



Double the trouble: giant proteins with dual kinase activity in the heart

Alyssa Grogan¹ · Panagiotis Tsakiroglou¹ · Aikaterini Kontrogianni-Konstantopoulos¹

Received: 16 June 2020 / Accepted: 2 July 2020 / Published online: 8 July 2020

© International Union for Pure and Applied Biophysics (IUPAB) and Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Obscurin and its homolog, striated muscle preferentially expressed gene (SPEG), constitute a unique group of proteins abundantly expressed in striated muscles that contain two tandemly arranged MLCK-like kinases. The physiological significance of the dual kinase motifs is largely understudied; however, a collection of recent studies characterizing their binding interactions, putative targets, and disease-linked mutations have begun to shed light on their potential roles in muscle pathophysiology. Specifically, obscurin kinase 1 is proposed to regulate cardiomyocyte adhesion via phosphorylating N-cadherin, whereas SPEG kinases 1 and 2 regulate Ca^{2+} cycling by phosphorylating junctophilin-2 and the sarcoendoplasmic Ca^{2+} ATPase 2 (SERCA2). Herein, we review what is currently known regarding the potential substrates, physiological roles, and disease associations of obscurin and SPEG tandem kinase domains and provide future directions that have yet to be investigated.

Keywords Obscurin · SPEG · UNC-89 · Myosin light chain kinase

Introduction

Myosin light chain kinases (MLCKs) comprise a multifaceted family of Ca^{2+} /calmodulin-dependent serine/threonine (Ser/Thr) kinases that play essential roles in a variety of cell types (Khapchaev and Shirinsky 2016). Ubiquitously expressed MLCKs participate in a number of important cellular processes such as cell adhesion and motility, proliferation, endo/exocytosis, and cytoskeletal stiffness by phosphorylating the regulatory light chain of myosin (Khapchaev and Shirinsky 2016). In a striated and smooth muscle, MLCKs primarily serve to regulate muscle contractility and the actomyosin cytoskeleton in response to cytosolic changes in Ca^{2+} (Kamm and Stull 2001).

Additional protein families that are expressed abundantly in muscle, such as titins, obscurins, and striated muscle preferentially expressed gene (SPEG), contain kinase domains that share significant homology to canonical MLCKs (Sutter et al. 2004; Gautel 2011; Russell et al. 2002). Obscurins, along with their invertebrate ortholog UNC-89 and vertebrate

paralog SPEG, represent a unique subfamily of MLCK-like protein kinases that contain two tandem Ser/Thr kinase motifs (Sutter et al. 2004; Russell et al. 2002). As giant modular proteins that interact with a diverse set of proteins, obscurins, UNC-89, and SPEG serve established roles in myofibril assembly, the integration of the sarcomere with the surrounding subcellular and membrane structures, and Ca^{2+} cycling (Wang et al. 2018b; Quan et al. 2019). Importantly though, the regulation, enzymatic activities, and substrate specificities of their dual kinase domains have remained highly enigmatic.

Herein, we present an overview of what is known regarding the phylogenetic association, catalytic regulation, potential substrates and/or binding partners, and disease implications of the tandem kinase domains of obscurin/UNC-89 and SPEG in the heart. Given that these proteins are preferentially expressed in striated muscles, it has been postulated that the presence of the dual kinase motifs could confer a specialized purpose within muscle pathophysiology (Sutter et al. 2004).

Obscurin

Domain architecture and cellular distribution

The *OBSCN* gene, spanning more than 170 kb on human chromosome 1q42.13, encodes 117 exons that are

✉ Aikaterini Kontrogianni-Konstantopoulos
akontrogianni@som.umaryland.edu

¹ Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, 108 N. Greene St, Baltimore, MD 21201, USA

alternatively spliced to give rise to the multitude of isoforms comprising the obscurin protein family, ranging in size between 50 and 870 kDa (Russell et al. 2002; Young et al. 2001; Fukuzawa et al. 2005; Ackermann et al. 2014; Bang et al. 2001). The prototypical obscurin isoform, obscurin A (~ 720 kDa), contains 65 immunoglobulin (Ig) and 2 fibronectin-III (FN-III) domains along with an array of COOH-terminal signaling domains, including a calmodulin-binding IQ motif, a Src homology-3 (SH3) domain, a Rho guanine nucleotide exchange factor (RhoGEF) domain, a pleckstrin homology (PH) domain, and a non-modular COOH-terminus that contains binding sites for ankyrins (Fukuzawa et al. 2005; Kontrogianni-Konstantopoulos et al. 2009). The largest obscurin isoform, obscurin B (~ 870 kDa), bears the same modular architecture as obscurin A but diverges COOH-terminal to Ig67, where it instead contains two Ser/Thr kinase domains referred to as kinase 1 and kinase 2 (Kontrogianni-Konstantopoulos et al. 2009; Fukuzawa et al. 2005) (Fig. 1). Kinase 1 is preceded by an additional Ig domain whereas kinase 2 is preceded by both an Ig and a FN-III domain, which is consistent with the domain arrangement of the tandem kinases present in the invertebrate obscurin ortholog UNC-89 and its closely related paralog SPEG (Fukuzawa et al. 2005; Sutter et al. 2004) (Fig. 1). Given this highly conserved organization and sequence similarity, it has been postulated that there could be significant overlap in the protein interactions, substrate specificity, targeting and functions of these three tandem kinase proteins (Sutter et al. 2004).

OBSCN also gives rise to two small obscurin kinase isoforms, termed double (~ 145 kDa) and single (~ 55 kDa) kinases (Russell et al. 2002; Borisov et al. 2008; Hu and Kontrogianni-Konstantopoulos 2013). The double kinase isoform contains partial kinase 1 and the entirety of kinase 2, whereas the single kinase isoform contains only kinase 2 and the Ig/FN-III module that precedes it (Russell et al. 2002; Borisov et al. 2008; Hu and Kontrogianni-Konstantopoulos 2013) (Fig. 1). Northern blotting and PCR experiments have indicated that the transcripts of both the double and single kinase obscurins are abundantly present in the heart; however, these variants have remained largely uncharacterized (Russell et al. 2002).

Initial studies demonstrated that giant obscurins localize to both the M-bands and Z-disks of sarcomeres, with predominant localization to the M-bands (Bang et al. 2001; Young et al. 2001; Kontrogianni-Konstantopoulos et al. 2003). Unlike other giant sarcomeric proteins which are integrated longitudinally within the myofibrillar cytoskeleton, giant obscurins wrap around the periphery of myofibrils where they link proteins of the sarcomere with the surrounding subcellular and membrane structures (Kontrogianni-Konstantopoulos et al. 2003; Wang et al. 2018b; Kontrogianni-Konstantopoulos et al. 2009). Previous work from our group has delineated the precise distribution of kinase-bearing

obscurin isoforms in the heart utilizing antibodies targeting different epitopes within kinase 2 or its preceding Ig/FN-III domains (Hu and Kontrogianni-Konstantopoulos 2013). Specifically, immunostaining of mouse myocardial sections and adult rat cardiomyocytes indicated that obscurin kinases localize at M-bands and Z-disks as well as the sarcolemma, the intercalated disc, and the nucleus (Hu and Kontrogianni-Konstantopoulos 2013). Whether the different obscurin kinase isoforms preferentially distribute to select subcellular compartments is not yet fully known due to the lack of unique epitopes among them; however, a ~ 70 kDa isoform proposed to correspond to the single kinase was shown to localize extracellularly where it possibly undergoes glycosylation and interacts with the β_1 -subunit of the N^+/K^+ ATPase (NKA β_1) (Hu and Kontrogianni-Konstantopoulos 2013).

