Synemin-related skeletal and cardiac myopathies: an overview of pathogenic variants

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Running title: Synemin polymorphism and related myopathies

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21 **List of abbreviations**

22 AKAP, A-kinase-anchoring protein

23 IF, Intermediate filament

24 IFAPs, IF associated proteins

25 GFAP, Glial fibrillary acidic protein

26 KO, Knocked-out

27 MAF, Minor allele frequency

28 PKA, Protein Kinase A

29 SNP, Single nucleotide polymorphism

30 SRF, Serum response factor

31

32 **Abstract**

33 This review analyzes data concerning patients with cardiomyopathies or skeletal myopathies
34 associated with a variation in the intermediate filament (IF) synemin gene (*SYNM*), also
35 referred to as desmuslin (*DMN*). Molecular studies demonstrate that synemin copolymerizes
36 with desmin and vimentin IF and interacts with vinculin, α -actinin, α -dystrobrevin, dystrophin,
37 talin and zyxin. It has been found that synemin is an A-kinase-anchoring protein (AKAP) that
38 anchors Protein Kinase A (PKA) and modulates the PKA-dependent phosphorylation of
39 several cytoskeletal substrates such as desmin. As several IF proteins, including desmin, have
40 been implicated in human genetic disorders such as dominant or recessive congenital and
41 adult onset myopathy, synemin becomes a significant candidate for cardiac and skeletal
42 myopathies of unknown etiology. Since *SYNM* is a new candidate gene that displays
43 numerous sequence polymorphisms, in this review, we summarize the genetic and clinical
44 literature about *SYNM* mutations. Protein changing variants (missense, frameshifts, nonsense)
45 were further evaluated based on structural modifications and amino acid interactions. We
46 present *in silico* modeling of helical salt-bridges between residues to evaluate the impact of
47 the synemin networks crucial to interactions with cytoskeletal proteins. Finally, a discussion
48 is featured regarding certain variants that may contribute to the disease state.

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50 **Keywords:** Intermediate Filament; Cytoskeleton; Desmuslin; Desmin; polymorphism;
51 genetic variant.

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56 **Introduction**

57 Intermediate filament (IF) proteins are constituents of the cytoskeleton encoded by a
58 dispersed multigene family of up to 70 genes. IFs are formed by nonglobular flexible protein
59 units, which consist mostly of α -helices. IF protein structure comprises a central α -helical rod
60 domain as its main part, flanked to a variable head and tail parts. IF proteins are chemically
61 heterogeneous, have different rates of assembly and produce discrete types of filaments (16).
62 In most vertebrate cells, cytoplasmic IFs are tethered to the nucleus. They extend into the
63 cytoplasm providing a scaffold for mitochondria, the Golgi complex, microtubule organizing
64 centers and other cytoskeletal elements (13). In the periphery, IFs associate with the plasma
65 membrane. The resulting network integrates and organizes the cytoplasm providing
66 mechanical integrity, which is essential to tissue function. This process has been highlighted
67 in more than 75 human genetic diseases. They are the result of deficiencies in this network
68 resulting in skin fragility and epidermolytic disorders, laminopathies, myopathies,
69 cardiomyopathies, neuropathies, cataracts and premature aging (Human Intermediate
70 Filament Mutation Database, <http://www.interfil.org>).

71 *SYNM* is a new candidate gene which shows numerous sequence polymorphisms. However,
72 few data have been available from pathologists with regards to synemin. Synemin is
73 considered as IF associated protein (IFAPs) (15). In this respect, synemin is associated with
74 multiple partners such as desmin, vimentin, keratins, glial fibrillary acidic protein (GFAP),
75 neurofilaments and is present in muscle cells, in a few neural cells and in various other
76 nonepithelial cell types. Accordingly, it can be responsible for human diseases of unknown
77 etiology. Data are summarized here concerning patients with cardiomyopathy or skeletal
78 myopathy in whom a variation in the *SYNM* gene exists. Moreover, *in silico* modeling of
79 helical salt-bridges between residues is presented, allowing for the evaluation of the impact of
80 synemin networks, which are crucial to interactions with cytoskeletal proteins.

