Whole Genome Sequencing Improves Clinical Diagnosis in Patients with a Suspected Genetic Disorder(s): Diagnostic yield from 386 cases

Ruby Liu, Justin Leighton, Leah Stansberry, Zeqiang Ma, Alka Chaubey, and Madhuri Hegde,
PerkinElmer Genomics, Pittsburgh, PA

BACKGROUND

- Whole genome sequencing (WGS) reduces amplification bias and increased more uniformed coverage of exonic and non-coding regions.
- WGS can identify previously unknown genes that may contribute to causative of disease also benefit in improving clinical sensitivity.
- The benefits of WGS promotes it a promising alternate as a first-tier diagnostic test for patients at early stage.
- WGS in our lab:
    - Performed by using the KAPA HyperPlus PCR-free library construction kit and sequenced on Illumina NovaSeq™ 6000 (2 x 150 bp mode).
    - Average coverage between 30-40X depth
    - Mitochondrial genome depth 1000x-1500x
    - Low Coverage nucleotides/ exons ranged from 1-2%
    - PKIG proprietary software for SNV and NxClinical 5.0 software (BioDiscovery, El Segundo, CA) were utilized for analysis, interpretation and reporting of CNVs and AOH. Multi Scale Reference algorithm is utilized by NxClinical 5.0.

RESULTS

Overall diagnostic yield

Figure 1: Diagnostic yield in pediatric cohort (n=386)

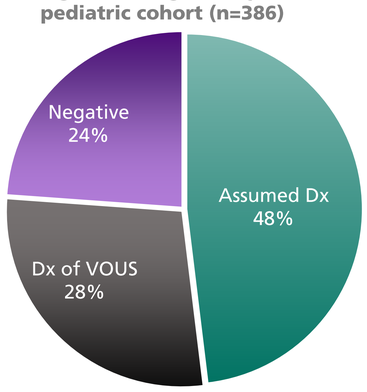


Figure 2: Diagnostic yield in mixed cohort of (n=~~386~~)

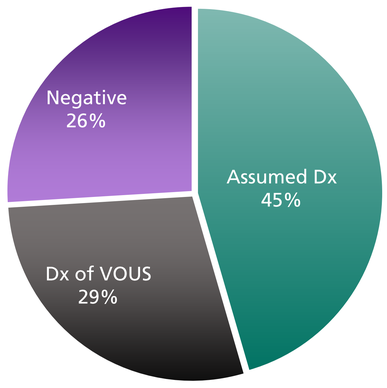
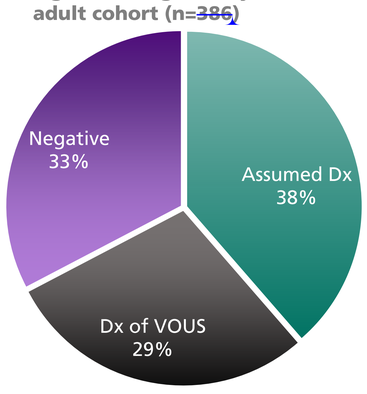
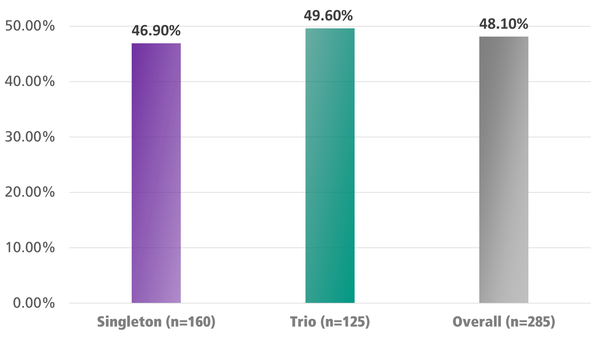


Figure 3: Diagnostic yield in adult cohort (n=~~386~~)



