Largest Gene-panel Sequencing Effort Including 4656 Patients Reveals Genetic Landscape of Limb-Girdle Muscular Dystrophies and Potential Multi-genic Inheritance

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Introduction

Limb-girdle muscular dystrophies (LGMD) are a group of heterogeneous genetic disorders involving predominantly proximal muscle weakness. Disease diagnosis and LGMD subtype identification is complicated by the clinical and genetic heterogeneity with overlap among the subtypes, which can lengthen the diagnostic odyssey and overall cost. To diagnose and also better understand the genetic basis of LGMD, we performed targeted next-generation gene-panel sequencing analysis on a large cohort of 4656 patients. Thirty-five (35) genes known to be associated with LGMD subtypes or LGMD-like neuromuscular disorders were investigated.

Materials and Methods

Muscular Dystrophy Association (MDA) and Jain Foundation each launched LGMD sequencing initiatives that consist of a 35 gene NGS panel through Emory Genetics Laboratory (EGL), following which a total of 4656 individuals underwent molecular testing. NGS was performed using a custom SureSelect capture library and short base pair read sequencing on Illumina HiSeq 2500. Alignment to the human reference genome (hg19) and variant calling was performed using NextGENe®.

LGMD Panel Results

Molecular diagnosis has been established in 27% of the patients with the majority having pathogenic variants identified in one of the following genes: *CAPN3* (17%), *DYSF* (16%), *FKRP* (9%) and *ANO5* (7%) indicating their major contribution to LGMD-like phenotypes.

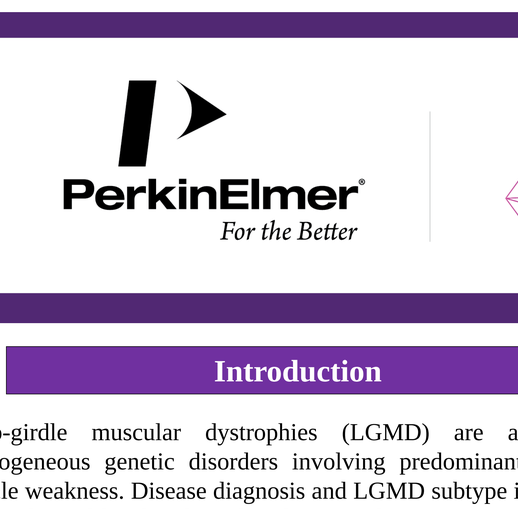

Figure1. Major contributing genes to LGMD phenotype. Majority of patients having pathogenic variants identified in one of the following genes *CAPN3* (17%), *DYSF* (16%), *FKRP* (9%) and *ANO5* (7%).

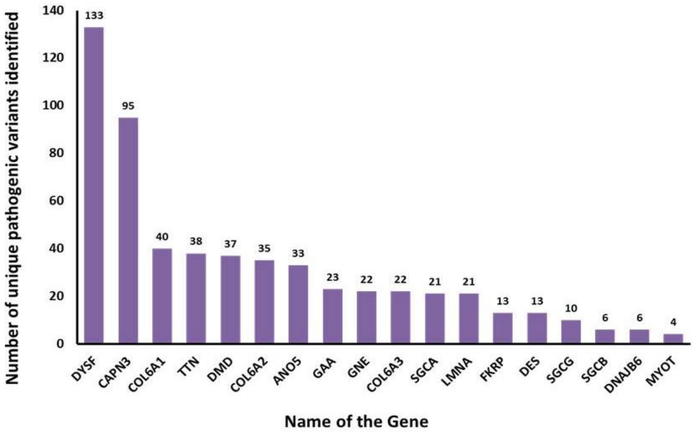

Figure 2. Number of unique pathogenic variants identified in different LGMD genes. A total of 133 unique pathogenic variants have been identified in *DYSF* followed by *CAPN3* with 95 variants and *COL6A1* with 40 variants signifying the high allelic heterogeneity in these LGMD genes.

Increased Prevalence: Late onset Pompe

Table1. Summary of *GAA* variants identified in late onset Pompe patients. Identification of 28 patients with two *GAA* pathogenic variants clearly indicate the increased prevalence of late onset Pompe disease in the current study.

