Shining a Light on Diagnosis of Rare Genetic Disorders: The Lantern Project

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Introduction

Sanofi Genzyme is partnering with Revvity Omics to offer a complimentary genetic testing program called the Lantern Project. The project aims in minimizing the barrier by providing a complementary biochemical and molecular genetic testing services for patients in the United States who are suffering from Gaucher disease, Fabry disease, Pompe disease, mucopolysaccharidosis type I (MPS I), or Niemann-Pick disease types A and B followed by appropriate genetic counseling and treatment information. Additionally, the testing also includes an enzyme panel for seven mucopolysaccharidoses and a 105 genes panel for limb-girdle muscular dystrophies (LGMD) and other myopathies (comprehensive neuromuscular gene panel).

Materials and Methods

The comprehensive neuromuscular gene panel used in this project allows rapid sequencing of multiple genes simultaneously, in addition to other diseases that may cause similar symptoms including Pompe disease and spinal muscular atrophy. Liquid Chromatography mass spectrometry (LC-MS/MS) is utilized with several multiplex enzymatic assays for the biochemical testing. NGS was performed using a custom SureSelect capture library and short base pair read sequencing on Illumina. Primary data analysis is performed using Illumina DRAGEN Bio-IT Platform v.2.03. Secondary and tertiary data analysis is performed using Revvity's internal ODIN v.1.01 software for SNVs and Biodiscovery's NxClinical v.4.3 or Illumina DRAGEN Bio-IT Platform v.2.03 for CNV and absence of heterozygosity (AOH).

Increased Prevalence: Late onset Pompe

Table1. Summary of *GAA* variants identified Pompe patients both by single gene testing and by panel. Identification of 8 patients out of 9 positive patients with two *GAA* pathogenic variants clearly indicate the increased prevalence of late onset Pompe disease in the current study.

Single Gene Testing					
Patient ID	Gender	Age (Yr)	Gene	Allele 1	Allele 2
PO1	F	55	*GAA*	c.2281delGinsAT	c.-32-13T>G
PO2	M	61	*GAA*	c.-32-13T>G	c.-32-13T>G
PO3	F	63	*GAA*	c.-32-13T>G	c.1438-1G>T
PO4	F	44	*GAA*	c.-32-13T>G	c.-32-13T>G
PO5	F	45	*GAA*	c.-32-13T>G	c.2140delC
PO6	M	22	*GAA*	c.-32-13T>G	c.1841C>A (p.T614K)
PO7	M	6 month	*GAA*	c.1559delA	c.2560C>T (R854X)
Panel					
P08	M	64	*GAA*	c.-32-13T>G	c.1841C>A (p.T614K)
PO9	F	71	*GAA*	c.-32-13T>G	c.1655T>C(p.L552P)

Result of Single Gene testing other than *GAA*

Table 2. Summary of positive samples for *GBA*, *GLA* by single gene testing

GBA Sequencing					
Patient ID	Gender	Age (yr)	Gene	Allele 1	Allele 2
GA1	F	4 month	*GBA*	c.1448T>C (p.L483P)	c.115+1G>A
GA2	F	4	*GBA*	c.1342G>C(D448H)	c.1448T>C (p.L483P)
GA3	M	41	*GBA*	c.1226A>G(p.N409S)	c.1448T>C (p.L483P)
GA4	F	61	*GBA*	c.1226A>G(p.N409S)	c.1226A>G(p.N409S)

GLA Sequencing					
Patient ID	Gender	Age (Yr)	Gene	Allele 1	Zygositiy
FA1	F	52	*GLA*	c.886A>G(p.M296V)	Heterozygous
FA2	F	40	*GLA*	c.646dupT	Heterozygous
FA3	M	63	*GLA*	c.640-1G>A	Hemizygous
FA4	M	61	*GLA*	c.548G>A(G183N)	Hemizygous

DMD gene deletion (Copy number variant)

DMD	Exons	Approximate Genomic Location	Predicted Frame
DMD1	45-47	g.32105254 (intron 44) – g.31928505 (intron 47)	In-frame
DMD2	45-49	g. 31999052 (intron 44) – g. 31840805 (intron 49)	In-frame
DMD3	63-79	g.31284954 – g.30863055	Difficult to predict
DMD4	45-47	g. 32053654 (intron 44) – g. 31897167 (intron 47)	In-frame
DMD5	45-48	g. 32050624 (intron 44) – g. 31891829 (intron 48)	In-frame

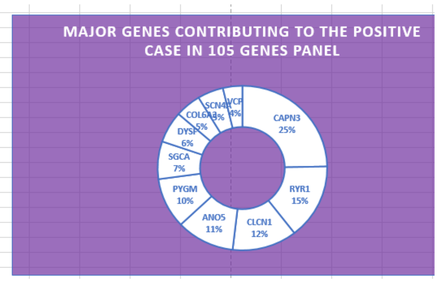
Result: Copy number variants detected in the panel in this study

Sample ID	Event	Chromosome Region	Cytoband	Length	Classification
CNV1	CN Loss	chr1:45,641,410-47,527,709	1p34.1 - p33 including *POMGNT1*	1886300	Pathogenic
CNV2	CN Loss	chr13:23,538,464-24,883,863	13q12.12 including *SGCG*	1345400	Pathogenic
*CNV3	CN Loss	chr13:23,500,814-24,937,513	13q12.12 including *SGCG*	1436700	Pathogenic
CNV4	CN Gain	chr19:10,939,566-10,942,766	19p13.2 including *DNM2*	3201	VOUS
CNV5	UPD1	Chr1: 1-249,250,621	1p36.33q44	Entire chromosome 1	AOH

* 2nd Pathogenic variant detected in the *SGCG* gene, confirm the diagnosis for this case.

Result: Single nucleotide variants detected in this study

The majority of pathogenic single nucleotide variants identified in one of the following genes: *CAPN3, RYR1, CLCN1, ANO5, PYGM, SGCA,DYSF, COL6A3, SCN4A and VCP* indicating their major contribution to LGMD-like phenotypes.



Summary

Positive case summary (Both Single gene and panel testing)			
Test			
GAA single gene sequencing			
	GAA Definite	GAA carriers	
	7	8	
GBA single gene sequencing			
	GAA Definite	GAA carriers	
	4	3	
GLA single gene sequencing			
	Affected Males	Female Carriers	
	2	2	
LGMD Panel			
	Total Positive Cases (all genes, both SNV and CNV)	GAA Definite	GAA Likely (carriers)
	50	2	7

Conclusions

- Early diagnosis can help patients to advance to more timely initiation of options in disease management and be enrolled in precision medicine initiatives.
- The study will help to identified late-onset Pompe disease patient and enrolled them from birth, this would have the potential to significantly increase life expectancy, as well as allowing for a better quality of life in patients.
- This project will help to identify a larger patient pool who has the potential to attract more interest in these rare disorders, increasing the developmental landscape of alternative therapies through company competition.
- Application of NGS panel testing to LGMD diagnosis has improved our understanding of the clinical spectrum of different LGMDs