The Clinical Exome: Personalis' Experience Using an Enhanced Exome and Genome-wide Structural Variant Detection for the Diagnosis of Diseases of Unknown Genetic Etiology

Sarah Garcia, Jeanie Tirch, Jason Harris, Anil Patwardhan, Stephen Chervitz, Deanna M. Church, Michael Clark, Gemma Chandratillake, Mark Pratt, John West, Richard Chen Personalis, Inc. | 1350 Willow Road, Suite 202 | Menlo Park, CA 94025

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

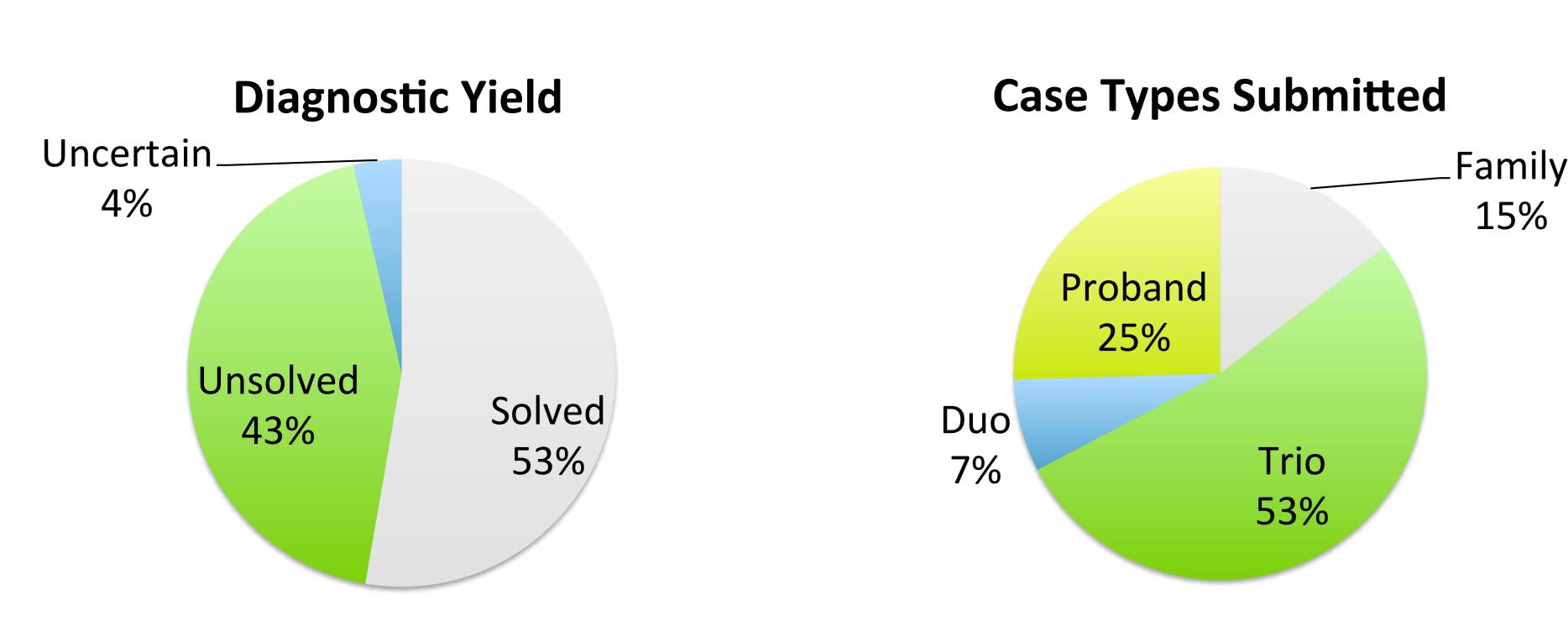
We used the ACE Exome[™] and/or ACE analytics for whole genome data (Personalis, Inc.) to sequence 55 cases of undiagnosed, presumed Mendelian, disease. 44% of cases had undergone array CGH prior to exome/genome sequencing. All participants provided written informed consent as part of their enrollment 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. Personalis ACE Exome[™] sequencing was performed in-house, variants were called and annotated with the Personalis ACE pipeline, and our knowledge-based ranking system approach was utilized to rank variants. Ten samples were subjected to both ACE Exome[™] and whole genome sequencing with ACE pipeline and analytics.

