Evidence of Complex Inheritance Patterns in Limb-Girdle and other Muscular Dystrophies: Synergistic Heterozygosity and Multigenic Inheritance

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INTRODUCTION

Alterations in a single gene or a unique locus is responsible for the expression phenotype in Mendelian inheritance. Whereas alterations in two or more genes are required to express the phenotype in digenic or multigenic inheritance. The first report of digenic inheritance in humans identified in retinitis pigmentosa with variants in two genes *ROM1* and *PRPH218*. Further studies conformed that both encoded proteins interact to form stable photoreceptor disc. Muscular dystrophies (MDs) are heterogeneous group of inherited disorders caused by pathogenic variants in a number of genes. The clinical and genetic heterogeneities of MDs make disease diagnosis complicated and expensive. Recent studies successfully reported digenic inheritance in different muscular dystrophies such as Emery-Dreifuss, limb-girdle, ullrich congenital and facioscapulohumeral, indicating more complex nature of disease presentation and progression in MD. To expand our understanding of the multigenic inheritance in limb-girdle and other overlapping MDs, we have studied cases with pathogenic variants in multiple genes using a comprehensive NGS-based multi-gene panel.

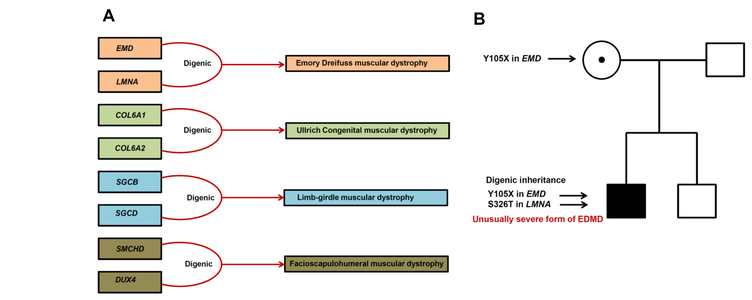

Figure 1. Digenic inheritance in muscular dystrophies. A) Digenic inheritance reported in Emery-Dreifuss, limb-girdle, ullrich congenital and facioscapulohumeral muscular dystrophies. B) Unusual disease progression in a patient diagnosed with X-linked Emery-Dreifuss muscular dystrophy (EDMD). Labelled patient has variants in EMD and LMNA genes.

MATERIALS AND METHODS

A custom Agilent SureSelect targeted sequence capture was used to enrich all 66 genes included in the panel, followed by NGS on an Illumina NovaSeq 6000 with 150-base pair paired-end reads. Sequence variants were assessed by our proprietary analysis and interpretation pipeline, Ordered Data Interpretation Network (ODIN). Variants were called at a minimum coverage of 8× and an alternate allele frequency of 20% or higher. Variants were evaluated by their frequency, as reported in public databases (gnomAD, dbSNP, EVS, and Leiden LOVD). Variants having a frequency greater than expected, given the prevalence of disease, were considered benign. Variant interpretations and classifications were performed using the American College of Medical Genetics (ACMG) standards and guidelines for the interpretation of sequence variants. Copy number variants (CNV) analysis was assessed using Biodiscovery's NxClinical software (BioDiscovery, El Segundo, CA). Male and female reference sets were created from healthy controls using NGS data with the help of BAM Multiscale Reference (MSR) Builder module. CNVs were detected using the Hidden Markov Model-based fast adaptive states segmentation technique algorithm.

RESULTS

Molecular diagnosis was established in 19.6% (1266) of 6473 cases with clinical suspicion of LGMD and other overlapping MDs using NGS based panel testing. The definitive molecular diagnosis increasingly suggests role of multiple genes in patients with muscular dystrophies. Pathogenic variants identified in two/multiple genes in these muscular dystrophy patients leading to digenic/multigenic inheritance along with unusual complex clinical presentation. This large-scale study further confirmed the possible role of digenic/multigenic inheritance by identifying 26 cases with pathogenic variants in multiple genes with combinations of dominant: dominant, dominant: recessive; recessive: recessive and dominant or recessive X-linked inheritance in *LDB3*, *DMD*, *TTN*, *DYSF*, *ANO5*, *PABPN1*, *COL6A1*, *FLNC*, *RYR1*, *GAA*, *LMNA*, *LYST*, *SYNE1*, *CLCN1*, *COL6A2* and *NEB* genes. These unusual clinical presentations were observed in patients with pathogenic variants in 16 genes which could lead to complex clinical features as a result of possible digenic/multigenic inheritance.

Table1. List of cases identified with possible multigenic inheritance in the current Study.

