King's Research Portal

DOI:

[10.1042/BST20170149](https://doi.org/10.1042/BST20170149)

[10.1042/BST20170149](https://doi.org/10.1042/BST20170149)

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Zhou, C., Rao, L., Shanahan, C. M., & Zhang, Q. (2018). Nesprin-1/2: roles in nuclear envelope organisation, myogenesis and muscle disease. *Biochemical Society Transactions*. <https://doi.org/10.1042/BST20170149>, <https://doi.org/10.1042/BST20170149>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

**Nesprin-1/2: roles in nuclear envelope organisation,
myogenesis and muscle disease**

Can Zhou^{1, 2}, Li Rao¹, Catherine M. Shanahan², Qiuping Zhang^{2*}

¹Dept. of Cardiology, West China Hospital of Sichuan University, Chengdu 610041, China

²King's College London British Heart Foundation Centre of Research Excellence, Cardiovascular
Division, London SE5 9NU, UK

*Corresponding author:

Dr. Qiuping Zhang

King's College London,

Cardiovascular Division,

James Black Centre,

125 Coldharbour Lane,

London SE5 9NU, UK

Tel: +44 20 78485222

Fax: +44 20 78485193

Email: qp.zhang@kcl.ac.uk

Abstract

Nesprins are multi-isomeric scaffolding proteins. Nesprin-1 and -2 are highly expressed in skeletal and cardiac muscle and together with SUN (Sad1p/UNC84)-domain containing proteins form the LInker of Nucleoskeleton and Cytoskeleton (LINC) complex at the nuclear envelope (NE) in association with lamin A/C and emerin. Mutations in nesprin-1/2 have been found in patients with autosomal dominant Emery–Dreifuss muscular dystrophy (EDMD) as well as dilated cardiomyopathy (DCM). Several lines of evidence indicate that compromised LINC complex function is the critical step leading to muscle disease. Herein we review recent advances in our understanding of the functions of nesprin-1/2 in the LINC complex and mechanistic insights into how mutations in nesprin-1/2 lead to nesprin-related muscle diseases, in particular DCM and EDMD.

Introduction

Nesprins (nuclear envelope spectrin repeat proteins) are a family of multi-isomeric scaffolding proteins [1]. To date, six Klarsicht/ANC-1/Syne Homology (KASH) domain containing proteins: nesprins-1, -2, -3, -4, KASH5 and Lymphoid-restricted membrane protein (LRMP) have been identified, which are encoded by *synaptic nuclear envelope (SYNE)-1*, -2, -3, -4, *KASH5* and *LRMP* genes [1-5]. Nesprin-1/2 localise at the nuclear envelope (NE) and interact with Sad1p/UNC84 (SUN) domain containing proteins, to form the linker of nucleoskeleton and cytoskeleton (LINC) complex in association with lamin A/C and emerin, thus providing a stable physical connection between the nucleus and the cytoskeleton. This complex participates in many cellular activities including maintaining nuclear morphology, regulating nuclear positioning and mechanical cell signalling as well as gene regulation [6-9]. Nesprin-1 and -2 are highly expressed

in heart and skeletal muscle [1]. A number of mutations in *SYNE1* and *SYNE2* genes encoding nesprin-1 and -2 have been identified that are associated with dilated cardiomyopathy (DCM) and autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD 4 and 5) [10-15]. In this review, we summarise all known nesprin-1/2-related muscle diseases as well as mouse models with cardiac and muscular phenotypes and discuss recent advances in our understanding of how nesprin dysfunction within the LINC complex causes nucleocytoskeletal uncoupling and chromatin disorganisation that manifests as defects in nuclear morphology, myonuclear positioning and distribution, aberrant mechanotransduction and gene regulation, leading to pathogenesis of these muscle disorders.

Nesprins and LINC complex

Nesprins-1 and -2 were first identified in screens for novel vascular smooth muscle cell (VSMC) differentiation markers [16]. Full-length human nesprin-1 and nesprin-2 are the second and third largest known proteins with molecular masses of ~1000kDa and ~800kDa respectively [1]. The typical structure of giant nesprin-1 and -2 consists of three major domains: A C-terminal KASH domain that is targeted to the NE, an N-terminal paired Calponin Homology (CH) domain that binds to the actin cytoskeleton, and a central rod domain containing multiple spectrin repeats (SRs) that link the CH and KASH domains and mediate protein-protein interactions [17]. Of note, there is a highly conserved adaptive domain (AD) at the C-terminus of nesprin-1 and -2, which acts to structurally stabilise the SRs in nesprin molecules [18, 19]. Extensive alternative transcription and splicing of *SYNE1* and *SYNE2* generate multiple nesprin-1 and -2 isoforms that vary greatly in size and those nesprin-1/2 isoforms exhibit multiple subcellular localisations [20] (Figure 1A). The giant isoforms localise at the outer nuclear membrane (ONM) and interact with SUN1/2 at the perinuclear space, forming the LINC complex that connects the nucleus to the actin

cytoskeleton [21-24]. Smaller isoforms localise at the inner nuclear membrane (INM) and interact with emerin, SUN1/2 and lamin A/C via their SRs [25]. Lamin A/C can bind to DNA and core histones, while emerin can interact with lamin A/C and DNA-associated proteins such as barrier to auto-integration factor (BAF), which are important for maintaining chromatin architecture, regulating cell signalling and gene expression [26-28]. Recently, studies have shown nesprin-2 and the small nesprin-1 isoform, nesprin-1 α , also localise at the ONM where they interact with the microtubule motor proteins dynein/dynactin, kinesin light chain-1/2 (KLC-1/2) and the centrosomal protein Akap450, a component of the microtubule-organising centre (MTOC) [12, 29-32].