Obscurin kinase domains phylogeny

Obscurin kinases 1 and 2 are members of the MLCK family (Russell et al. 2002; Sutter et al. 2004) (Fig. 2). Detailed phylogenetic analysis has established their close relation to UNC-89 and SPEG, which also contain dual MLCK-like kinase activity (Sutter et al. 2004) (Fig. 3), as well as other proteins which contain single MLCK-like domains, such as titin and its invertebrate orthologs and the death-associated protein kinases (DAPK) trio and duet (Russell et al. 2002; Sutter et al. 2004; Gautel 2011). Canonically, MLCK-like kinases are regulated by Ca^{2+} /calmodulin (CaM) where binding of CaM to the autoinhibitory region of the regulatory domain confers activation (Kamm and Stull 2001; Stull et al. 1998). It has not yet been experimentally determined whether obscurin kinases 1 and 2 are regulated via CaM; however, the presence of a putative CaM-binding regulatory motif immediately downstream of the catalytic portion of kinase 1 highly supports this possibility (Fukuzawa et al. 2005; Hu and Kontrogianni-Konstantopoulos 2013). Interestingly though, CaM-dependent activation is not an entirely conserved feature among all MLCK-like family members. Titin kinase, for instance, sharing ~ 28 and ~ 27% homology to kinases 1 and 2 respectively (Fukuzawa et al. 2005), undergoes an alternative activation mechanism mediated by mechanical unfolding of the autoinhibitory region in response to stretch and subsequent phosphorylation of an inhibitory tyrosine residue (Tyr170) which exposes the catalytic aspartate (Gautel 2011; Grater et al. 2005; Mayans et al. 1998; Puchner et al. 2008). Intriguingly, both titin kinase and obscurin kinase 2 share a unique arginine residue in proximity to the catalytic aspartate (Fig. 2) that participates in the autoinhibitory mechanism mediated by Tyr170 in titin kinase (Fukuzawa et al. 2005). Therefore, it is possible that obscurin kinases 1 and 2 are regulated distinctly, and thus possess different physiological functions. In support of this notion, obscurin kinases 1 and 2

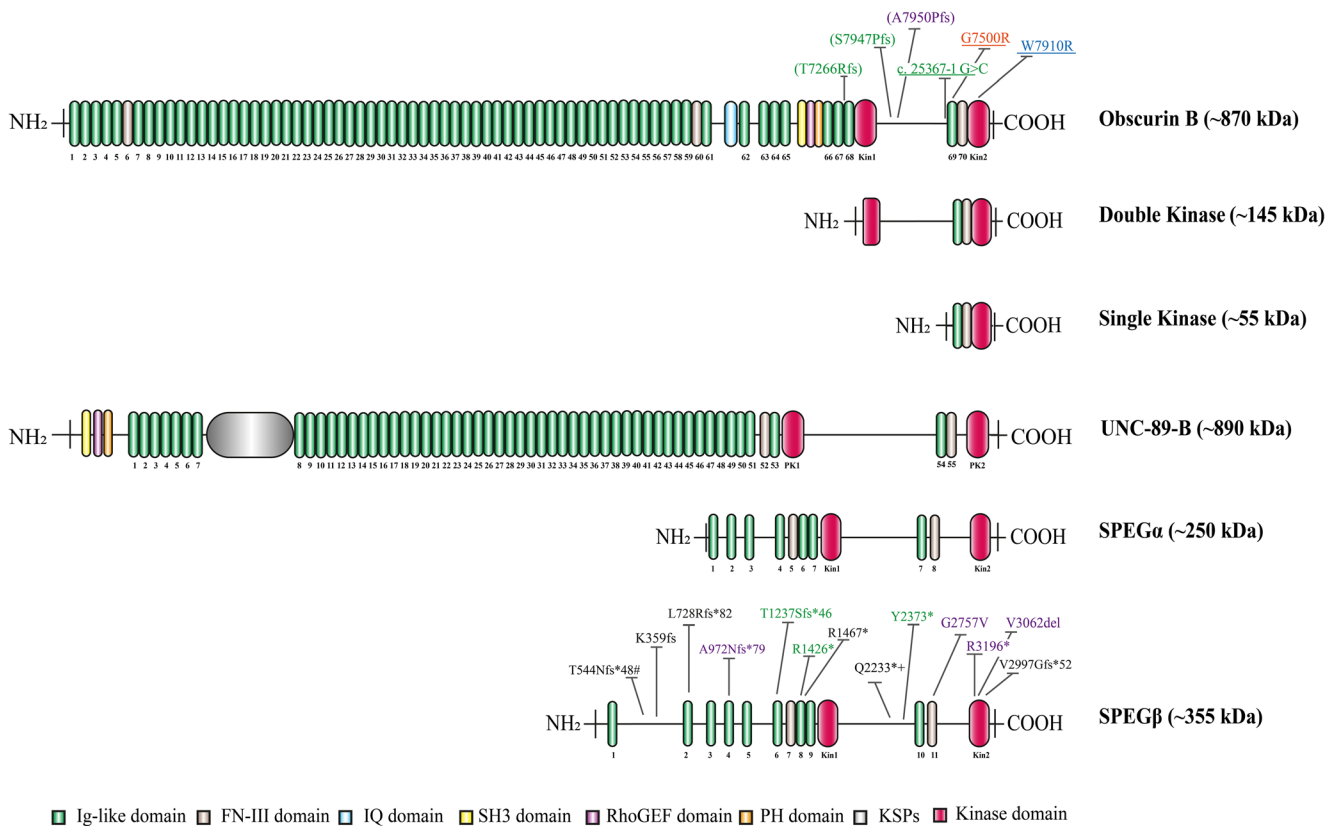


Fig. 1 Schematic representation of the modular domain architecture of giant obscurin B, obscurin double kinase, obscurin single kinase, UNC-89-B, SPEG α , and SPEG β . The currently known pathogenic mutations localized in the vicinity of the kinase domains are also shown; mutations present in obscurin are denoted according to accession numbers NP_001092093 (underlined) and NP_001258152 (in parenthesis), while mutations in SPEG are denoted according to accession numbers NM_005876.4 (marked with +) or NM_005876 (marked with #). Mutations are depicted in different colors to indicated their association with different forms of cardiomyopathy, as follows: LVNC-associated mutations in green; DCM-associated mutations in purple; HCM-associated mutations in orange; and systolic heart failure with hereditary ataxia-associated mutations in blue; while mutations not associated with cardiomyopathy are shown in black

are only 30% identical to each other (Fukuzawa et al. 2005) (Fig. 2).

are only 30% identical to each other (Fukuzawa et al. 2005) (Fig. 2).

Putative substrates of obscurin kinases

Recent findings from our group have demonstrated that both obscurin kinases 1 and 2 are enzymatically active and undergo autophosphorylation *in vitro* (Hu and Kontrogianni-Konstantopoulos 2013). Yeast two-hybrid screening experiments utilizing kinases 1 or 2 as bait identified several putative binding partners and/or substrates from an adult human cardiac muscle cDNA library. In particular, N-cadherin, galectin, the immunoglobulin superfamily with leucine repeats protein, and titin were identified as potential ligands of kinase 1, whereas NKA β_1 , galectin, fibronectin-1, γ -filamin, and four and a half LIM domain-2 protein (FHL2) were identified as potential ligands of kinase 2 (Hu and Kontrogianni-Konstantopoulos 2013). With the exception of N-cadherin and NKA β_1 (described in more detail below), these potential binding interactions have not been further confirmed or characterized. Given the identification of major components of the

cytoskeleton (i.e., titin, γ -filamin, and FHL2) or extracellular matrix (i.e., fibronectin-1 and galectin) as potential ligands and/or substrates, it is tempting to speculate that the obscurin kinases may be essential in regulating cytoskeletal stability and/or remodeling, mechanotransduction, or cell-cell or cell-extracellular matrix communication.

