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

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3 Denise Paulin<sup>1</sup>, Yeranuhi Hovannisyanyan<sup>1</sup>, Serdar Kasakyan<sup>2</sup>, Onnik Agbulut<sup>1</sup>, Zhenlin Li<sup>1\*</sup>,  
4 Zhigang Xue<sup>1</sup>

5

6 <sup>1</sup> Sorbonne Université, Institut de Biologie Paris-Seine (IBPS), CNRS UMR 8256, INSERM  
7 ERL U1164, Biological Adaptation and Ageing, 75005, Paris, France.

8 <sup>2</sup> Duzen Laboratories Group, Center of Genetic Diagnosis, 34394, Istanbul, Turkey.

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11 Running title: Synemin polymorphism and related myopathies

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15 \*Corresponding Author:

16 Dr Zhenlin Li, Sorbonne Université, Institut de Biologie Paris-Seine, UMR CNRS 8256,  
17 INSERM ERL U1164, 7, quai St Bernard - case 256 - 75005 Paris-France.

18 Phone : +33-144273421, Email : zhenlin.li@sorbonne-universite.fr

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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,  $\alpha$ -actinin,  $\alpha$ -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.

49

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  $\alpha$ -helices. IF protein structure comprises a central  $\alpha$ -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  $\alpha$   
91 (170 kDa), middle (M) or  $\beta$  (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).

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

### 118 **Synemin interactions with other proteins**

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

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

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

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

#### 184 **Synemin-related disorders**

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

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

205 creates a premature stop codon in the first exon of *SYNM* and was present in a patient with  
206 nemaline myopathy (NCBI36/Ensembl54: chr15:97463527, exon 1, c.598C>T, p.Q200\*,  
207 non-conservative). This SNP was detected in the unaffected father of the patient. Neither the  
208 mother nor any of the other patients were carriers of this genetic variation. This variant is  
209 currently cataloged in gnomAD\_v2.1.1 database (rs5030689) as a rare stop-gain variant  
210 presenting in 1/14494 African, 3/24992 Latino, 68/8400 Ashkenazi Jewish, 39/63602  
211 European (non-Finnish), 5/5074 Other origins of alleles. PolyPhen2 and SIFT prediction  
212 analysis did not yield any predictions. Mutation Taster predicted this variant as being “disease  
213 causing”.

214 **Synemin variants leading to cardiomyopathies:** In 2006, Zeller et al. (54) reported the first  
215 *SYNM* mutation (c.2356G>A, p.V746M) associated with dilated cardiomyopathy. Zeller et al.  
216 features a simple and reliable protocol for mutation screening in 25 known and putative  
217 candidate genes for familial cardiomyopathy by heteroduplex analysis (54). Among the  
218 screened families, one family carried a synemin mutation. Two simultaneous missense  
219 mutations were identified in the patient suffering from dilated cardiomyopathy, one in *SYNM*  
220 (c.2356G>A, p.V746M) and the other in the VEGF receptor 1 (*FLT1*) (c.411G>C, p.R54S)  
221 gene. Both mutations were also found in the patient’s mother and daughter. The daughter  
222 showed a borderline left ventricular ejection fraction with a normal left ventricular dimension  
223 and was clinically asymptomatic. The mother had regular echocardiographic findings and was  
224 also asymptomatic (54). This variant is currently cataloged in gnomAD\_v2.1.1 database as a  
225 rare missense variant (rs142594053). This variant has an overall minor allele frequency (MAF)  
226 of 0.0010476, meaning it is still a rare variant (gnomAD allele frequency <0,5%). It has been  
227 reported that this mutation of *SYNM* and *FLT1* is involved in the VEGF-PLC gamma 1  
228 signaling pathway that controls cardiac contractility in the embryonic heart (39). Disturbance

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

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

239 Based on its tissue-specific expression profiles and the available data on KO mice phenotypes,  
240 Zlotina et al (56) suggested *SYNM* as a candidate gene contributing to the Ulnar-mammary  
241 syndrome-like phenotype, a group of rare congenital conditions that combine cardiac  
242 pathology (structural defect or arrhythmic disorder) and limb abnormality. Employing whole-  
243 exome sequencing and bioinformatics strategies, a novel heterozygous missense variant in  
244 *SYNM* was identified (GRCh37/hg19: chr15:99645578, exon 1, c.173C>T, p.A58V,  
245 conservative) (56). Further comprehensive functional studies are required to evaluate possible  
246 involvement of *SYNM* in genesis of complex heart-limb pathology. That variant is currently  
247 cataloged in the gnomAD\_v2.1.1 database as a rare missense variant presented only in one  
248 allele of African origin among 3938 African alleles (1/3938). No accounts of the same  
249 variation have been found in other populations studied and accepted into the gnomAD  
250 database to date. That variant, having no “rs” accession number assigned yet, presents a minor  
251 allele frequency (MAF) of 0.00000811. When analyzed as a single event, PolyPhen-2 and  
252 SIFT prediction algorithm analyses qualified this variant as “benign/neutral”, respectively.  
253 Mutation Taster predicted the variant as being “polymorphism”.

