Calpainopathy – Changing Paradigm of Mode of Inheritance

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Introduction

Next Generation Sequencing (NGS) is beginning to show its full potential for diagnostic and therapeutic applications. In particular, we are beginning to see revolution in understanding genes role in the disease physiology and inheritance of previously identified genes and discovering new genes, for clinically heterogeneous disorders eg. muscular dystrophy. Allelic disorders with both dominant and recessive modes of inheritance have been reported for a number of muscle diseases, such as myotonia congenita, desminopathies and collagen 6-related myopathies. Until 2016, calpainopathy was considered an autosomal recessive limb girdle muscular dystrophy (LGMD) subtype 2A. Recently autosomal dominant LGMD1I, co-segregating with a 21 bp in-frame deletion (c.643_663del21), has been established, in addition to autosomal recessive LGMD2A.

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®. Comprehensive analysis was conducted with the calpainopathy cases to understand the complex inheritance patterns of *CAPN3* gene.

Mutation Spectrum of *CAPN3*

Total 428 variants have been reported in *CAPN3* in Human Gene Mutation Database. Wide variety of mutations including missense, nonsense, splice, small deletions and small indels have been reported throughout the *CAPN3* gene (Figure 1). Majority of the reported variants (59%) are missense and nonsense variants. Pathogenic variants in *CAPN3* are associated with limb girdle muscular dystrophy subtype 2A (LGMD2A). It has been strictly considered as an autosomal recessive LGMD subtype for many years, but patients carrying a single pathogenic variant in the CAPN3 gene have been recently reported to have an autosomal dominant subtype LGMD1I.

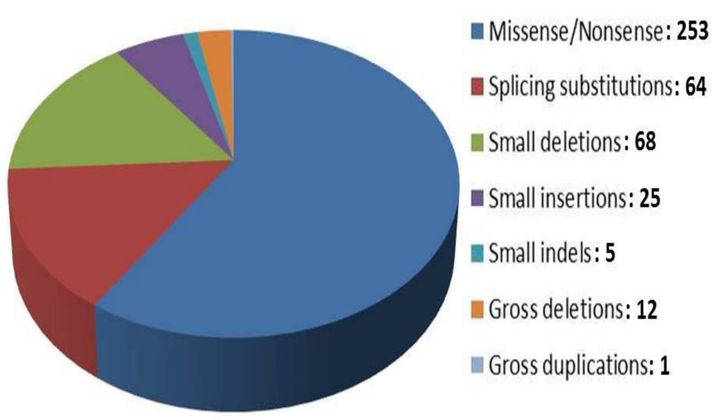

Figure1. Mutation Spectrum of *CAPN3* gene. Diverse mutation spectrum has been documented for *CAPN3*. Total 428 *CAPN3* variants have been reported in Human Gene Mutation Database. Wide variety of mutations including missense, nonsense, splice, small deletions and small indels have been reported throughout the *CAPN3* gene. Majority of the reported variants (59%) are missense and nonsense variants.

Improved Understanding of mode of Inheritance in Calpainopathy

Calpainopathy (LGMD2A) is characterized by symmetric and progressive weakness of proximal limb-girdle muscles. The age of onset ranges from 2 to 40 years. The clinical phenotype shows intra- and interfamilial variability ranging from severe to mild. LGMD2A has been considered strictly an autosomal recessive LGMD subtype for many years, but patients carrying a single pathogenic variant in the calpain 3 gene have been reported recently (Vissing et al., 2016). A 21 bp in-frame deletion c.643_663del21 co-segregated in 37 individuals from 10 families with muscle disease and autosomal dominant transmission in several generations(Vissing et al., 2016). The normal expression of mutated mRNA and the severe loss of calpain 3 on western blotting were observed in patients with this 21 bp in-frame deletion, suggesting a dominant negative mechanism affecting the calpain 3 homodimer. In the current study, the same 21 bp in-frame deletion c.643_663del21 was identified in 17 individuals with ages ranging from 13 -78 years (Table 1). There was no reportable second pathogenic variant identified in these 17 patients in the targeted LGMD panel sequencing, although further deletion-duplication analysis was not conducted. These results further confirm the 21 bp in-frame deletion c.643_663del21 as a common dominant mutation.

Table 1. List of 17 calpainopathy patients with 21 bp in-frame deletion. No second pathogenic variant was identified in these patients.

Patient ID	Gender	Age (Years)	*CAPN3* variant 1	*CAPN3* variant 2
C100	Male	24	c.643_663del21	-
C200	Male	72	c.643_663del21	-
C300	Male	44	c.643_663del21	-
C400	Female	70	c.643_663del21	-
C500	Male	58	c.643_663del21	c.584A>C (p.N195T), VUS
C600	Female	73	c.643_663del21	-
C700	Male	57	c.643_663del21	-
C800	Male	60	c.643_663del21	-
C900	Female	61	c.643_663del21	-
C111	Male	69	c.643_663del21	-
C222	Female	17	c.643_663del21	-
C333	Male	57	c.643_663del21	c.640G>A (p.G214S), VUS
C444	Female	34	c.643_663del21	-
C555	Male	45	c.643_663del21	-
C666	Female	13	c.643_663del21	-
C777	Female	78	c.643_663del21	-
C888	Female	73	c.643_663del21	-

We thoroughly investigated for any other possible in-frame deletions that could be responsible for the autosomal dominant form of calpainopathy. We report a similar 15 bp in-frame deletion c.598_612del15 in 16 patients with ages ranging from 48-76 years (Table 2). A second pathogenic variant was not identified in these 16 patients indicating the possible role of this 15 bp in-frame deletions in causing the autosomal dominant form of calpainopathy. All these cases had late onset of disease and a milder phenotype compared to that reported in the previously described dominant variant. Parental segregation studies are required to further confirm this variant's role in a dominant subtype. The same 15 bp in-frame deletion c.598_612del15 as a single *CAPN3* variant has been previously described in one patient but dominant inheritance was not suggested (Chrobakova et al., 2004; Stehlikova et al., 2007).

Table 2. List of 16 calpainopathy patients with 15 bp in-frame deletion. No second pathogenic variant was identified in these patients.

Patient ID	Gender	Age (Years)	*CAPN3* variant 1	*CAPN3* variant 2
D001	Male	59	c.598_612del15	-
D002	Female	57	c.598_612del15	-
D003	Female	48	c.598_612del15	-
D004	Male	69	c.598_612del15	-
D005	Female	59	c.598_612del15	-
D006	Male	57	c.598_612del15	c.794C>T (p.S265F), VUS
D007	Female	58	c.598_612del15	c.1477C>T (p.R493W), VUS
D008	Female	54	c.598_612del15	-
D009	Female	57	c.598_612del15	-
D010	Male	76	c.598_612del15	-
D011	Female	53	c.598_612del15	c.1505T>C (p.I502T), VUS
D012	Female	54	c.598_612del15	-
D013	Male	39	c.598_612del15	-
D014	Female	59	c.598_612del15	-
D015	Female	69	c.598_612del15	-
D016	Male	66	c.598_612del15	-

Conclusions

- This study further supports the evidence of both dominant and recessive form of the calpainopathy and has improved our understanding of the clinical spectrum of different types and inheritance patterns of muscular dystrophy.
- These findings should be taken into consideration in the diagnostic work up and genetic counselling of patients with only one heterozygous allele in *CAPN3* gene. New clinical trials are opening for muscular dystrophies and this paradigm shift needs to be taken into consideration for enrolling patients in clinical trials.