81 **Gene expression**

82 In humans, *SYNM*, also known as desmuslin (*DMN*), was localized to chromosome 15q26.3
83 by electronic screening of the human DNA sequence database and using the GB4 radiation
84 hybrid panel (29). It was located in a 4-cM interval flanked by the microsatellite markers
85 D15S212 and D15S642, and close to D15S120 (48), (GRCh38:chr15:99098217-99135593).
86 Synemin was first identified as a high-molecular-mass protein associated with desmin and
87 vimentin filaments in chicken muscles (14). Synemin expression was later detected in
88 different tissues, including the nervous system, endothelial cells, retinal cells and hepatic
89 stellate cells (18–20, 41, 47, 50, 53). The *SYNM* gene encodes three isoforms in mice and
90 humans via an alternative splicing mechanism giving rise to three isoforms: high (H) or α
91 (170 kDa), middle (M) or β (140 kDa), and low (L) (41 kDa) synemin with the same head and
92 rod domains but differing in the tail domain of the protein (see Figure 1) (29, 48, 53). *SYNM*
93 expression is regulated in an isoform specific manner during the development and
94 regeneration of different tissues.

95 During mouse embryo development, synemin M was present as early as in 5-day-old embryo
96 (E5) of mice with vimentin and nestin. Synemin H was found later at E9 in the nervous
97 system and mesodermic derivatives concomitantly with angiogenesis and the migration of
98 neural crest cells. Synemin L appeared later in neurons at E15 (20). Moreover, de Souza
99 Martins et al. demonstrated that synemin M is expressed in mouse pluripotent embryonic
100 stem cells while synemin H was produced only in neural precursors when neural
101 differentiation began, concurrently with synemin M, nestin and glial fibrillary acidic protein
102 (GFAP) (10). The expression of synemin H and M was reported in multipotent neural stem
103 cells, particularly in the subventricular zone of the lateral ventricle of the adult brain; a
104 neurogenic germinal niche of mice (19).

The expression of synemin has also been demonstrated in skeletal muscle satellite cells (26). Synemin M was present in all types of smooth muscles (blood vessels, bladder, and stomach) whereas synemin H and L were detected solely in the bladder (53). Furthermore, a study identified synemin as a novel serum response factor (SRF)-independent target of myocardin that is abundantly expressed in all smooth muscle cells (46). In striated muscles, the predominant synemin isoforms was M but all three synemin isoforms were produced by an alternative splicing of the synemin pre-mRNA and their expression was regulated in a developmentally specific manner (53). The temporal distributions of the three synemin isoforms in striated muscles were distinct. The H/M isoforms were detected in the epaxial muscles (myotomes) and the heart as early as E10 in mice, and they persisted throughout all embryo stages and in the adult. In contrast, the L isoform was found only in a subset of adult muscles (53). These differences probably reflect differing functions of the synemin isoforms during development.

Synemin interactions with other proteins

Synemin consists of a short head domain, a missing conserved TALL sequence in the 2A subdomain that is characteristic of all IFs, and the TYRKLLEGEE motif in the 2B subdomain that differs from all other IFs (24). In this respect, synemin is associated with multiple partners such as desmin, vimentin, keratins, GFAP, neurofilaments to form the filament in different tissues. Synemin co-assembles with desmin and vimentin to become heteropolymers in striated and smooth muscle tissues (53). It interconnects Z-discs to costamere in skeletal muscle (1) and to desmosomes in the heart (27) as well as participates in mitochondria positioning, a process that probably involves plectin (25). The synemin protein interacts with and is closely related to desmin (34); a protein encoded by a locus mutated in some forms of hereditary distal myopathy (28). In myopathies caused by desmin mutation, synemin is associated with cytoplasmic desmin aggregates in skeletal muscles (4, 5, 7, 32, 34, 48).

130 Interestingly, in desmin mutations located into the highly conserved amino acid motif of helix
131 2B (E401K, R406W, E413K), synemin is excluded from desmin containing aggregates (7). In
132 this study, using *in silico* constructs the authors demonstrated that such behavior with these
133 mutants was correlated to the abnormal salt-bridges of desmin-dimer which prevent
134 longitudinal annealing and/or radial compaction during desmin filament formation. This
135 perturbs synemin-desmin heteropolymer filament assembly (7). Co-transfection and
136 immunolabeling experiments indicate that both synemin H and M isoforms are incorporated
137 to desmin to form heteropolymeric IFs. Furthermore, using desmin- and vimentin-deficient
138 mice whose synemin was unstable and delocalized in cells, there is direct evidence of
139 association with desmin or vimentin in skeletal and smooth muscles (6, 19, 22, 53). Synemin
140 also interacts with plectin, zyxin, α -actinin, dystrophin, utrophin, α -dystrobrevin, vinculin and
141 metavinculin, which highlights its role as a bridging protein (2, 3, 17, 21, 25, 29, 33, 44, 45)
142 (see Figure 2).