254 The study of Zhang and al. (55) concerns the investigation of a Chinese family of three  
255 generations in which four patients had dilated cardiomyopathy. Employing whole-exome  
256 sequencing and bioinformatics strategies, a novel heterozygous missense mutation of *SYNM*  
257 gene was identified (GRCh37/hg19: chr15:99670180, exon 4, c.1612T>C; p.W538R) and co-  
258 segregated in the affected family members (55). The heterozygous variant was detected in all  
259 living patients in this family and all family members carrying the wild-type genotype were  
260 healthy. The missense mutation resulting in a substitution of tryptophan by arginine is located  
261 in the C-terminal domain of synemin, known as the region close to the encoding AKAP site  
262 of synemin which could be crucial for kinase activity. Cross-species alignment analysis of  
263 synemin amino acid sequences revealed that this mutated site was highly conserved in  
264 mammals, which suggests that the modification of tryptophan at position 538 may affect the  
265 structure of synemin. As we demonstrated in Figure 3 using *in silico* modeling, this missense  
266 mutation may cause structural changes in the synemin protein (see Figure 3). This variant is  
267 currently cataloged in gnomAD\_v2.1.1 database as a rare missense variant (rs551837344)  
268 presenting in 8/17520 alleles of East Asian origin and 4/110162 alleles of European (non-  
269 Finnish) origin. This variant has a minor allele frequency -10-(MAF) of 0.0000491. (see table  
270 1: in PolyPhen2, probably damaging; in SIFT, deleterious; in Mutation Taster, disease  
271 causing).

## 272 ***In silico* modeling the structure of synemin protein and characterization of** 273 **synemin variants**

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

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

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

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

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

315 **Concluding remarks: Pathogenic mutations or uncertain significant**  
316 **variants?**

317 To date, among the variants described of the *SYNM* gene, 7 could be associated in human to a  
318 cardiomyopathy or a skeletal myopathy. Data on synemin KO mice suggest that synemin  
319 plays an important role in skeletal muscle and in the heart. Synemin is thought to  
320 mechanically stabilize the cardiomyocyte by linking the extracellular matrix to the Z-disc.  
321 Synemin interacts with type III IFs desmin and vimentin, through the head and rod domain,  
322 and interacts with membrane proteins through its long C-terminal tail. Furthermore, through  
323 its interaction with cytoskeletal proteins dystrophin, utrophin and vinculin, synemin is able to  
324 link those heteropolymers to adherent-type junctions within striated muscle cells, suggesting  
325 that it plays an important role in the component assembly of the cytoskeleton and in  
326 morphogenesis. In addition, we emphasize that *in silico* modeling of the helical salt-bridge  
327 between residues could evaluate the impact of synemin on network organization or integration  
328 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.

346

347

348 **Statement of author contributions**

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

351

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361 synemin and desmin presented in Figure 1.

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368 **References**

- 369 1. **Bellin RM, Huiatt TW, Critchley DR, Robson RM.** Synemin may function to  
370 directly link muscle cell intermediate filaments to both myofibrillar Z-lines and costameres. *J*  
371 *Biol Chem* 276: 32330–32337, 2001.
- 372 2. **Bellin RM, Sernett SW, Becker B, Ip W, Huiatt TW, Robson RM.** Molecular  
373 characteristics and interactions of the intermediate filament protein synemin. Interactions with  
374 alpha-actinin may anchor synemin-containing heterofilaments. *J Biol Chem* 274: 29493–  
375 29499, 1999.
- 376 3. **Bhosle RC, Michele DE, Campbell KP, Li Z, Robson RM.** Interactions of  
377 intermediate filament protein synemin with dystrophin and utrophin. *Biochem Biophys Res*  
378 *Commun* 346: 768–777, 2006.
- 379 4. **Capetanaki Y, Papathanasiou S, Diokmetzidou A, Vatsellas G, Tsikitis M.**  
380 Desmin related disease: a matter of cell survival failure. *Curr Opin Cell Biol* 32: 113–120,  
381 2015.
- 382 5. **Carlsson L, Fischer C, Sjöberg G, Robson RM, Sejersen T, Thornell L-E.**  
383 Cytoskeletal derangements in hereditary myopathy with a desmin L345P mutation. *Acta*  
384 *Neuropathol* 104: 493–504, 2002.
- 385 6. **Carlsson L, Li ZL, Paulin D, Price MG, Breckler J, Robson RM, Wiche G,**  
386 **Thornell LE.** Differences in the distribution of synemin, paranemin, and plectin in skeletal  
387 muscles of wild-type and desmin knock-out mice. *Histochem Cell Biol* 114: 39–47, 2000.
- 388 7. **Chourbagi O, Bruston F, Carinci M, Xue Z, Vicart P, Paulin D, Agbulut O.**  
389 Desmin mutations in the terminal consensus motif prevent synemin-desmin heteropolymer  
390 filament assembly. *Exp Cell Res* 317: 886–897, 2011.
- 391 8. **Clemen CS, Herrmann H, Strelkov SV, Schröder R.** Desminopathies: pathology  
392 and mechanisms. *Acta Neuropathol* 125: 47–75, 2013.