Deletion analysis indicated that both intracellular and extracellular portions of N-cadherin are required for binding to the catalytic region of kinase 1 (Hu and Kontrogianni-Konstantopoulos 2013). Moreover, proximity ligation assay (PLA) and immunofluorescent microscopy confirmed that kinase-bearing obscurins and N-cadherin localize in proximity at the intercalated disc and the sarcolemma. In line with their direct binding and co-localization, these studies further demonstrated that kinase 1 can specifically phosphorylate the cytoplasmic domain of N-cadherin *in vitro*. Importantly, N-cadherin is the only confirmed substrate of an obscurin kinase to date. Although the exact phosphorylation site and physiological impact of this modification has yet to be elucidated, this implicates obscurin as a mediator of cardiomyocyte adhesion via the kinase 1 activity.

sp MLCK	-----VCQHEVL-GGGRFGQVHRCTEKSTGL--	25
sp ObscurinKin1	-----YEVKEEI-GRGVFGVQRVQHKGNKI--	25
sp ObscurinKin2	-----FAFQTQI-QRGRFSVVRQCWEKASGR--	25
sp UNC89 PK1	SCTVTVEAEGDYNDELPRRRVTIESRRVRELYEISEKDEKLAAGAPFRVKEKATGR--	58
sp UNC89 PK2	-----FQIGGLK-FKGRFSVIRDAVDSTTEGHA	27
sp Unc89 PK1	-----YDIGDEL-GRGTQGITYHAVERSSSGD--	25
sp Unc89 PK2	-----YSFISEI-ARGEFSTIVKGIQKSTDT--	25
sp SPEGSK1	-----YDIHQEI-GRGAFSYLRRIVERSSGL--	25
sp SPEGSK2	-----YTFLEEK-ARGRFGVVRACRENATGR--	25
	
sp MLCK	PLAAKTIKVKSAKDREDVKNEINIMNQLSHVNLIQLYDAFESKH--SCTLVMEYVDG-GE	82
sp ObscurinKin1	LCAAKFIPL-RSRTRAQAYRERDILAALSHPLVTGLDQFETRK--TLILILELCCSS-EE	81
sp ObscurinKin2	ALAAKIIPY-HPKDKTAVLREYEALKGLRHPHLAQLHAAVLSR- -HLVLILELCSG-PE	81
sp UNC89 PK1	EFLAQLRPI-----DDALMRHVDIHNSLDHPGIVQMHRVLRDEKL--ALVVFDNANSTID	111
sp UNC89 PK2	HCAVKIRHP-----SSEATSEYSLRDGQHENVQRLIAAFNNSN--FLYLLSERLY--ED	78
sp Unc89 PK1	NYAAKIMYG-RPELRPFMLNELEMMNTFNHKNLRPYDAYDTR--SVTLIMELAAG-GE	81
sp Unc89 PK2	VVAKILEV-TDENEDNVVAEFDNFKLRRHERIPALFSAYKPLNVPIAIFVMEKLGQ-AD	83
sp SPEGSK1	EFAAKFIPS-QAKPKASARREARLLARLQHDCVLYFHEAFERRR--GLVIVTELCT--EE	80
sp SPEGSK2	TFVAKIVPY-AAEGKRRVLQYEYVRLTLLHHERIMSLHEAYITPR--YLVLIAESCGN-RE	81
	. : : . * : . : : :	
sp MLCK	-----LFDRLTDEKYHLTELDVLFTRQICEGVHYLHQHYLHLDLKPENILCVNQT-	134
sp ObscurinKin1	-----LLDRL-YRKGVVTEAEVKVYIQQLVEGLHYLHSHGVLHLDLTKPSNILMVHPA-	132
sp ObscurinKin2	-----LLPCL-AERASYSSEVKDYLLWQMSATQYLHNQILHLDLRSNMIITEYN-	132
sp UNC89 PK1	GLSSLAHPGVEIAEPKGVNRETCVRFVVRQLLLALKHMHDLRIAHLDRPETILLQDD--	169
sp UNC89 PK2	-----VFSRF-VFNDYYTEEQVALTMRQVTSALHFLHFKGIAHLDMVPHNIMFQSKR-	129
sp Unc89 PK1	-----LVRDNLRRDYTERDIAHYIRQTLWGLEHMHMGVGHMGLTIKDLLISVVG-	133
sp Unc89 PK2	-----VLTYF-SSRHEYSQMVATVVTQLLDALQYLHWRGYCHLNIQPDNVVMASVR-	134
sp SPEGSK1	-----LLERI-ARKPTVCESEIRAYMRQVLEGIHYLHQSHVHLHLDVKNPILLVWDGAA	132
sp SPEGSK2	-----LLCGL-SDRFRYSDDVATVMVQLLQGLDYLHGHHVHLHLDIKPDNLLLAPDN-	132
	. * : * . . . * * : : :	
sp MLCK	-GHQIKIIDFGLARRYKPREKL---KVNFGTPEFLAPEVVNVEFVSPFTDMWSVGVITYM	190
sp ObscurinKin1	-REDIKICDFGFAQNIPTAELQ---FSQYGSPEFVSPETIQNPVSEASDIWAMGVISYL	188
sp ObscurinKin2	---LLKVVDLGNASLSQEKVL-PSDKFKDYLETMAPELLLEGQAVPQTDIWAIGVTAFI	188
sp UNC89 PK1	---KLLADFGQARRLLRGLIT---GEIKGSPEFVSPETVRSYPLTLATDMWSTGVLTYY	223
sp UNC89 PK2	-SWVVKLVDFGRAQKVSGAVKPVDFDTKVASPEFHIFE---TPVTVQSDMWGMGVVTF	184
sp Unc89 PK1	-GDIKVSDFGLSRKINRHNS---TLDYGMPEFVSPVVKVGNVSHDMWTVGLITYV	189
sp Unc89 PK2	-SIQVKLVDFGSAKKVNLGMM---VTPCGSLDFQPPEMINDEPIFPQSDIWSLALTYL	190
sp SPEGSK1	GEQQVRIKDFGNAQELTPGEPQ---YCQYGTPEFVAPEIVNQSPVSGVTDIWPVGVVAFL	189
sp SPEGSK2	---ALKIVDFGSAQPYNPQALR-PLGHRGTLEFMAPEMVKGEPIGSATDIWAGVLTYYI	188
	: : * : * : : : * * * : * * : :	
sp MLCK	LLSGLSPFLGETDAETMNFIVNCSWDFDA-DTFEGLSEEAKDFVSRLLVKEKSCRMSATQ	249
sp ObscurinKin1	SLTCSSPFAGESDRATLLNVLEGRVSWSS-PMAAHLSEDAKDFIKATLQRPQARPSAAQ	247
sp ObscurinKin2	MLSAEYVPSSEGARDLQRGLRGLVRLS--RCYAGLSGGAVAFRLSTLCAQPWGRPCASS	246
sp UNC89 PK1	LLTGLSPFHGDNDNETLANVDSQCFDS---SPLGNFSYDAGDFVKLLTEIPVSRLTVDE	280
sp UNC89 PK2	LLAGFHPFTSEYDREEIKENVINVKCDPNLIPVNASQECLSFATWALKKSPVRRMTDE	244
sp Unc89 PK1	LLGGHNPFLGIDDRDLTKIREGRWDFKD-EIWITHISDDGRDFISRLLLYSPEERMDVKT	248
sp Unc89 PK2	LLSGCSPFRGADEYETKQNISFVRYRFE--NLFKEVTPEATRFIMLLFKRHPTKRPYTED	248
sp SPEGSK1	CLTGISPFVGENDRITLMNIRNYNVAFEE-TTFLSLSREARGFLIKVLV-QDRLRPTAEE	247
sp SPEGSK2	MLSGRSPFYEPDPQETEARIVGGRFADF--QLYPNTSQSATLFLRKVLSVHPWSRPSLQD	246
	* * . : * : *	
sp MLCK	CLKHEWL	256
sp ObscurinKin1	CLSHPW	254
sp ObscurinKin2	CLQCPWL	253
sp UNC89 PK1	ALDHPWI	287
sp UNC89 PK2	ALSHKFL	251
sp Unc89 PK1	ALKHPWF	255
sp Unc89 PK2	CLEHRWL	255
sp SPEGSK1	TLEHPWF	254
sp SPEGSK2	CLAHPWL	253
	* : :	