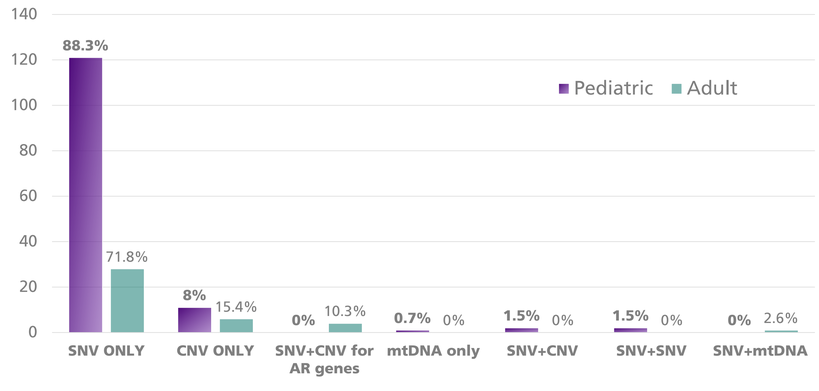
Diagnostic yield in pediatric cases

Figure 4: Diagnostic yield in trio vs singleton



Types of variants identified

Figure 5: Breakdown of variants identified and % of total cohort



SELECT CASE EXAMPLES

Case	Clinical Information	Previous Genetic Studies	WGS Result
1	• 3 months old female • Lethargy, vomiting, hypoglycemia, hyperammonemia • Biochemical testing suggestive of VLCAD deficiency	• No variants detected in *ACADVL, CPT2, HANDHA, SLC25A2D* by sequencing and del/dup • Looking for intronic variants	• *ETFDH* homozygous pathogenic variant • Glutaric acidemia IIC; Multiple Acyl-CoA dehydrogenation deficiency
2	• Newborn male • Lactic acidosis, hypoglycemia • hypotonia • Sibling died at 3 weeks of age • Mom with cardiomyopathy • Mom's sister died at 10 months of age • One maternal aunt with cardiomyopathy	• *SLC25A4* mutation was found in deceased sibling per submitted clinical info. • No detail	• Homoplastic MT-ATP8 pathogenic variant • Negative for any reportable variants in *SLC25A4*
3	• 15 year-old male • intractable frontal lobe epilepsy (onset at 3.5 years old), focal seizure, tonic seizure • ADHD, learning disability, • persistent baby teeth	117 gene epilepsy panel without copy number variants analysis	Pathogenic XYY syndrome (Jacob's syndrome)
4	• 3 year-old female • severe global DD, minimal verbal, severe autism • unsteady gait, hypotonia, severe behavior concerns, head banging • myopia, astigmatism, recurrent ear infection, exotropia, bilateral hearing loss, feeding problem, • facial dysmorphism, large tongue	Negative CMA	Pathogenic intragenic deletion of *MED13L* exons 3-4
5	• 4 year-old male • infantile spasms, epilepsy, • global DD • hypogenesis of corpus callosum, delayed myelination, hypomyelination • hypsarrhythmia	Negative for epilepsy panel, muscular dystrophy & myopathy panel, Prader Will syndrome and Angelman syndrome	Likely pathogenic variant in *SLC16A2* (X-linked)

MULTIPLE DIAGNOSES EXAMPLES

Case	Clinical Information	WGS Result
1	• 19 years old female • Achondroplasia, hypochodroplasia, short stature, rhizomatic shortening of long bones, short neck, narrow thorax, brachydactyly, macrocephaly, lumbar kyphosis • Febrile seizure	• *COL2A1* pathogenic variant • *PCDH19* Pathogenic variant
2	• 60 years old male • Hereditary ataxia, Neuropathy in association with hereditary ataxia, Dysarthria and anarthria, spinocerebellar disease, polyneuropathy, dystonia, torsion dystonia, wheelchair dependence, gait abnormalities/instability, neurogenic bladder and bowel, muscle weakness and cramping, tremor	• *SPG7* pathogenic variant • *TRNS1* (MT-TS1) pathogenic variant
3	• 6 years old male • Macrocephaly, tall stature, hypertrichosis, minor anomalies, musculoskeletal system, Multiple congenital malformations	• *PTEN* pathogenic variant • *PRKAG2* pathogenic variant

POTENTIAL NEW DISEASE-GENE ASSOCIATIONS

IQGAP3, GRIA1, PZP, SYNJ2, ZNF44, DRGX, DLX6, CAPN9, DSCAM, CNTNAP4, ZIC4, GOLGA2, TANC2, XIRP1, OBSCN, SHMT1, TAF3, TNFAUP3, MYF6