The LINC complex and human disease

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Abstract
The LINC ( linker of nucleoskeleton and cytoskeleton) complex is a proposed mechanical link tethering the nucleo- and cyto-skeleton via the NE (nuclear envelope). The LINC components emerin, lamina A/C, SUN1, SUN2, nesprin-1 and nesprin-2 interact with each other at the NE and also with other binding partners including actin filaments and B-type lamins. Besides the mechanosstructural functions, the LINC complex is also involved in signalling pathways and gene regulation. Emerin was the first LINC component associated with a human disease, namely EDMD (Emery–Dreifuss muscular dystrophy). Later on, other components of the LINC complex, such as lamins A/C and small isoforms of nesprin-1 and nesprin-2, were found to be associated with EDMD, reflecting a genetic heterogeneity that has not been resolved so far. Only approximately 46% of the EDMD patients can be linked to genes of LINC and non-LINC components, pointing to further genes involved in the pathology of EDMD. Obvious candidates are the LINC proteins SUN1 and SUN2. Recently, screening of binding partners of LINC components as candidates identified LUMA (TMEM43), encoding a binding partner of emerin and lamins, as a gene involved in atypical EDMD. Nevertheless, such mutations contribute only to a very small fraction of EDMD patients. EDMD-causing mutations in STA/EMD (encoding emerin) that disrupt emerin binding to Btf (Bcl-2-associated transcription factor), GCL (germ cell-less) and BAF (barrier to autointegration factor) provide the first glimpses into LINC being involved in gene regulation and thus opening new avenues for functional studies. Thus the association of LINC with human disease provides tools for understanding its functions within the cell.

Key words: Emery-Dreifuss muscular dystrophy (EDMD), linker of nucleoskeleton and cytoskeleton complex (LINC complex), neuromuscular disorder.

Abbreviations used: EDMD, Emery-Dreifuss muscular dystrophy; FHA, four and a half lamin domain; LINC, linker of nucleoskeleton and cytoskeleton; LMNA, lamina A/C; NE, nuclear envelope; SUN2, synaptic nuclear envelope; XMPMA, X-linked myopathy with proximal muscle atrophy.

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The LINC ( linker of nucleoskeleton and cytoskeleton) complex

The NE (nuclear envelope) defines the barrier between the nucleus and cytoplasm and features inner and outer membranes separated by a perinuclear space. The inner nuclear membrane contains specific integral and associated proteins, including emerin, lamina A, lamina C, SUN1, SUN2, nesprin-1 and nesprin-2 that are proposed to form a mechanical link, called the LINC complex, tethering the nucleo- and cyto-skeleton via the NE [1] (Figure 1). The LINC components interact with each other at the NE and also with other binding partners, including actin filaments and B-type lamins [2]. Disruption of nesprin-2 giant, SUN2 or actin prevents nuclear movement and centrosome reorientation. The coupling of actin cables to the nuclear membrane for nuclear movement via specific membrane proteins indicates that, like plasma membrane integrins, nuclear membrane proteins assemble into actin-dependent arrays for force transduction. Many nuclear movements are microtubule-dependent. However, nuclear movement to reorient the centrosome in migrating fibroblasts occurs through an actin-dependent mechanism. Linear arrays of outer (nesprin-2 giant) and inner (SUN2) nuclear membrane proteins assembled on and moved with actin cables participate in nuclear movement in polarizing cells [3]. The nucleus is the largest and most rigid cell organelle. Therefore its physical properties contribute critically to the biomechanical behaviour of contractile cells, e.g. during amoeboid migration or perfusion through narrow capillaries. Furthermore, it has been speculated that nuclear deformations could allow cells to sense mechanical stress directly, e.g. by modulating the access of specific transcription factors to their binding sites. Defects in nuclear mechanics have also been reported in a variety of muscular dystrophies, particularly EDMD (Emery–Dreifuss muscular dystrophy) caused by mutations in NE proteins. These findings have provided important insights into the mechanical behaviour of the nucleus under physiological conditions, the distinct mechanical contributions of the nuclear lamina and interior, and how mutations in NE proteins associated with a variety of human diseases can cause distinct alterations in the physical properties of the nucleus and contribute to the disease mechanism [4].