143 Phosphorylation of IF proteins in serine and threonine residues causes rearrangements and
144 interactions with other cellular proteins (8, 49, 52). It has been demonstrated that synemin is
145 an A-kinase-anchoring protein (AKAP) (40) that anchors protein kinase A (PKA) and
146 modulates the PKA-dependent phosphorylation of several cytoskeletal substrates such as
147 desmin and as such, may influence muscle growth and repair by regulating the
148 phosphorylation of proteins of the Z-disks and sarcolemma (12, 26). In addition, synemin also
149 interacts with Akt (36); a kinase important for the normal growth of fast twitch muscles.
150 These observations collectively reinforce the idea that synemin has dual functions acting both
151 as a bridging cytoskeletal protein and as a regulator of signal transduction pathways.

152 **Loss of synemin in mice leads to cardiac and skeletal myopathies**

153 Two independent synemin deficient mice were generated using homologous recombination
154 method, one of them using the Cre-loxP mediated system to remove exon 1 (26), the second

model was a constitutive knocked-out (KO) by removing all exons (12). The KO mice are viable, fertile and were born at expected Mendelian ratio. Synemin is not required for embryonic development, myogenesis or for the development of the cardiovascular system (26). No anatomic and behavioral defects were observed at birth. However, mice lacking synemin developed a myopathic phenotype, possibly due to its role in linking desmin filaments to costameres and sarcomeres (12, 26) and presented a reduced bone mass which underlines that synemin plays also a role in bone homeostasis (30, 42). In muscle, synemin inactivation leads to a decrease in maximal force, fatigue resistance and alteration of membrane integrity associated with an occurrence of focal muscle degeneration and regeneration. Synemin may play this role through its demonstrated binding to costameric and sarcolemmal proteins, such as α -actinin, vinculin (1, 44) and members of the dystroglycan complex (29). In desmin KO mice, synemin no longer associates with Z-discs but remains present in myotendinous and neuromuscular junctions, suggesting that synemin association with junctional complexes, but not with Z-discs, is desmin-independent (6). This phenomenon could be due to differential synemin isoform distribution since synemin H preferentially localizes in cell-cell junctions while synemin M predominates in Z-discs (27). Moreover, synemin H localizes to the M-band of the sarcomere through interaction with the M10 region of titin (38). Synemin also affects the behavior of satellite cells. The balance between self-renewal and differentiation of muscle satellite cells might be compromised in KO mice (26). The authors suggested that synemin might influence satellite cell behavior through its role in the regulation of PKA activity, which is important for myogenesis.

Loss of synemin also leads to cardiomyopathy. As described by Garcia-Pelagio et al. (11), the genetic silencing of synemin in mice causes left ventricular systolic dysfunction as early as the age of 3 months old. Although contractile and costameric proteins remained unchanged in the old synemin KO hearts, some alterations were identified in several signaling proteins

(PKA-RII, ERK and p70S6K) critical to cardiomyocyte function. The mice also present a left ventricular hypertrophy and eventual dilation over time. Alterations in calcium handling indicate that abnormalities originate specifically from cardiomyocytes and not from other cardiac cells (11).

Synemin-related disorders

Synemin has also been linked to human diseases including heart disease, cancer, and neurodegenerative disorders (21, 23, 31, 35, 37). Its expression is upregulated in pathological conditions such as neurotrauma, Alexandre disease and myofibrillar myopathies (9, 35). The complete sequence of the human *SYNM* gene was reported by Titeux et al. and Mizuno et al. in 2001 (29, 48). As a result of their studies, analyses were performed based on the full transcript of the *SYNM* gene (transcript ID: ENST00000336292) in an effort to list all protein changing variations (missense, frameshifts, nonsense). The Human Gene Mutation Database and Clin Var database report the disease-causing mutations over the *SYNM* gene (see Table 1). In this review, we analyzed the data concerning seven cases with skeletal myopathy or cardiomyopathy associated with a variation in *SYNM*: Variant c.173C>T (p.A58V) (56); variant c.598C>T (p.Q200*) (28); variant c.815C>T (p.A272V) (28); variant c.1612T>C (p.W538R) (55); variant c.2356G>A (p.V746M) (54); variant c.2456_2457delAG (p.E819fs) (51); variant c.4555C>T (p.Q1519*) (51) (see Table 2).

