12G Exome A	93.4%
12G Exome B	92.9%
96G Whole Genome	88.3%
12G ACE Exome™	99.3%

Conclusion

We demonstrate the ACE Exome diagnostic yield in cases of unknown genetic etiology. The ACE Exome sequencing approach improved sequence coverage in genes with reported variation and detected SVs causative of disease. Due to prior aCGH testing, our pilot set was depleted for cases likely to be explained by large SVs, and thus diagnostic yield may be further improved in an unselected patient cohort. An observational case study is underway to assess the diagnostic performance of the ACE Exome and other diagnostic testing strategies in a consecutively referred patient population.

Acknowledgments

We are grateful to the families, our collaborators, and our early access participants.

References

Yang *et al.*, N Engl J Med. 2013;369(16):1502-11.

Discussion of Selected Cases

Genes without Clinical Testing

History
- Bilateral cataracts requiring surgical treatment
- Unilateral microtia and conductive deafness
- Previously reported array finding of 1q41 copy gain containing *DISP1, TLR5, SUSD4*. Significance unknown.
- 3-yr-old sibling with bilateral cataracts, aphakic glaucoma
- Hispanic ethnicity, no known consanguinity

Findings
- 1q41 copy gain inherited
- Homozygosity for previously described missense variant in *GCNT2*, p.Y347C
- *GCNT2* previously described in association with congenital cataracts (no clinical testing available)
- Present in constitutive *GCNT2* exon near previously described variants

Variant Types Not Typically Detectable by Exome

History
- Extensive family history of co-segregation of neurofibromatosis with melanoma, neural tumors
- Unable to obtain clinical *CDKN2A* testing, research testing never returned

Findings
- Deletion of exon 1b in *CDKN2A*
- Variant previously described in association with melanoma and neural system tumors

Panels Quickly Become Out-of-Date

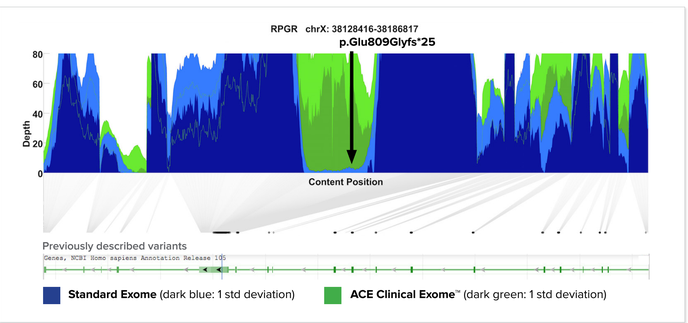
History
- Diagnosis of Leber Congenital Amaurosis
- No family history
- Panel negative

Findings
- Compound heterozygosity for two previously described missense variants in *NMNAT1*, p.N18S and p.R188W
- *NMNAT1*-associated Leber Congenital Amaurosis described in 2012, after the patient had received negative panel results

Exomes Miss Exons

History
- Male, diagnosis of retinitis pigmentosa
- No family history

Findings
- Novel frameshifting indel in *RPGR* exon ORF15, p.Glu809Glyfs*25
- Most pathogenic variants occur in ORF15, an exon poorly covered in standard exome sequencing



Further Information

Personalis @ NSGC 2014

Booth 205

- **The ACE Clinical Exome Test: Advanced Diagnostic Test for Genetic Disease**
  Thursday, 9/18 @ 11:30 AM in VSP Pavilion, Exhibitor Suite, Hall B
- **Application of an Enhanced Exome in the Diagnosis of Rare Genetic Diseases**
  Friday, 9/19 @ 7:00 AM (CEU-approved) in Great Hall B & C
- **Gemma Chandratillake**: *"Revised Diagnosis Through Exome Sequencing of an Infant With Congenital Cataracts Expands Phenotypic Spectrum of COL4A1-associated Disorders"* **Concurrent Session: Genetic Testing I, Friday 8.15 AM**
- **Jeanie Tirch** *et al.*, *"Successful Utilization of Enhanced Exome Sequencing to Identify the Genetic Cause of Retinal Disorders in a Case Series"* **Poster #148**
- **Gemma Chandratillake** *et al.*, *"A Negative Result on Exome Sequencing: What a Genetic Counselor Should Know"* **Poster #119**
- **Sarah Garcia** *et al.*, *"Homocystinuria Diagnosed by Whole Exome Sequencing in Siblings from an Isolated Central American Village"* **Poster #123**