Case ID	Age	Sex	Gene 1					Gene 2				
			Gene	Variant	Exon	Zygosity	Class	Gene	Variant	Exon	Zygosity	Class
OP5	76	M	**LDB3**	c.439G>A (p.Ala147Thr)	5	Het	P	**PABPN1**	12 GCN repeat allele	1	Het	P
XLMD46	21	M	**DMD**	Deletion	5 to 9	Hemi	P	**LMNA**	c.1634G>A (p.Arg545His) & c.1584G>C (p.Thr528=)	10 & 9	Het & Het	LP & VUS
XLMD92	54	M	**DMD**	Deletion	45 to 57	Hemi	P	**RYR1**	c.1840C>T (p.Arg614Cys)	17	Het	P
ARMD484	46	F	**TTN**	c.87422C>G (p.Pro29141Arg) & c.2998C>T (p.Leu1000Phe)	292 & 18	Het & Het	LP & VUS	**LYST**	c.9939_9940insTTAG & c.9938G>A (p.Gly3313Asp H)	44 & 44	Het & Het	P & VUS
ARMD155	31	M	**DYSF**	c.3367_3368del	31	Hom	LP	**TTN**	c.56984dup	259	Het	LP
ARMD42	50	F	**ANO5**	c.1899-2A>C & c.368C>T (p.Ser123Leu)	IVS17 & 7	Het & Het	LP & VUS	**SYNE1**	c.25862_25871del	144	Het	LP
OP51	72	F	**PABPN1**	13 GCN repeat allele	1	Het	P	**TTN**	c.88716C>A (p.Tyr29572Ter) & c.70579C>G (p.Pro23527Ala)	296 & 275	Het & Het	LP & VUS
OP78	66	F	**PABPN1**	13 GCN repeat allele	1	Het	P	**CLCN1**	c.1129C>T (p.Arg377Ter)	10	Het	P
ADMD216	57	M	**ANO5**	c.172C>T (p.Arg58Trp)	4	Het	P	**COL6A2**	deletion	1 to 17	Het	LP
ADMD204	7	M	**COL6A1**	c.1003-2del	13	Het	P	**SYNE1**	c.1933C>T (p.Gln645Ter)	18	Het	P
ADMD205	11	M	**COL6A1**	c.1003-2del	13	Het	P	**SYNE1**	c.1933C>T (p.Gln645Ter)	18	Het	P
ADMD249	38	M	**FLNC**	c.577G>A (p.Ala193Thr)	2	Het	P	**NEB**	c.24094C>T (p.Arg8032Ter) & c.20098C>A (p.Leu6700Ile)	169 & 131	Het & Het	P & VUS
ARMD493	51	M	**TTN**	c.97718dup & c.33605C>T (p.Thr11202Met)	307 & 174	Het & Het	LP & VUS	**RYR1**	c.6617C>T (p.Thr2206Met)	40	Het	P
XLMD56	57	F	**DMD**	Deletion	44	Het	P	**ANO5**	c.692G>T (p.Gly231Val)	8	Het	P
OP28	58	F	**PABPN1**	13 GCN repeat allele	1	Het	P	**NEB**	c.25441C>T (p.Arg8481Ter) & c.9071C>T (p.Ala3024Val)	182 & 64	Het & Het	P & VUS
XLMD76	64	M	**DMD**	Deletion	45 to 48	Hemi	P	**ANO5**	c.191dup	5	Het	P
XLMD67	20	M	**DMD**	Deletion	45 to 47	Hemi	P	**GAA**	c.-32-13T>G & c.510C>T (p.Asp170=)	IVS1 & 2	Het & Het	P & VUS
ARMD420	43	F	**RYR1**	c.14209C>T (p.Arg4737Trp) & c.12884C>T (p.Ala4295Val)	98 & 91	Het & Het	P & VUS	**LMNA**	c.1634G>A (p.Arg545His)	10	Het	LP
ARMD280	45	M	**GAA**	c.-32-13T>G & c.1114C>T (p.His372Tyr)	IVS1 & 7	Het & Het	P & VUS	**ANO5**	c.692G>T (p.Gly231Val)	8	Het	P
ARMD241	69	F	**GAA**	c.-32-13T>G & c.525del	IVS1 & 2	Het & Het	P & P	**CLCN1**	c.979G>A (p.Val327Ile)	8	Het	LP
OP22	70	F	**PABPN1**	13 GCN repeat allele	1	Het	P	**ANO5**	c.191dup	5	Het	P
ADMD55	49	M	**ANO5**	c.191dup	5	Het	P	**RYR1**	c.6274+1G>A	38	Het	P
ADMD129	28	F	**CLCN1**	c.689G>A (p.Gly230Glu)	5	Het	P	**SELENON**	c.683_689dup	5	Het	P
ADMD411	44	M	**SCN4A**	c.4428G>A (p.Met1476Ile)	24	Het	P	**ANO5**	c.1898+1G>A	17	Het	P
ADMD491	37	F	**VCP**	c.464G>A (p.Arg155His)	5	Het	P	**CLCN1**	c.2680C>T (p.Arg894Ter)	23	Het	LP
ARMD59	2	M	**CAPN3**	c.2306G>A (p.Arg769Gln)	22	Hom	P		**XXY** - Klinefelter syndrome		Het	P

F = female; Hemi = hemizygous; Het = heterozygous; Hom = homozygous; LP = likely pathogenic; M = male; P = pathogenic; VUS = variant of uncertain significance.

CONCLUSION

- We identified a high prevalence of patients with pathogenic variants in more than one MD gene suggesting possible multigenic contribution to disease presentation that needs consideration as a part of diagnostic modality.
- Overall, this largescale study clearly indicated the importance of further genotype-phenotype correlation studies in other family members to completely understand the complex inheritance mechanism involved in the different muscular dystrophy subtypes.