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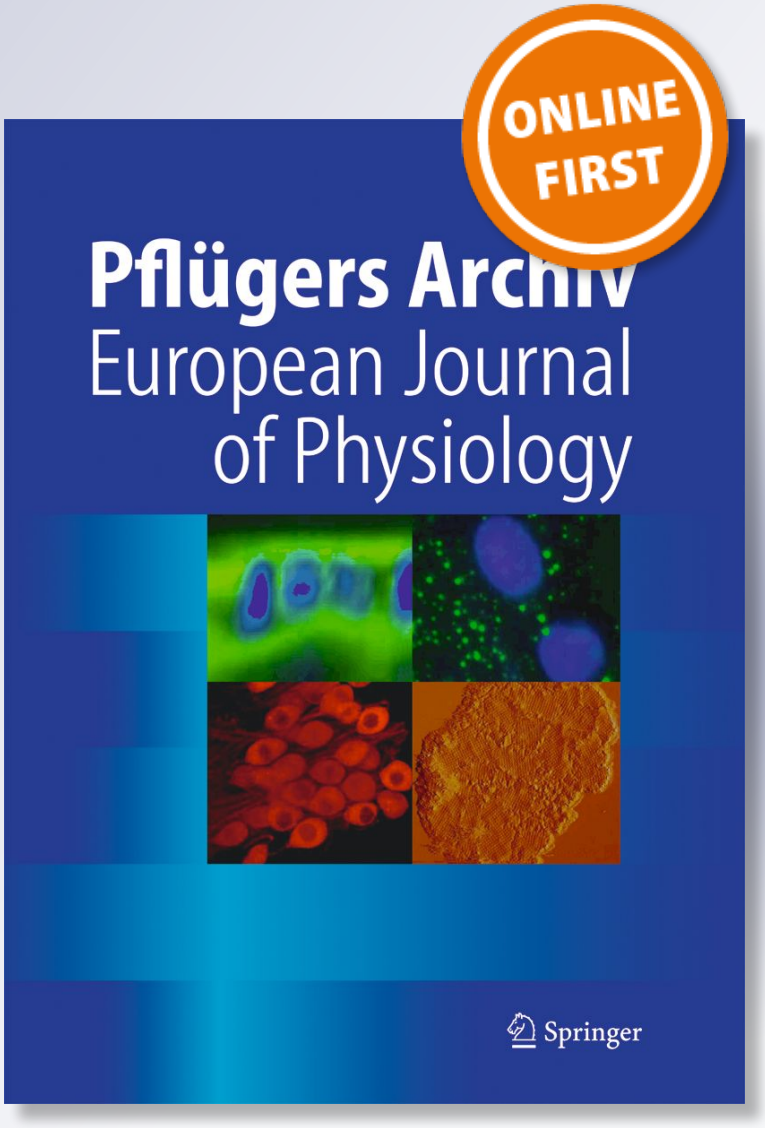

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INVITED REVIEW

Unraveling obscurins in heart disease

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Abstract

Obscurins, expressed from the single *OBSCN* gene, are a family of giant, modular, cytoskeletal proteins that play key structural and regulatory roles in striated muscles. They were first implicated in the development of heart disease in 2007 when two missense mutations were found in a patient diagnosed with hypertrophic cardiomyopathy (HCM). Since then, the discovery of over a dozen missense, frameshift, and splicing mutations that are linked to various forms of cardiomyopathy, including HCM, dilated cardiomyopathy (DCM), and left ventricular non-compaction (LVNC), has highlighted *OBSCN* as a potential disease-causing gene. At this time, the functional consequences of the identified mutations remain largely elusive, and much work has yet to be done to characterize the disease mechanisms of pathological *OBSCN* variants. Herein, we describe the *OBSCN* mutations known to date, discuss their potential impact on disease development, and provide future directions in order to better understand the involvement of obscurins in heart disease.