Nesprin-1/2 are ubiquitously expressed, and are highly abundant in skeletal and cardiac muscle, in particular, smaller isoforms nesprin-1 α_2 and nesprin-2 α_1 [1, 33]. *SYNE1* and *SYNE2* genes share 64% homology [1]. Nesprin-1 is more conserved than nesprin-2 as the latter is relatively divergent in length and the first half of its amino acid sequence. However, both giant nesprin-1 and -2 are highly conserved in their CH domains and C-terminal region of the SR block, especially SRs69-71 of nesprin-1 and SRs51-53 of nesprin-2, as well as the adjacent unstructured AD domain, corresponding to small skeletal muscle and cardiac specific isoforms: nesprin-1 α_2 , nesprin-2 α_1 and nesprin-2 ϵ_2 [18, 34], suggesting they may play similar roles in muscle cell functions. The remaining portions of giant nesprin-1 and -2 rod domains may be more structural and have different roles in other tissues. Nesprin-3 is also ubiquitously expressed, and mainly interacts with plectin, the intermediate filaments cytolinker [2, 35]. Nesprin-4 is predominately expressed in epithelial cells [3, 36]. LRMP is expressed in a subset of taste receptor cells in mammals [37] and enriched in zebrafish zygotes [38], whereas KASH5 is limited to meiotic cells [5, 39]. Both nesprin-4 and KASH5 can interact with microtubule motor proteins kinesin-1 or

dynein/dynactin [3, 5]. In addition to forming the microtubule LINC complex, LRMP also binds calcium channel IP3 receptors to regulate taste signal transduction [37, 38]. Thus nesprins form a variety of LINC complexes that strengthen the connection between the nucleus and filamentous cytoskeletal networks.

Roles of nesprin-1/2 in muscle cell differentiation

The LINC complex has been revealed to play a major role in maintaining the position of the nucleus and its movements during cell migration and differentiation [40, 41]. A dynamic change of small and giant nesprin-1/2 isoforms has been shown to be involved in muscle differentiation although current knowledge in cardiac muscle cell differentiation is limited due to technical issues. In human myoblasts, the expression of giant nesprin-1 is present and nesprin-1 α_2 is relatively low. During myogenesis, nesprin-1 α_2 is upregulated and accompanied by a two-fold increase in nesprin-1 giant [12, 42]. Knockdown of nesprin-1 prevents mouse embryonic stem cells from differentiating into cardiomyocytes (CMs) [43]. *In vitro*, differentiation models have shown nesprin-2 isoforms re-localise from the NE of C2C12 myoblasts to the sarcomere of myotubes [25]. *In vivo*, during the transition from immature to mature muscle fibres, nesprin-2 giant partly replaces nesprin-1 giant at the NE and the short isoform, nesprin-1 α_2 , becomes dominant [44].

During myoblast differentiation, the expression levels of myogenic regulatory factors (MRFs) including myoblast determination protein (MyoD), myogenin and myosin heavy chain (MHC); microtubule (MT) motor protein KLC-1/2; as well as centrosomal proteins including Akap450, pericentriolar material 1 (PCM1) and pericentrin are increased in association with upregulated nesprin-1 α in differentiated myotubes [12, 30, 45]. Proper nuclear positioning and movement is critical in muscle cell differentiation and development, and is driven by cytoskeletal

networks of MTs, actin and/or intermediate filaments, and involve a connection between the cytoskeleton and the NE, mediated by the LINC complex [29, 46]. Both nesprin-1 and -2 interact with KLC-1/2 through a highly conserved 'LEWD' motif at the AD region near the C-terminus of nesprin-1/2 and this interaction drives nuclear distribution during skeletal muscle cell differentiation [12, 29]. Furthermore, nesprin-1 α has been shown to serve as a key factor to facilitate switching the MTOC from the centrosome in myoblasts to the cytoplasmic surface of the NE in myotubes by recruiting Akap450 to the NE to allow efficient microtubule nucleation and subsequent nuclear positioning, which is a critical step for nuclear spreading during muscle cell differentiation [30, 47]. These data suggest the combined actions of nesprin-1 α -mediated KLC-1/2 recruitment and microtubule nucleation at the nucleus via Akap450 is essential for proper nuclear positioning in myotubes.

Nesprin-1/2 mutations and muscle disease

A wide range of cardiac and skeletal myopathies have been linked to mutations in LINC complex proteins [48]. DCM is characterised by dilatation and impaired contraction of the left or both ventricles, and is an important cause of heart failure and sudden cardiac death, particularly in the young [49]. EDMD manifests with skeletal muscle wasting with a distinctive humeroperoneal distribution early in the course of the disease and later extends to the proximal limb girdle musculature. Cardiac dysfunction is another feature of EDMD including heart conduction defects (CD) and DCM. Although CD and DCM always develop in a later stage of life, they are the main causes of mortality for these disorders [50]. Literature shows mutations in the *LMNA* gene (encoding protein lamin A/C), account for 5% of familial DCM [49, 51] and mutations in both *LMNA* and *EMD* genes together account for 40% of EDMD [52, 53]. How mutations in both lamin A/C and emerin, which are ubiquitously expressed proteins, can lead to muscle-specific diseases

has been a subject of debate for some time. Evidence suggests that their binding partners nesprin-1/2 and SUN1/2 are also involved in these diseases. Recently a total of 15 nesprin-1 and 6 nesprin-2 mutants as well as 7 SUN1/2 mutants were identified in EDMD and DCM patients [10-15, 54-56]. Moreover, nesprin-1 mutants are identified in other muscular disorders such as congenital muscular dystrophy (CMD) and arthrogryposis multiplex congenita (AMC) [57-60] (Figure 2).