Clinical picture of EDMD
EDMD is clinically very variable and genetically heterogeneous [5]. Emery [6] adopted the term Emery–Dreifuss ‘syndrome’, recognizing that the clinical features may cover more than one disease entity and, more recently, the term...
Figure 1 | Schematic representation of the LINC complex
NPC, nuclear pore complex.

LINC components and EDMD
Emerin, encoded by STA/EMD, was the first LINC component associated with EDMD [16]. STA is located on Xq28. Thus mutations in STA result in X-linked EMD (EDMD1, OMIM #310300). Emerin is anchored with its C-terminal domain in the nuclear membrane [17]. Its precise function is unclear, but working hypotheses include, as a LINC component, effects on nuclear structural integrity, increased cellular susceptibility to mechanical stress damage, alterations in gene expression in response to NE changes and effects on cell proliferation and differentiation [18]. A clear effect of defective emerin on nuclear signalling has been demonstrated [19]. Since the discovery of the gene, more than 100 STA mutations have been identified (http://www.dmd.nl; http://www.hgmd.cf.ac.uk; and http://www.umd.be/EMD/). The mutations are quite evenly distributed over the gene, but seem to occur more frequently at the 3′ end coding for the C-terminal anchoring domain. Most of the mutations (approximately 86%) cause a total lack of emerin. Missense mutations usually result in a milder phenotype.

The LMNA (lamin A/C) gene associated with autosomal dominant and recessive EDMD (EDMD2, OMIM #181350; EDMD3, OMIM #604929) [20,21] is localized on chromosome 1q21-q23. The gene consists of 12 exons distributed over 24 kb of genomic DNA. Alternative splicing in exon 10 results in two different forms: lamin A and lamin C. Furthermore, lamins C2 and Δ10 are also derived from LMNA by alternative splicing. To date, more than 200 different, mostly missense, mutations of LMNA have been reported and collected in locus-specific databases (http://www.hgmd.cf.ac.uk; http://www.umd.be). Lamins, including lamin A, B1, B2 and C, are preferentially localized in the nuclear lamina adjacent to the inner nuclear membrane. Lamins are implicated in DNA replication, organization of chromatin, mechanical stabilization of the nucleus, positioning of nuclear pores and anchoring of nuclear membrane components.

The multiple functions of lamins may explain the clinical variability produced by particular mutations [22,23] and the wide variety of disease phenotypes, collectively called laminopathies, caused by pleiotropic mutations in LMNA [24,25]. Besides EDMD, limb-girdle muscular dystrophy type 1B, Charcot–Marie–Tooth neuropathy type 2B, dilated cardiomyopathy type 1A, familial partial lipodystrophy type Dunnigan, mandibulofacial dysplasia, Hutchinson–Gilford progeria syndrome and restrictive dermopathy also belong to the laminopathies [25].

SYNE1 (SYNE is synaptic nuclear envelope) and SYNE2 are located on chromosomes 6q25 and 14q23 respectively, encoding nesprin 1 and nesprin 2. Nesprin-1 and -2 are multi-isomeric spectrin-repeat proteins that bind both emerin and lamins A/C and form a network in muscle linking the nucleoskeleton to the inner nuclear membrane, the outer nuclear membrane, membranous organelles, the sarcomere and the actin cytoskeleton [26–28]. Thus disruptions in nesprin–lamin–emerin interactions might play a role in the muscle-specific pathogenesis of EDMD. Thus a functional candidate gene approach was applied to associate SYNE1 and SYNE2 to EDMD (EDMD4, OMIM #612998; EDMD5, OMIM #612999) [27]. So far DNA variations in nesprin-1α and nesprin-2β have been found in EMD patients, resulting in amino acid exchanges in SYNE1 as well as in SYNE2. All amino acid exchanges were found in the evolutionarily highly conserved emerin- and lamin-binding domains of the nesprins. Fibroblasts from these patients exhibited