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 31 (56%) cases, with the majority having causative/likely causative variants in genes known to cause a phenotype consistent with the described presentation. 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 (3 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

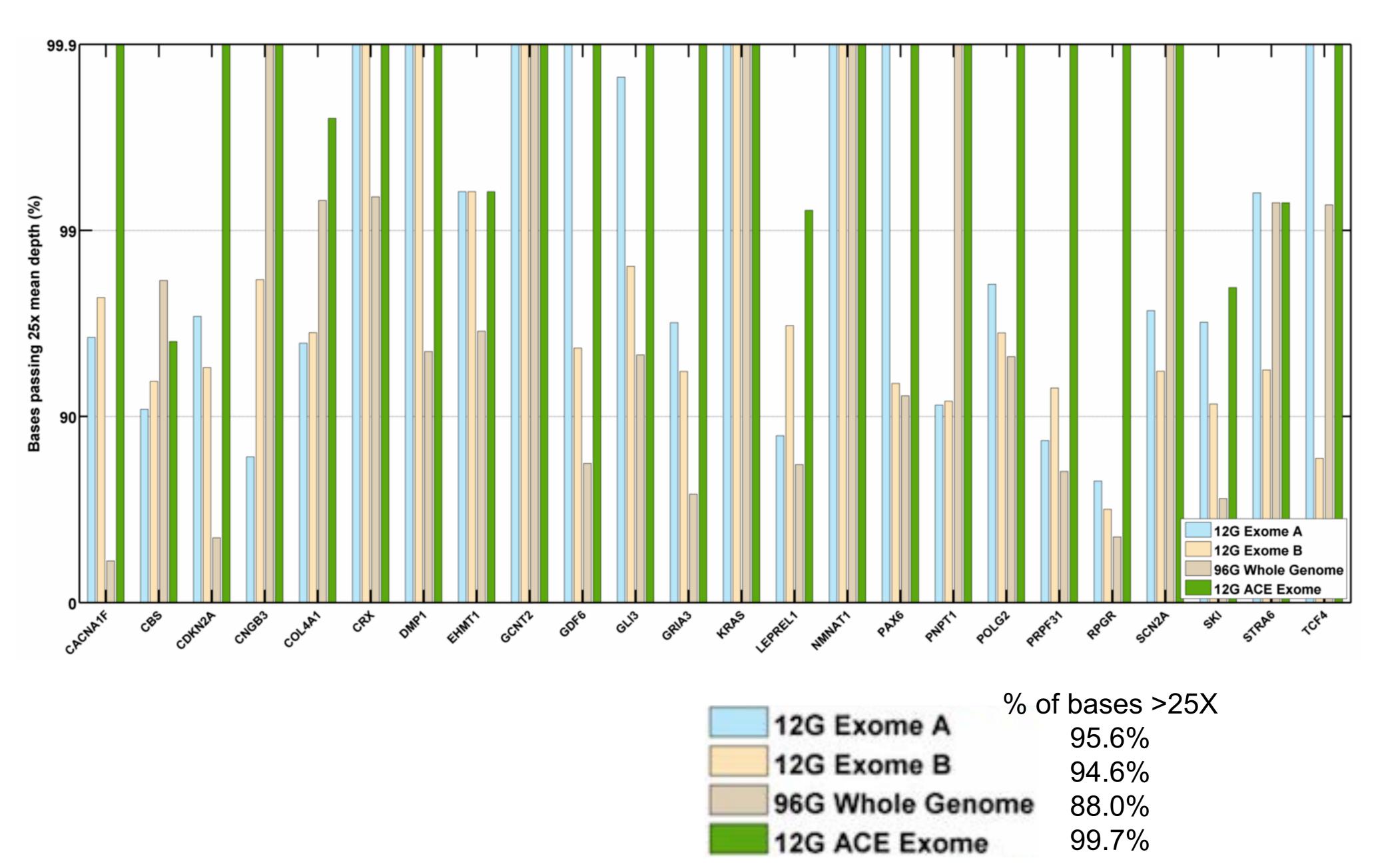


Case Type	Diagnostic Yield
Family	64.5%
Trio	48.3%
Duo	75.0%
Proband	61.5%

Results (continued)

Average performance over the genes reported (all 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 22/24 reported genes. By comparison, only 11/24 and 6/24 genes reached target threshold in two standardly utilized exome kits. A 96G whole genome achieved coverage threshold on 10/24 genes.

FIGURE 2: Enhanced Gene Coverage with ACE Exome™



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.

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.