14 out of 25 experimentally validated and characterised nesprin-1/2 mutants occur in the C-terminus of nesprin-1/2 giant, within the nesprin-1 α and 2 β region, which were identified in patients with muscle specific disorders, including several small family pedigrees as well as in sporadic EDMD patients with DCM [10, 12-15, 42, 56, 58, 60]. In these studies, nuclear morphology changes were commonly observed in mutant cells. Convolved nuclear appearance as well as micronuclei, giant and fragmented nuclei were observed in patient fibroblasts or lymphoblasts [13, 14]. The perturbed localisation of LINC complex components and altered binding with nesprin-1/2 are another featured observation. Both lamin A/C and SUN2 were mislocalised from the NE in fibroblasts derived from EDMD patients or neonatal rat cardiomyocytes (NRCs) transfected with constructs encoding nesprin-1 mutations identified in DCM patients [12, 14]. The association between nesprin and lamin A/C, SUN and the microtubule motor protein KLC-1/2 were all disrupted by these nesprin mutations [12, 14]. Furthermore, during myoblast differentiation, reduced fusion capacity with decreased expression of myogenin and MHC was observed in C2C12 cells infected with nesprin-1 mutations identified in DCM patients [12]. Centralised nuclei was another feature in EDMD patients accompanied by increased fibre size variability, fibrosis and apparent fat tissue in the skeletal muscle biopsy [15, 56]. In addition, expression levels of nesprin-1 were increased in fibroblasts derived from DCM patients and the localisation of nesprin-1 at the NE was totally absent in myoblasts derived from CMD patients

[10, 30]. Disrupted LINC complex, altered nuclear morphology and perturbed myogenesis were also reported in EDMD/DCM disorders caused by lamin A/C or emerin mutations, suggesting that an intact LINC complex is critical for maintaining muscular physiological functions [61-65].

In addition to muscular disorders, nesprin-1 mutations have been shown to cause the neurological disorder autosomal recessive cerebellar ataxia type 1 (ARCA1) [66-69]. ARCA1 is characterised by progressive problems with cerebellar dysarthria, limb and gait ataxia and diffuse cerebellar atrophy [70]. It is intriguing that the nesprin-1/2 mutations result in such distinctly different diseases affecting either muscle or the central nervous system (CNS).

Comparing the mutations of *SYNE1/2* that affect both muscle and CNS systems, it is apparent that the majority of nesprin-1 mutations associated with muscle diseases, especially those experimentally validated and characterised, are heterozygous missense mutations at the C-terminus of nesprin-1 giant, and are all within the muscle specific isoform nesprin-1 α_2 . These mutants cause amino acid substitutions, resulting in changes in charge or hydrophilic/hydrophobic properties of conserved amino acids and are likely to affect the structure/flexibility of the functional regions of both nesprin-1 and -2 due to the high homology and conservation in their C-terminal regions [34]. In contrast, mutations affecting CNS development are homozygous and scatter along the whole *SYNE1* gene. They are nonsense mutations and introduce a stop codon. This premature stop codon results in many truncated nesprin-1 variants lacking either SRs and/or KASH domains or total loss of nesprin-1 due to nonsense-mediated decay of mutant mRNA [69, 71]. The varied positions of *SYNE1/2* mutations and their related diseases indicate: 1) the KASH domain and CH domain containing isoforms have tissue specific scaffolding functions in muscle and CNS respectively due to the differential homology and conservation of their amino acid sequence and structure in these isoforms. Mutations near/in the KASH domain of nesprin-1 or -2

may affect the muscle functions in both nesprin-1 and -2 while mutations in CH domains/N-terminal and central part of nesprin-1 mainly cause defects in neural/brain isoforms of nesprin-1, which could partly explain why the mutations causing ARCA1 were only reported in *SYNE1* rather than *SYNE2*. 2) In muscle disease, mutations in *SYNE1/2* similar to *LMNA* or *EMD*, are mainly independent muscular disorder-causing genes [14]. In contrast, *SUN1/2* are thought to be disease modifier genes in individuals with co-segregating mutations in other EDMD causing-genes such as *LMNA*, *EMD*, and *LAP2α* [54, 55]. 3) The different localisations of nesprin mutations may impair different functions of the LINC complex. Nesprin mutations in the SR regions at the C-terminus are more likely to induce nuclear morphology defects and disrupt associations with the nesprin binding proteins lamin A/C, emerin and SUN at the NE, promoting LINC complex instability. Changes in LINC complex stability perturb mechanotransduction in muscle cells especially when subject to mechanical strain [72]. Nesprin mutations in the AD region have more effects on the interaction with microtubule motor protein KLC-1/2, resulting in defects in nuclear migration and positioning, perturbing myogenesis and muscle maturation [12].

Nesprin-1/2 mouse models with cardiac/skeletal muscle phenotypes

To further understand the roles of nesprins in the LINC complex and in nesprin-related muscle diseases, more than 10 nesprin mouse models have been generated. Two of these spontaneously developed cardiac phenotypes and nearly half showed skeletal muscular disorders.

Nesprin-1/2 mouse models with cardiac phenotypes

A cardiomyopathy phenotype was observed in global KASH1 knockout (KO) and nesprin-1/2 double knockout (DKO) mouse models [10, 73, 74]. The global KASH1 KO mouse was generated via deletion of the last exon of *SYNE1* through inserted LoxP sites, leading to removal

of the last two exons including the KASH domain. Of note, unexpected insertion of an extra 61 amino acids occurred during KASH1 deletion, although this sequence was not homologous to any known proteins. Homozygous KASH1 KO mice exhibited lethality, with approximately 50% mortality at or near birth due to un-inflated lungs causing respiratory failure. The surviving KASH1 KO mice developed CD at an early age (<32 weeks) and cardiomyopathy later in life (>52weeks). Isolated CMs displayed elongated nuclear shape with large invaginations of the NE, and reduced heterochromatin. The LINC complex was shown to be disrupted due to the abolished interaction between nesprin-1 and SUN2. Cell signalling was perturbed as mitogen-activated protein kinases (MAPKs) were hyper-activated in heart tissue derived from both young and old KASH1 KO mouse [12].