Keywords Obscurin · Sarcomeric mutations · Cardiomyopathy · Heart failure

Introduction

Cardiomyopathies comprise a heterogeneous group of myocardial disorders that are characterized by abnormal pumping of blood and/or the inability to maintain normal electrical rhythm [13]. They are routinely grouped into six major subtypes according to the clinical presentation of patients and the structural and functional maladaptations that manifest in the diseased myocardium [12, 16, 45, 75]. The most common subtype, hypertrophic cardiomyopathy (HCM), occurs with an incidence of 1 in 500 and is the leading cause of sudden cardiac death in young athletes. Dilated cardiomyopathy (DCM) affects 1 in 2500 individuals and is the third leading cause of heart failure in the USA after coronary artery disease and hypertension. The remaining subtypes are more rare forms of heart disease and include restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), left ventricular non-compaction (LVNC), and inflammatory cardiomyopathy (IC) [12, 16, 45, 75].

The causes of cardiomyopathy are variable and complex, often driven by environmental factors (e.g., industrialism, pollution), lifestyle choices (e.g., alcohol or drug abuse, obesity), other diseases (e.g., viral or bacterial infections, diabetes, thyroid, or autoimmune disease), and idiosyncratic factors (e.g., age, gender, and race), as well as genetic etiologies (e.g., inherited mutations and/or genetic predisposition). Over the last three decades, particular emphasis has been given on the familial transmission of mutations in genes encoding sarcomeric, Ca^{2+} sensitive and Ca^{2+} cycling, signaling, storage, and metabolic proteins highlighting the importance of genetics in heart disease [13, 18, 37, 48, 68]. As such, remarkable progress has been made towards the identification of genetically based cardiomyopathies that had been previously classified as idiopathic.

The discovery of hundreds of pathogenic mutations in genes encoding sarcomeric proteins has established "sarcomeric cardiomyopathies" as an entity. Depending on the severity of the mutation, sarcomeric cardiomyopathies can develop either early in life or later in adulthood. Early onset cases represent a common cause of pediatric cardiomyopathy often associated with sudden cardiac death, whereas adult onset cases develop between 20 and 50 years of age [71]. The clinical presentation of affected patients is highly diverse, exhibiting hypertrophic (the most commonly observed pathology), dilated, non-compaction, or restrictive cardiac phenotypes [13, 69, 71].

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OBSCN, encoding obscurins, has been recently added to the long list of affected sarcomeric genes due to the identification of missense, frameshift, and splicing mutations associated with different forms of cardiomyopathy [46, 74]. Obscurins comprise a multifaceted family of modular proteins that range in size between 50 and 870 kDa [3]. Although the molecular architecture of the giant isoforms has been well characterized, the structural composition of the intermediate and small obscurins is still unclear [3]. Nevertheless, giant obscurins, referred to as obscurin-A (~ 720 kDa) and obscurin-B (~ 870 kDa), consist of a series of immunoglobulin (Ig) and fibronectin-III (Fn-III) domains followed by a tandem array of signaling motifs, including two Ser/Thr kinase domains, referred to as Kin1 and Kin2, that play key structural and regulatory roles (Fig. 1; for a detailed description of the structure, localization, and proposed roles of obscurins, we refer the interested reader to the following recent reviews: [39, 43, 73]).

Herein, we present a synopsis of the currently known mutations identified in the *OBSCN* gene linked to heart disease, and how we envision the involvement of mutant or truncated obscurins in the development of cardiomyopathy given their roles as structural and signaling mediators in the sarcomeric cytoskeleton.

Obscurins and cardiomyopathy

Despite the major technological advancements in high-throughput DNA sequencing methodologies, the involvement of *OBSCN* in the development of sarcomeric cardiomyopathies has only begun to be interrogated. There are likely several reasons for this prior oversight in including *OBSCN* in large-scale genetic screens for familial heart disease. First, the relatively recent discovery of *OBSCN* in 2001 [78], especially compared to other heavily mutated sarcomeric genes such as *MYBPC3*, *TTN*, and *MYH7* that have been known and investigated for several decades, has obviously contributed to *OBSCN* being understudied [73]. Second, its large size (> 170 kb) and molecular complexity arising from the presence of at least two promoters, three possible translation initiation sites, and multiple transcript variants [3, 64] may have also deterred cardiovascular geneticists from examining whether mutations in *OBSCN* are causatively linked to familial cardiomyopathy. Third, although many reports over the past 15 years have demonstrated that obscurins may act as structural and signaling mediators in muscle cells [1, 2, 6, 11, 17, 28, 34–36, 38, 54, 56, 58, 59, 78], their exact roles in muscle pathophysiology are still unclear. Neglecting to include *OBSCN* in the genetic evaluation of heart failure patients was slowly reversed when obscurin transcript levels were found increased in a mouse HCM model [9] and a canine tachycardia-induced DCM model [76], and isoform switching was reported in the left ventricles of severely diseased DCM patients [41]. Following these early observations, a number of studies have identified the presence of mutations in *OBSCN* that are associated with different forms of cardiomyopathy.

