Compound heterozygous variants in *MYBPC1* lead to severe distal arthrogryposis type-1 manifestations

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Abstract

Dominant missense variants in *MYBPC1* encoding slow Myosin Binding Protein-C (sMyBP-C) have been increasingly linked to arthrogryposis syndromes and congenital myopathy with tremor. Herein, we describe novel compound heterozygous variants – NM_002465.4:[c.2486_2492del];[c.2663A>G] – present in fibronectin-III (Fn-III) C7 and immunoglobulin (Ig) C8 domains, respectively, manifesting as severe, early-onset distal arthrogryposis type-1, with the carrier requiring intensive care and several surgical interventions at an early age. Computational modeling predicts that the c.2486_2492del p.(Lys829IlefsTer7) variant destabilizes the structure of the Fn-III C7 domain, while the c.2663A>G p.(Asp888Gly) variant causes minimal structural alterations in the Ig C8 domain. Although the parents of the proband are heterozygous carriers for a single variant, they exhibit no musculoskeletal defects, suggesting a complex interplay between the two mutant alleles underlying this disorder. As emerging novel variants in *MYBPC1* are shown to be causatively associated with musculoskeletal disease, it becomes clear that *MYBPC1* should be included in relevant genetic screenings.

Key words: *MYBPC1*, distal arthrogryposis type-1, autosomal recessive inheritance

1 Introduction

Distal arthrogryposis (DA) disorders comprise a heterogeneous group of arthrogryposis multiplex congenita (AMC) syndromes, all of which manifest with joint contractures of the distal extremities and are further classified into ten subtypes based on the genetic etiology and clinical presentation[1]. Distal arthrogryposis type-1 (DA-1), a congenital disorder predominantly characterized by camptodactyly and clubfoot, has been commonly associated with mutations in *TPM2* and *MYBPC1*[2,3]. *MYBPC1* encodes slow Myosin Binding Protein-C (sMyBP-C), an essential sarcomeric accessory protein that harbors structural and regulatory functions in skeletal myofibers[4]. The COOH-terminus of sMyBP-C constitutively interacts with the light meromyosin portion of myosin and the gigantic protein titin, contributing to thick filament organization and integrity, while the NH_2-terminus dynamically interacts with both the heavy meromyosin portion of

myosin, harboring ATPase activity, and actin filaments to regulate actomyosin crossbridge formation and kinetics[5-9].

Of the handful of previously identified variants in *MYBPC1* that result in arthrogryposis syndromes, only two (W236R and Y856H) have been associated with DA-1, both of which exhibit an autosomal dominant inheritance pattern[2]. Conversely, biallelic homozygous variants in *MYBPC1* have been linked to lethal congenital contracture syndrome-4 (LCCS-4) – a neonatally lethal form of arthrogryposis – and AMC of variable severity[10,11]. Herein, we describe novel biallelic compound heterozygous *MYBPC1* variants, c.2486_2492del p.(K829Ifs*7) and c.2663A>G p.(D888G), that result in DA-1.

2 Material and Methods

2.1 Clinical evaluation and identification of variants

Clinical and genetic examination of the proband and family, residing in Czech Republic, was performed by a pediatric neurologist and a clinical geneticist, respectively, with informed consent from all subjects and/or their parents. Genetic variants were identified via next generation sequencing (NGS) of a modified neuromuscular gene panel (**Supplemental Table 1**), as previously described[12]. The novel NM_002465.4: c.[2486_2492del];[2663A>G] variants have been submitted to the LOVD database under individual #00442606[13].

2.2 Molecular dynamics modeling

Models of the sMyBP-C (NP_002456.2) C7 (residues 743-843) and C8 (residues 844-939) domains were generated using Alphafold and visualized in PyMol[14,15]. All models had positional predicted local distance difference test scores of >90 and were thus deemed ‘high confidence’. Models of two splice variations of C7 were performed, corresponding to *MYBPC1* human variant 1 (hv-1; Uniprot Q00872-4) and 3 (hv-3; Uniprot Q00872-1)[16]. These models were then equilibrated in YASARA, as previously described[17]. WT simulations ran for 140-200 nanoseconds (ns) for C7 and 90 ns for C8, with the root mean square deviation stabilizing after ~5 ns. Mutations were introduced to these equilibrated structures using the YASARA ‘swap’

command; the resulting mutant models ran in duplicates, for ~200-220 ns for C7 and ~200 ns for C8.