The nesprin DKO mouse line was generated by breeding the cardiac specific nesprin-1 deletion mouse with the global nesprin-2 deletion mouse, both targeted to the SRs near the C-terminus of nesprin-1 and nesprin-2. The nesprin DKO mice survived over 1 year, but displayed early onset cardiomyopathy with thinner left ventricle walls and decreased fractional shortening from 10 weeks, which deteriorated over time. The CMs derived from these DKO mice had dramatic nuclear deformation including an increased nuclear area, length and perimeter; decreased nuclear circularity and distance; and less condensed heterochromatin. Subcellular localisation of lamin A/C and emerin at the NE was altered while expression levels of LINC complex components lamin A/C, emerin and SUN were unchanged. These data strengthen the hypothesis that the ablation of nesprins compromises the LINC complex. In addition, biochemical stimulation of CMs led to attenuated response of biomechanical signalling genes such as *iex-1*, *egr-1*, *c-jun*, *c-fos* and *c-myc*, further suggesting disruption of the LINC complex leads to impaired mechanotransduction in these muscle cells.

Nesprin-1/2 mouse models with skeletal muscle phenotypes

Other nesprin mouse models such as nesprin-1 or 2 KASH overexpression, nesprin-1 or 2 KO, and nesprin-1 α_2 KO mice all exhibited the skeletal muscle defects [73, 75-78]. One of the main features in these mice was abnormal nuclear positioning along skeletal myofibers. The variability of muscle fibre size was also increased [77]. Myonuclei clusters or arrays were observed in the longitudinal section [76-78], whilst centralised myonuclei were present in the cross section of muscle fibres [73, 77]. The function of muscle fibres was also compromised in nesprin-1 mutant mice. Myonuclei were detached when the sarcolemma was peeled off from muscle fibres, and nuclear aspect ratio (length divided by width) showed less change with fibre strain in nesprin-1 KO mice when compared with wild type (WT), indicating defective nuclear anchorage and decreased strain transmission from perinuclear regions to the nuclei [77]. Moreover, absence or overexpression of the KASH domain resulted in the expulsion of synaptic nuclei from the neuromuscular junction (NMJ) to the peripheral region, causing longer motor nerve branches, which was important for proper motor neuron innervation and respiration [75, 76]. In addition to the muscular and cardiac phenotypes, the literature also reported misshapen nuclei in epithelial cells and defective cell migration in the retina caused by deletion of nesprin-2 CH domain or KASH2 respectively [32, 79].

The data generated from these mouse models suggest the C-terminal region of giant nesprin-1/2 and nesprin-1 α_2 isoforms play an important role in muscle cell function, especially in nuclear positioning in developing skeletal muscle.

Pathogenic mechanism of nesprin-1/2 dysfunction

Nesprin-1/2 localise at the NE, forming the LINC complex with SUN1/2, lamin A/C and emerin. This complex is integrated for linking the nucleus to actin cytoskeleton. Currently there are two key hypotheses that have been proposed for the pathological mechanisms underlying these muscle diseases. **The structural hypothesis** (transcriptional independent): suggesting the LINC complex plays a role in nucleocytoskeletal coupling by regulating both force transmission and balance of the relations between the nuclear lamina, microtubules, kinesin-dynein motor counteraction, myosin and other biological processes. **The gene regulation hypothesis** (transcriptional regulation): suggesting structural changes in the LINC complex may uncouple chromatin from the NE and cytoskeleton and affect mechanotransduction/signalling which may eventually lead to genome reorganisation and transcriptional dysregulation. In muscle diseases, the two hypotheses are not mutually exclusive and the consequence of any defects can be summarised into the following detailed aspects: (Figure 1B).

Firstly, nesprin-1 mutations alter nuclear morphology [10, 12, 14, 74], mis-localise its binding partners lamin A/C, emerin and SUN2 from the NE [12, 14, 74] and perturb their interactions [12, 14]. This causes disruption of the LINC complex and thereby compromises its functions as a physical linker and force transmission propagator. Meanwhile, mutated nesprin-1 also perturbs the interaction with the microtubule motor proteins KLC-1/2 and centrosomal protein Akap450 at the ONM [12, 29, 30], leading to disassembly of the link between the nucleus and the microtubule.

Secondly, nesprin is the key element to ensure proper nuclear positioning and migration which was initially examined in lower organisms. In *C. elegans*, mutated ANC-1 (a nesprin orthologue) results in freely floating and grouped nuclei in the cytoplasm of hypodermal syncytium [80]. In *Drosophila melanogaster*, mutated MSP-300 (a nesprin orthologue) leads to mis-localised

nuclei in oocytes [81]. Similarly, in mammalian myoblasts, the nesprin-1 mutation (E7854X) abolishes the recruitment of Akap450 to the NE during muscle cell differentiation and another nesprin-1 mutation (R8272Q) near the 'LEWD' motif disrupts its binding with KLC-1/2 [12, 29]. These nesprin-1 mutations affect microtubule-mediated nucleation and nuclear migration/positioning, leading to defects in myogenesis.