- 393 9. **Clemen CS, Stöckigt F, Strucksberg K-H, Chevessier F, Winter L, Schütz J,**  
394 **Bauer R, Thorweihe J-M, Wenzel D, Schlötzer-Schrehardt U, Rasche V, Krsmanovic P,**  
395 **Katus HA, Rottbauer W, Just S, Müller OJ, Friedrich O, Meyer R, Herrmann H,**  
396 **Schrickel JW, Schröder R.** The toxic effect of R350P mutant desmin in striated muscle of  
397 man and mouse. *Acta Neuropathol* 129: 297–315, 2015.
- 398 10. **de Souza Martins SC, Agbulut O, Diguët N, Larcher J-C, Paulsen BS, Rehen SK,**  
399 **Moura-Neto V, Paulin D, Li Z, Xue Z.** Dynamic expression of synemin isoforms in mouse  
400 embryonic stem cells and neural derivatives. *BMC Cell Biol* 12: 51, 2011.
- 401 11. **García-Pelagio KP, Chen L, Joca HC, Ward C, Jonathan Lederer W, Bloch RJ.**  
402 Absence of synemin in mice causes structural and functional abnormalities in heart. *J Mol*  
403 *Cell Cardiol* 114: 354–363, 2018.
- 404 12. **García-Pelagio KP, Muriel J, O’Neill A, Desmond PF, Lovering RM, Lund L,**  
405 **Bond M, Bloch RJ.** Myopathic changes in murine skeletal muscle lacking synemin. *Am J*  
406 *Physiol, Cell Physiol* 308: C448-462, 2015.
- 407 13. **Godsel LM, Hobbs RP, Green KJ.** Intermediate filament assembly: dynamics to  
408 disease. *Trends Cell Biol* 18: 28–37, 2008.
- 409 14. **Granger BL, Lazarides E.** Synemin: a new high molecular weight protein associated  
410 with desmin and vimentin filaments in muscle. *Cell* 22: 727–738, 1980.
- 411 15. **Green KJ, Böhringer M, Gocken T, Jones JCR.** Intermediate filament associated  
412 proteins. *Adv Protein Chem* 70: 143–202, 2005.
- 413 16. **Herrmann H, Aebi U.** Intermediate filaments: molecular structure, assembly  
414 mechanism, and integration into functionally distinct intracellular Scaffolds. *Annu Rev*  
415 *Biochem* 73: 749–789, 2004.
- 416 17. **Hijikata T, Nakamura A, Isokawa K, Imamura M, Yuasa K, Ishikawa R,**  
417 **Kohama K, Takeda S, Yorifuji H.** Plectin 1 links intermediate filaments to costameric

418 sarcolemma through beta-synemin, alpha-dystrobrevin and actin. *J Cell Sci* 121: 2062–2074,  
419 2008.

420 18. **Hirako Y, Yamakawa H, Tsujimura Y, Nishizawa Y, Okumura M, Usukura J,**  
421 **Matsumoto H, Jackson KW, Owaribe K, Ohara O.** Characterization of mammalian  
422 synemin, an intermediate filament protein present in all four classes of muscle cells and some  
423 neuroglial cells: co-localization and interaction with type III intermediate filament proteins  
424 and keratins. *Cell Tissue Res* 313: 195–207, 2003.

425 19. **Izmiryan A, Cheraud Y, Khanamiryan L, Leterrier JF, Federici T, Peltekian E,**  
426 **Moura-Neto V, Paulin D, Li Z, Xue ZG.** Different expression of synemin isoforms in glia  
427 and neurons during nervous system development. *Glia* 54: 204–213, 2006.

428 20. **Izmiryan A, Peltekian E, Federici T, Paulin D, Li ZL, Xue ZG.** Synemin isoforms  
429 in astroglial and neuronal cells from human central nervous system. *Neurochem Res* 35: 881–  
430 887, 2010.

431 21. **Jing R, Pizzolato G, Robson RM, Gabbiani G, Skalli O.** Intermediate filament  
432 protein synemin is present in human reactive and malignant astrocytes and associates with  
433 ruffled membranes in astrocytoma cells. *Glia* 50: 107–120, 2005.

434 22. **Jing R, Wilhelmsson U, Goodwill W, Li L, Pan Y, Pekny M, Skalli O.** Synemin is  
435 expressed in reactive astrocytes in neurotrauma and interacts differentially with vimentin and  
436 GFAP intermediate filament networks. *J Cell Sci* 120: 1267–1277, 2007.

437 23. **Kapoor S.** Synemin: an evolving role in tumor growth and progression. *J Cachexia*  
438 *Sarcopenia Muscle* 5: 347–348, 2014.

439 24. **Khanamiryan L, Li Z, Paulin D, Xue Z.** Self-assembly incompetence of synemin is  
440 related to the property of its head and rod domains. *Biochemistry* 47: 9531–9539, 2008.

441 25. **Konieczny P, Fuchs P, Reipert S, Kunz WS, Zeöld A, Fischer I, Paulin D,**  
442 **Schröder R, Wiche G.** Myofiber integrity depends on desmin network targeting to Z-disks

443 and costameres via distinct plectin isoforms. *J Cell Biol* 181: 667–681, 2008.

