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Identification of potential genetic modifiers underlying phenotypic variability in a French family with striated muscle laminopathies

L. Benarroch [1], A. Bertrand [1], M. Beuvin [1], I. Nelson [1], N. Naouar [2], F. Simonet [3], C. Dina [3], C. Pionneau [4], J. Schott [3], R. Ben Yaou [1], G. Bonne [1]
[1] *Sorbonne Université, Inserm, Institut de Myologie, Paris, France;* [2] *ARTbio Bioinformatics Analysis Facility, CNRS, Paris, France;* [3] *Institut du Thorax, Université de Nantes, Nantes, France;* [4] *Sorbonne Université, Inserm, UMS Pass-P3S, Paris, France*

LMNA gene mutations are responsible for a wide spectrum of disorders called laminopathies, the majority of which affecting striated muscles. Among them, Emery-Dreifuss muscular dystrophy (EDMD) and limb-girdle muscular type 1B (LGMD1B) show skeletal muscle involvement of different severity but share the same cardiac involvement, i.e., dilated cardiomyopathy with conduction system disease (DCM-CD) that can also be present in an isolated manner. Clinical heterogeneity is well known among the *LMNA* mutation carriers. Modifier genes have been suggested to explain such variability. The *LMNA* mutation (p.Gln6*), identified in a large French family (named here EMD1), is associated with a wide range of age at onset of myopathic symptoms (AOMS). According to this latter, three phenotypic subgroups have been described within the family: AOMS before 20 years (early AOMS), AOMS after 30 years (late AOMS) and isolated cardiac disease without musculo-skeletal symptoms. Our objective was to identify genetic modifiers underlying the intrafamilial phenotypic variability within EMD1 family. Whole genome sequencing (WGS) was performed in 16 *LMNA*-mutation carriers exhibiting the 3 phenotypic subgroups in EMD1 family. Among the 12 million variants annotated, 2 splice variants with a potential aggravating effect and 1 intronic variant with a potential protective effect have been identified and are currently under functional validation. Moreover, 4 structural variants have been detected only in early AOMS patients. An identity by descent analysis specific to phenotypic subgroups was performed and identified one region shared on chromosome 1, containing the *LMNA* gene. Our results suggest that a single genetic modifier may not be solely responsible for phenotypic variability in this family, but that a combination of several factors is more likely.

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Characterising the molecular consequences of *LMNA*-related congenital muscular dystrophy in patient myoblasts

E. Storey [1], I. Holt [1], S. Owen [1], S. Synowsky [2], S. Shirran [2], G. Morris [1], H. Fuller [1]
[1] *Keele University and RJAH Orthopaedic Hospital, Oswestry, UK;* [2] *University of St Andrews, St Andrews, UK*

Mutations in *LMNA*, encoding nuclear envelope (NE) protein lamin A/C, can cause congenital muscular dystrophy (L-CMD), but the downstream molecular mechanisms that give rise to L-CMD are unclear. Using quantitative western blotting and immunofluorescence microscopy, we characterised the expression and localization of NE proteins during myoblast differentiation in healthy cells, and cells from L-CMD patients. Potential lamin A interactors were then identified in the cells using immunoprecipitation followed by mass spectrometry (MS) analysis, and DIA-MS analysis was used to quantitatively compare the proteome of the L-CMD cells against controls. Although there were no differences in NE protein expression, the L-CMD cells had an abnormal nuclear morphology, whilst emerin appeared to be mislocalized from the NE to the cytoplasm. We identified enrichment of known interactors of healthy lamin A and several new putative interactors, in addition to possible mutant lamin A interactors that were undetected in the healthy cells. DIA-MS analysis revealed differential expression of 124 and 228 proteins in L-CMD myoblasts and myotubes, respectively, compared to control myoblasts and myotubes. Ingenuity pathway analysis revealed relevant enriched canonical pathways associated with the differentially expressed proteins including the synaptogenesis signalling and necroptosis signalling pathways in L-CMD versus control myoblasts, and Huntington's disease signalling, xenobiotic metabolism signalling and insulin secretion signalling pathways in L-CMD *versus* control myotubes. Verification and further study of candidate lamin A interactors will allow us to widen our understanding of lamin A functions in healthy cells and L-CMD patient cells and may indicate what aberrant molecular mechanisms are involved in L-CMD. Additionally, DIA-MS analysis has elucidated proteins and molecular pathways downstream of the genetic mutations which may represent targets for future development of therapies.