Synemin variants leading to skeletal myopathy: First, Mizuno et al. (28) suggested that synemin could be a new candidate gene for myopathies. In that study, *SYNM* was screened for mutations in 71 patients with various forms of myopathy for which a genetic cause could not be identified. The authors reported 9 single-nucleotide polymorphisms (SNPs) in *SYNM* that do not change the amino acid sequence and 12 SNPs that do. Nine silent SNPs, which did not modify the amino acid sequence, were also identified in the patient population. Among twelve modifier SNPs, 10 SNPs are common but the remaining 2 are rare. One of these rare SNPs

creates a premature stop codon in the first exon of *SYNM* and was present in a patient with nemaline myopathy (NCBI36/Ensembl54: chr15:97463527, exon 1, c.598C>T, p.Q200*, non-conservative). This SNP was detected in the unaffected father of the patient. Neither the mother nor any of the other patients were carriers of this genetic variation. This variant is currently cataloged in gnomAD_v2.1.1 database (rs5030689) as a rare stop-gain variant presenting in 1/14494 African, 3/24992 Latino, 68/8400 Ashkenazi Jewish, 39/63602 European (non-Finnish), 5/5074 Other origins of alleles. PolyPhen2 and SIFT prediction analysis did not yield any predictions. Mutation Taster predicted this variant as being “disease causing”.

Synemin variants leading to cardiomyopathies: In 2006, Zeller et al. (54) reported the first *SYNM* mutation (c.2356G>A, p.V746M) associated with dilated cardiomyopathy. Zeller et al. features a simple and reliable protocol for mutation screening in 25 known and putative candidate genes for familial cardiomyopathy by heteroduplex analysis (54). Among the screened families, one family carried a synemin mutation. Two simultaneous missense mutations were identified in the patient suffering from dilated cardiomyopathy, one in *SYNM* (c.2356G>A, p.V746M) and the other in the VEGF receptor 1 (*FLT1*) (c.411G>C, p.R54S) gene. Both mutations were also found in the patient’s mother and daughter. The daughter showed a borderline left ventricular ejection fraction with a normal left ventricular dimension and was clinically asymptomatic. The mother had regular echocardiographic findings and was also asymptomatic (54). This variant is currently cataloged in gnomAD_v2.1.1 database as a rare missense variant (rs142594053). This variant has an overall minor allele frequency (MAF) of 0.0010476, meaning it is still a rare variant (gnomAD allele frequency <0,5%). It has been reported that this mutation of *SYNM* and *FLT1* is involved in the VEGF-PLC gamma 1 signaling pathway that controls cardiac contractility in the embryonic heart (39). Disturbance

of both mechanical integrity and calcium homeostasis of the cardiomyocyte is known to cause cardiomyopathy.

In 2016, two variants of synemin were reported in dilated cardiomyopathy patients (c.2456_2457delAG, p.E819fs; c.4555C>T, p.Q1519*) by Ware et al. (51). This study shows the identification of 26 distinct rare heterozygous truncating variants in eight genes. Of the 26 truncated variants, 2 *SYNM* variants were identified; one being a missense mutation that creates a premature stop codon (c.4555C>T, p.Q1519*) and the second being a frameshift mutation (c.2456_2457delAG, p.E819fs). Mutation Taster predicted the variant c.4555C>T (p.Q1519*) as being “disease causing”: it results in the loss of protein tail interacting with Dystrophin (*DMD*) and Utrophin (*UTRN*).

Based on its tissue-specific expression profiles and the available data on KO mice phenotypes, Zlotina et al (56) suggested *SYNM* as a candidate gene contributing to the Ulnar-mammary syndrome-like phenotype, a group of rare congenital conditions that combine cardiac pathology (structural defect or arrhythmic disorder) and limb abnormality. Employing whole-exome sequencing and bioinformatics strategies, a novel heterozygous missense variant in *SYNM* was identified (GRCh37/hg19: chr15:99645578, exon 1, c.173C>T, p.A58V, conservative) (56). Further comprehensive functional studies are required to evaluate possible involvement of *SYNM* in genesis of complex heart-limb pathology. That variant is currently cataloged in the gnomAD_v2.1.1 database as a rare missense variant presented only in one allele of African origin among 3938 African alleles (1/3938). No accounts of the same variation have been found in other populations studied and accepted into the gnomAD database to date. That variant, having no “rs” accession number assigned yet, presents a minor allele frequency (MAF) of 0.00000811. When analyzed as a single event, PolyPhen-2 and SIFT prediction algorithm analyses qualified this variant as “benign/neutral”, respectively. Mutation Taster predicted the variant as being “polymorphism”.