444 26. **Li Z, Parlakian A, Coletti D, Alonso-Martin S, Hourdé C, Joanne P, Gao-Li J,**  
445 **Blanc J, Ferry A, Paulin D, Xue Z, Agbulut O.** Synemin acts as a regulator of signalling  
446 molecules during skeletal muscle hypertrophy. *J Cell Sci* 127: 4589–4601, 2014.

447 27. **Lund LM, Kerr JP, Lupinetti J, Zhang Y, Russell MA, Bloch RJ, Bond M.**  
448 Synemin isoforms differentially organize cell junctions and desmin filaments in neonatal  
449 cardiomyocytes. *FASEB J* 26: 137–148, 2012.

450 28. **Mizuno Y, Puca AA, O’Brien KF, Beggs AH, Kunkel LM.** Genomic organization  
451 and single-nucleotide polymorphism map of desmuslin, a novel intermediate filament protein  
452 on chromosome 15q26.3. *BMC Genet* 2: 8, 2001.

453 29. **Mizuno Y, Thompson TG, Guyon JR, Lidov HG, Brosius M, Imamura M, Ozawa**  
454 **E, Watkins SC, Kunkel LM.** Desmuslin, an intermediate filament protein that interacts with  
455 alpha -dystrobrevin and desmin. *Proc Natl Acad Sci USA* 98: 6156–6161, 2001.

456 30. **Moorer MC, Buo AM, Garcia-Pelagio KP, Stains JP, Bloch RJ.** Deficiency of the  
457 intermediate filament synemin reduces bone mass in vivo. *Am J Physiol, Cell Physiol* 311:  
458 C839–C845, 2016.

459 31. **Noetzel E, Rose M, Sevinc E, Hilgers R-D, Hartmann A, Naami A, Knüchel R,**  
460 **Dahl E.** Intermediate filament dynamics and breast cancer: aberrant promoter methylation of  
461 the Synemin gene is associated with early tumor relapse. *Oncogene* 29: 4814–4825, 2010.

462 32. **Olivé M, Goldfarb L, Dagvadorj A, Sambuughin N, Paulin D, Li Z, Goudeau B,**  
463 **Vicart P, Ferrer I.** Expression of the intermediate filament protein synemin in myofibrillar  
464 myopathies and other muscle diseases. *Acta Neuropathol* 106: 1–7, 2003.

465 33. **Paul M, Skalli O.** Synemin: Molecular Features and the Use of Proximity Ligation  
466 Assay to Study Its Interactions. *Meth Enzymol* 568: 537–555, 2016.

467 34. **Paulin D, Huet A, Khanamyrian L, Xue Z.** Desminopathies in muscle disease. *J*

468 *Pathol* 204: 418–427, 2004.

469 35. **Pekny T, Faiz M, Wilhelmsson U, Curtis MA, Matej R, Skalli O, Pekny M.**  
470 Synemin is expressed in reactive astrocytes and Rosenthal fibers in Alexander disease.  
471 *APMIS* 122: 76–80, 2014.

472 36. **Pitre A, Davis N, Paul M, Orr AW, Skalli O.** Synemin promotes AKT-dependent  
473 glioblastoma cell proliferation by antagonizing PP2A. *Mol Biol Cell* 23: 1243–1253, 2012.

474 37. **Poot M, Verrijn Stuart AA, van Daalen E, van Iperen A, van Binsbergen E,**  
475 **Hochstenbach R.** Variable behavioural phenotypes of patients with monosomies of 15q26  
476 and a review of 16 cases. *Eur J Med Genet* 56: 346–350, 2013.

477 38. **Prudner BC, Roy PS, Damron DS, Russell MA.**  $\alpha$ -Synemin localizes to the M-band  
478 of the sarcomere through interaction with the M10 region of titin. *FEBS Lett* 588: 4625–4630,  
479 2014.

480 39. **Rottbauer W, Just S, Wessels G, Trano N, Most P, Katus HA, Fishman MC.**  
481 VEGF-PLC $\gamma$ 1 pathway controls cardiac contractility in the embryonic heart. *Genes Dev*  
482 19: 1624–1634, 2005.

483 40. **Russell MA, Lund LM, Haber R, McKeegan K, Cianciola N, Bond M.** The  
484 intermediate filament protein, synemin, is an AKAP in the heart. *Arch Biochem Biophys* 456:  
485 204–215, 2006.

486 41. **Schmitt-Graeff A, Jing R, Nitschke R, Desmoulière A, Skalli O.** Synemin  
487 expression is widespread in liver fibrosis and is induced in proliferating and malignant biliary  
488 epithelial cells. *Hum Pathol* 37: 1200–1210, 2006.

489 42. **Skalli O.** The cytoskeleton meets the skeleton. Focus on “Deficiency of the  
490 intermediate filament synemin reduces bone mass in vivo.” *Am J Physiol, Cell Physiol* 311:  
491 C837–C838, 2016.

492 43. **Steinert PM, Zimmerman SB, Starger JM, Goldman RD.** Ten-nanometer filaments

493 of hamster BHK-21 cells and epidermal keratin filaments have similar structures. *Proc Natl*  
494 *Acad Sci USA* 75: 6098–6101, 1978.

495 44. **Sun N, Critchley DR, Paulin D, Li Z, Robson RM.** Human alpha-synemin interacts  
496 directly with vinculin and metavinculin. *Biochem J* 409: 657–667, 2008.