Thirdly, mutations in *SYNE1/2* that disrupt muscle specific nesprin-1 and -2 isoforms, or mutations in other components (*LMNA*, *EMD* and *SUN1/2*) of the LINC complex that disrupt their binding with muscle specific nesprin-1 and -2 isoforms, lead to defects in nuclear morphology and nucleocytoskeletal coupling [10, 14, 54, 55, 82]. They are also likely to influence chromatin structural integrity as mis-localised emerin and lamin A/C could also alter binding with DNA, core histones and the chromatin-associated proteins BAF [26-28, 83]. Disruption of these interactions would affect cell signalling and gene regulation including: augmented cell signalling (hyperactivation of MAPK kinase) [12], dysregulated gene expression (perturbed expression of MRFs in muscle cell differentiation *in vitro* and cardiac foetal genes and fibrotic genes in heart *in vivo*) [12, 74] and abnormal mechanotransduction (altered mechanosensitive genes in CMs derived from nesprin-1/2 DKO mouse) [74]. These aspects of compromised functions of the LINC complex further support the structural and gene regulation hypotheses, contributing to pathogenesis of EDMD and DCM.

Concluding remarks

Over past decades, nesprin associated muscular diseases have emerged and nesprin mouse models with skeletal and/or cardiac muscle phenotypes have been generated. Our knowledge of the roles of nesprin-1/2 in NE organisation and myogenesis have expanded dramatically, which

has aided our understanding of how nesprin-1/2 mutants contribute to DCM and EDMD via the LINC complex associated structural disruption and gene dysregulation. However, we still lack a complete understanding of the diverse nesprin isoforms, the myriad of nesprin binding partners and their interactions/functions with the LINC complex in muscle cells. Additionally, increased evidence has implied that nesprin-1 and -2 also participate in formation of the NE-MTOC, Ca^{2+} /Calmodulin mediated nuclear trafficking and mitochondria positioning [30, 84, 85]. Interestingly, they are also present at the sarcomere in addition to their localisation at the NE [14, 25]. Thus, future studies focusing on these aspects of nesprin-1/2 functions and utilizing derived induced Pluripotent Stem Cells (iPSCs) and/or myoblasts derived from patients, as well as generating cardiac or muscle specific nesprin mutant knock-in mouse models will help to decipher the underlying mechanisms enabling the development of potential diagnostic and therapeutic targets for these muscular disorders.

Acknowledgements

We thank Dr. Derek T. Warren (University of East Anglia) and Dr. Andrew Cobb (King's College London) for critical reading of this manuscript.

Conflict of Interest statement. None declared.

Funding

This work was supported by the British Heart Foundation (BHF) [PG/11/58/29004 to Q.P.Z., RG/11/14/29056 to C.M.S.] and the National Natural Science Foundation of China [81270289 to L.R.].

References:

1. Zhang, Q., et al., *Nesprins: a novel family of spectrin-repeat-containing proteins that localize to the nuclear membrane in multiple tissues*. J Cell Sci, 2001. **114**(Pt 24): p. 4485-98.
2. Wilhelmsen, K., et al., *Nesprin-3, a novel outer nuclear membrane protein, associates with the cytoskeletal linker protein plectin*. J Cell Biol, 2005. **171**(5): p. 799-810.
3. Roux, K.J., et al., *Nesprin 4 is an outer nuclear membrane protein that can induce kinesin-mediated cell polarization*. Proc Natl Acad Sci U S A, 2009. **106**(7): p. 2194-9.
4. Behrens, T.W., et al., *Jaw1, A lymphoid-restricted membrane protein localized to the endoplasmic reticulum*. J Immunol, 1994. **153**(2): p. 682-90.
5. Morimoto, A., et al., *A conserved KASH domain protein associates with telomeres, SUN1, and dynactin during mammalian meiosis*. J Cell Biol, 2012. **198**(2): p. 165-72.
6. Crisp, M., et al., *Coupling of the nucleus and cytoplasm: role of the LINC complex*. J Cell Biol, 2006. **172**(1): p. 41-53.
7. Lombardi, M.L. and J. Lammerding, *Keeping the LINC: the importance of nucleocytoplasmic coupling in intracellular force transmission and cellular function*. Biochem Soc Trans, 2011. **39**(6): p. 1729-34.
8. Mellad, J.A., D.T. Warren, and C.M. Shanahan, *Nesprins LINC the nucleus and cytoskeleton*. Curr Opin Cell Biol, 2011. **23**(1): p. 47-54.
9. Stroud, M.J., et al., *Linker of nucleoskeleton and cytoskeleton complex proteins in cardiac structure, function, and disease*. Circ Res, 2014. **114**(3): p. 538-48.
10. Puckelwartz, M.J., et al., *Nesprin-1 mutations in human and murine cardiomyopathy*. J Mol Cell Cardiol, 2010. **48**(4): p. 600-8.
11. Akinrinade, O., et al., *Genetics and genotype-phenotype correlations in Finnish patients with dilated cardiomyopathy*. Eur Heart J, 2015. **36**(34): p. 2327-37.
12. Zhou, C., et al., *Novel nesprin-1 mutations associated with dilated cardiomyopathy cause nuclear envelope disruption and defects in myogenesis*. Hum Mol Genet, 2017. **26**(12): p. 2258-2276.
13. Haskell, G.T., et al., *Whole Exome Sequencing Identifies Truncating Variants in Nuclear Envelope Genes in Patients With Cardiovascular Disease*. Circ Cardiovasc Genet, 2017. **10**(3).
14. Zhang, Q., et al., *Nesprin-1 and -2 are involved in the pathogenesis of Emery Dreifuss muscular dystrophy and are critical for nuclear envelope integrity*. Hum Mol Genet, 2007. **16**(23): p. 2816-33.
15. Chen, Z., et al., *A novel SYNE1 gene mutation in a Chinese family of Emery-Dreifuss muscular dystrophy-like*. BMC Med Genet, 2017. **18**(1): p. 63.
16. Shanahan, C.M., P.L. Weissberg, and J.C. Metcalfe, *Isolation of gene markers of differentiated and proliferating vascular smooth muscle cells*. Circ Res, 1993. **73**(1): p. 193-204.
17. Rajgor, D. and C.M. Shanahan, *Nesprins: from the nuclear envelope and beyond*. Expert Rev Mol Med, 2013. **15**: p. e5.
18. Simpson, J.G. and R.G. Roberts, *Patterns of evolutionary conservation in the nesprin genes highlight probable functionally important protein domains and isoforms*. Biochem Soc Trans, 2008. **36**(Pt 6): p. 1359-67.
19. Zhong, Z., et al., *Stabilization of the spectrin-like domains of nesprin-1alpha by the evolutionarily conserved "adaptive" domain*. Cell Mol Bioeng, 2010. **3**(2): p. 139-150.
20. Rajgor, D., et al., *Multiple novel nesprin-1 and nesprin-2 variants act as versatile tissue-specific intracellular scaffolds*. PLoS One, 2012. **7**(7): p. e40098.
21. Razafsky, D. and D. Hodzic, *Bringing KASH under the SUN: the many faces of nucleo-cytoskeletal connections*. J Cell Biol, 2009. **186**(4): p. 461-72.

