

## Short Report

# *LMNA* mutation position predicts organ system involvement in laminopathies

Hegele RA. *LMNA* mutation position predicts organ system involvement in laminopathies.  
Clin Genet 2005; 68: 31–34. © Blackwell Munksgaard, 2005

Laminopathies are multisystem diseases caused by mutations in *LMNA* encoding lamins A and C. The underlying disease mechanisms likely include mutation effects on the nuclear envelope and on interactions between lamins and transcription factors. At the same time, can a simple genomic attribute – for instance, mutation position within the *LMNA* sequence – predict the complex phenotypic effects? In order to assess this, hierarchical cluster analysis (HCA) was used for assembling 16 laminopathies into two classes based on organ system involvement. Ninety-one reported causative *LMNA* mutations in these laminopathies were then classified according to their position upstream or downstream of the nuclear localization signal sequence (NLS). Contingency analysis was used in order to assess a non-random relationship between HCA laminopathy class and *LMNA* mutation position relative to the NLS. HCA laminopathy class and *LMNA* mutation position were strongly associated ( $p < 0.0001$ ). The odds ratio for general association between an HCA class 1 laminopathy and a mutation upstream of the NLS sequence was 8.4 (95% confidence interval = 2.9 – 24.7,  $p < 0.0001$ ). Although the underlying molecular biology is complex, the findings support the hypothesis that laminopathy phenotype and *LMNA* genotype are non-randomly associated. Furthermore, HCA may be a tool to help with the study of phenotype – genotype associations, or ‘phenomics’.

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Key words: cardiovascular disease – hierarchical cluster analysis – neurological disease – nuclear envelope – phenotype–genotype association

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Received 9 December 2004, revised and accepted for publication 1 March 2005

Systematic correlation of phenotype with genotype is a key goal of the emerging field of phenomics, which is expected to help define complex diseases (1, 2). Lessons from phenotype – genotype associations in monogenic phenotypes might provide strategies to deal with such correlations for complex traits. However, even in monogenic diseases, phenotype – genotype associations can often be complex and unpredictable (3). Sometimes, the mutation type is related to quantitative and/or qualitative differences in phenotype, such as a more severe phenotype associated with nonsense mutations than with missense mutations (4, 5), or variable organ involvement depending on which specific amino acid is substituted (6). However, the position of the mutation within the primary nucleotide sequence is usually poorly correlated with phenotype, and *in vitro* functional studies are required in order to resolve the phenotype – genotype associations

(7 – 9). Furthermore, statistical assessment of the phenotype – genotype correlation can be hampered by the relatively narrow ranges of variation in quantitative and qualitative phenotypes in many monogenic diseases, because mutations in most genes are associated with relatively subtle phenotypic differences.

Laminopathies are a family of monogenic multi-system disorders that result from mutation in *LMNA* (MIM 150330) on chromosome 1q21, which encodes nuclear lamins A and C. To date, 16 distinct disease phenotypes have been shown to result from scores of various *LMNA* mutations, including 12 autosomal dominant (AD) and four autosomal recessive (AR) phenotypes (Table 1). Thus, the spectrum of laminopathy syndromes defined by specific tissue and organ system involvement and the number of disease-causing *LMNA* mutations are both large, potentially affording sufficient power for

Table 1. Laminopathies

	MIM number
Autosomal dominant (AD) disorders	
Emery–Dreifuss muscular dystrophy (AD-EMD2)	181350
Dilated cardiomyopathy with CCA (CMD1A)	115200
Early onset atrial fibrillation (EOAF)	607554
Familial partial lipodystrophy – Dunnigan type (FPLD2)	151660
Hutchinson–Gilford progeria syndrome (HGPS)	176670
Atypical progeria syndromes	
Atypical HGPS (AHGPS)	150330
Atypical Werner syndrome (AWRN)	150330.0030
Overlapping syndromes (OLS)	
OLS1 (LIRLLC)	608056
OLS2 (LD + MW + DCM + CCA)	*R28W
OLS3 (LD + DCM + CCA)	150330.0005
OLS4 (CMD1A + QM)	150330.0017
OLS5 (LD + MD + CCA)	150330.0003
Autosomal recessive (AR) disorders	
Emery–Dreifuss muscular dystrophy (AR-EMD2)	604929
Charcot–Marie–Tooth disease (AR-CMT)	605588
Limb-girdle muscular dystrophy (LGMD1B)	159001
Mandibuloacral dysplasia (MAD)	248370

Specific individual mutations can be found under MIM numbers, except for \*R28W (13).

MIM, Mendelian inheritance in man; LD, lipodystrophy; MW, muscle weakness; DCM, dilated cardiomyopathy; CCA, cardiac conduction abnormalities; QM, quadriceps myopathy; MD, muscular dystrophy; LIRLLC, syndrome of lipodystrophy, insulin-resistant diabetes, disseminated leucomelanodermic papules, liver steatosis and cardiomyopathy.

statistical evaluation of phenotype – genotype association. In a small analysis, mutation position within the lamin A tail tertiary structure appeared to be related to either muscle involvement in Emery – Dreifuss muscular dystrophy or adipose tissue involvement in familial partial lipodystrophy (10). However, *LMNA* mutations are considered to be spread homogeneously across the molecule and do not predict obvious dysfunction or a specific phenotype (10). At the same time, the hypothesis that *LMNA* mutation position determines the laminopathy phenotype has not been formally tested.