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

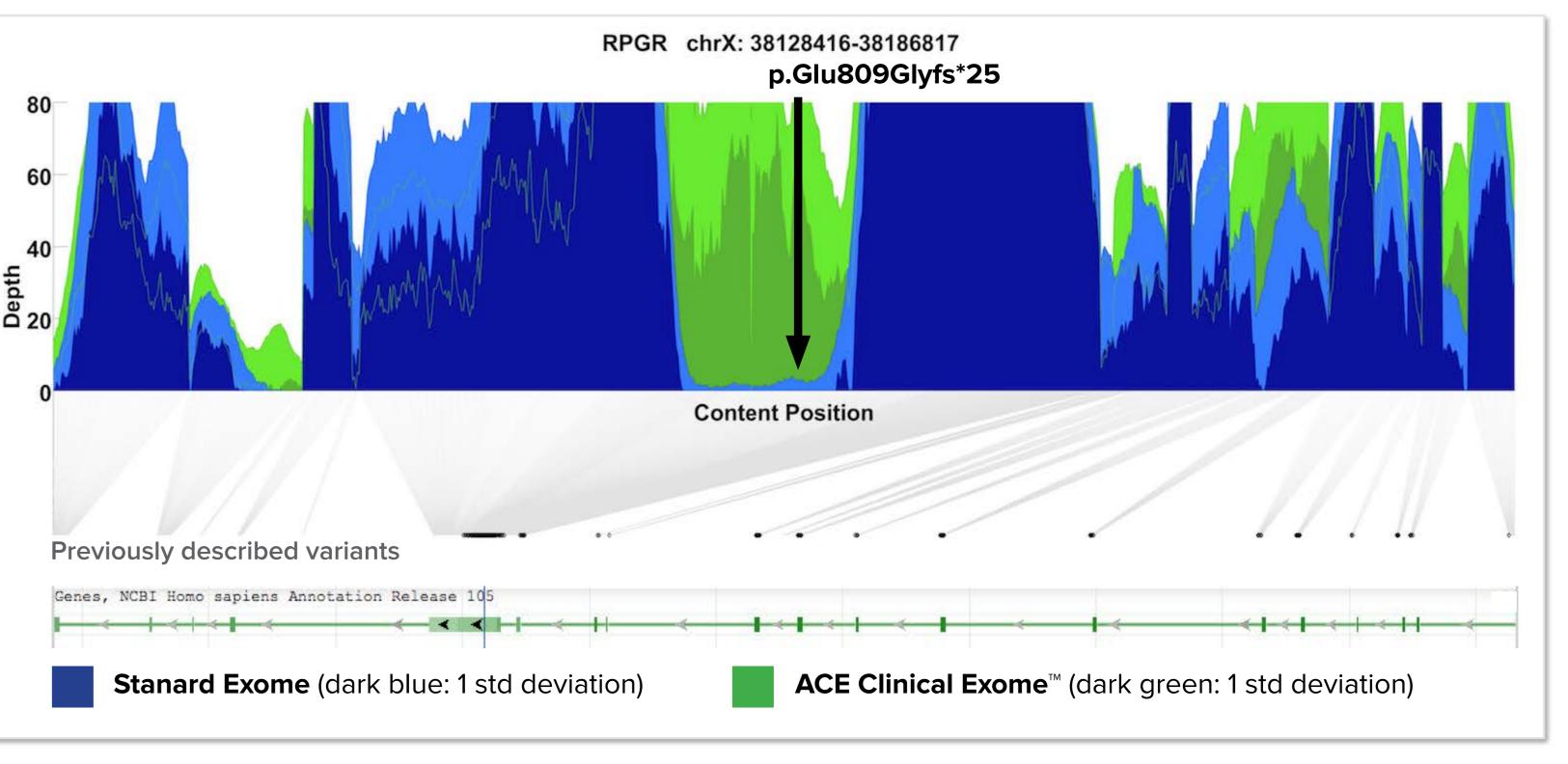
Exomes Miss Exons

History

- Male, diagnosis of retinitis pigmentosa
- No family history

Finding

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



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

Further Information from Personalis Wednesday, June 11

3:30 - 5:00 PM

POS-EESG-1.0 June 2014

Panels vs. Exomes: An Interactive Panel Discussion

Church et al., Impacts of Updating the Reference Assembly on Genome Interpretation

Chen et al., Top 10 Misconceptions of Clinical NGS Sequencing