‘Emery–Dreifuss-like syndromes’ has been employed [7]. Clinically, EDMD is characterized by early contractures of elbow and Achilles tendons as well as postcervical muscles that lead to rigidity of the spine and the neck, also known as rigid spine. Frequently, contractures of the Achilles tendons without muscle involvement in the first decade of life are observed as the first signs of the disease. Contractures of the elbows and rigid spine appear mostly in early adolescence. Slowly progressive muscle weakness and wasting may occur in early childhood, typically affecting particular muscle groups. Initially, humero-peroneal distribution can be observed: proximally the upper extremities and distally the lower extremities are affected. In later stages of the disease, the proximal lower extremities will also be affected. Usually, the patients remain ambulant, but, in severe cases, patients may become wheelchair-bound [8]. Cardiomyopathy is clinically the most important aspect of the disease, usually starting after the occurrence of muscular weakness in early adulthood. At onset, conduction defects can be observed, which lead to a high risk of sudden heart death and require pacemaker or defibrillator implantation and, in rare cases, heart transplantation [9]. Muscle biopsies show mild to moderate myopathic features with variation in fibre size and mild fibrosis, but rarely fibre necrosis [10,11]. Nuclear shape changes, including lobulation, herniation and micronuclei, can be observed [12–14]. Electron microscopy can reveal nuclear changes in the form of chromatin clumping and detachment from the nuclear membrane [10,13,15].
morphological nuclear defects and specific patterns of emerin mislocalization. In addition, diminished NE localization of nesprins and impaired nesprin–emerin–lamin binding interactions were a common feature of all EDMD patient fibroblasts. siRNA (small interfering RNA) knockdown of nesprin-1 or -2 in normal fibroblasts reproduced the nuclear morphological changes and mislocalization of emerin observed in patient fibroblasts. Taken together, these data suggest that uncoupling of the nucleoskeleton and cytoskeleton due to perturbed nesprin–emerin–lamin interactions may cause EDMD. The inheritance follows an autosomal dominant pattern. However, in one family, a digenic inheritance was observed. The clinical expression of nesprin mutations vary from asymptomatic, through slightly increased creatine kinase to dilated cardiomyopathy, limb-girdle muscular dystrophy and muscular dystrophy with severe dilated cardiomyopathy requiring heart transplantation at age 26 [27]. Nesprin mutations affecting the giant isoforms have also been shown to be associated with a severe recessive form of myogenic arthrogryposis [29].

Digenic pathogenesis involving components of the LINC complex have also been observed in EDMD patients [30,31]. Digenic mutations in STA and LMNA such as STA c.1A>G, p.0 and LMNA c.1044G>T, p.M348I recently found in a Belgian family (Figure 2) may help to explain the clinical variability in EDMD.

The mutation STA c.1A>G, p.0 leading to a loss of emerin has been shown previously to be causative for X-linked recessive EDMD [16] and would explain the lack of emerin and a pathogenic effect found in the male patient G-13730 by itself (Figure 2). Obviously, the additional presence of LMNA c.1044G>T, p.M348I seems to worsen the phenotypic expression in G-13730. However, the index case’s elder sister, G-14537, is heterozygous only for the STA c.1A>G, p.0 mutation, while clinically showing very mild conduction defects, but no skeletal muscle involvement, which has occasionally reported from female carriers of STA mutations and can be explained by unequal X-inactivation. Conduction defects, without muscle impairment, but on a more advanced level requiring pacemaker implantation, was observed in the LMNA c.1044G>T, p.M348I/STA c.1A>G, p.0 double heterozygous mother G-14538. Considering STA c.1A>G, p.0 as an X-linked recessive mutation [16] means that it probably has no clinical effect on the carrier, G-14538. Thus the dominant LMNA c.1044G>T, p.M348I mutation should explain the cardiac conduction defect in G-14538. On the basis of these findings, LMNA c.1044G>T, p.M348I should be considered causative for a dilated cardiomyopathy (CMD1A) phenotype.