The study of Zhang and al. (55) concerns the investigation of a Chinese family of three generations in which four patients had dilated cardiomyopathy. Employing whole-exome sequencing and bioinformatics strategies, a novel heterozygous missense mutation of *SYNM* gene was identified (GRCh37/hg19: chr15:99670180, exon 4, c.1612T>C; p.W538R) and co-segregated in the affected family members (55). The heterozygous variant was detected in all living patients in this family and all family members carrying the wild-type genotype were healthy. The missense mutation resulting in a substitution of tryptophan by arginine is located in the C-terminal domain of synemin, known as the region close to the encoding AKAP site of synemin which could be crucial for kinase activity. Cross-species alignment analysis of synemin amino acid sequences revealed that this mutated site was highly conserved in mammals, which suggests that the modification of tryptophan at position 538 may affect the structure of synemin. As we demonstrated in Figure 3 using *in silico* modeling, this missense mutation may cause structural changes in the synemin protein (see Figure 3). This variant is currently cataloged in gnomAD_v2.1.1 database as a rare missense variant (rs551837344) presenting in 8/17520 alleles of East Asian origin and 4/110162 alleles of European (non-Finnish) origin. This variant has a minor allele frequency -10-(MAF) of 0.0000491. (see table 1: in PolyPhen2, probably damaging; in SIFT, deleterious; in Mutation Taster, disease causing).

***In silico* modeling the structure of synemin protein and characterization of synemin variants**

It has been known for several years that all IFs are α -helix type fibrous proteins; that is, they contain coiled-coil helices (43). An amino acid sequence of the rod domain can assume this structure. It contains a succession of seven residue peptides (heptads) (a-b-c-d-e-f-g), where a and d are usually apolar residues, and are normally buried inside the protein core (16). Four segments, widely referred to as 1A, 1B, 2A, and 2B, are separated by linkers. Linker L1

connects 1A to 1B; linker L2 joins 2A to 2B. These are interspersed by short linkers that cannot form a coiled-coil sequence of the form (hydrophilic-apolar). Linker L12 joins the segments 1 and 2 (16).

Table 1 describes the different types of changes (conservative, missense, or stop) for 24 *SYNM* variant. Among them, 9 variants lead to conservative amino acids, 12 missense amino acids, 2 premature stops, and one is a deletion. Moreover, Table 1 and Figure 2 report the position of the amino acid variants with potential pathogenicity. Variants A58V, Q200*, A272V related to cardiomyopathy or skeletal myopathy are located in the rod domain, characterized by interactions with desmin, vimentin, vinculin, α -dystrobrevin, dystrophin and utrophin. Variant W538R is found near the region 556 to 707 with α -helices described by Russell et al. (40) while also potentially being anchored to PKA. The missense variant V746M is located in the tail region of the M and H synemin but not of the L synemin, which is shorter. The tail region from 909 to 1564, which is known to interact with zyxin, talin, vinculin, α -actinin and dystrophin, includes the variant Q1519* that causes the integration of a premature stop codon.

Helical Wheel analyses performed by Khanamiryan et al (24) as well as by Russell et al (40) show that certain regions of synemin could form an amphipathic structure. The software Helical Wheel Custom Images and Interactive Java Applet software were used to search for potential α -helices within the synemin protein and for modifications of amino acid interactions between the variants. It can be hypothesized that mutations leading to a change of charge, distance and interactions between the amino acids of the helix are deleterious and modify an association with their partners. In order to support our hypothesis, the interactions between amino-acid residues were examined using *in silico* modeling. The modeling of mutated synemin for missense residue 538 shows that the replacement of a tryptophan neutral apolar hydrophobic residue by a positive hydrophilic arginine residue causes the loss of the

hydrophobic interaction between residue 538 and residue leucine 541 (W--L--L vs R L--L) (Table 2, Figure 3A). For missense mutation V746M, a nonpolar hydrophobic residue valine changed to a nonpolar hydrophobic residue methionine does not change the interaction. However, although variant 272 is a conservative mutation, the change of a nonpolar hydrophobic residue alanine residue with a nonpolar hydrophobic residue valine creates a new interaction between residue 272 valine and residue 276 leucine: (I--L A vs I--L--V) (Table 2, Figure 3B). By contrast, for the conservative mutation of A58V, a nonpolar hydrophobic residue alanine to a nonpolar hydrophobic residue valine does not modify the interactions (Table 2, Figure 3B). It is evident that the change of hydrophobic interaction between amino acids in the helix depends not only on the characteristic of the mutant residue, but also on the property of its partner residue.