Fig. 2 Sequence alignment of the kinase domains of human cardiac MLCK (UniProt Q32MK0; aa 515-770), human obscurin kinase 1 (UniProt Q5VST9; aa 6468-6721) and kinase 2 (UniProt Q5VST9; aa 7672-7924), *C. elegans* UNC-89 PK1 (UniProt O01761; aa 6592-6878) and PK2 (O01761, aa 7285-8035), *Drosophila* Unc89 PK1 (UniProt A8DYP0; aa 3186-3440) and PK2 (UniProt A8DYP0; aa 3897-4151), and human SPEG α/β kinase 1 (UniProt Q15772; aa 1601-1854) and kinase 2 (UniProt Q15772; aa 2966-3218). Domains were aligned using the CLUSTALW software (<http://www.ebi.ac.uk/clustalw/>). Residues identical in all kinases are denoted with an asterisk (*), residues conserved in closely related kinases with similar properties are marked with colon (:), and residues shared by kinases with weakly similar properties are indicated with a dot (.), while gaps in residue alignment are represented by dashes (-). The highly conserved lysine (K) residue within the ATP-binding site is denoted with an arrow, while the catalytic aspartate (D) is marked with an arrowhead; the arginine (R) at position + 2 from the catalytic aspartate (D) present in obscurin kinase 2 (similar to titin-MLCK) is underlined

The binding interaction between obscurin kinase 2 and NKA β_1 generated novel insights on the possible roles of obscurin kinases in the heart, since the minimal interacting region that is necessary and sufficient to support binding to the catalytic subunit of kinase 2 resides within the extracellular domain of NKA β_1 (Hu and Kontrogianni-Konstantopoulos 2013). Prior to these findings, obscurins had not been shown to localize extracellularly. Experiments utilizing nonpermeabilized cardiomyocytes identified a small (~ 70 kDa) obscurin kinase variant proposed to encode the single obscurin kinase isoform that localizes extracellularly where it possibly undergoes glycosylation (Hu and Kontrogianni-Konstantopoulos 2013). Consistent with a possible interaction between obscurin kinase 2 and NKA β_1 , obscurin kinase-bearing proteins and NKA β_1

localize in proximity at the sarcolemma and the intercalated disc in cardiomyocytes. However, unlike the phosphorylation event mediated by kinase 1 on N-cadherin, in vitro kinase assays could not confirm NKA β_1 as a substrate of kinase 2 (Hu and Kontrogianni-Konstantopoulos 2013). It remains to be clarified whether this lack of phosphorylation on NKA β_1 is due to limitations of the in vitro expression system used, or if NKA β_1 is not actually a substrate (but possibly a binding partner) of obscurin kinase 2.

Disease implications of obscurin kinases

To date, approximately ten missense, one splicing, and seven frameshift mutations have been identified in *OBSCN* that are linked to various forms of cardiomyopathy in humans, including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and left ventricular non-compaction (LVNC) (Grogan and Kontrogianni-Konstantopoulos 2019). Importantly, six of these mutations localize at the COOH-terminus of obscurin B where they reside within close proximity to kinases 1 and/or 2 (Fig. 1). Their close association to one or both kinases raises the possibility for pathogenic mechanisms related to the altered targeting, regulation, or enzymatic activity of obscurin kinases.

In particular, whole exome sequencing analysis performed on a cohort of 74 Chinese patients in 2015 discovered six novel *OBSCN* variants linked to HCM (Xu et al. 2015). Of these six variants, one missense mutation, G7500R, was identified within Ig69 (Fig. 1). Given that Ig69 is a component of the Ig/FN-III module immediately preceding kinase 2, the presence of

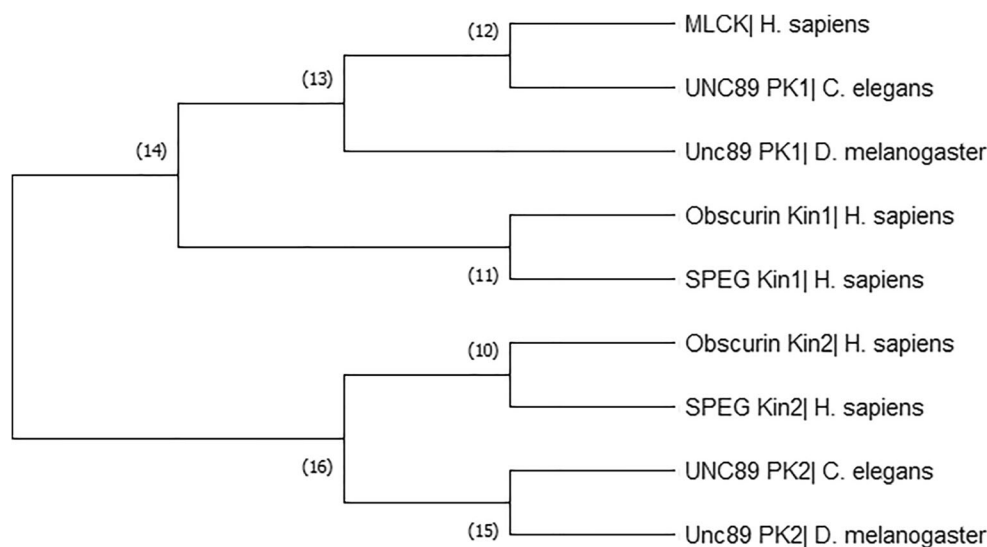


Fig. 3 Phylogenetic tree showing the evolutionary relationships between the kinase domains of human cardiac MLCK, human obscurin B, human SPEG α/β , *C. elegans* UNC-89, and *D. melanogaster* Unc-89. The phylogenetic tree was produced using the neighbor-joining algorithm. The evolutionary distances were computed using the Poisson correction method. Bootstrap values are shown on the branches, based on 2000

bootstrap replications. The calculated distance values are displayed in parenthesis. The optimal tree amino acids were aligned using MEGA X software, as in (Kumar et al. 2018). Note that the human obscurin kinases 1 and 2 domains are most closely related to the human SPEG α/β kinases 1 and 2 domains, respectively, consistent with the notion that *SPEG* arose from *OBSCN* via a gene duplication event

G7500R could potentially impact the targeting and/or substrate specificity of the dual kinase domains. In the following year, Rowland and colleagues identified four frameshift mutations in *OBSCN* from a cohort of 10 LVNC and 325 DCM patients that all localize to the COOH-terminal region of obscurin B (Rowland et al. 2016) (Fig. 1). Although these mutations remain uncharacterized, their positioning immediately upstream of one or both kinases could lead to the loss of kinases 1 and/or 2 expressions due to frameshifts. These findings were particularly intriguing since three of these mutations (T7266Rfs*53; S7947Pfs*82; 25367-1 G>C) were associated with LVNC while the fourth mutation (A7950Pfs*79) was identified in a patient with DCM. Given that LVNC is a relatively rare form of cardiomyopathy and only represented ~ 3% of the tested cohort, *OBSCN* mutations that cluster around dual kinase domains could be more prevalent in the development of LVNC compared to HCM or DCM. Lastly, a missense mutation W7910R localized to kinase 2 was recently identified in a patient with chronic systolic heart failure and hereditary ataxia (Catalano et al. 2018) (Fig. 1). This represents the only known mutation residing directly within an obscurin kinase domain, potentially affecting the binding affinity to its substrates and/or enzymatic activity. Given that biochemical and functional analyses of these disease-linked *OBSCN* mutations residing in the vicinity of the dual kinase domains are considerably lacking at this time, their pathophysiological impact and molecular manifestations remain speculative.

In addition to the known mutations, previous studies have also reported upregulation of obscurin kinase transcripts and/or protein in various animal models of cardiac disease. In particular, Borisov and colleagues reported up to an 18-fold increase in transcripts encoding kinase 1 approximately 48 h after the induction of myocardial hypertrophy via aortic constriction in mice (Borisov et al. 2003). Consistent with this, a mouse model carrying the HCM-linked R4344Q mutation in Ig58 exhibited an increase in the expression of giant obscurin B in response to chronic pressure overload (Hu et al. 2017). Lastly, recent work from our lab utilizing a constitutive deletion mouse model (*Obscn-ΔIg58/59*) expressing obscurin lacking the Ig58/59 module exhibited an upregulation of obscurin B prior to, and during, progressive ventricular remodeling from HCM to DCM through aging (Grogan et al. 2019). These studies collectively signify that the upregulation of obscurin kinases could play a role in the hypertrophic response, provide a form of molecular compensation during stress, or serve as a marker of early manifestation of cardiac disease. Therefore, elucidating the precise roles and signaling pathways regulated by obscurin kinases 1 and 2 in the heart is essential.