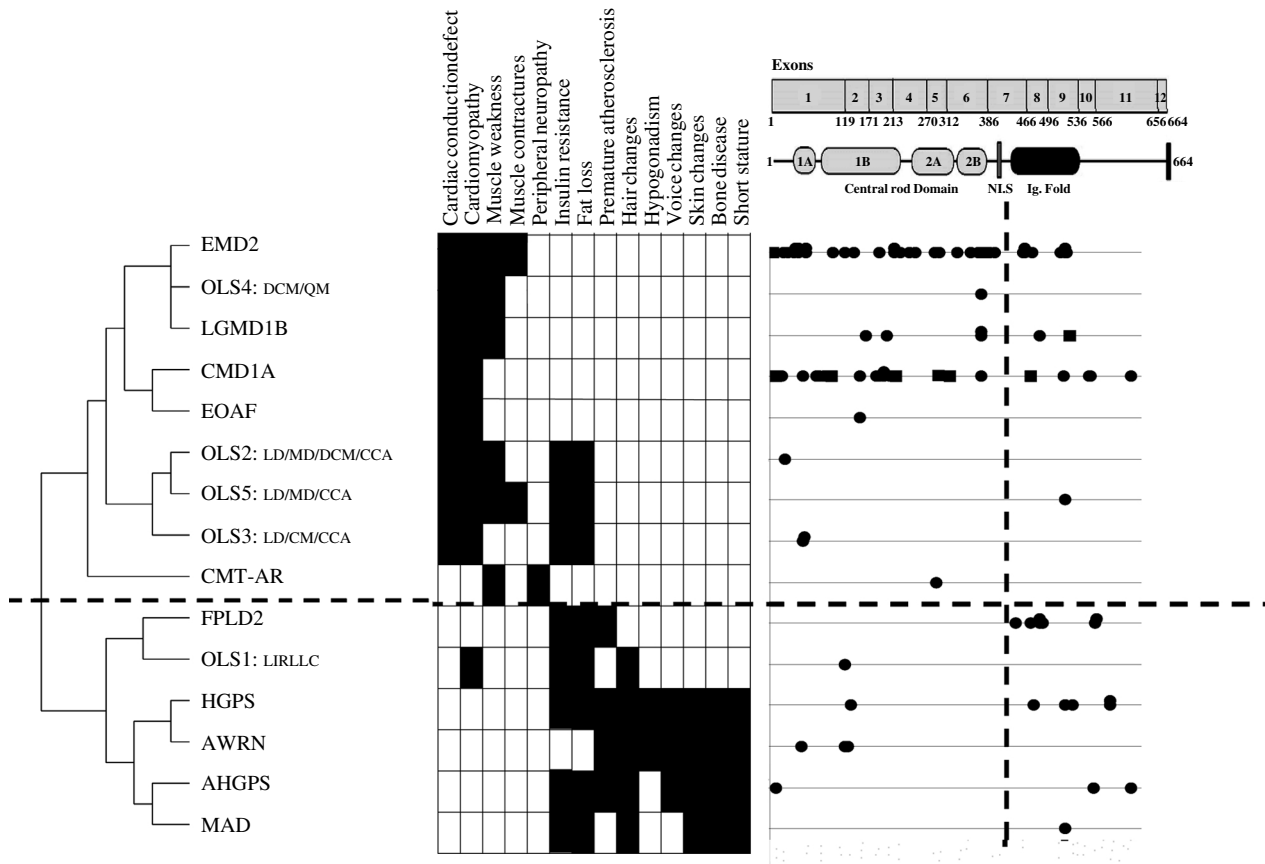
## Methods

### Analytic strategy

Laminopathies were classified according to phenotypic similarity by using hierarchical cluster analysis (HCA). Figure 1 shows a grid with the columns specifying organ and system involvement in laminopathies and the rows specifying the laminopathy type. AR and AD Emery – Dreifuss muscular dystrophy (EMD2) clustered to the same rank and were considered to be the same phenotype for this analysis. This grid was assembled with HCA in *SPSS* v11.5, by using a model assuming complete linkage between groups. A single dendrogram explained the data, with the horizontal branch lengths indicating the correlation coefficient distances between

the laminopathies defined by the organ system involvement. The HCA identified two main classes of laminopathies, as specified by the left-most branch points of the dendrogram. HCA ‘class 1 laminopathies’ included those with cardiac, skeletal muscle and neurological involvement, and in addition several complex overlapping syndromes, whereas HCA ‘class 2 laminopathies’ included simple partial lipodystrophy, progeria syndromes and mandibuloacral dysplasia.