Concluding remarks: Pathogenic mutations or uncertain significant variants?

To date, among the variants described of the *SYNM* gene, 7 could be associated in human to a cardiomyopathy or a skeletal myopathy. Data on synemin KO mice suggest that synemin plays an important role in skeletal muscle and in the heart. Synemin is thought to mechanically stabilize the cardiomyocyte by linking the extracellular matrix to the Z-disc. Synemin interacts with type III IFs desmin and vimentin, through the head and rod domain, and interacts with membrane proteins through its long C-terminal tail. Furthermore, through its interaction with cytoskeletal proteins dystrophin, utrophin and vinculin, synemin is able to link those heteropolymers to adherent-type junctions within striated muscle cells, suggesting that it plays an important role in the component assembly of the cytoskeleton and in morphogenesis. In addition, we emphasize that *in silico* modeling of the helical salt-bridge between residues could evaluate the impact of synemin on network organization or integration into desmin filaments and be crucial to its interaction with cytoskeletal proteins. Two

329 mutations in position 272 or in position 538, corresponding to patients with respectively
330 myopathy or cardiomyopathy, showed a change in neighbor amino acid interactions. The
331 position 538 near the encoding AKAP site of synemin could be crucial for kinase activity and
332 synemin-desmin heteropolymer filament assembly. Taken together, all data support a role for
333 synemin in the integrity of the intermediate filaments.

334 In this review, we summarized several studies indicate that a single mutant allele of *SYNM*
335 may cause disease as an autosomal dominant manner. In spite of the amount of accumulated
336 animal model research data and the information directly provided by medical genetic
337 diagnostic studies on human samples, we cannot correlate all *SYNM* variants related to
338 cardiomyopathy or skeletal myopathies. Like other rare variants, they may contribute to the
339 penetrance of potentially pathogenic structural or nucleotide variants of the genome, leading
340 to disease. Given that current state of exome data lacks the gene network analysis for
341 polymorphisms in myopathies, we propose a careful analysis and interpretation of additional
342 variants in *SYNM* and other related genes to be considered when evaluating a phenotype, both
343 in research and clinical context. Many studies including animal models, *in vitro* cell culture
344 assay, as well as *in silico* modeling of helical salt-bridges should be performed in order to
345 validate the clinical significance of *SYNM* variants.

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Statement of author contributions

ZX, YH performed *in silico* modeling of synemin protein; SK, DP, ZX contributed to genetic variant analysis; DP, OA, ZL and ZX wrote the manuscript.

Fundings

Financial support has been provided by Sorbonne Université, CNRS, INSERM, the AFM-Téléthon [contract numbers: 21833 and 22142] and the Fédération Française de Cardiologie. Y.H. was supported by a fellowship from the AFM-Téléthon.

Acknowledgments

We thank Dr Serge Fermandjian for valuable suggestions, Pr Ali Ladram for helpful advices, Dr Ara Parlakian for critical reading of the manuscript and Sareen Kardjian for linguistic improvements. We express our thanks to Pr L.E.Thornell for the gift of immunolabeling of synemin and desmin presented in Figure 1.

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Figure Legends

Figure 1. Schematic representation of the human synemin gene (*SYNM*) and protein

isoforms (*SYNM*). (A) Organization of synemin gene. The *SYNM* encodes three RNA corresponding respectively to M (exons 1-2-3-4-5), H (exons 1-2-3-4a) and L (exons 1-2-3-5) through an alternative splicing mechanism, giving rise to three proteins. (B) Organisation of synemin isoforms. High (H) or α -synemin (170 kDa), Middle (M) or β -synemin (140 kDa), and Low (L) synemin (41 kDa) with the same head (10 amino acids) and rod domains (312 amino acid) but with different lengths of the C-terminal end (respectively 1243, 931 and 6 amino-acids). AA: amino acid. (C) Immunofluorescence detection of desmin (green) and synemin (red) in human skeletal muscle (from Lars-Eric Thornell, Umeå University, Sweden). Synemin co-localizes with desmin at the myofibrillar Z-lines. Bar: 5 μ m.