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Deflazacort treatment in *LMNA*-related congenital muscular dystrophy: an ongoing Italian cohort pilot study

G. Ricci [1], L. Maggi [2], A. D'Amico [3], C. Fiorillo [4], E. Schirinzi [1], A. Pini [5], E. Pegoraro [6], E. Bertini [3], P. Bernascono [2], G. Lattanzi [7], A. Lo Gerfo [1], G. Siciliano [1]
[1] *University of Pisa, Pisa, Italy;* [2] *IRCCS Carlo Besta, Milano, Italy;* [3] *Bambino Gesù Children's Hospital, Roma, Italy;* [4] *IRCCS Gaslini, Genova, Italy;* [5] *IRCCS Istituto delle Scienze Neurologiche, Bologna, Italy;* [6] *University of Padua, Padova, Italy;* [7] *CNR National Research, Bologna, Italy*

LMNA-related congenital muscular dystrophy (L-CMD) and *LMNA*-linked Emery-Dreifuss muscular dystrophy (EDMD2) with early onset (<5 years) may be considered a continuum phenotype. There is no cure for patients with these neuromuscular diseases. Literature data and ongoing clinical approaches suggest the use of steroids. Starting from the collaborative approach and the multidisciplinary effort of the Italian network for laminopathies, we are planning an open-label prospective cohort pilot study aimed to: *1)* evaluate the effect of the treatment with deflazacort in a cohort of 20 L-CMD or EDMD2 patients with infantile onset, aged 3-30 years; *2)* analyze the secretome profile at basal condition and during steroid treatment, to evaluate variations and establish a correlation between steroid treatment and clinical outcome; *3)* validate selected cytokines as biomarkers for L-CMD and EDMD2. Study protocol: Patients will be monitored for a period of six months. Then, they will start therapy with Deflazacort for 12 months. After 12 months, the treatment will be stopped and the patients will continue to be clinically followed every 3 months until the end of the study. Overall, we expect to evaluate whether a 12-month-treatment with deflazacort is effective in improving clinical outcome measures in L-CMD patients. We also expect to identify a panel of cytokines altered in L-CMD that can be considered as biomarkers of disease.

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Genotype-phenotype correlations in human diseases caused by mutations of LINC complex-associated genes: a systematic review and meta-summary

E. Storey, H. Fuller
Keele University and RJAH Orthopaedic Hospital, Oswestry, UK

The linker of nucleoskeleton and cytoskeleton (LINC) complex is a structure that physically connects the nucleus to the cytoskeleton. Mutations in the genes encoding LINC complex proteins give rise to different human diseases with varying phenotypes including cardiac, skeletal muscle, metabolic, or nervous system pathologies. Some of the mechanisms underlying the genotypes and associated phenotypes remain unclear, making it difficult to identify targets for the development of therapies. We systematically reviewed and analysed published mutations affecting LINC complex-associated proteins to determine whether any patterns exist between the genetic sequence variants and clinical phenotypes. This study revealed that *LMNA*, encoding lamin A/C, is the only LINC-complex associated gene for which mutations frequently cause distinct conditions. We identified a total of 37 different diseases linked to mutations in *LMNA*. Although there appears to be no obvious genotype-phenotype correlations, clusters of *LMNA* variants causing striated muscle diseases were found to occur most often in exons 1 and 6, which may disrupt nuclear lamin assembly and lead to a compromised nuclear lamina. We also found that metabolic disease-associated *LMNA* variants frequently affect the tail domain of lamin A/C, which may hinder interactions between lamin A/C and other proteins. Additionally, a mutation "hot-spot" was identified at exon 6 of *EMD*, the gene encoding emerin. *SYNE1* nonsense mutations also appear to most often cause spinocerebellar ataxia, highlighting the potential for the development of a stop-codon readthrough therapy for the treatment of this disorder. These results provide insight into the varied roles of LINC-complex proteins in human disease and provide direction for clinical diagnosis and future gene-targeted therapy development. Moreover, identifying conditions affected by mutations in different NE proteins may allow for sharing of therapies or therapeutic development strategies.

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