HCM-linked OBSCN mutations

HCM is characterized by left ventricular hypertrophy, reduced chamber size, fibrosis, impaired relaxation, and increased susceptibility to arrhythmia [13, 18]. In the absence of pathologies such as hypertension or aortic stenosis that may underlie or contribute to disease development, HCM is considered a monogenic disorder caused by dominant mutations in genes encoding sarcomeric proteins [69, 71]. It is therefore regarded as the most common cardiac manifestation of sarcomeric cardiomyopathy.

The first indication that obscurins may be causatively involved in the development of familial sarcomeric cardiomyopathy came in 2007 with the discovery of two *OBSCN* mutations in a 19-year-old patient with HCM from Japan [5]. Genomic

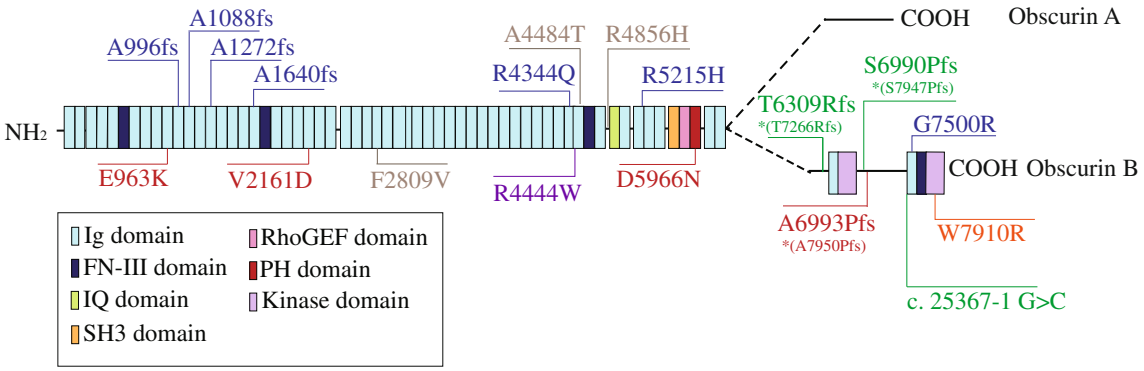

Fig. 1 Domain schematic of giant obscurins and localization of known *OBSCN* mutations linked to myopathy (based on accession numbers NP-001092093 or NP-001258152 when mutations are marked with an asterisk, *). To date, there are seven known mutations associated with HCM (shown in blue), four mutations associated with DCM (shown in red), three mutations associated with LVNC (shown in green), one mutation associated with a case of systolic heart failure with hereditary ataxia (shown in orange), and one mutation linked to distal myopathy (shown in purple). Three polymorphisms that have been classified as non-pathogenic are shown in gray

linkage analysis identified two heterozygous missense mutations, R4344Q (c.13031 G>A in exon 51) and A4484T (c.13450 G>A in exon 52) in obscurin Ig58 and Ig59 domains, respectively, that were most likely inherited from the mother of the proband who was also diagnosed with HCM (Fig. 1 and Table 1) [5]. Neither of these mutations was present in a cohort of 288 normal subjects of Japanese origin [5].