Figure 2. Localization of synemin interacting regions with its partners. Tripartite structure

of synemin comprising a central α -helical rod domain (amino acid position from 11 to 322) flanked by non-helical head (amino acid position from 1 to 10) and tail domains (amino acid position from 323 to 1565). Note that the amino acids 1151 to 1463 are absent of M synemin (48). Protein interactions with parts of rod domain of synemin are presented: Desmin (7), Vimentin (3), Vinculin (44), α -Dystrobrevin (29), Dystrophin (3), Utrophin (3) interacting site. Interactions with parts of the tail domain of M and H synemin (from 909 to 1564 amino acid): Zyxin (45), Talin (44), Vinculin (44), α -Actinin (2), Dystrophin (3). Triangles show the positions of 7 variants examined in this review; numbers correspond to the position of mutated amino acids.

Figure 3. Schematic representation of helical part of synemin variants: Computer analyses (DNASTAR) were used to search for potential α -helices and the modifications of amino acid interactions in the variants. (A) The modeling of mutated synemin for residue 538 shows that the replacement of a neutral apolar hydrophobic tryptophan residue by a positive hydrophilic arginine residue causes a suppression of the hydrophobic interactions between residue 538 and leucine 541. (B) Schematic presentation of interactions for mutants: (A58V) conservative without modification, (A272V) with a new hydrophobic bond, (W538R) missense with loss of the hydrophobic bond or (V746M) without modification.

Table 1. *SYNM* polymorphism: variants, prediction and functional consequences of selected 24 *SYNM* variants.

1	2	3	4	5	6	7	8	9	10	11
Genomic Coordinates	Codon Change	Base Variant	Amino acid change	Effect	Phenotype (Associated Disease)	References	SIFT prediction	PolyPhen 2 prediction	Mutation taster prediction	dbSNP accession nr.
99645578	CAG G[C/T]C CGC	173C>T	A58V	C	Ulnar-Mammary-Like syndrome, Cardiomyopathy	(56)	damaging	benign / possibly damaging	polymorphism	rs1367107502
99646003	GTG [C/T]AG CTG	598C>T	Q200*	Stop	Nemaline Myopathy	(28)	n/a	n/a	disease causing	rs5030689
99653800	AGA G[T/C]G ATT	815C>T	A272V	C	nd	(28)	tolerated	benign	polymorphism	rs2305445
99666979	CAC [G/A]TT GAA	988G>A	V330I	C	nd	(28)	tolerated	benign	polymorphism	rs5030691
99669628	GAA [A/T]GG AAT	1063A>T	R335W	M	nd	(28)	damaging	possibly damaging	polymorphism	rs3743242
99669628	GAA [A/T]GG AAT	1065T>A	W355R	M	nd	unpublished	damaging	possibly damaging	polymorphism	rs3743242
99669684	TCG GC[A/G] CTG	1119T>C	V373A	C	nd	unpublished	tolerated	n/a	polymorphism	rs1555485392
99670180	AGA [T/C]GG GAA	1612T>C	W538R	M	Cardiomyopathy	(55)	damaging	damaging	disease	rs551837344
99670265	TCA C[C/T]G AAG	1700C>T	P567L	C	nd	(28)	tolerated	benign	polymorphism	rs3743244
99670400	GCA G[A/C]A GCA	1835A>C	E612A	M	nd	(28)	damaging	benign	polymorphism	rs5030692
99670624	ATA [G/C]TT GTG	2061C>G	L687V	C	nd	unpublished	tolerated	benign	polymorphism	-
99670725	GGC AA[G/C] TCA	2160G>C	K720N	C	nd	unpublished	damaging	benign / possibly damaging	polymorphism	-
99670801	AAC [G/A]TG GAG	2356G>A	V746M	M	Cardiomyopathy	(54)	damaging	probably damaging	disease causing	rs142594053
99670847	AAG C[C/T]G GAG	2282C>T	P761L	C	nd	(28)	tolerated	benign	polymorphism	rs3743247
99671012	GTG G[A/G]A GAA	2445A>G	E815G	M	nd	unpublished	damaging	probably damaging	disease causing	-
99671022- 23	N/A	2456_2457delAG	E819fs	FS	Cardiomyopathy	(51)	n/a	n/a	disease causing	rs1555485680
99671401	CCC [C/T]GG CAG	2836C>T	R946W	M	nd	(28)	damaging	possibly damaging	polymorphism	rs5030694
99671492	GGG C[A/G]G GGT	2927A>G	Q976R	M	nd	(28)	tolerated	benign	polymorphism	rs5030695
99671741	GCC C[C/T]G GCT	3176C>T	P1059L	C	nd	(28)	damaging	benign	polymorphism	rs5030697
99671765	AGG C[G/C]T TGG	3200G>C	R1067P	M	nd	(28)	damaging	benign	polymorphism	rs5030698
99671795	CCT T[C/T]A GGC	3230C>T	S1077L	M	nd	(28)	damaging	benign	polymorphism	rs5030699
99672599	CAC G[G/C]A GAG	4032G>C	G1345A	M	nd	unpublished	tolerated	benign	polymorphism	rs7167599
99672722	GCA G[A/G]G GAC	4155A>G	E1386G	M	nd	unpublished	tolerated	benign	polymorphism	rs2292288
99673120	GAA [C/T]AG GGC	4555C>T	Q1519*	Stop	Cardiomyopathy	(51)	n/a	n/a	disease causing	-