Invertebrate obscurin homolog UNC-89

As there is still a lot to learn regarding the substrates, signaling events, and physiological functions of mammalian obscurin

kinases 1 and 2, considering the regulation, established binding partners and purported roles of obscurin's closely related invertebrate ortholog, UNC-89, has been proven highly informative. Similar to obscurin, UNC-89 is a giant modular protein composed of tandem Ig and FN-III domains along with an NH₂-terminal signaling region consisting of an SH3 domain, a RhoGEF domain, a PH domain, and KSP motif (Benian et al. 1996). The largest isoforms, UNC-89-B and -F (750-900 kDa), contain two COOH-terminal Ser/Thr kinase domains termed PK1 and PK2 that are preceded by an Ig domain or Ig/FN-III module, respectively (Small et al. 2004; Ferrara et al. 2005) (Fig. 1). The *unc-89* gene also gives rise to two smaller kinase isoforms, UNC-89-C and -D (~ 156 kDa), that both encompass partial PK1 through PK2, but differ in their NH₂-termini where they contain a segment of 8 (UNC-89-C) or 11 (UNC-89-D) unique amino acids (Small et al. 2004).

The dual kinases of UNC-89 are also members of the MLCK protein family (Small et al. 2004) (Fig. 2). Based on homology modeling to other known kinases and members of the DAPK subfamily, PK2 is predicted to be catalytically active, whereas PK1 is considered to be enzymatically inactive due to the lack of several critical amino acids within the active site that are important for ATP or Mg²⁺ binding (Small et al. 2004). The potential activities of PK1 or 2 have not been experimentally proven yet, and there are no currently known substrates for either kinase, although Gieseler and colleagues mentioned (as unpublished data) that PK2 can phosphorylate several model peptide substrates, indicating that it is enzymatically active (Gieseler et al. 2017). Nevertheless, the identification of several proteins that bind to the kinase domains has provided insight on their potential cellular functions. In particular, a series of studies demonstrated that small C-terminal domain phosphatase-like 1 (SCPL-1), a novel protein phosphatase, and LIM-9, the nematode ortholog of FHL2, form a ternary complex with UNC-89 in *C. elegans*, where either one or both of the tandem kinases bind to SCPL-1 or LIM-9 directly (Xiong et al. 2009; Qadota et al. 2008). It was proposed that this complex could facilitate UNC-89 dimerization, or serve to link the M-band to the extracellular matrix via LIM-9's indirect association with integrins (Xiong et al. 2009). Given that FHL2 was also identified as a putative binding partner of obscurin kinase 2 (Hu and Kontrogianni-Konstantopoulos 2013), it is tempting to speculate that these complexes could also exist in vertebrates. Lastly, PK1 binds Ball, an active Ser/Thr kinase, and both PK1 and PK2 support binding to the multiple ankyrin repeats single KH domain protein (MASK) in *Drosophila* (Katzemich et al. 2015). Experiments utilizing siRNA-mediated knockdown of Ball or MASK in indirect flight muscles (IFM) resulted in major sarcomeric disorganization, suggesting a potential role for these proteins in sarcomeric stability. Given that UNC-89 was properly localized to the M-bands in Ball and MASK knockdown IFMs, whereas the reciprocal knockdown of

UNC-89 resulted in the improper localization of both Ball and Mask, it was proposed that UNC-89 kinases serve as signaling scaffolds for proteins involved in the assembly and maintenance of the sarcomere (Katzemich et al. 2015).

Importantly, the distance between the two kinase domains is highly conserved between vertebrate obscurin and nematode UNC-89 (Sutter et al. 2004). The interkinase region, consisting of a ~ 700 amino acid segment that lacks secondary structure, was recently demonstrated via single molecular force spectroscopy to exhibit properties of a random coil similar to the elastic spring elements found in titin (Qadota et al. 2020). Given that deletion of this region led to disorganized A-bands in *C. elegans*, it was proposed to serve important roles in sarcomeric assembly and stability of the thick filament (Qadota et al. 2020). It will therefore be important to also consider the potential roles of the interkinase region within vertebrate obscurins as well.

Striated Preferentially Expressed Gene

Domain architecture and cellular distribution

The *SPEG* gene encodes four distinct tissue-specific isoforms, APEG, BPEG, SPEG α and SPEG β , which are generated by alternative splicing and two separate transcription initiation sites (Hsieh et al. 1999; Hsieh et al. 2000). APEG and BPEG are preferentially expressed in aortic/vascular smooth muscle and brain respectively, whereas SPEG α and β are specific to striated muscles where they are co-expressed (Hsieh et al. 2000; Hsieh et al. 1999). SPEG α (~ 250 kDa) and β (~ 355 kDa) represent the largest of the isoforms and are unique among the other members of their protein family in containing two Ser/Thr kinase domains at their COOH-terminus (Hsieh et al. 2000) (Fig. 1). In addition to the kinase domains, referred to herein as kinases 1 (internal kinase) and 2 (COOH-terminal kinase), are a total of 9 Ig domains and 2 FN-III domains that make up the SPEG β isoform. SPEG α is identical to SPEG β , but is transcribed by a separate intronic promoter and lacks the first two NH₂-terminal Ig domains of SPEG β (Hsieh et al. 2000; Agrawal et al. 2014). Importantly, the tandem kinases of SPEG α/β contain the same arrangement of preceding Ig and Ig/FN-III modules as described above for obscurin and UNC-89, alluding to their high homology and potentially conserved functions (Sutter et al. 2004).

Early studies identified SPEG α/β at the Z-disks of sarcomeres in both the atria and ventricles of immunostained mouse hearts (Hsieh et al. 2000). Similar to obscurins, they were also revealed to exhibit a reticular distribution surrounding myofibrils and co-localizing with proteins of the sarcoplasmic reticulum (SR) (Agrawal et al. 2014). Moreover, a series of recent studies identified SPEG α/β at the cardiac diads as well, where they interact with junctophilin-2 (JPH2), the ryanodine receptor 2

(RyR2) (Quick et al. 2017), and the sarcoendoplasmic reticulum Ca²⁺ ATPase 2 (SERCA2) (Quan et al. 2019), suggesting their involvement in Ca²⁺ cycling and excitation-contraction coupling.

SPEG kinase domains phylogeny

Given the conserved exon structure between obscurin and SPEG tandem kinases, and the lack of an invertebrate SPEG ortholog, it is generally believed that *SPEG* arose from *OBSCN* by a gene duplication event (Sutter et al. 2004) (Fig. 3). The most striking difference between SPEG and obscurin, however, is that the exon immediately preceding SPEG kinase 2 contains an additional ~ 500 nucleotides, leading to increased separation between SPEG kinase 2 and the preceding Ig/FN-III module (Sutter et al. 2004). Although the distance between the NH₂-terminal kinase 1 and its preceding Ig domain remains conserved, this suggests potential divergence in the binding interaction profiles of obscurin and SPEG kinases (Sutter et al. 2004).

Similar to obscurin kinases 1 and 2, SPEG kinases also show significant homology to members of the MLCK family (Hsieh et al. 2000) (Fig. 2). In particular, SPEG kinase 1 shows ~ 37% sequence identity to other MLCK-like kinases, including smooth muscle MLCK, titin and its invertebrate orthologs, and DAPKs (Hsieh et al. 2000). It also contains 14 of the 15 canonically conserved amino acid residues present in most protein kinases, providing evidence that it is catalytically active (Hsieh et al. 2000). Accordingly, in vitro kinase assays demonstrated that SPEG kinase 1 is an active kinase that undergoes autophosphorylation (Hsieh et al. 2000). In contrast, SPEG kinase 2 only shares ~ 24% sequence identity with the other MLCK-like proteins due to divergence within its ATP-binding region (Hsieh et al. 2000) (Fig. 3). Hsieh and colleagues therefore initially predicted that SPEG kinase 2 would be catalytically inactive (Hsieh et al. 2000); though recent evidence that SERCA2a is a substrate of SPEG kinase 2 (described in detail below) has suggested otherwise (Quan et al. 2019).