The Ig58/Ig59 module of obscurin, where the R4344Q and A4484T mutations reside, has been shown to support binding to the Ig9/Ig10 domains of titin [78]. Titin is a modular 3–4-MDa protein that extends longitudinally across a half-sarcomere, and functions as a molecular blueprint, regulator of passive stiffness, and mechanosensor [19, 21–23, 40]. In vitro studies indicated that the presence of R4344Q, but not A4484T, modestly diminishes binding of the obscurin Ig58/59 module to the titin Ig9/10 domains [5]. Furthermore, immunofluorescence experiments and modeling analyses indicated that R4344Q also affects the Z-disk incorporation of mutant Ig58/Ig59 and interferes with the structure of Ig58 [5]. Due to the lack of a functional effect observed from the presence of A4484T alone, Arimura and colleagues suggested that the A4484T variation might not be pathogenic. This is consistent with its presence in the genome of wild-type C57Bl6/J mice, further lending credence to the classification of the A4484T variation as a polymorphism rather than a driving mutation [27].

Further evaluation of the frequency of the R4344Q variant in larger and more diverse databases resulted in its reclassification from "hypertrophic cardiomyopathy-causing" to "hypertrophic cardiomyopathy-causing?" and later to "benign" due to its high prevalence (~ 15%) among African Americans (Human Gene Mutation Database 2017.4) [42]. Notably though, its prevalence remained relatively low among white Americans (~ 0.3%). Although minor allele frequency is a key determinant of classifying a variant as pathogenic or non-pathogenic, one could argue that given that the African American population exhibits considerably higher rates of heart disease compared to white Americans (44.4 versus 36.6% for males and 48.9 versus 32.4% for females, respectively) [20], the R4344Q variant may contribute or predispose carriers to

Table 1 *OBSCN* mutations linked to myopathy

OBSCN (NP-001092093)		*OBSCN* (NP-001258152)				
Mutation	Domain	Mutation	Domain	Disease	Genotype	Reference
Missense mutations						
E963K	Ig10	E1055K	Ig11	DCM	Het	Marston et al. 2015
V2161D	Ig23	V2536D	Ig27	DCM	Compound Het	Marston et al. 2015
F2809V	Ig29	F3238 V	Ig34	DCM	Compound Het	Marston et al. 2015
R4344Q	Ig47	R5304Q	Ig58	HCM	Compound Het	Arimura et al. 2007
R4444W	Ig48	R5401W	Ig59	Distal muscular dystrophy	Compound Het	Rossi et al. 2017
A4484T	Ig48	A5441T	Ig59	HCM	Compound Het	Arimura et al. 2007
R4856H	Between Ig50 and IQ	R5813H	Between Ig61 and Ig62	DCM	Het	Marston et al. 2015
R5215H	Ig52	R6172H	Ig63	HCM	Het	Xu et al. 2015
G7500R	Ig58	G8457R	Ig69	HCM	Het	Xu et al. 2015
D5966N	PH	D6923N	PH	DCM	Het	Marston et al. 2015
W7910R	Kin2	W8865R	Kin2	Systolic HF/ hereditary ataxia	Het	Catalano et al. 2018
Frameshift mutations						
A996fs	Ig11	A1088fs	Ig12	HCM	Het	Xu et al. 2015
A1088fs	Ig12	A1180fs	Ig13	HCM	Het	Xu et al. 2015
A1272fs	Ig14	A1364fs	Ig15	HCM	Het	Xu et al. 2015
A1640fs	Ig18	A2015fs	Ig22	HCM	Het	Xu et al. 2015
T6309Rfs*53	Between Ig56 and Ig57	T7266Rfs*53	Between Ig67 and Ig68	LVNC	Het	Rowland et al. 2015
S6990Pfs*82	Between Kin1 and Ig58	S7947Pfs*82	Between Kin1 and Ig69	LVNC	Het	Rowland et al. 2015
A6993Pfs*79	Between Kin1 and Ig58	A7950Pfs*79	Between Kin1 and Ig69	DCM	Het	Rowland et al. 2015
Splicing mutations						
c. 25367-1 G>C	Ig58	c. 25367-1 G>C	Ig58	LVNC	Het	Rowland et al. 2015

Ig immunoglobulin, *IQ* isoleucine/glutamine, *PH* pleckstrin homology, *Kin1* obscurin kinase 1, *Kin2* obscurin kinase 2, *HCM* hypertrophic cardiomyopathy, *DCM* dilated cardiomyopathy, *HF* heart failure, *LVNC* left ventricular non-compaction

cardiomyopathy along with other influences, such as idiosyncratic factors, lifestyle choices, and socio-economical factors.