Patient ID	Gender	Age	Gene	Variant 1	Variant 2
AOP1	Female	61	*GAA*	c.-32-13T>G	c.1124G>T (p.R375L)
AOP2	Female	79	*GAA*	c.-32-13T>G	c.2140delC
AOP3	Female	33	*GAA*	c.-32-13T>G	c.525delT
AOP4	Male	71	*GAA*	c.-32-13T>G	c.1912G>T (p.G638W)
AOP5	Unknown	54	*GAA*	c.-32-13T>G	c.2512C>T(p.Q838X)
AOP6	Male	66	*GAA*	c.-32-13T>G	c.2481+102_2646+31del
AOP7	Male	70	*GAA*	c.-32-13T>G	c.2481+102_2646+31del
AOP8	Female	44	*GAA*	c.-32-13T>G	c.2481+102_2646+31del
AOP9	Male	18	*GAA*	c.-32-13T>G	c.2481+102_2646+31del
AOP10	Male	40	*GAA*	c.-32-13T>G	c.2238G>A(p.W746X)
AOP11	Male	59	*GAA*	c.-32-13T>G	c.1655T>C(p.L552P)
AOP12	Male	70	*GAA*	c.736delC	c.546G>A(p.T183T)
AOP13	Female	53	*GAA*	c.-32-13T>G	c.1841C>A(p.T614K)
AOP14	Male	68	*GAA*	c.-32-13T>G	c.1143delC
AOP15	Female	40	*GAA*	c.853C>T	c.2560C>T(p.R854X)
AOP16	Male	41	*GAA*	c.-32-13T>G	c.2560C>T(p.R854X)
AOP17	Male	44	*GAA*	c.-32-13T>G	c.655G>A(p.G219R)
AOP18	Male	70	*GAA*	c.-32-13T>G	c.1064T>C(p.L355P)
AOP19	Female	49	*GAA*	c.-32-13T>G	c.1655T>C(p.L552P)
AOP20	Female	56	*GAA*	c.-32-13T>G	c.525delT
AOP21	Female	36	*GAA*	c.-32-13T>G	c.1827delC
AOP22	Male	80	*GAA*	c.-32-13T>G	c.525delT
AOP23	Male	33	*GAA*	c.-32-13T>G	c.258dupC
AOP24	Female	46	*GAA*	c.-32-13T>G	c.766_785delinsC
AOP25	Female	61	*GAA*	c.-32-13T>G	c.2481+102_2646+31del
AOP26	Male	18	*GAA*	c.-32-13T>G	c.525delT
AOP27	Female	56	*GAA*	c.-32-13T>G	c.525delT
AOP28	Female	8	*GAA*	c.-32-13T>G	c.2242dupG

Multigene Inheritance in LGMD

More interestingly, we also identified high prevalence of patients with pathogenic variants in more than one LGMD gene suggesting a possible role of synergistic heterozygosity and digenic/multigenic contribution to disease presentation that needs to be considered as a part of inheritance modality in the clinic**.**

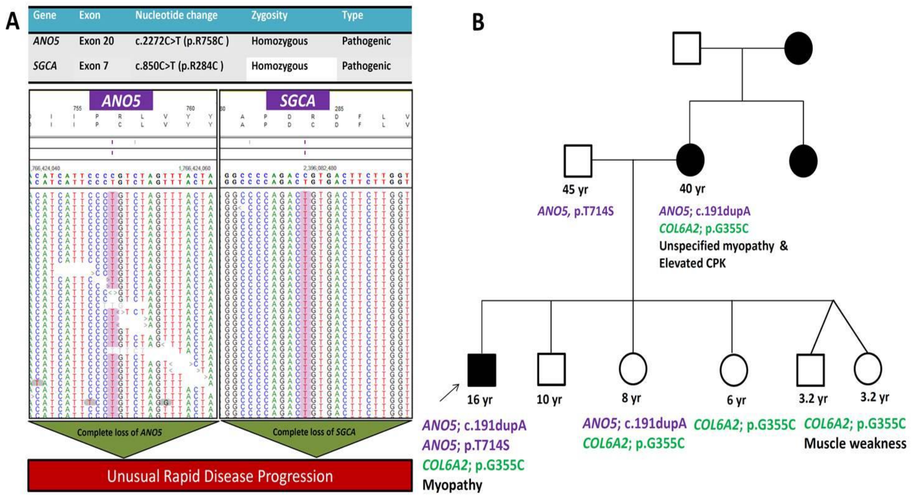

Figure 3. Multigenic inheritance in LGMD. Pathogenic variants identified in more than one LGMD genes in two patients with unusual disease presentation and progression indicating complex inheritance patterns of LGMD.
A. Patient with homozygous variants in both *ANO5* and *SGCA* genes. NGS reads clearly indicated the identification of homozygous missense pathogenic variants c.2272C>T (p.R758C) and c.850C>T (R284C) in *ANO5* and *SGCA* genes respectively.
B. Rapid disease progression was observed in 16 year old male with two pathogenic variants in *ANO5* gene and one pathogenic variant in *COL6A2* gene indicating multiple gene contribution for unusual presentation.