3 Results

3. 1 Clinical Case Presentation

Manifestation of this disease in the proband dates to the prenatal period, evident by severe polyhydramnios *in utero*, likely from impaired swallowing and thus ineffective recirculation of the amniotic fluid, requiring amnioreduction. Craniofacial anomalies, hypotonia, clubfoot and camptodactyly were present at birth, leading to the diagnosis of DA-1. A nasogastric tube was placed for initial feeding to ameliorate sucking and swallowing difficulties. Episodes of desaturation and inspiratory stridor prompted endoscopic examination and further diagnosis of laryngomalacia.

Psychomotor development was delayed, but milestones were eventually met with qualitative deficits in fine motor skills. The patient was sitting, crawling, and standing by 13, 15, and 18 months, respectively. She underwent operative release of hip contractures at age two, after which she was able to walk independently, and later Achilles tenotomy and surgical correction of knee contractures at age three. Cognitive milestones during this period progressed normally.

Pulmonary function tests demonstrated reduced forced expiratory volume (FEV; 54%) and forced vital capacity (FVC; 54%) due to diaphragmatic and intercostal muscle weakness, but normal peak expiratory flow (80%). However, results may have been confounded by inappropriate spirometry technique due to decreased facial muscle tone. Episodes of accelerated breathing were observed but resolved soon after without recurrence. Polysomnography, nocturnal desaturation screenings, and cardiac examination concluded with normal findings.

Currently at seven-years-old, the proband presents with dystrophic, small body habitus (**Supplemental Fig. 1**) and dysmorphic craniofacial features, including hypomimia and bilateral ptosis. Feeding is normal, though with smaller meal portions and assistance necessary to cut food

of tougher consistency. Thoracic and lumbar spinal rigidity, along with mild thoracic scoliosis, contribute to an abnormal body posture (**Supplemental Fig. 1**). Mild contractures of proximal interphalangeal (PIP) joints, knees, and feet remain post-surgical intervention. General hypotonia and muscle weakness are apparent, with maximum weakness localized in the axial regions as evident by the poor cervical flexion in a supine position, and mild weakness in the limb girdles of both upper and lower limbs. Muscle weakness and feet deformities contribute to a myopathic gait. Though she can walk independently and continuously for 4.5 kilometers, she experiences increased fatigability, falls three to four times weekly, and often utilizes upper limb support when climbing stairs. Episodes of postural tremor of the lower and upper limbs are present upon instances of prolonged standing and holding heavy objects, respectively. Brain MRI, sensory and proprioception examinations, and cognitive development are normal. Although hearing loss was suspected, she currently does not present with any hearing disabilities. The family did not consent to a muscle biopsy.

Targeted NGS sequencing analysis revealed compound heterozygous variations in *MYBPC1* – NM_002465.4:c.[2486_2492del];[2663A>G] – inherited from her mother and father, respectively (**Fig 1A**). The maternal variant p.(K829Ifs*7) is predicted to result in frameshift termination of the C7 Fn-III domain resulting in addition of six new residues and introduction of an early stop codon, while the paternal variant p.(D888G) is predicted to result in a missense mutation in the C8 Ig domain (**Fig 1B**). Additionally, a heterozygous variant of unknown significance (VUS) –NM_001378183.1:c.6895G>A p.(Val2299Met)– was detected in *PIEZO2*, inherited from the mother. Neither the mother nor the father demonstrates any neuromuscular deficits.

3.2 Variant-specific *in-silico* effects on respective sMyBP-C domain structures

To probe the molecular alterations potentially elicited by each inherited variant, we performed molecular dynamics (MD) simulations. Both the C7 and C8 domain models adopt classical Fn-III and Ig folds, respectively, typical of β-sandwich structures[18,19].

As alternative splicing takes place within the Fn-III C7 domain, we performed MD simulations using both splice forms, hv-1 and hv-3, with the latter including a spliced sequence (residues 761-779; Q00872-1)[16]. MD simulations of the K829Ifs*7 variant in hv-1 C7 showed that the β-strand in which it resides is structurally altered due to the introduction of six new amino acids followed by a premature stop codon (**Fig. 2A**). Accordingly, these six altered residues result in the exposure of the C7 hydrophobic core, consequently promoting domain instability (**Fig. 2B-C and Supplemental Figs 2 and 3**). Moreover, the presence of the premature stop codon results in loss of the terminal β-strand within C7 (**Fig. 2A**) and of the ensuing C8-C10 domains that mediate binding to light meromyosin and titin[20-22]. Of note, computational simulations yielded comparable K829Ifs*7-induced alterations in the hv-3 C7 structure, indicating that the impact of the variant is independent of the exclusion or inclusion of the spliced region (**Supplemental Fig. 4**).