497 45. **Sun N, Huiatt TW, Paulin D, Li Z, Robson RM.** Synemin interacts with the LIM  
498 domain protein zyxin and is essential for cell adhesion and migration. *Exp Cell Res* 316: 491–  
499 505, 2010.

500 46. **Swärd K, Krawczyk KK, Morén B, Zhu B, Matic L, Holmberg J, Hedin U,**  
501 **Uvelius B, Stenkula K, Rippe C.** Identification of the intermediate filament protein  
502 synemin/SYNM as a target of myocardin family coactivators. *Am. J. Physiol., Cell Physiol.*  
503 317: C1128-C1142, 2019.

504 47. **Tawk M, Titeux M, Fallet C, Li Z, Daumas-Duport C, Cavalcante LA, Paulin D,**  
505 **Moura-Neto V.** Synemin expression in developing normal and pathological human retina and  
506 lens. *Exp Neurol* 183: 499–507, 2003.

507 48. **Titeux M, Brocheriou V, Xue Z, Gao J, Pellissier JF, Guicheney P, Paulin D, Li Z.**  
508 Human synemin gene generates splice variants encoding two distinct intermediate filament  
509 proteins. *Eur J Biochem* 268: 6435–6449, 2001.

510 49. **Tsikitis M, Galata Z, Mavroidis M, Psarras S, Capetanaki Y.** Intermediate  
511 filaments in cardiomyopathy. *Biophys Rev* 10: 1007–1031, 2018.

512 50. **Uyama N, Zhao L, Van Rossen E, Hirako Y, Reynaert H, Adams DH, Xue Z, Li**  
513 **Z, Robson R, Pekny M, Geerts A.** Hepatic stellate cells express synemin, a protein bridging  
514 intermediate filaments to focal adhesions. *Gut* 55: 1276–1289, 2006.

515 51. **Ware JS, Li J, Mazaika E, Yasso CM, DeSouza T, Cappola TP, Tsai EJ, Hilfiker-**  
516 **Kleiner D, Kamiya CA, Mazzarotto F, Cook SA, Halder I, Prasad SK, Pisarcik J,**  
517 **Hanley-Yanez K, Alharethi R, Damp J, Hsich E, Elkayam U, Sheppard R, Kealey A,**

518 **Alexis J, Ramani G, Safirstein J, Boehmer J, Pauly DF, Wittstein IS, Thohan V, Zucker**  
519 **MJ, Liu P, Gorcsan J, McNamara DM, Seidman CE, Seidman JG, Arany Z, IMAC-2**  
520 **and IPAC Investigators.** Shared Genetic Predisposition in Peripartum and Dilated  
521 Cardiomyopathies. *N Engl J Med* 374: 233–241, 2016.

522 52. **Winter DL, Paulin D, Mericskay M, Li Z.** Posttranslational modifications of desmin  
523 and their implication in biological processes and pathologies. *Histochem Cell Biol* 141: 1–16,  
524 2014.

525 53. **Xue ZG, Cheraud Y, Brocheriou V, Izmiryan A, Titeux M, Paulin D, Li Z.** The  
526 mouse synemin gene encodes three intermediate filament proteins generated by alternative  
527 exon usage and different open reading frames. *Exp Cell Res* 298: 431–444, 2004.

528 54. **Zeller R, Ivandic BT, Ehlermann P, Mücke O, Zugck C, Remppis A, Giannitsis E,**  
529 **Katus HA, Weichenhan D.** Large-scale mutation screening in patients with dilated or  
530 hypertrophic cardiomyopathy: a pilot study using DGGE. *J Mol Med* 84: 682–691, 2006.

531 55. **Zhang S-B, Liu Y-X, Fan L-L, Huang H, Li J-J, Jin J-Y, Xiang R.** A novel  
532 heterozygous variant p.(Trp538Arg) of SYNM is identified by whole-exome sequencing in a  
533 Chinese family with dilated cardiomyopathy. *Ann Hum Genet* 83: 95–99, 2019.

534 56. **Zlotina A, Kiselev A, Sergushichev A, Parmon E, Kostareva A.** Rare Case of  
535 Ulnar-Mammary-Like Syndrome With Left Ventricular Tachycardia and Lack of TBX3  
536 Mutation. *Front Genet* 9: 209, 2018.

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543 **Figure Legends**

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

554

555 **Figure 2. Localization of synemin interacting regions with its partners.** Tripartite structure  
556 of synemin comprising a central  $\alpha$ -helical rod domain (amino acid position from 11 to 322)  
557 flanked by non-helical head (amino acid position from 1 to 10) and tail domains (amino acid  
558 position from 323 to 1565). Note that the amino acids 1151 to 1463 are absent of M synemin  
559 (48). Protein interactions with parts of rod domain of synemin are presented: Desmin (7),  
560 Vimentin (3), Vinculin (44),  $\alpha$ -Dystrobrevin (29), Dystrophin (3), Utrophin (3) interacting  
561 site. Interactions with parts of the tail domain of M and H synemin (from 909 to 1564 amino  
562 acid): Zyxin (45), Talin (44), Vinculin (44),  $\alpha$ -Actinin (2), Dystrophin (3). Triangles show the  
563 positions of 7 variants examined in this review; numbers correspond to the position of  
564 mutated amino acids.