Columns 1, 2, 11, data from NCBI; <https://www.ncbi.nlm.nih.gov/projects/sviewer>. Column 1 indicates genomic coordinates; column 2 indicates codon change; and column 11 indicates db SNP (single nucleotide polymorphism) accession numbers of selected 24 *SYNM* variants. Column 3 indicates base variant; and column 4 indicates amino acid change from published (28, 51, 54-56) and unpublished data (Z Li, Sorbonne Université, France). For amino acid changes analyses, the sequence of synemin from Titeux et al (48) were used (GenBank under accession numbers AJ310522 for the α isoform (Synemin H) and AJ310521 for the β isoform (Synemin M). Column 5 indicates effect of mutations: (C) Conservative, (M) Missense, (FS) Frame Shift, Stop. Column 6 indicates the phenotype or associated disease from published data (28, 51, 54-56). nd: non-determined. Column 8 to 10 indicate prediction analyses; SIFT prediction: consequences of mutations: damaging, tolerated, n/a: not available; PolyPhen2 prediction: consequence of mutations for synemin protein NP_663780 (isoform α /H) / NP_056101 (isoform β /M); benign, probably damaging, possibly damaging; Mutation taster prediction: consequence of mutations polymorphism or disease causing. Gray rows indicate the seven variants selected in this review.

Table 2. Synemin variants from patients with skeletal myopathy or cardiomyopathy.

Location	Amino Acid Change	Mutation Site	Changes in Amino Acid Interaction (wild-type/mutant)	Modification	Associated Disease (References)
Exon 1	Conservative	58 REGLWAEGQ <u>A</u> RCAEEARS REGLWAEGQ <u>V</u> RCAEEARS	A A L / V A L	No modification	Cardiomyopathy (56)
Exon 1	Stop	200 VAESWRET <u>Q</u> LYEDEVRE VAESWRET <u>*</u>	n/a	-	Nemaline Myopathy (28)
Exon 2	Conservative	272 GIQAEERQR <u>A</u> IDCLEDEK GIQAEERQR <u>V</u> IDCLEDEK	I--L A / I--L--V	New hydrophobic bond creation	nd (28)
Exon 4	Missense	538 KASEERNL <u>R</u> WEELTKLDK KASEERNL <u>R</u> WEELTKLDK	W--L--L / R L--L	Hydrophobic bond lost	Cardiomyopathy (55)
Exon 4	Missense	746 REGRAKVVN <u>V</u> EIVEEPVS REGRAKVVN <u>M</u> EIVEEPVS	V--V--V--V / V--M--V--V	No modification	Cardiomyopathy (54)
Exon 4	Frameshift	819 NTTHVEEVT <u>E</u> AGDSEGEQ NTTHVEEVT <u>fs</u>	n/a	-	Cardiomyopathy (51)
Exon 5	Stop	1519 GEGDQAHRE <u>Q</u> GKEQAMFD GEGDQAHRE <u>*</u>	n/a	-	Cardiomyopathy (51)

Different *SYNM* variants and their consequences: A58V (Ulnar-mammary syndrome) (56), Q200* (Nemaline myopathy) (28), A272V (nd: no-determined) (28), W538R (Dilated cardiopathy) (55), V746M (Dilated cardiopathy) (54), E819fs (Cardiomyopathy) (51), Q1519* (Cardiomyopathy) (51). Different types of amino acid interaction changes are indicated for four variants: (A58V) conservative without modification, (A272V) with a new hydrophobic bond, (W538R) missense with loss of the hydrophobic bond or (V746M) without modification. (--) indicates hydrophobic interaction. n/a: no applicable (see figure 3).

A

Synemin Gene (*SYNM*)

B

Synemin Protein (*SYNM*)

C

A**missense mutation W538R**

KASEERNLR**W**EELTKLDK
WT

KASEERNLR**R**EELTKLDK
mutant

B**A58V** **A272V** **W538R** **V746M**