Putative substrates of SPEG kinases

Unlike obscurin and UNC-89, both kinase domains of SPEG have confirmed substrates where the physiological functions of these phosphorylation events have been characterized in relative detail. In an attempt to identify novel proteins of the junctional membrane complex (JMC), Quick and colleagues performed immunoprecipitation experiments followed by proteomics analysis and identified SPEG as a binding partner of both RyR2 and JPH2 in the heart (Quick et al. 2017). Although the minimal interacting domains of these protein complexes were not determined, these findings suggested that RyR2 and/or JPH2 could potentially serve as substrates of SPEG kinases. Using an inducible, cardiac-specific SPEG

knockout mouse model, which develops ventricular dilation and contractile dysfunction leading to premature death, it was further demonstrated that SPEG deficiency is associated with enhanced SR Ca^{2+} leak by RyR2, and severely disrupted t-tubules consequent of reduced phosphorylation of JPH2. Importantly, t-tubule disarray preceded the onset of heart failure in SPEG-deficient mice, suggesting that the loss of phosphorylation of JPH2 was a direct effect of the loss of SPEG and causal in the development of disease (Quick et al. 2017). The exact phosphorylation site has not been confirmed yet; however, additional studies validated JPH2 as a direct substrate of SPEG kinase 1 in vitro (Quan et al. 2019).

In contrast to JPH2, the effect of SPEG knockout on RyR2 function is less clear at this time since the canonical RyR2 phosphorylation sites (Ser2808, Ser2814) were unaffected in SPEG-deficient mice (Quick et al. 2017). Moreover, unlike the impact on t-tubule organization, the observed alterations in diastolic Ca^{2+} handling did not occur prior to the onset of disease following SPEG knockout. It therefore remains unknown whether RyR2 function is directly regulated by SPEG or if these pathologies develop as a downstream consequence of cardiomyopathy.

Recent studies also employing immunoprecipitation and proteomics techniques identified SERCA2a as a binding partner of SPEG kinase 2 (Quan et al. 2019). In vitro kinase assays demonstrated that kinase 2, but not kinase 1, is capable of phosphorylating SERCA2a specifically on Thr484. This novel SERCA2a phosphorylation site was shown to increase SERCA2a oligomerization in vitro, which was associated with enhanced Ca^{2+} reuptake into the SR. Utilizing the inducible, cardiac-specific SPEG knockout mouse, Quan and colleagues revealed that loss of SPEG resulted in decreased SERCA2a phosphorylation on Thr484, providing evidence for this regulation in vivo. In addition, reduced Thr484 phosphorylation in SPEG-deficient mice preceded the development of ventricular dilation and dysfunction, suggesting that the loss of SERCA2a phosphorylation by SPEG contributes to disease development (Quan et al. 2019).

Collectively, these studies demonstrate that SPEG kinases serve important roles in JMC integrity, Ca^{2+} cycling, and excitation-contraction coupling through phosphorylation of major Ca^{2+} handling proteins.

Disease implications of SPEG kinases

Early studies interrogating the precise roles of SPEG revealed the presence of a severe cardiac phenotype in SPEG-deficient mice manifested as significant dilation of the ventricles and atria accompanied by decreased contractility and cardiac function occurring in the developing heart by 18.5 days post-coitum and leading to prominent neonatal mortality (Liu et al. 2009). Additional studies found that SPEG mRNA levels

are significantly reduced in mouse models of heart failure induced by isoproterenol or transaortic constriction (Quan et al. 2019). Lastly, an 83% reduction of SPEG transcripts were reported in human heart failure patients compared to non-failing hearts (Quick et al. 2017). Together, these findings implicate the loss of SPEG, and likely SPEG kinases, in the development of cardiomyopathy. Accordingly, a total of 13 mutations have been identified in *SPEG* to date that are all recessively inherited. Notably, seven of these mutations are found in patients with DCM or LVNC. Although only three of the currently known mutations localize directly within a kinase domain, most are predicted to cause significant truncations of SPEG where either one or both kinase domains are lost (Fig. 1).

Whole exome sequencing analysis on a cohort of 29 patients with centronuclear myopathy (CNM) identified three unrelated patients carrying mutations in *SPEG*, two of which also exhibited DCM (Agrawal et al. 2014). In particular, a total of five mutations that affected both SPEG α and β isoforms were found in either a homozygous or compound heterozygous state. The first patient carried a homozygous truncating mutation (Q2233*) that localized to the interkinase region. Given that this patient did not undergo a cardiac evaluation before dying at 3 weeks of age, it is not confirmed whether this mutation affected the heart. A second patient was compound heterozygous for a frameshift (T1237Sfs*46) and a nonsense (R1426*) mutation in Ig6 and Ig8 respectively. This patient developed a dilated left ventricle with severe cardiac dysfunction by 2 months of age. The third patient, carrying a frameshift mutation in Ig4 (A972Nfs*79) and a missense mutation in FN-III1 (G2757V), developed dilated cardiomyopathy by 1 month. Notably, skeletal muscle biopsies obtained from patients 2 and 3 demonstrated a significant reduction in both SPEG α and β , suggesting pathogenic mechanisms that are mediated by the loss of function of both isoforms (Agrawal et al. 2014).

Recently, two novel homozygous *SPEG* mutations were discovered in additional patients with CNM (Wang et al. 2017). The first patient, who did not develop cardiomyopathy, carried a frameshift mutation (T544Nfs*48) localized between Ig1 and Ig2 that introduced a premature stop codon. Importantly, this mutation is localized upstream of the SPEG α transcription initiation site and is predicted to only affect SPEG β , suggesting that a functional SPEG α isoform can compensate for the loss of SPEG β in the heart. The second CNM patient, who presented with ventricular dilation and reduced ejection fraction, carried a truncating mutation (R3196*) localized within kinase 2 that is predicted to cause partial loss of the kinase in both SPEG α and β . Similarly, another study by the same group reported the presence of a homozygous truncating mutation (Y2373*) that resulted in loss of the COOH-terminal Ig/FN-III and kinase 2 module in a CNM patient with LVNC (Wang et al. 2018a).

Lastly, two recent studies identified an additional five *SPEG* mutations that affect either one or both *SPEG* isoforms. Specifically, a truncating mutation localized between Ig1 and Ig2 (K359fs) and another localized to Ig8 (R1467*) were identified in a patient with congenital myopathy that did not show any apparent signs of cardiac disease (Lornage et al. 2018). Another patient presenting with CNM but no cardiac pathology carried two compound heterozygous truncating mutations localized to Ig2 (L728Rfs*82) and kinase 2 (V2997Gfs*52), respectively (Qualls et al. 2019). Given that *SPEG* α lacks Ig1 and Ig2, each of these patients only carries one (heterozygous) mutation affecting both isoforms, which further suggests that the presence of a single wild-type *SPEG* α allele can compensate for the presence of truncated *SPEG* β in the heart. Consistent with this assertion, another CNM patient that was homozygous for a truncating mutation (V3062del) localized within Kin2 and present in both *SPEG* α and β , developed DCM (Qualls et al. 2019).

Taken together, these findings suggest that mutations truncating both *SPEG* isoforms and therefore resulting in the loss of kinases 1 and 2 are associated with the development of DCM and LVNC.

What we know and future perspectives

There is much left to discover regarding the potential roles of obscurin/UNC-89 and *SPEG* tandem kinases in the heart. Nonetheless, the currently identified binding partners, putative substrates, and disease associations have implicated them in a number of essential physiological processes, from cell adhesion, cytoskeletal and myofibrillar stability, to mechanotransduction, Ca^{2+} signaling, and excitation-contraction coupling. Despite having highly conserved domain architectures, the presence of unique substrates that are located in distinct cellular compartments suggest that obscurin and *SPEG* kinases serve specialized purposes. Accordingly, although both proteins appear to overlap at the Z-disk, *SPEG*'s unique positioning at the JMC and the distribution of obscurins primarily to the M-band could reflect that these tandem kinase proteins serve different roles depending on their localization. In addition, the varying length of the COOH-terminal Ig/FN-III module with kinase 2 further supports the notion that obscurin and *SPEG* kinases could form interactions with different protein complexes (Sutter et al. 2004).