Consistent with this notion, homozygous knock-in mice carrying the R4344Q mutation develop arrhythmia by 1 year of age under sedentary conditions, consisting of significant tachycardia and frequent episodes of premature ventricular contractions [27]. Isolated cardiomyocytes exhibit enhanced Ca^{2+} transients and accelerated contractility kinetics due to increased expression and activity of SERCA2. Detailed structural and biochemical work demonstrated that enhanced SERCA2 activity might result from sequestration of phospholamban, the major SERCA2 regulator in cardiac cells, via enhanced binding to mutant obscurin [27]. Of note, the expression and localization of titin were indistinguishable between wild-type and homozygous knock-in mice. Moreover, young adult homozygous knock-in mice subjected to sustained pathological mechanical stress via transaortic constriction develop a DCM-like phenotype characterized by left ventricular dilation and fibrosis [27]. Taken together, these findings indicate that the presence of the R4344Q mutation leads to pathogenicity and disease development by interfering with binding interactions of the Ig58 domain that contribute to the regulation of Ca^{2+} cycling.

Almost a decade after the identification of the R4344Q and A4484T variants, Xu and colleagues reported the presence of six novel, rare, pathogenic *OBSCN* variants linked to HCM [77]. These variants, identified by whole exome sequencing (WES) analysis in a cohort of 74 Chinese patients diagnosed with sporadic HCM, were absent from 2000 control subjects [77]. Remarkably, *OBSCN* was identified in the top 10 putative HCM-associated genes out of 92 candidate genes interrogated [77]. All six variants were present in a dominant fashion and included four frameshift and two missense mutations (Fig. 1 and Table 1). Notably, the four frameshift mutations, Ala996fs, Ala1088fs, Ala1272fs, Ala1640fs, cluster in the NH_2-terminal Ig12, Ig13, Ig15, and Ig22 domains that are shared by both obscurin-A and obscurin-B. These mutations likely result in truncated proteins and thus "loss of function." The two missense mutations that were identified, R5215H and G7500R, localize to the Ig63 and Ig69 domains, respectively. The Ig63 domain is present in both giant isoforms, whereas the Ig69 domain precedes the most COOH-terminal Ser/Thr kinase domain and is restricted to obscurin-B and the smaller obscurin-kinase isoforms. At this time, the molecular, biochemical, and functional implications of these mutations are largely unknown, which appears to be the case for almost all of the *OBSCN* mutations known to date (with the exception of R4344Q discussed above).

DCM-linked OBSCN mutations

Mutations in *OBSCN* have also been associated with the development of DCM. Left ventricular dilation accompanied by reduced wall thickness and systolic dysfunction is the hallmark of DCM [13, 18]. Approximately 40% of all DCM cases have been attributed to monogenic causes, involving ~50 genes mainly encoding sarcomeric proteins [26, 44]. Notably, truncating mutations in *TTN* account for ~25% of all inherited DCM cases identified to date [25, 61].

OBSCN was recently included in the list of sarcomeric genes linked to DCM when Marston and colleagues identified five novel heterozygous missense mutations, E963K, V2161D, F2809V, R4856H, and D5966N, via WES in 4 out of 30 patients diagnosed with end-stage heart failure and familial DCM (Fig. 1 and Table 1) [47]. Of these five mutations, V2161D and F2809V were found in a compound heterozygous state. Moreover, F2809V and R4856H were classified as non-pathogenic polymorphisms due to high prevalence in reference databases and lack of conservation among species, respectively [47]. The three potentially pathogenic mutations E963K, V2161D, and D5966N are spread throughout the length of giant obscurins in domains that are shared by both giant isoforms, localizing in Ig11, Ig27, and the PH domain, respectively. Interestingly, DCM biopsies carrying the three pathogenic mutations exhibited reduced expression levels of mutant obscurins compared to DCM samples without *OBSCN* mutations, HCM samples, and healthy controls, suggesting that these mutations may function via haploinsufficiency [47].