In contrast to the K829Ifs*7-prompted structural alterations predicted in Fn-III C7, MD simulations of the D888G variant located in Ig C8 revealed that the overall structure of the domain is minimally affected (**Fig 3A**), with the resulting glycine in the flexible loop exhibiting comparable flexibility to the original aspartic acid (**Fig 3B**).

4 Discussion

Herein we describe, for the first time, two novel *MYBPC1* compound heterozygous variants in a pediatric patient resulting in severe DA-1 neither of which have been described in the gnomAD database individually or together (**Supplemental Table 2**)[23]. In addition to harboring typical DA-1 features of camptodactyly and clubfoot, the patient presents with manifestations not previously reported in individuals diagnosed with DA-1, including facial muscle weakness and episodes of postural tremor upon exertion[2].

Both variants are located at the COOH-terminus of the protein, specifically in the C7 and C8 domains that contribute to the targeting of sMyBP-C to the sarcomeric C-zone through interactions with thick filament proteins. Specifically, the sMyBP-C C7-C10 and C8-C10 modules

mediate binding to light meromyosin and titin, respectively[5,9,20]. Our modeling analysis of the K829Ifs*7 variant clearly predicts not only the loss of the downstream C8-C10 domains, but also truncation and misfolding of the C7 domain containing the mutation. Notably, the structures of both sMyBP-C C7 splice forms are predicted to be affected similarly, as the presence of the spliced region does not augment or alleviate the impact of the mutation. This is consistent with the lack of proximity of the spliced region to the location of the mutation in the indicated β-strand. Although *in-silico* findings do not predict the D888G mutation to significantly alter the structure or dynamics of the C8 domain, it may affect interactions with key binding partners (i.e., titin and light meromyosin)[20-22].

NGS analysis also revealed a heterozygous VUS in *PIEZO2*, which encodes a mechanosensitive ion channel mediating signal transduction in sensory and proprioception processes[24]. Mutations in *PIEZO2* predominantly exhibit dominant inheritance and have been associated with contracture syndromes, including DA-3 (Gordon Syndrome), DA-5, and Marden-Walker syndrome. In addition to malformations of the distal extremities, other common clinical findings include short stature, ophthalmoplegia, cleft palate, and/or cerebellar malformation[25]. Given that the proband does not present with any of these manifestations and has normal sensory and proprioception functions, coupled with the lack of neuromuscular abnormalities in the mother carrying the *PIEZO2* VUS, strongly suggest that the DA-1 phenotype is a consequence of the compounding *MYBPC1* variants. However, we cannot preclude the possibility that the *PIEZO* VUS may be a contributing factor to the clinical presentation of the patient.

It is important to note that the parents, each of whom are carriers for one of the reported *MYBPC1* variants, do not show signs of arthrogryposis or muscle weakness, do not present with tremor, have no history of difficulties with physical activities, and have had normal psychomotor development. Interestingly though, when these two variants are inherited concurrently, a pathogenic phenotype emerges. This suggests that one functional copy of *MYBPC1* may be sufficient to compensate for the presence of each of these COOH-terminal mutations in the other

allele. Future studies are necessary to elucidate the mechanisms by which each of these novel *MYBPC1* variants contributes to the observed arthrogryposis phenotype accompanied by myopathy and tremor when compounded.

Herein, we describe a novel set of compound heterozygous variants in *MYBPC1* that results in severe DA-1 manifestations beginning *in utero*. Interestingly, this patient shares numerous manifestations with a newly identified form of myopathy – *MYBPC1*-linked congenital myopathy with tremor (MYOTREM) – including facial dysmorphia, hypotonia, and postural tremor following exertion[26-29]. This highlights the complexity of sMyBP-C in muscle pathophysiology and a need for improved understanding of the molecular etiologies of *MYBPC1*-associated diseases.

5 Acknowledgements

We would like to sincerely thank the family for their willingness and participation.

6 Funding Sources: This work was supported by the NIH (T32 DK067872 to AI, T32 AR007592 to JM, and R01 AR076373), NSF:2024182 (NTW) and Charles University Project GA UK No. 388422 (BL).

7 Author Contribution Statement

AI, BL, and AKK wrote the manuscript. NW performed the modeling and analysis; AI and JM aided in *in-silco* experimental design and analysis. BL and JH performed the clinical evaluation; JZ and PL performed the molecular diagnosis.