In order to identify a relationship between HCA-defined laminopathy class and the mutation site within the primary nucleotide sequence, *LMNA* was divided into two regions based on nuclear localization signal (NLS) sequence, which spans residues 416 – 423, and within which no mutations have yet been found. The region upstream of codon 416 encoded the central alpha-helical rod domain, whereas the region downstream of codon 423 encoded the ‘homology box’, including the all-beta immunoglobulin-like fold (10) and a DNA-binding region (11). For each laminopathy, missense, nonsense and splicing mutation positions were obtained from human disease gene mutation databases (<http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=150330> and <http://uwcmlls.uwcm.ac.uk/uwcm/mg/search/132146.html>). Mutations were mapped along the linear cDNA sequence, as showed in the right side of Fig. 1, and could be unequivocally classified as being upstream or downstream of the NLS sequence. A total of 91 mutations (nine nonsense or splicing, four small



**Fig. 1.** Schematic diagram of laminopathy–*LMNA* phenotype–genotype association. The grid shows organ system involvement in laminopathies, with darkened cells indicating the presence of the subphenotype. The re-ordering of the diseases compared to Table 1 was the result of the hierarchical cluster analysis (HCA) that clustered the diseases based on similarities in organ system involvement. Horizontal dendrogram branch lengths are proportional to HCA correlation coefficient distances between the laminopathies. ‘Class 1’ and ‘class 2’ laminopathies are above and below the thick horizontal dashed line, respectively. *LMNA* cDNA sequence map is at the top right; thin vertical lines demarcate exon boundaries and the numbers refer to amino acid sequence. Lamin A/C protein structure has been indicated, together with the rod domain, nuclear localization signal (NLS) and immunoglobulin (Ig) fold. Circles and squares show positions of missense/in-frame deletion and missense/splicing mutations, respectively. For mutations, vertical position is collinear with the associated laminopathy, whereas horizontal position indicates linear distance from the cDNA transcription start site. Thick dashed vertical line divides mutations into those upstream or downstream of the NLS. There are, respectively, 54, 16, 6 and 15 mutations in the upper left, upper right, lower left and lower right quadrants formed by intersection of the two dashed lines.

in-frame deletions and 78 missense) were then analysed using  $2 \times 2$  contingency analysis in order to assess the relationship between HCA laminopathy class and *LMNA* mutation site relative to the NLS.

## Results

HCA laminopathy class was significantly associated with mutation site in the *LMNA* nucleotide sequence; 77.1 and 28.6%, respectively, of mutations in HCA class 1 and class 2 laminopathies were upstream of the NLS ( $p < 0.0001$ , Fisher exact test). The odds ratio (OR) for a general association between an HCA class 1 laminopathy and a mutation upstream of the NLS of *LMNA* was 8.4 (95% confidence interval (CI) = 2.9 – 24.7). When

only missense mutations were considered, this OR was also 8.4 (95% CI = 2.8 – 25.1,  $p < 0.0001$ ).

## Discussion

Laminopathies assemble into two HCA phenotype classes based on organ system involvement. Furthermore, HCA laminopathy class has a strong, non-random association with the *LMNA* mutation position relative to the NLS. This phenotype – genotype relationship appears to be more systematic than was previously believed (10). While the phenotype – genotype association does not address mechanism, it can suggest hypotheses for subsequent functional studies. For instance, most of the region upstream of the NLS harbours the conserved rod domain

that is common to intermediate filament proteins and is an essential building block that contributes to the structure and integrity of the nucleoskeleton (10). By contrast, the region downstream of the NLS may interact more closely with non-nucleoskeleton elements, such as DNA, chromatin and/or transcription factors (10). This distinction may be important considering that *in vivo* induced mutant mouse models suggest that *LMNA* mutations can affect either nuclear structure or interactions with transcription factors, or both (12). Various associations with primary structure could underlie differences in pathogenic mechanisms and abnormal function in specific diseases.

This analysis did not account for such important attributes as the existence of lamin A and C isoforms, nor could it begin to reflect the mutational effect on the tertiary structure of lamin A/C or the possible effects on interacting molecules. Nevertheless, HCA appears to classify complex monogenic phenotypes into groupings that might not have been immediately obvious. These groupings were non-randomly associated with a fundamental genomic feature, namely the mutation position in the nucleotide sequence. Because complex disease phenotype databases are increasing in size, HCA might similarly help deconvolute relationships between subphenotypes, in turn helping detect phenotype – genotype associations and generating hypothesis for mechanistic studies.

### Acknowledgements

Matthew Ban and Dr Hafiz Khan assisted with analysis and manuscript preparation. Supported by a Canada Research Chair (Tier I) in Human Genetics and a Career Investigator award from the Heart and Stroke Foundation of Ontario. Laboratory support has come from the Canadian Institutes for Health Research, the Heart and Stroke Foundation of Ontario, the Canadian Genetic Diseases Network, the Canadian Diabetes Association, the Ontario Research and Development Challenge Fund (TFAGA) and the Blackburn group.

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