In addition to the DCM associated *OBSCN* mutations identified by Marston et al., Rowland and colleagues also reported a dominant frameshift mutation, A7950Pfs*ter79, residing between Kin1 and Ig69, which is restricted to kinase-bearing obscurins (Fig. 1 and Table 1) [63]. No biochemical work followed the identification of this mutation, and it is therefore unknown whether it may also act through haploinsufficiency.

LVNC-linked OBSCN mutations

In addition to HCM and DCM, mutations in *OBSCN* have also been linked to LVNC [63]. LVNC is a relatively rare form of cardiomyopathy (0.014–1.3% occurrence in the general population). Although it can be sporadic, 40–50% of cases exhibit a familial cause [15]. LVNC is characterized by the presence of prominent left ventricular trabeculation and deep intertrabecular recesses, most frequently in the apex that are filled with blood with no evidence of communication with the epicardial coronary artery system [4, 70]. Mutations in ~10 sarcomeric genes, including *MYH7*, *MYBPC3*, *ACTC*, *TNNT2*, and *TPM1*, have been associated with the development of LVNC [15, 29, 50]. Using the TruSight One-Sequence panel, Rowland and coworkers interrogated over 4800 genes associated with clinical phenotypes in a population of 335 cardiomyopathy patients, 325 of which were diagnosed with DCM and the remaining 10 with LVNC [63]. Three novel, dominant, variants in *OBSCN* were identified in the 10 LVNC patients that all cluster in the unique COOH-

terminus of obscurin-B (Fig. 1 and Table 1). These variants include two frameshift mutations, T7266Rfs*ter53 and S7947Pfs*ter82, and one splicing mutation, c. 25367-1 G>C, which localize between Ig67 and Ig68, between Kin1 and Ig69, and Ig69, respectively. Given the prevalence of DCM samples in the cohort, it is tempting to speculate that *OBSCN* mutations affecting the expression, regulation, and/or enzymatic activity of the two obscurin Ser/Thr kinase domains may be more commonly associated with the pathogenesis of LVNC rather than DCM or HCM.

Additional myopathy-linked OBSCN mutations

OBSCN mutations associated with other muscle diseases have also been reported, although the details regarding the clinical presentation of the carriers are currently missing. A novel dominant missense mutation, W7910R, was identified in Kin2 via next-generation sequencing in a patient with chronic systolic heart failure, unspecified hereditary ataxia, and a family history of heart failure (Fig. 1 and Table 1) [14]. In another study, WES and gene-based association analysis revealed nominal significant association of *OBSCN* variants (along with *MYLK*, *DYNC2H1*, and *RNF213* variants) with the development of multifocal fibromuscular dysplasia (FMD) [30]. FMD comprises a group of non-atherosclerotic and non-inflammatory vascular diseases leading to stenosis, aneurysm, and dissection primarily of renal arteries and carotids, which manifests as hypertension, dizziness, transient ischemic attack, or stroke [49]. Lastly, a missense mutation identified in Ig59, c.13330C>T (p.R4444W) in combination with the *FLNC* c.5161delG frameshift mutation, may underlie the development and/or increase the penetrance of distal muscular dystrophy (Fig. 1 and Table 1) [62]. Contrary to the A4484T polymorphism that also resides in Ig59, the R4444W mutation significantly reduces binding of the obscurin Ig58/Ig59 module to titin Ig9/Ig10 region by ~15-fold [62].

Future directions: what we know and what we need to learn

The roles of obscurins in healthy muscle have been heavily investigated since their discovery in 2001, though their involvement in heart disease has only begun to be interrogated. Through their diverse binding partners and different subcellular locations, obscurins have been implicated in several cellular processes [33, 60, 73]. In particular, it has been postulated that obscurins serve as structural linkers between internal membranes and the sarcomeric cytoskeleton as well as the sarcolemma and superficial myofibrils, maintain the subsarcolemmal microtubule network, contribute to the targeting of dystrophin to costameres, play key roles in the assembly and stabilization of sarcomeric M- and A-bands and the SR membranes, participate in the regulation of Ca^{2+} homeostasis, mediate RhoA and PI3K signaling cascades, and are involved in cell adhesion and mechanotransduction pathways [2, 6, 8, 10, 17, 28, 34, 36, 38, 52, 53, 55, 58, 59, 65]. Given the many processes that obscurins are implicated within a healthy cell, it is reasonable to speculate that mutations in *OBSCN* may lead to structural and/or signaling alterations that result in disease development.