8 Ethical Approval

Informed consent was provided by the proband's parents to publish pertinent clinical information, including photos/videos, and results of genetic analysis. Not for human research (NHSR) approval was granted (#HP-00107425, UMSOM).

9 Competing Interests

The authors declare no competing interests.

10 Data Availability

The data is not available publicly due to privacy and ethical limitations. Therefore, data that does not infringe on privacy and ethical obligations to the patient's family, are available upon reasonable requests to the research team.

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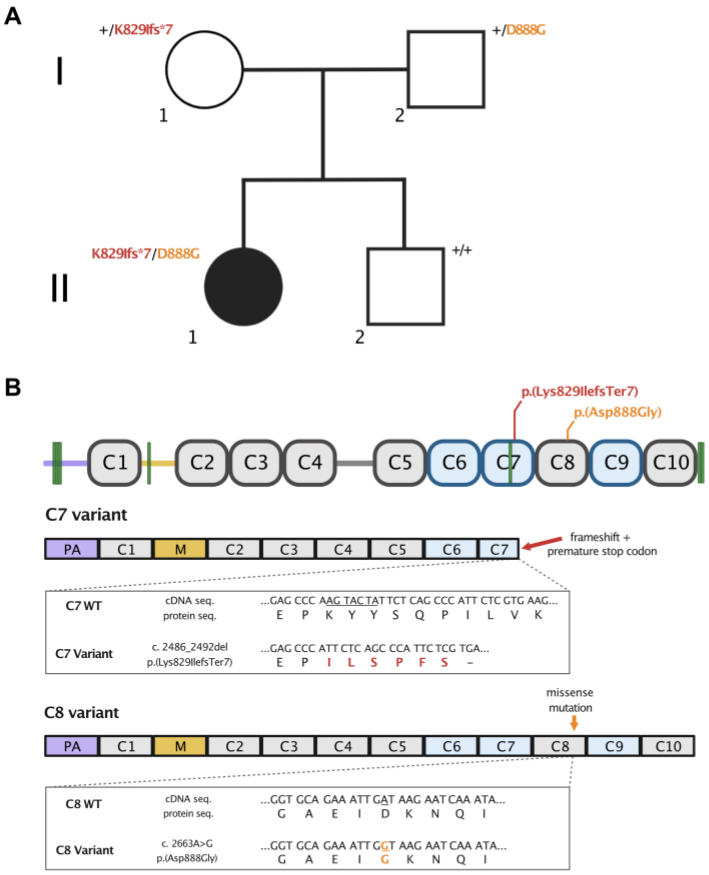

Figure 1

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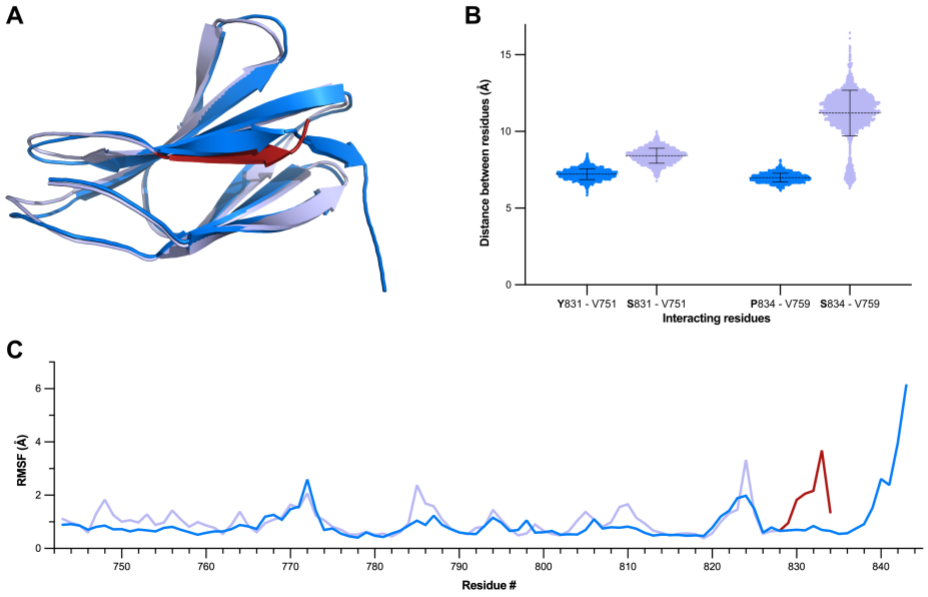
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Figure 2

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