565

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

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

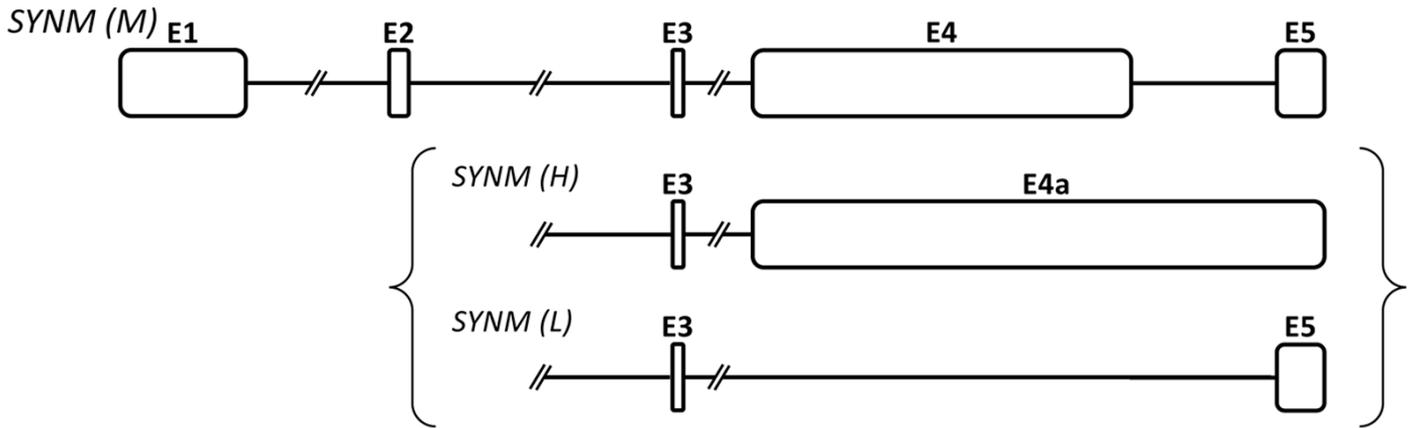
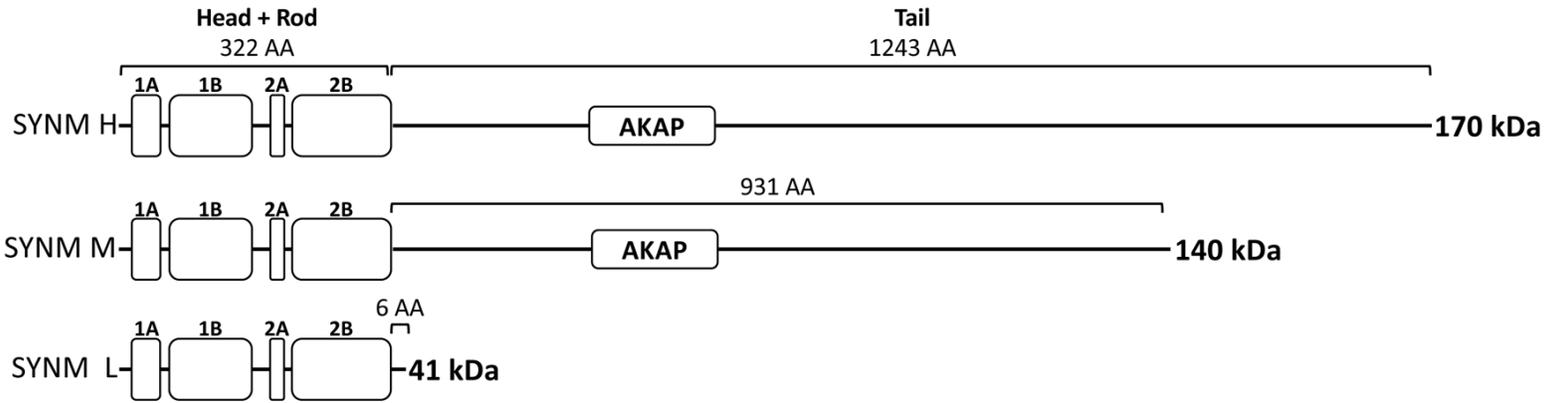
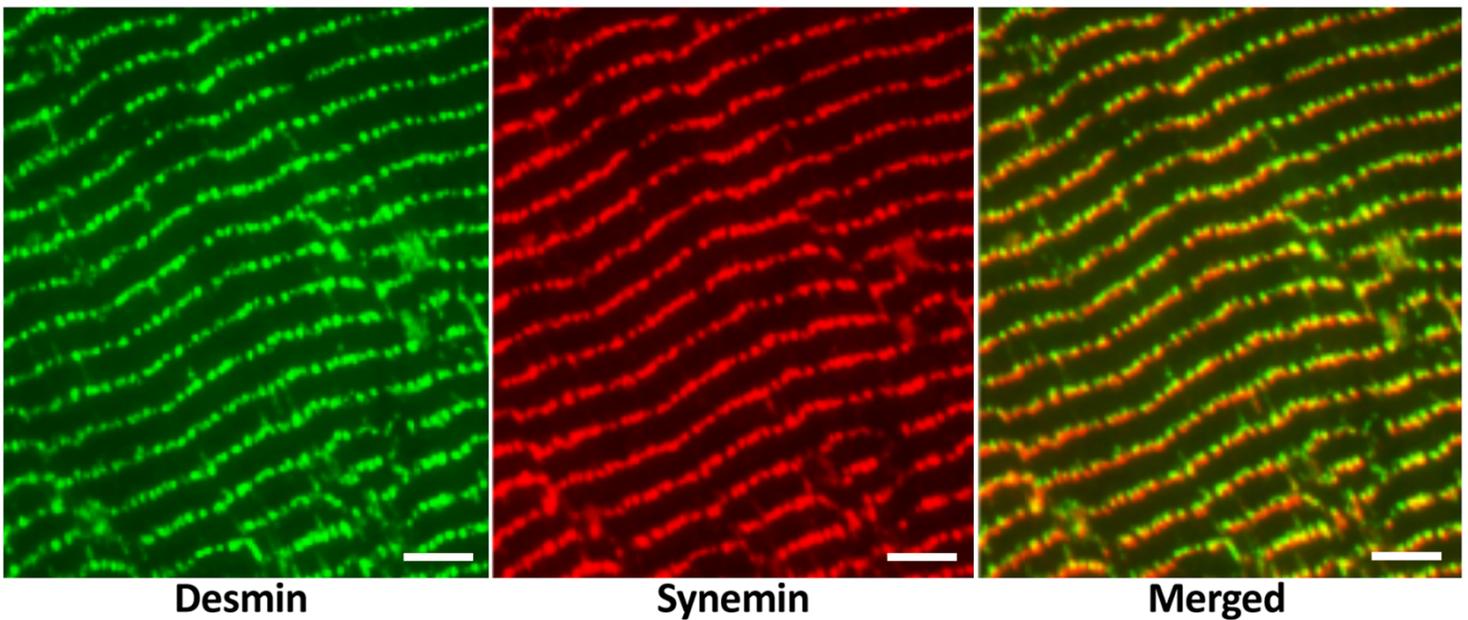
1	2	3	4	5	6	7	8	9	10	11
Genomic Coordinates	Codon Change	Base Variant	Amino acid change	Effect	Phenotype (Associated Disease)	References	SIFT prediction	PolyPhen 2 prediction	Mutation taster prediction	dbSNP accession nr.
99645578	CAG G[C/T]C CGC	173C>T	A58V	C	Ulnar-Mammary-Like syndrome, Cardiomyopathy	(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	rs5156187344
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]TTGG	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	-