To date, a total of 10 missense, 1 splicing, and 7 frameshift mutations have been identified in *OBSCN* that are linked to various forms of cardiomyopathy (Fig. 1 and Table 1). Similar to the majority of sarcomeric mutations, mutations in *OBSCN* are inherited in an autosomal dominant fashion. Mutations that result in frameshift and premature chain terminations are more obviously pathogenic, as they result in truncated proteins and thus "loss of function" or "dominant negative" effects. On the other hand, it is less clear how missense mutations lead to disease development. Similar to missense mutations in *TTN*, encoding titin, and *NEB*, encoding nebulin, it is simultaneously astonishing and puzzling how a single amino acid substitution in a protein that contains from ~8000 (obscurin and nebulin) up to ~34,000 (titin) residues leads to pathogenicity.

There are several possible explanations for the detrimental effects of missense mutations, including alterations in the targeting and incorporation of the mutant protein into sarcomeres, altered stability at the mRNA or protein level, or changes in the binding interactions of the specific domain containing the mutation. In agreement with these hypotheses, Marston and colleagues showed that although the targeting of mutant missense obscurins at sarcomeric M-bands and Z-disks was unaffected, their expression levels were significantly diminished in cardiac biopsies from DCM patients, suggesting haploinsufficiency as a possible pathogenic mechanism [47]. Although it is currently unknown how obscurin transcripts and proteins are targeted for degradation in normal cardiac cells, work from breast cancer epithelial cells, in which obscurins are heavily mutated and expressed in low levels, demonstrated that they undergo lysosomal degradation [51]. Conversely, missense mutations may stabilize mutant obscurins either at the mRNA or protein level resulting in allelic imbalance and therefore affecting disease progression and severity.

The presence of pathogenic substitutions could also affect the folding and/or surface charge distribution of the specific domain that carries the mutation. This could render strong binding interactions into weak and unfavorable, or transient and dynamic binding interactions into stable ones. In support of this mechanism, recent work utilizing a knock-in mouse model carrying the HCM-linked R4344Q substitution demonstrated that the presence of R4344Q alters the topography of the electrostatic charges on the surface of Ig58 leading to enhanced binding between mutant Ig58 and PLN [27]. The sequestration of PLN by mutant obscurin Ig58 leads to

deregulation of Ca^{2+} cycling via enhanced SERCA activity and thus the development of arrhythmia [27].

Moreover, an area that has remained unexplored concerns the potential correlation of disease development with direct or indirect alterations in post-translational modifications of obscurin. As such, obscurins appear to be regulated through phosphorylation [32, 78], and their phosphorylation status changes drastically in response to endurance exercise [24] or following electrically evoked maximal intensity contractions [53]. Future work should investigate the effect of mutations on the post-translational modification profile of obscurins as a possible mechanism of pathogenicity, in addition to those discussed above.

Although no hot spots have been identified along the length of obscurins, it is notable that 6 out of the 18 currently known mutations associated with cardiomyopathy are restricted to obscurin-B, suggesting disease mechanisms that are driven by alterations in kinase-mediated cascades. Earlier work has shown that both kinases are enzymatically active and undergo autophosphorylation, which might regulate their activity [28]. While Kin1 binds and phosphorylates N-cadherin, Kin2 binds to Na/K ATPase subunit 1; at this time, it is unknown whether Na/K ATPase subunit 1 is also a substrate of Kin2. Given the presence of kinase-bearing obscurins at the intercalated disc [28], alterations in the regulation and/or enzymatic activity of Kin1 and Kin2 could perhaps affect the mechanical and electrical coupling of neighboring cardiomyocytes resulting in cardiac remodeling, contractile impairment, and the development of arrhythmias ultimately leading to cardiomyopathy and heart failure.

