A Diverse Set of Case Presentation Highlight the Power of Whole Genome Sequencing – What Next?

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BACKGROUND

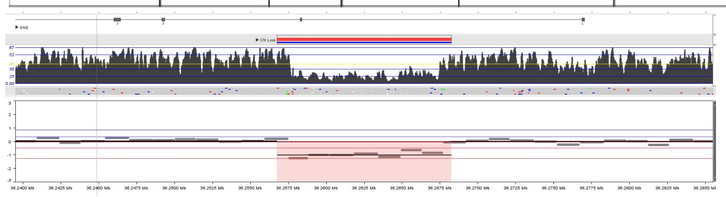
- The progression of high throughput sequencing technologies in the past decades has promoted whole genome sequencing (WGS) as the most comprehensive method to scrutinize the entire human genome to identify sequencing and structural variants in coding and non-coding regions of the genome.
- Emerging data have demonstrated that WGS has become the first test to be considered for critically sick children and WGS trio analysis helped clinical diagnosis. Additionally, WGS has been used as a screening tool that allows healthy individuals to learn their potential risks of developing medically actionable conditions as well as carrier status.
- WGS conducted in our laboratory showed a diagnostic yield ranging from 38% to 48% in adult and pediatric patients, respectively (ACMG 2021 abstract).
- Despite the strengths of WGS in clinical testing and research, the weakness of WGS including misalignment due to repetitive sequences, limited knowledge to fully understand variants of uncertain significance cannot be neglected.

RESULTS

Case 1

- 20-year-old South Asian male
- Distal muscle weakness, myopathy, muscle atrophy, hyperCKemia, Gait difficulty
- Clinical suspicion of Nonaka myopathy

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



Same intron 1 deletion along with the same pathogenic GNE variant were detected in two additional South Asians in our laboratory with clinical suspicion of muscular dystrophy.

Deletion of *GNE intron 1* (11.5Kb)

Case 2

- 2-year-old female
- Cerebellar ataxia, cerebellar atrophy, leukodystrophy, con-rod dystrophy, vision loss, developmental delay and intellectual disability
- Negative SNV and CNV by WGS
- *ATXN7* GCA repeat expansion of 48-66 through WGS screening. Confirmatory test is required.

Gene	Disorder	Locus Structure	Nucleotide Repeat	Reference Region	Supporting Reads Types	Locus Coverage	Zygosity	Normal Repeat Number	Full-Penetrance Pathogenic Repeat Number	Allele1/Allele2 Repeat	Confidence Interval	Allele1/Allele2 Category
ATXN7 (AD)	Spinocerebellar ataxia type 7	(GCA)*(GCC)+	GCA	3:63898360-63898390	SPANNING/INREPEAT	58.55	Het	<=27	>=36	10/58	10-10/48-66	Normal/Inconclusive

Case 3

- 5-years old female
- Limb girdle weakness, positive Gower's sign, idiopathic gait dysfunction, femoral anteversion, hypermobility, proximal muscle weakness
- Neuromuscular panel is negative
- WGS screening revealed homozygous deletion of *SMN1* exon 7, confirmed by MLPA

WGS Screening

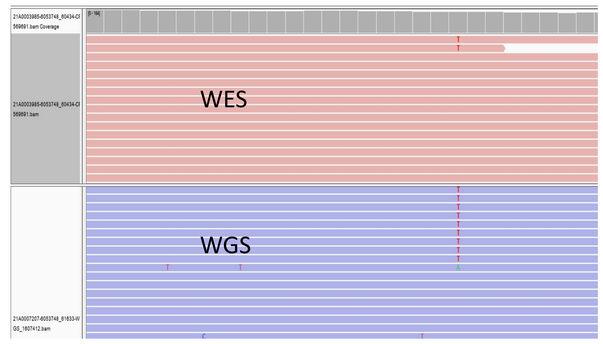
Estimated SMN1 Copy	Estimated SMN2 Copy
0.00	3.77

D [nt]	Gene-Exon	Chr.band	hg18 loc.	Height	Area	Ratio H	Stdev	[REF]	Width	d[nt]	[Mut details]
274	SMN1-7	05q13.2	05-070.283489	666	3445	5%	0	<<*	25	0.0	-
295	SMN1-8	05q13.2	05-070.284168	0	0	0%	0	<<**	0	0.0	-
281	SMN2-7	05q13.2	05-069.408017	24908	102958	1.74	0.08	>>*	37	0.0	-
301	SMN2-8	05q13.2	05-069.408800	23798	106080	1.81	0.07	>>*	45	0.0	-

MLPA Confirmation

Case 4

- 6 months old female
- retinoblastoma and extra-ocular features
- *RB1* negative
- WGS detected a pathogenic mtDNA variant that was missed by WES due to low coverage



Sequence variants:

Classification	Gene	Exon/ Intron	DNA Change	Protein Change	Zygosity	Inheritance	OMIM	Associated Disease
Pathogenic	*MT-ND6*	1	m.14568C>T	-	Heteroplasmic (de novo)	Mitochondrial	516006	Leber's hereditary optic neuropathy

Case 5

- Family with two similarly affected preteen sisters
- Spastic diplegia, diplegic cerebral palsy, developmental delay, intellectual disability, midbrain lesion, macrocephaly, encephalopathy.
- Multiple variants reported by WES and microarray and yet non-diagnostic
- Two variants in *trans* in the newly defined *HPDL* gene associated with neurological disorder detected by WGS quad analysis, in both sisters

Classification	Gene	Exon/ Intron	DNA Change	Protein Change	Zygosity	Inheritance	OMIM	Associated Disease
Uncertain Significance	*HPDL*	1	c.469T>C	p.Trp157Arg	Heterozygous (Paternal)	Autosomal Recessive	618994	Neurodevelopmental disorder with progressive spasticity and brain white matter abnormalities; Spastic paraplegia 83
Uncertain Significance	*HPDL*	1	c.816_817del	-	Heterozygous (Maternal)	Autosomal Recessive	618994	Neurodevelopmental disorder with progressive spasticity and brain white matter abnormalities; Spastic paraplegia 83

CONCLUSION

- Albeit the advantages of WGS to capture multiple types of genetic variation, provide complete picture of the individual's genetic makeup, large data set and variant/gene of uncertain significance also leads to difficulties in interpreting the test results.
- It necessitates to integrate genomic data with other levels of information including transcription, translation, epigenomics and metabolism, to delineate disease-causing mechanisms and genotype-phenotype correlation.

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