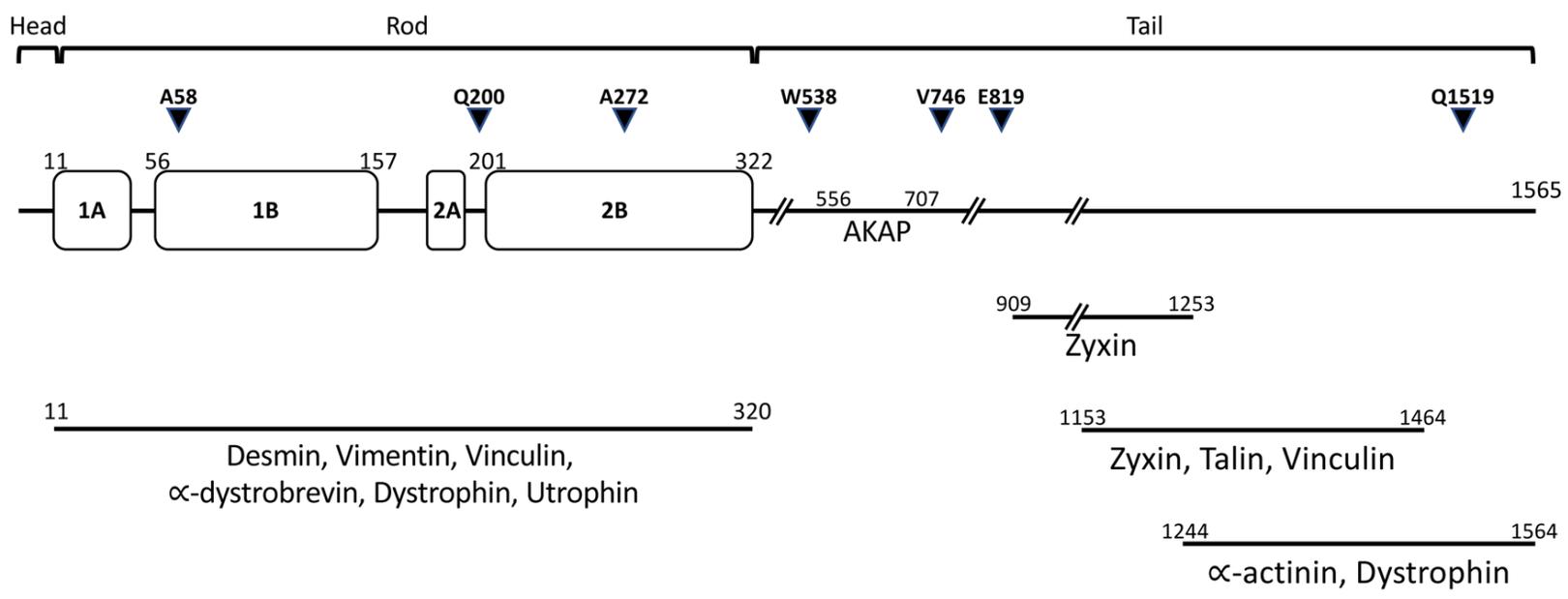
Columns 1, 2, 11, data from NCBI; <https://www.ncbi.nlm.nih.gov/projects/sviewer>. Column 1 indicates genomic coordinates; column 2 indicates codon change; and column 11 indicates db SNP (single nucleotide polymorphism) accession numbers of selected 24 *SYNM* variants. Column 3 indicates base variant; 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  $\alpha$  isoform (Synemin H) and AJ310521 for the  $\beta$  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  $\alpha$ /H) / NP\_056101 (isoform  $\beta$ /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.

