P157

Efficiency of Genome Sequencing in Establishing Molecular Diagnosis in Undiagnosed Patients

Arul Joseph Duraisamy[1], Lakshmanan Jagannathan[1], Shruti Sureshkumar[1], Rajiv Rose[1], Babi Ramesh Reddy Nallamilli[2], Christin Collins[2], Madhuri Hegde[2].
[1]PerkinElmer Health Sciences Pvt. Ltd., Chennai, India, [2]PerkinElmer Genomics, Duluth, GA, USA

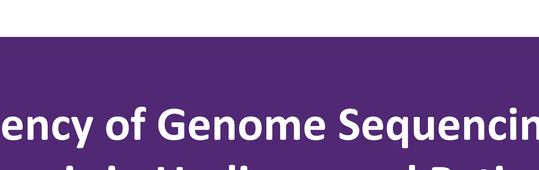
BACKGROUND

- Advancements in the field of next generation sequencing has made molecular diagnosis and management possible in many patients with inherited genetic diseases.
- Understanding individual test limitations, can help clinical genetics providers choose the assay that is most suitable for the diagnosis of their patient.
- Here we demonstrate the clinical utility of genome sequencing (GS) in identifying clinically relevant variants in previously undiagnosed patients, some of which had previous negative/inconclusive exome sequencing (ES) results.

CASE PRESENTATIONS

Patient 1 — 3-year-old female with inconclusive previous ES

- Phenotype: Global developmental delay, delayed motor and language development, developmental regression, intellectual disability, muscular hypotonia, spasticity, epileptic activity, microcephaly, difficulty chewing, difficulty swallowing, poor sucking, severe constipation, gastroesophageal reflux disease (GERD), allergies, behavioral issues, grinding, hand biting, repetitive behavior, hyperopia, ankyloglossia.
- We identified a pathogenic single exon copy number loss in the *MECP2* gene.
- The uniform coverage of GS was helpful in the identification of this deletion. A comparison of coverages in ES and GS for the exon 3 of *MECP2* gene is shown below.



Coverage by GS

Coverage by ES

Patient 2 — Fetus Sample

- From terminated pregnancy.
- Phenotype: Increased NT with septations.
- Negative SNV and CNV by GS.
- Possible trinucleotide repeat (TNR) expansions in the pathogenic range of 49-55 GCN repeats were identified in *ZIC2* gene.

Patient 3 — 2-year-old female

- Phenotype: Cerebellar ataxia, cerebellar atrophy, leukodystrophy, cone-rod dystrophy, vision loss, developmental delay and intellectual disability.
- Negative SNV and CNV by GS.
- Possible TNR in the pathogenic range of 48-66 GCA repeats were identified in *ATXN7* gene.

PKIG GS covers TNRs. Confirmatory testing by an orthogonal method is recommended.

Patient 4 — 5-year-old male

- Phenotype: Suspected lysosomal storage disease; MPSII – Hunter Syndrome.
- Biochemical results showed reduced iduronate 2-sulfatase levels.
- Previously underwent Sanger sequencing and MLPA of the *IDS* gene, which were both negative.
- Intronic variant in the *IDS* gene was identified: c.880-72A>G, through GS's uniform coverage even in the intronic regions.
- Although the variant was classified as a VOUS, it does fit the clinical description, and provides a potential answer for the family.
- RNA sequencing demonstrated splice site defect to move to pathogenic with supporting biochemical diagnosis.

Patient 5 — 20-year-old male with inconclusive previous ES

- Phenotype: Distal muscle weakness, muscle atrophy, increased creatine kinase, gait difficulty, Clinical suspicion of Nonaka myopathy.
- *GNE* c.2179G>A (p.Val727Met) and CN loss in *GNE* intron 1 were identified.
- Similar deletions in this region were found as compound heterozygous with *GNE* c.2179G>A (p.Val727Met) pathogenic variant in one individual affected with GNE myopathy in the literature (PMID: 30990900 , 33031330) and in two individuals tested in our laboratory with clinical suspicion of GNE myopathy. This deletion was classified as likely pathogenic.

Classification	Gene	Exon/ Intron	DNA Change	Protein Change	Zygosity	Inheritance	OMIM	Associated Disease
Pathogenic	*GNE*	12	c.2179G>A	p.Val727Met	Heterozygous	Autosomal Recessive	603824	Nonaka myopathy

11.5 kb deletion in *GNE* intron 1

Patient 6 — 3-years-old female with inconclusive previous ES

- Phenotype: Short stature, low set dysplastic ears, micro retrognathia, microstomia, short neck, developmental delay, poor palmar creases, small hands, joint contractures, suspected syndromic arthrogryposis, antenatal polyhydramnios, fetal dyskinesia, laxity at wrist joint, decreased myelination for the age, sensory polyneuropathy.
- Identified a heterozygous missense variant in *ZC4H2* gene. Phenotypic variability and disease manifestations in female carriers of *ZC4H2* variants results from skewed X-inactivation (PMID: 31206972).

Patient 7 — 10-years-old female with inconclusive previous ES/GS

- Phenotype: Global developmental delay, failure to thrive, spastic diplegia, celiac disease, oxygen dependent sleep, bronchiectasis secondary to chronic GERD/ aspiration syndrome, clubbing, laryngomalacia, subglottic stenosis, hypo/ hyperpigmentation, recurrent chest infection, muscle wasting, spastic gait, increased reflexes, clinical suspicion of Krabbe Disease.
- Identified two homozygous VUS variants in *SELENON* gene and two VUS variants in *trans* in the *DNAAF1* variants through Trio GS testing.

INFERENCE

- Patients 1 – 5 had a genetic diagnosis that was possible only because of uniform coverage throughout the genome achieved by GS that would not have been possible with coverage of only the exons and flanking regions, as provided by ES.
- Patients 6 and 7 had inconclusive ES results and with additional detailed clinical information, clinically relevant variants were identified through GS.

CONCLUSION

- We recommend GS as the comprehensive test to identify SNVs and CNVs in coding / non-coding regions to establish molecular diagnosis.
- We further emphasize the importance of detailed clinical information to provide better analysis of genomic data.