As a testament to the diversity of human diseases causatively linked to *OBSCN* mutations, *OBSCN* single nucleotide polymorphisms (SNP) may underlie the pathogenesis of aspirin exacerbated respiratory disease (AERD) by contributing to aspirin sensitivity in asthmatics [31]. Kim and colleagues suggested that altered sarcoplasmic reticulum (SR) structure in airway smooth muscle cells bearing variant *OBSCN* alleles may result in defective Ca^{2+} signaling and bronchoconstriction upon aspirin ingestion. Moreover, a number of solid tumors have been shown to possess mutant obscurins [7, 57, 67]. The first indication that mutant obscurins may be associated with tumor formation and progression came with the observation that the *OBSCN* gene is bisected within the first intron by a chromosomal translocation associated with the childhood kidney disease, Wilms' tumor [72]. Recent data further suggests that *OBSCN* mutations may contribute to the formation of several solid cancers. During large-scale sequencing efforts aiming to identify commonly mutated genes in breast and colorectal cancers, *OBSCN* was identified as one of only two genes (the other one being *TP53*) which, when mutated, could drive the formation of both tumor types [67]. In a follow-up study, *OBSCN* mutations were also detected in glioblastoma and melanoma [7]. This accumulated evidence led our group to investigate the role of obscurins in cancer. We found that the expression of giant obscurins is dramatically reduced in breast, skin, and colon cancer cells relative to their nonmalignant counterparts [51], resulting in increased tumorigenicity and metastasis via deregulation of the RhoA and PI3K signaling cascades [52, 65]. Thus, similar to the haploinsufficiency observed in DCM samples by Marston and colleagues [47], epithelial cancer cell lines and advanced stage human biopsies containing mutant obscurins express considerably low levels, and this phenotype correlates with disease progression [51, 66].

Though there are increasing reports of *OBSCN* mutations linked to disease, a major caveat of the current research is the lack of evidence for co-segregation with disease development among blood-related family members. This is obviously due to the types of analyses that have been performed, having mainly used biobank samples obtained during transplantation surgery following end-stage heart failure. Moreover, cardiac biopsies are often unavailable, and thus, the genetic characterization originates from analysis of blood samples. Inclusion of *OBSCN* as a potential pathological gene in large-scale genetic screenings could potential remedy the fact that it was previously somewhat neglected.

Furthermore, with the exception of the HCM-linked R4344Q mutation, which our lab has recently started to characterize after generating the respective knock-in mouse model (please see above) [27], there are no preclinical animal models at this time carrying known *OBSCN* mutations. Generating animal models that express the identified *OBSCN* mutations, or lack specific domains that are potential hot spots, will be crucial for understanding the pathogenic mechanisms underlying *OBSCN* mutations linked to cardiomyopathy.

There is still much to learn about the potential involvement of obscurins in the development of heart disease. It is currently unknown whether *OBSCN* variants may be monogenic causes of cardiomyopathy or whether they contribute to disease development via compound heterozygosity with variations in other genes [46]. It is therefore our anticipation and prediction that more research will focus on *OBSCN* and its causative association with disease development in the near future. Inclusion of *OBSCN* in the list of clinically pathogenic genes, co-segregation analysis, biochemical and biophysical studies, the generation of preclinical animal models, and the use of human biopsies (Table 2) are therefore essential in order to interrogate the functional consequences of known *OBSCN* mutations and further identify novel *OBSCN* mutations and their potentially causative association to heart disease.

Table 2 Future directions for deciphering the involvement of *OBSCN* in cardiomyopathy

Future directions
• Inclusion of *OBSCN* in large-scale genetic screens to identify novel disease-causing variants
• Co-segregation analyses of *OBSCN* variants in blood-related family members
• Biochemical, biophysical, and functional studies of known *OBSCN* variants
• Investigation of post-translational modification status as a potential disease mechanism
• Generation of preclinical animal models carrying known *OBSCN* mutations

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Compliance with ethical standards

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

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