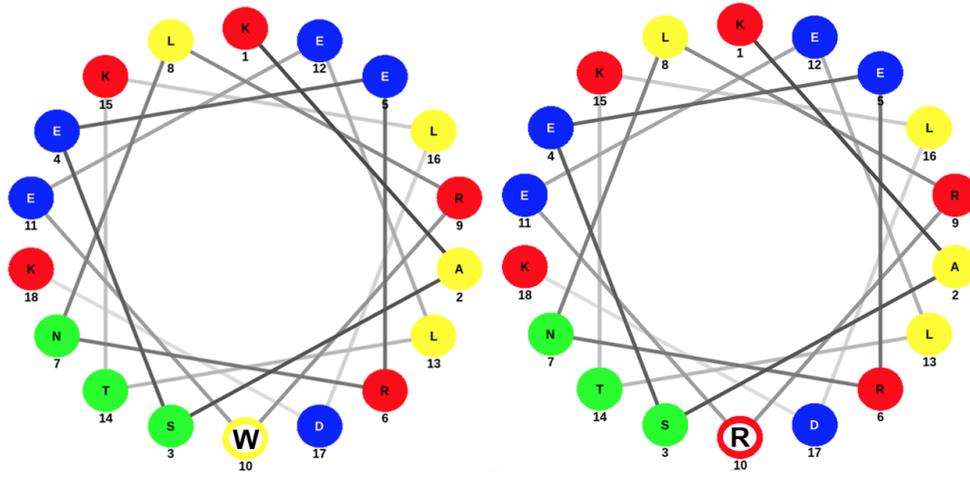
**Table 2. Synemin variants from patients with skeletal myopathy or cardiomyopathy.**

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> RCAEEAR REGLWAEGQ <u>V</u> RCAEEAR	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> W EELTKLDK KASEERNL <u>R</u> E EELTKLDK	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).

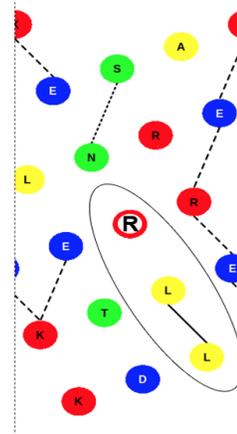
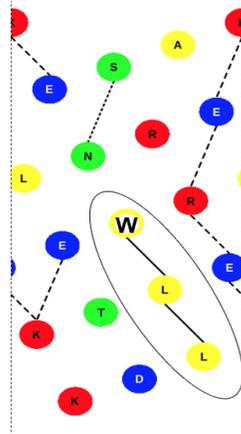
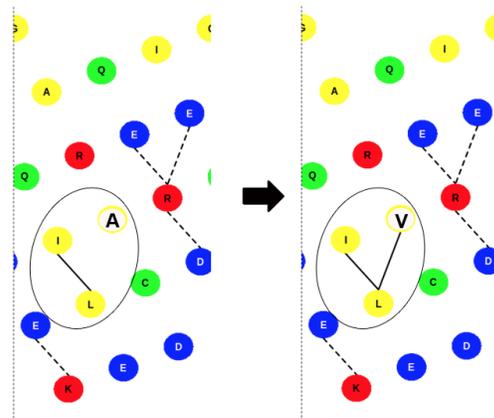
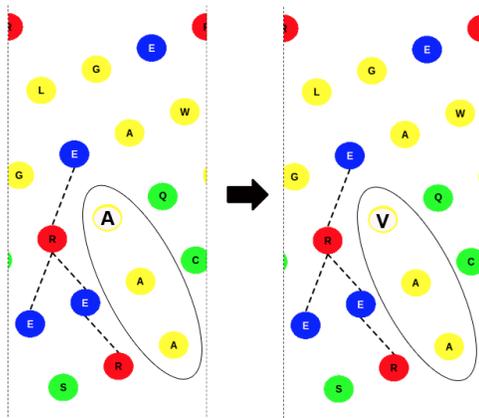
**A****Synemin Gene (*SYNM*)****B****Synemin Protein (*SYNM*)****C**



**A****missense mutation W538R**

KASEERNLR**W**EELTKLDK  
WT

KASEERNLR**R**EELTKLDK  
mutant

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