Similarly, digenic pathogenic effects have been shown for mutations in SYNE1 and SYNE2 [27] or in desmin and LMNA [30]. Such cases highlight that digenic inheritance should be considered when the clinical features are atypical or quite variable. All of the LINC components associated with EDMD interact with each other at the inner nuclear membrane [17,32]. But, unlike the majority of muscular dystrophies, which are caused by changes at the sarcolemma, LINC links the nuclear membrane to the actin cytoskeleton, just as dystrophin links the sarcolemma to the actin
cytoskeleton, and this may explain why both nuclear and sarcolemmal protein changes can cause similar muscular disorders.

**Non-LINC components and EDMD**

An example for a non-LINC component is the FHL1 (four and a half LIM domain 1) encoded by the X-linked gene [33] that is associated with EDMD (EDMD6, OMIM #300696). All FHL1 mutations associated with EDMD cause changes in the distal region of the gene and putatively destroy one of the four LIM domains or an NLS (nuclear localization signal) of the three known isoforms of the protein [34]. Surprisingly, to date, no functional relationships or interactions have been found between FHL1 isoforms and lamin, emerin or nesprins. However, the X-linked EDMD associated with FHL1 mutations shows some phenotypic peculiarities specific for XMPMA (X-linked myopathy with postural muscle atrophy) [34]. Besides the typical variable age of onset and clinical intrafamilial variability of EDMD, the FHL1 mutations specifically show atrophy of postural muscles, while other muscle groups are hypertrophic, which may lead to an athletic constitution of the patient. Moreover, hypertrophy of the heart muscle can be observed, finally leading to a hypertrophic cardiomyopathy. Sometimes patients suffer scoliosis. Thus the FHL1-associated X-linkedEDMD shows a substantial phenotypic overlap with XMPMA [34]. FHL1 mutations can also produce other myopathic phenotypes [35].

Recently, LUMA, another nuclear membrane protein and binding partner of emerin and SUN2, encoded by TMEM43, was identified as a component involved in atypical EDMD [36].

**Outlook**

In our cohort of 195 EDMD patients, only approximately 46% of the EDMD patients can be linked to LINC and non-LINC components (Table 1). Thus one can speculate that further genes, each probably less than 5% of all patients, are involved in the molecular pathogenesis of EDMD.

Other LINC components such as SUN1 and SUN2 are promising candidates to be associated with EDMD [37]. In the search for further candidates, non-LINC components, but interacting with individual LINC proteins, should be considered, as shown for LUMA [36]. EDMD-causing mutations in STA/EMD, disrupting emerin binding to the transcriptional repressor Btf (Bel-2-associated transcription factor), GCL (germ cell-less) and BAF (barrier to autointegration factor) provide the first glimpses into LINC being involved in gene regulation and thus opening a new avenue for functional studies [38]. Finally, the association of LINC and LINC-related components with human disease helps us to resolve genetic heterogeneity and clinical variability and provides tools for understanding their functions within the cell as well.

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### Table 1 | Patients identified with mutations in genes associated with EDMD

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<thead>
<tr>
<th>Gene mutated</th>
<th>Proportion of EDMD patients (%)</th>
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<tbody>
<tr>
<td>STA/EMD</td>
<td>19</td>
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<tr>
<td>LMNA</td>
<td>22</td>
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<tr>
<td>SYNE1/SYNE2</td>
<td>2</td>
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<td>FHL1</td>
<td>3</td>
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### References