It remains to be clarified how exactly the kinase domains of obscurin and *SPEG* are regulated. Canonically, MLCK-like kinases are activated via CaM binding to the regulatory domain which functions to relieve autoinhibition. However, other proteins containing MLCK-like domains that are regulated distinctly, such as titin kinase, signify that this regulatory mechanism is not entirely conserved. Whether or not obscurin

and *SPEG* kinases 1 and 2 are regulated by CaM or through an alternative mechanism represents an important question that remains to be experimentally tested. Moreover, the recent discovery that the interkinase region of UNC-89 is an elastic random coil (Qadota et al. 2020) points to another form of potential regulation of kinase activity. Given that this region shares many physical properties with the elastic domains of titin (Qadota et al. 2020), it is conceivable that the interkinase region could mediate mechanical signals to kinases 1 and/or 2.

Lastly, the exact physiological significance of having two closely associated kinase domains within the same protein molecule is largely unknown. One possibility is that kinases 1 and 2 regulate each other through direct phosphorylation events, or by indirect mechanisms such as modulating other kinases and phosphatases that act on the other. Alternatively, given their large size and distribution to multiple cellular compartments, the presence of tandem kinase domains could function to spatially integrate multiple signaling pathways together. Therefore, detailed investigations of their individual targets, physiological roles, and disease mechanisms are essential in order to shed light on these intriguing questions.

Funding information This work was supported by the National Institutes of Health (Training Program in Muscle Biology, T32 AR007592-17 to A.G. and P.T.) and the American Heart Association (Grant In Aid 16GRNT31290010 to A.K.K.).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Ackermann MA, Shriver M, Perry NA, Hu LY, Kontrogianni-Konstantopoulos A (2014) Obscurins: Goliaths and Davids take over non-muscle tissues. *PLoS One* 9(2):e88162. <https://doi.org/10.1371/journal.pone.0088162>
- Agrawal PB, Pierson CR, Joshi M, Liu X, Ravenscroft G, Moghadaszadeh B, Talabere T, Viola M, Swanson LC, Haliloglu G, Talim B, Yau KS, Allcock RJ, Laing NG, Perrella MA, Beggs AH (2014) *SPEG* interacts with myotubularin, and its deficiency causes centronuclear myopathy with dilated cardiomyopathy. *Am J Hum Genet* 95(2):218–226. <https://doi.org/10.1016/j.ajhg.2014.07.004>
- Bang ML, Centner T, Fornoff F, Geach AJ, Gotthardt M, McNabb M, Witt CC, Labeit D, Gregorio CC, Granzier H, Labeit S (2001) The complete gene sequence of titin, expression of an unusual approximately 700-kDa titin isoform, and its interaction with obscurin identify a novel Z-line to I-band linking system. *Circ Res* 89(11):1065–1072. <https://doi.org/10.1161/hh2301.100981>
- Benian GM, Tinley TL, Tang X, Borodovsky M (1996) The *Caenorhabditis elegans* gene unc-89, required for muscle M-line assembly, encodes a giant modular protein composed of Ig and signal transduction domains. *J Cell Biol* 132(5):835–848. <https://doi.org/10.1083/jcb.132.5.835>

- Borisov AB, Raeker MO, Kontrogianni-Konstantopoulos A, Yang K, Kumit DM, Bloch RJ, Russell MW (2003) Rapid response of cardiac obscurin gene cluster to aortic stenosis: differential activation of Rho-GEF and MLCK and involvement in hypertrophic growth. *Biochem Biophys Res Commun* 310(3):910–918. <https://doi.org/10.1016/j.bbrc.2003.09.035>
- Borisov AB, Raeker MO, Russell MW (2008) Developmental expression and differential cellular localization of obscurin and obscurin-associated kinase in cardiac muscle cells. *J Cell Biochem* 103(5):1621–1635. <https://doi.org/10.1002/jcb.21551>
- Catalano J, Paynton B, Kaniper S, Gerhard G, Alvarez R (2018) Identification of a novel obscurin protein variant in nonischemic cardiomyopathy. *J Am Coll Cardiol* 71(11)
- Ferrara TM, Flaherty DB, Benian GM (2005) Titin/connectin-related proteins in *C. elegans*: a review and new findings. *J Muscle Res Cell Motil* 26(6-8):435–447. <https://doi.org/10.1007/s10974-005-9027-4>
- Fukuzawa A, Idowu S, Gautel M (2005) Complete human gene structure of obscurin: implications for isoform generation by differential splicing. *J Muscle Res Cell Motil* 26(6-8):427–434. <https://doi.org/10.1007/s10974-005-9025-6>
- Gautel M (2011) Cytoskeletal protein kinases: titin and its relations in mechanosensing. *Pflugers Arch* 462(1):119–134. <https://doi.org/10.1007/s00424-011-0946-1>
- Gieseler K, Qadota H, Benian GM (2017) Development, structure, and maintenance of *C. elegans* body wall muscle. *WormBook* 2017:1–59. <https://doi.org/10.1895/wormbook.1.81.2>
- Grater F, Shen J, Jiang H, Gautel M, Grubmuller H (2005) Mechanically induced titin kinase activation studied by force-probe molecular dynamics simulations. *Biophys J* 88(2):790–804. <https://doi.org/10.1529/biophysj.104.052423>
- Grogan A, Kontrogianni-Konstantopoulos A (2019) Unraveling obscurins in heart disease. *Pflugers Arch* 471(5):735–743. <https://doi.org/10.1007/s00424-018-2191-3>
- Grogan A, Hu L-YR, Ward C, Kontrogianni-Konstantopou A (2019) Loss of binding between giant obscurin and titin results in cardiac maladaptation. *Biophys J* 116:115a
- Hsieh CM, Yet SF, Layne MD, Watanabe M, Hong AM, Perrella MA, Lee ME (1999) Genomic cloning and promoter analysis of aortic preferentially expressed gene-1. Identification of a vascular smooth muscle-specific promoter mediated by an E box motif. *J Biol Chem* 274(20):14344–14351. <https://doi.org/10.1074/jbc.274.20.14344>
- Hsieh CM, Fukumoto S, Layne MD, Maemura K, Charles H, Patel A, Perrella MA, Lee ME (2000) Striated muscle preferentially expressed genes alpha and beta are two serine/threonine protein kinases derived from the same gene as the aortic preferentially expressed gene-1. *J Biol Chem* 275(47):36966–36973. <https://doi.org/10.1074/jbc.M006028200>
- Hu LY, Kontrogianni-Konstantopoulos A (2013) The kinase domains of obscurin interact with intercellular adhesion proteins. *FASEB J* 27(5):2001–2012. <https://doi.org/10.1096/fj.12-221317>
- Hu LR, Ackermann MA, Hecker PA, Prosser BL, King B, O'Connell KA, Grogan A, Meyer LC, Berndsen CE, Wright NT, Jonathan Lederer W, Kontrogianni-Konstantopoulos A (2017) Deregulated Ca(2+) cycling underlies the development of arrhythmia and heart disease due to mutant obscurin. *Sci Adv* 3(6):e1603081. <https://doi.org/10.1126/sciadv.1603081>
- Kamm KE, Stull JT (2001) Dedicated myosin light chain kinases with diverse cellular functions. *J Biol Chem* 276(7):4527–4530. <https://doi.org/10.1074/jbc.R000028200>
- Katzemich A, West RJ, Fukuzawa A, Sweeney ST, Gautel M, Sparrow J, Bullard B (2015) Binding partners of the kinase domains in *Drosophila* obscurin and their effect on the structure of the flight muscle. *J Cell Sci* 128(18):3386–3397. <https://doi.org/10.1242/jcs.170639>
- Khapchaeve AY, Shirinsky VP (2016) Myosin light chain kinase MYLK1: anatomy, interactions, functions, and regulation. *Biochemistry (Mosc)* 81(13):1676–1697. <https://doi.org/10.1134/S000629791613006X>
- Kontrogianni-Konstantopoulos A, Jones EM, Van Rossum DB, Bloch RJ (2003) Obscurin is a ligand for small ankyrin 1 in skeletal muscle. *Mol Biol Cell* 14(3):1138–1148. <https://doi.org/10.1091/mbc.e02-07-0411>
- Kontrogianni-Konstantopoulos A, Ackermann MA, Bowman AL, Yap SV, Bloch RJ (2009) Muscle giants: molecular scaffolds in sarcomerogenesis. *Physiol Rev* 89(4):1217–1267. <https://doi.org/10.1152/physrev.00017.2009>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35(6):1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Liu X, Ramjiganesh T, Chen YH, Chung SW, Hall SR, Schissel SL, Padera RF Jr, Liao R, Ackerman KG, Kajstura J, Leri A, Anversa P, Yet SF, Layne MD, Perrella MA (2009) Disruption of striated preferentially expressed gene locus leads to dilated cardiomyopathy in mice. *Circulation* 119(2):261–268. <https://doi.org/10.1161/CIRCULATIONAHA.108.799536>
- Lornage X, Sabouraud P, Lannes B, Gaillard D, Schneider R, Deleuze JF, Boland A, Thompson J, Bohm J, Biancalana V, Laporte J (2018) Novel SPEG mutations in congenital myopathy without centralized nuclei. *J Neuromuscul Dis* 5(2):257–260. <https://doi.org/10.3233/JND-170265>
- Mayans O, van der Ven PF, Wilm M, Mues A, Young P, Furst DO, Wilmanns M, Gautel M (1998) Structural basis for activation of the titin kinase domain during myofibrillogenesis. *Nature* 395(6705):863–869. <https://doi.org/10.1038/27603>
- Puchner EM, Alexandrovich A, Kho AL, Hensen U, Schafer LV, Brandmeier B, Grater F, Grubmuller H, Gaub HE, Gautel M (2008) Mechanoenzymatics of titin kinase. *Proc Natl Acad Sci U S A* 105(36):13385–13390. <https://doi.org/10.1073/pnas.0805034105>
- Qadota H, McGaha LA, Mercer KB, Stark TJ, Ferrara TM, Benian GM (2008) A novel protein phosphatase is a binding partner for the protein kinase domains of UNC-89 (Obscurin) in *Caenorhabditis elegans*. *Mol Biol Cell* 19(6):2424–2432. <https://doi.org/10.1091/mbc.E08-01-0053>
- Qadota H, Moody JC, Lesanpezeski L, Moncrief T, Kitzler D, Bhat PD, Vanapalli SA, Oberhauser AF, Benian GM (2020) A region of UNC-89 (obscurin) lying between two protein kinase domains is a highly elastic spring required for proper sarcomere assembly. [bioRxiv:2020.04.03.023374](https://doi.org/10.1101/2020.04.03.023374). <https://doi.org/10.1101/2020.04.03.023374>
- Qualls AE, Donkervoort S, Herkert JC, D'Gama AM, Bharucha-Goebel D, Collins J, Chao KR, Foley AR, Schoots MH, Jongbloed JDH, Bonnemann CG, Agrawal PB (2019) Novel SPEG mutations in congenital myopathies: genotype-phenotype correlations. *Muscle Nerve* 59(3):357–362. <https://doi.org/10.1002/mus.26378>
- Quan C, Li M, Du Q, Chen Q, Wang H, Campbell D, Fang L, Xue B, MacKintosh C, Gao X, Ouyang K, Wang HY, Chen S (2019) SPEG controls calcium reuptake into the sarcoplasmic reticulum through regulating SERCA2a by its second kinase-domain. *Circ Res* 124(5):712–726. <https://doi.org/10.1161/CIRCRESAHA.118.313916>
- Quick AP, Wang Q, Philippen LE, Barreto-Torres G, Chiang DY, Beavers D, Wang G, Khalid M, Reynolds JO, Campbell HM, Showell J, McCauley MD, Scholten A, Wehrens XH (2017) SPEG (striated muscle preferentially expressed protein kinase) is essential for cardiac function by regulating junctional membrane complex activity. *Circ Res* 120(1):110–119. <https://doi.org/10.1161/CIRCRESAHA.116.309977>
- Rowland TJ, Graw SL, Sweet ME, Gigli M, Taylor MR, Mestroni L (2016) Obscurin variants in patients with left ventricular