22. Sosa, B.A., et al., *LINC complexes form by binding of three KASH peptides to domain interfaces of trimeric SUN proteins*. Cell, 2012. **149**(5): p. 1035-47.
23. Zhou, Z., et al., *Structure of Sad1-UNC84 homology (SUN) domain defines features of molecular bridge in nuclear envelope*. J Biol Chem, 2012. **287**(8): p. 5317-26.
24. Zhang, Q., et al., *The nesprins are giant actin-binding proteins, orthologous to Drosophila melanogaster muscle protein MSP-300*. Genomics, 2002. **80**(5): p. 473-81.
25. Zhang, Q., et al., *Nesprin-2 is a multi-isomeric protein that binds lamin and emerin at the nuclear envelope and forms a subcellular network in skeletal muscle*. J Cell Sci, 2005. **118**(Pt 4): p. 673-87.
26. Taniura, H., C. Glass, and L. Gerace, *A chromatin binding site in the tail domain of nuclear lamins that interacts with core histones*. J Cell Biol, 1995. **131**(1): p. 33-44.
27. Stierle, V., et al., *The carboxyl-terminal region common to lamins A and C contains a DNA binding domain*. Biochemistry, 2003. **42**(17): p. 4819-28.
28. Lee, K.K., et al., *Distinct functional domains in emerin bind lamin A and DNA-bridging protein BAF*. J Cell Sci, 2001. **114**(Pt 24): p. 4567-73.
29. Wilson, M.H. and E.L. Holzbaur, *Nesprins anchor kinesin-1 motors to the nucleus to drive nuclear distribution in muscle cells*. Development, 2015. **142**(1): p. 218-28.
30. Gimpel, P., et al., *Nesprin-1alpha-Dependent Microtubule Nucleation from the Nuclear Envelope via Akap450 Is Necessary for Nuclear Positioning in Muscle Cells*. Curr Biol, 2017.
31. Zhang, X., et al., *SUN1/2 and Syne/Nesprin-1/2 complexes connect centrosome to the nucleus during neurogenesis and neuronal migration in mice*. Neuron, 2009. **64**(2): p. 173-87.
32. Yu, J., et al., *KASH protein Syne-2/Nesprin-2 and SUN proteins SUN1/2 mediate nuclear migration during mammalian retinal development*. Hum Mol Genet, 2011. **20**(6): p. 1061-73.
33. Duong, N.T., et al., *Nesprins: tissue-specific expression of epsilon and other short isoforms*. PLoS One, 2014. **9**(4): p. e94380.
34. Autore, F., et al., *Large-scale modelling of the divergent spectrin repeats in nesprins: giant modular proteins*. PLoS One, 2013. **8**(5): p. e63633.
35. Ketema, M., et al., *Nesprin-3 connects plectin and vimentin to the nuclear envelope of Sertoli cells but is not required for Sertoli cell function in spermatogenesis*. Mol Biol Cell, 2013. **24**(15): p. 2454-66.
36. Horn, H.F., et al., *The LINC complex is essential for hearing*. J Clin Invest, 2013. **123**(2): p. 740-50.
37. Shindo, Y., et al., *Lrmp/Jaw1 is expressed in sweet, bitter, and umami receptor-expressing cells*. Chem Senses, 2010. **35**(2): p. 171-7.
38. Lindeman, R.E. and F. Pelegri, *Localized products of futile cycle/lrmp promote centrosome-nucleus attachment in the zebrafish zygote*. Curr Biol, 2012. **22**(10): p. 843-51.
39. Stewart, C.L. and B. Burke, *The missing LINC: a mammalian KASH-domain protein coupling meiotic chromosomes to the cytoskeleton*. Nucleus, 2014. **5**(1): p. 3-10.
40. Dupin, I. and S. Etienne-Manneville, *Nuclear positioning: mechanisms and functions*. Int J Biochem Cell Biol, 2011. **43**(12): p. 1698-707.
41. Gundersen, G.G. and H.J. Worman, *Nuclear positioning*. Cell, 2013. **152**(6): p. 1376-89.
42. Holt, I., et al., *Specific localization of nesprin-1-alpha2, the short isoform of nesprin-1 with a KASH domain, in developing, fetal and regenerating muscle, using a new monoclonal antibody*. BMC Cell Biol, 2016. **17**(1): p. 26.
43. Gao, F., et al., *[Effect of nesprin-1 in the differentiation of mouse embryonic stem cells into cardiomyocytes]*. Sichuan Da Xue Xue Bao Yi Xue Ban, 2012. **43**(2): p. 161-5.
44. Randles, K.N., et al., *Nesprins, but not sun proteins, switch isoforms at the nuclear envelope during muscle development*. Dev Dyn, 2010. **239**(3): p. 998-1009.