DNAJB6 Associated LGMD Subtype 1E

Table 2. Summary of patients with *DNAJB6* variants. We identified 29 cases with pathogenic variants and 13 additional cases with novel variants in *DNAJB6* which stresses the importance of LGMD1E.

Patient ID	Gender	Age	Gene	Exon	Nucleotide change	AA change	Classification
JB1	Female	51	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB2	Male	49	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB3	Male	75	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB4	Female	51	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB5	Male	20	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB6	Male	55	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB7	Female	45	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB8	Male	43	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB9	Female	45	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB10	Male	69	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB11	Female	55	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB12	Male	63	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB13	Female	25	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB14	Male	41	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB15	Female	39	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB16	Female	57	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB17	Female	79	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB18	Male	46	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB19	Male	59	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB20	Female	68	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB21	Female	43	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB22	Female	59	*DNAJB6*	5	c.265T>A	p.F89I	Pathogenic
JB23	Male	51	*DNAJB6*	5	c.271T>G	p.F91V	Pathogenic
JB24	Male	16	*DNAJB6*	5	c.273C>G	p.F91L	Pathogenic
JB25	Male	63	*DNAJB6*	5	c.279C>A	p.F93L	Pathogenic
JB26	Male	53	*DNAJB6*	5	c.279C>A	p.F93L	Pathogenic
JB27	Male	59	*DNAJB6*	5	c.279C>A	p.F93L	Pathogenic
JB28	Male	69	*DNAJB6*	5	c.279C>G	p.F93L	Pathogenic
JB29	Male	57	*DNAJB6*	5	c.279C>G	p.F93L	Pathogenic
J909	Male	8	*DNAJB6*	4	c.230G>T	p.G77V	VOUS
J111	Female	30	*DNAJB6*	10	c.947C>G	p.S316W	VOUS
J222	Female	67	*DNAJB6*	4	c.184C>T	p.R62W	VOUS
J333	Female	76	*DNAJB6*	9	c.832G>C	p.E278Q	VOUS
J444	Male	73	*DNAJB6*	9	c.891C>T	p.S297S	VOUS
M444	Male	33	*DNAJB6*	10	c.962C>T	p.S321L	VOUS
M555	Male	70	*DNAJB6*	6	c.410C>T	p.T137M	VOUS
M666	Female	65	*DNAJB6*	10	c.962C>T	p.S321L	VOUS
M777	Female	38	*DNAJB6*	7	c.547A>G	p.S183G	VOUS
M888	Female	73	*DNAJB6*	8	c.661G>A	p.D221N	VOUS
M999	Male	18	*DNAJB6*	7	c.571A>G	p.I191V	VOUS
M200	Female	58	*DNAJB6*	9	c.721G>A	p.E241K	VOUS
M300	Female	29	*DNAJB6*	9	c.706G>A	p.D236N	VOUS

Deep Intronic Variant in *DYSF* gene

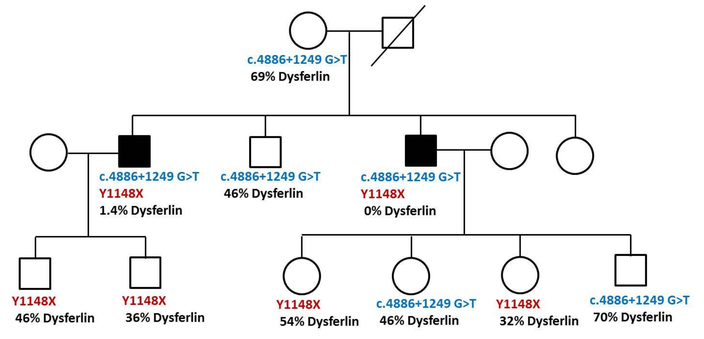

Figure4. Segregation analysis of deep intronic variant in *DYSF* gene in a large family with LGMD2B. Deep intronic variant in *DYSF* gene alters mRNA splicing and ultimately results in inframe insertion of new pseudo exon in dysferlin..

Conclusions

- Application of NGS panel testing to LGMD diagnosis has improved our understanding of the clinical spectrum of different LGMDs.
- Molecular diagnosis has been established in 27% of the patients with the majority having pathogenic variants identified in one of the following major genes: *CAPN3* (17%), *DYSF* (16%), *FKRP* (9%) and *ANO5* (7%).
- Identification of pathogenic variants in more than one LGMD genes in at least 31 individuals suggested possible digenic and multiple genes contribution to LGMD disease presentation and progression.
- Increased prevalence of *DNAJB6* associated LGMD1E was observed indicating the importance of autosomal dominant LGMD genes.
- Current study emphasized the prevalence of late onset Pompe disease.