- noncompaction. *J Am Coll Cardiol* 68(20):2237–2238. <https://doi.org/10.1016/j.jacc.2016.08.052>
- Russell MW, Raeker MO, Korytkowski KA, Sonneman KJ (2002) Identification, tissue expression and chromosomal localization of human Obscurin-MLCK, a member of the titin and Dbl families of myosin light chain kinases. *Gene* 282(1-2):237–246. [https://doi.org/10.1016/s0378-1119\(01\)00795-8](https://doi.org/10.1016/s0378-1119(01)00795-8)
- Small TM, Gernert KM, Flaherty DB, Mercer KB, Borodovsky M, Benian GM (2004) Three new isoforms of *Caenorhabditis elegans* UNC-89 containing MLCK-like protein kinase domains. *J Mol Biol* 342(1):91–108. <https://doi.org/10.1016/j.jmb.2004.07.006>
- Stull JT, Lin PJ, Krueger JK, Trewhella J, Zhi G (1998) Myosin light chain kinase: functional domains and structural motifs. *Acta Physiol Scand* 164(4):471–482. <https://doi.org/10.1111/j.1365-201x.1998.tb10699.x>
- Sutter SB, Raeker MO, Borisov AB, Russell MW (2004) Orthologous relationship of obscurin and Unc-89: phylogeny of a novel family of tandem myosin light chain kinases. *Dev Genes Evol* 214(7):352–359. <https://doi.org/10.1007/s00427-004-0413-5>
- Wang H, Castiglioni C, Kacar Bayram A, Fattori F, Pekuz S, Araneda D, Per H, Erazo R, Gumus H, Zorludemir S, Becker K, Ortega X, Bevilacqua JA, Bertini E, Cirak S (2017) Insights from genotype-phenotype correlations by novel SPEG mutations causing centronuclear myopathy. *Neuromuscul Disord* 27(9):836–842. <https://doi.org/10.1016/j.nmd.2017.05.014>
- Wang H, Schanzer A, Kampschulte B, Daimaguler HS, Logeswaran T, Schlierbach H, Petzinger J, Ehrhardt H, Hahn A, Cirak S (2018a) A novel SPEG mutation causes non-compaction cardiomyopathy and neuropathy in a floppy infant with centronuclear myopathy. *Acta Neuropathol Commun* 6(1):83. <https://doi.org/10.1186/s40478-018-0589-y>
- Wang L, Geist J, Grogan A, Hu LR, Kontrogianni-Konstantopoulos A (2018b) Thick filament protein network, functions, and disease association. *Compr Physiol* 8(2):631–709. <https://doi.org/10.1002/cphy.c170023>
- Xiong G, Qadota H, Mercer KB, McGaha LA, Oberhauser AF, Benian GM (2009) A LIM-9 (FHL)/SCPL-1 (SCP) complex interacts with the C-terminal protein kinase regions of UNC-89 (obscurin) in *Caenorhabditis elegans* muscle. *J Mol Biol* 386(4):976–988. <https://doi.org/10.1016/j.jmb.2009.01.016>
- Xu J, Li Z, Ren X, Dong M, Li J, Shi X, Zhang Y, Xie W, Sun Z, Liu X, Dai Q (2015) Investigation of pathogenic genes in Chinese sporadic hypertrophic cardiomyopathy patients by whole exome sequencing. *Sci Rep* 5:16609. <https://doi.org/10.1038/srep16609>
- Young P, Ehler E, Gautel M (2001) Obscurin, a giant sarcomeric Rho guanine nucleotide exchange factor protein involved in sarcomere assembly. *J Cell Biol* 154(1):123–136. <https://doi.org/10.1083/jcb.200102110>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.