45. Charge, S.B. and M.A. Rudnicki, *Cellular and molecular regulation of muscle regeneration*. *Physiol Rev*, 2004. **84**(1): p. 209-38.
46. Chang, W., et al., *Linker of nucleoskeleton and cytoskeleton (LINC) complex-mediated actin-dependent nuclear positioning orients centrosomes in migrating myoblasts*. *Nucleus*, 2015. **6**(1): p. 77-88.
47. Sanchez, A.D. and J.L. Feldman, *Microtubule-organizing centers: from the centrosome to non-centrosomal sites*. *Curr Opin Cell Biol*, 2017. **44**: p. 93-101.
48. Meinke, P., T.D. Nguyen, and M.S. Wehnert, *The LINC complex and human disease*. *Biochem Soc Trans*, 2011. **39**(6): p. 1693-7.
49. Weintraub, R.G., C. Semsarian, and P. Macdonald, *Dilated cardiomyopathy*. *Lancet*, 2017. **390**(10092): p. 400-414.
50. Helbling-Leclerc, A., G. Bonne, and K. Schwartz, *Emery-Dreifuss muscular dystrophy*. *Eur J Hum Genet*, 2002. **10**(3): p. 157-61.
51. Hershberger, R.E., D.J. Hedges, and A. Morales, *Dilated cardiomyopathy: the complexity of a diverse genetic architecture*. *Nat Rev Cardiol*, 2013. **10**(9): p. 531-47.
52. Bonne, G., et al., *108th ENMC International Workshop, 3rd Workshop of the MYO-CLUSTER project: EUROMEN, 7th International Emery-Dreifuss Muscular Dystrophy (EDMD) Workshop, 13-15 September 2002, Naarden, The Netherlands*. *Neuromuscul Disord*, 2003. **13**(6): p. 508-15.
53. Gotzmann, J. and R.P. Foisner, *Lamins and Emerin in muscular dystrophy: the nuclear envelope connection*. 2013.
54. Meinke, P., et al., *Muscular dystrophy-associated SUN1 and SUN2 variants disrupt nuclear-cytoskeletal connections and myonuclear organization*. *PLoS Genet*, 2014. **10**(9): p. e1004605.
55. Li, P., et al., *Contribution of SUN1 mutations to the pathomechanism in muscular dystrophies*. *Hum Mutat*, 2014. **35**(4): p. 452-61.
56. Fanin, M., et al., *Dominant muscular dystrophy with a novel SYNE1 gene mutation*. *Muscle Nerve*, 2015. **51**(1): p. 145-7.
57. Voit, T., et al., *C.O.4 Congenital muscular dystrophy with adducted thumbs, mental retardation, cerebellar hypoplasia and cataracts is caused by mutation of Enaptin (Nesprin-1): The third nuclear envelopathy with muscular dystrophy*. *Neuromuscular Disorders*. **17**(9): p. 833-834.
58. Attali, R., et al., *Mutation of SYNE-1, encoding an essential component of the nuclear lamina, is responsible for autosomal recessive arthrogryposis*. *Hum Mol Genet*, 2009. **18**(18): p. 3462-9.
59. Laquerriere, A., et al., *Mutations in CNTNAP1 and ADCY6 are responsible for severe arthrogryposis multiplex congenita with axoglial defects*. *Hum Mol Genet*, 2014. **23**(9): p. 2279-89.
60. Baumann, M., et al., *Homozygous SYNE1 mutation causes congenital onset of muscular weakness with distal arthrogryposis: a genotype-phenotype correlation*. *Eur J Hum Genet*, 2017. **25**(2): p. 262-266.
61. Reichart, B., et al., *Expression and localization of nuclear proteins in autosomal-dominant Emery-Dreifuss muscular dystrophy with LMNA R377H mutation*. *BMC Cell Biol*, 2004. **5**: p. 12.
62. Frock, R.L., et al., *Lamin A/C and emerin are critical for skeletal muscle satellite cell differentiation*. *Genes Dev*, 2006. **20**(4): p. 486-500.
63. Taranum, S., et al., *LINC complex alterations in DMD and EDMD/CMT fibroblasts*. *Eur J Cell Biol*, 2012. **91**(8): p. 614-28.
64. Nagano, A., et al., *Emerin deficiency at the nuclear membrane in patients with Emery-Dreifuss muscular dystrophy*. *Nat Genet*, 1996. **12**(3): p. 254-9.
65. Shimojima, M., et al., *Emerin plays a crucial role in nuclear invagination and in the nuclear calcium transient*. *Sci Rep*, 2017. **7**: p. 44312.

66. Gros-Louis, F., et al., *Mutations in SYNE1 lead to a newly discovered form of autosomal recessive cerebellar ataxia*. Nat Genet, 2007. **39**(1): p. 80-5.
67. Noreau, A., et al., *SYNE1 mutations in autosomal recessive cerebellar ataxia*. JAMA Neurol, 2013. **70**(10): p. 1296-31.
68. Izumi, Y., et al., *Cerebellar ataxia with SYNE1 mutation accompanying motor neuron disease*. Neurology, 2013. **80**(6): p. 600-1.
69. Synofzik, M., et al., *SYNE1 ataxia is a common recessive ataxia with major non-cerebellar features: a large multi-centre study*. Brain, 2016. **139**(Pt 5): p. 1378-93.
70. Fogel, B.L. and S. Perlman, *Clinical features and molecular genetics of autosomal recessive cerebellar ataxias*. Lancet Neurol, 2007. **6**(3): p. 245-57.
71. Brogna, S. and J. Wen, *Nonsense-mediated mRNA decay (NMD) mechanisms*. Nat Struct Mol Biol, 2009. **16**(2): p. 107-13.
72. Brosig, M., et al., *Interfering with the connection between the nucleus and the cytoskeleton affects nuclear rotation, mechanotransduction and myogenesis*. Int J Biochem Cell Biol, 2010. **42**(10): p. 1717-28.
73. Puckelwartz, M.J., et al., *Disruption of nesprin-1 produces an Emery Dreifuss muscular dystrophy-like phenotype in mice*. Hum Mol Genet, 2009. **18**(4): p. 607-20.
74. Banerjee, I., et al., *Targeted ablation of nesprin 1 and nesprin 2 from murine myocardium results in cardiomyopathy, altered nuclear morphology and inhibition of the biomechanical gene response*. PLoS Genet, 2014. **10**(2): p. e1004114.
75. Grady, R.M., et al., *Syne proteins anchor muscle nuclei at the neuromuscular junction*. Proc Natl Acad Sci U S A, 2005. **102**(12): p. 4359-64.
76. Zhang, X., et al., *Syne-1 and Syne-2 play crucial roles in myonuclear anchorage and motor neuron innervation*. Development, 2007. **134**(5): p. 901-8.
77. Zhang, J., et al., *Nesprin 1 is critical for nuclear positioning and anchorage*. Hum Mol Genet, 2010. **19**(2): p. 329-41.
78. Stroud, M.J., et al., *Nesprin 1alpha2 is essential for mouse postnatal viability and nuclear positioning in skeletal muscle*. J Cell Biol, 2017. **216**(7): p. 1915-1924.
79. Luke, Y., et al., *Nesprin-2 Giant (NUANCE) maintains nuclear envelope architecture and composition in skin*. J Cell Sci, 2008. **121**(11): p. 1887-98.
80. Starr, D.A. and H.N. Fridolfsson, *Interactions between nuclei and the cytoskeleton are mediated by SUN-KASH nuclear-envelope bridges*. Annu Rev Cell Dev Biol, 2010. **26**: p. 421-44.
81. Yu, J., et al., *The KASH domain protein MSP-300 plays an essential role in nuclear anchoring during Drosophila oogenesis*. Dev Biol, 2006. **289**(2): p. 336-45.
82. Raharjo, W.H., et al., *Nuclear envelope defects associated with LMNA mutations cause dilated cardiomyopathy and Emery-Dreifuss muscular dystrophy*. J Cell Sci, 2001. **114**(Pt 24): p. 4447-57.
83. Ye, Q. and H.J. Worman, *Interaction between an integral protein of the nuclear envelope inner membrane and human chromodomain proteins homologous to Drosophila HP1*. J Biol Chem, 1996. **271**(25): p. 14653-6.
84. Kelkar, P., et al., *Nesprin-2 mediated nuclear trafficking and its clinical implications*. Nucleus, 2015. **6**(6): p. 479-89.
85. Starr, D.A. and M. Han, *Role of ANC-1 in tethering nuclei to the actin cytoskeleton*. Science, 2002. **298**(5592): p. 406-9.

Legends to Figures:

Figure 1. Potential roles of nesprin-1/2 at the LINC complex

The schematic shows (A) nesprin-1/2 play multiple functions at both INM and ONM. Large nesprin-1/2 isoforms localise at the ONM, forming the SUN-KASH bridge at the perinuclear space, and linking the nucleus to the actin cytoskeleton via the N-terminal CH domains. Small nesprin isoforms lacking the CH domains such as nesprin-1 α also localise at the ONM, forming the microtubule LINC via interaction with microtubule motor proteins KLC-1/2 and centrosomal protein Akap450. Meanwhile, small nesprin-1/2 isoforms interact with NE proteins: emerin, SUN1/2 and lamin A/C at the INM. The LINC architecture strengthens the connection from inside of the nucleus to the cytoskeleton and maintain the cellular homeostasis in muscle cells. (B) How nesprin-1/2 mutants disrupt the LINC complex, contributing to pathogenesis of muscle disease. Red* indicates where the nesprin-1 mutants are; number 1-3 indicate dysfunctions of the disrupted NE-LINC complex caused by nesprin mutants: 1. structural defect; 2. defects in nuclear migration and positioning; 3. dysregulated cell signalling and gene expression. NE: nuclear envelope; LINC: linker of nucleoskeleton and cytoskeleton; ONM: outer nuclear membrane; INM: inner nuclear membrane; KASH domain: Klarsicht/ANC-1/Syne Homology; CH domain: calponin homology domain; SUN: Sad1p/UNC84.

Figure 2. Nesprin-1/2 mutations in muscle diseases

The validated nesprin-1/2 mutations associated with human muscular disorders (DCM-red, EDMD-dark blue, CMD-light blue and AMC-violet) are presented on the nesprin-1/2 giant isoforms respectively. Mutations identified in Finnish DCM patients, which have not been experimentally validated and characterized, are shown in grey shadow. DCM: dilated

cardiomyopathy; EDMD: Emery-Dreifuss muscular dystrophy; CMD: congenital muscular dystrophy; AMC: arthrogryposis multiplex congenita.

A.

B.

Nesprin-1 giant

Nesprin-2 giant

Calponin Homology domain

Spectrin Repeat

KASH domain

DCM

EDMD

CMD

AMC