The effects of dilated cardiomyopathy and atrial fibrillation lamin A/C mutations on phosphorylated kinase C alpha cellular distribution and activity.

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Abstract

Dilated Cardiomyopathy (DCM) with conduction disease and Atrial Fibrillation (AF) are the two cardiac-specific diseases associated with lamin A/C gene (*LMNA*) mutations. Protein Kinase C Alpha, (PKCα) functions as a nodal integrator of cardiac contractility by "sensing" intracellular calcium and signal transduction. PKCα has been implicated in heart failure and cardiac hypertrophy. Moreover, abnormal PKCα function results in irregular atrial potassium channel activity associated with chronic AF PKCα is a lamin A/C binding partner. Thus, the deregulation of PKCα signaling can contribute to the development of DCM and AF.

Our hypothesis is that the AF (Thr528Met), DCM-associated (Arg541Cys) and (Arg541Gly) and DCM/AF-associated (Tyr481Stop) *LMNA* variants will disrupt the cellular distribution of PKCα therefore resulting in impaired PKCα function.

The first objective was to phenotypically characterise Arg541Cys *LMNA* variant in murine skeletal myoblasts cell line (C2C12) in comparison to cellular phenotypes induced by *LMNA* variants associated with AF, DCM and DCM with AF. Arg541Cys lamin A and C variants formed circular and sickle-shaped lamin A/C in the nucleus of C2C12 cells.

The second objective was to determine the effect of these lamin variants on cellular distribution of PKCα in C2C12 cells. PKCα mislocalized into the nucleus of C2C12 cells transfected with AF and DCM-associated variants (Thr528Met and Arg541Cys). Colocalization analysis showed significant increase in PKCα in the nucleus of AF (Thr528Met) and DCM (Arg541Cys) variants when lamin A and C, were co-transfected compared to wild-type, DCM (Arg541Gly) and DCM/AF (Tyr481Stop) variants.

Densitometry analysis showed statistically significant increase in phosphorylated PKCα, the active form of PKCα, in nuclear and cytoplasmic extracts of C2C12 cells expressing Arg541Cys variant. Densitometry analysis also showed statistically significant increase in non-phosphorylated PKCα in the nuclear extract of Thr528Met variant expressing cells.

The third objective was to determine the effect of AF and DCM-associated variants on the activity of PKCα. PKCα activity is quantified by measuring the phosphorylation of a known phosphorylated PKCα substrate. Alpha-6-tubulin phospho (Ser165) is phosphorylated by PKCα. Hence, this was used to quantify PKCα activity. No statistical significance was observed in the level of phosphorylated alpha-6-tubulin at (Ser165) in the C2C12 cells that were transfected with lamin A and C variants compared to wild type. Furthermore, PKCα phosphorylation state is cyclic in nature and this could have had an impact on the phosphorylation state of the chosen substrate in this study.

The functional consequence of nuclear translocation of PKCα with respect to laminopathies is unknown. Abnormal activation of the Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK1/2) which are branches of the mitogen-activated protein kinase (MAPK) signalling cascade in hearts of mice, and humans prior to the onset of cardiomyopathy. These findings have been associated to cardiac disease-causing lamin A/C alteration to signal transduction pathways implicated in heart function and cardiomyopathy. Human LMNA cardiomyopathy, could lead to abnormal activation of MAPK signalling pathways via abnormal PKCα activation in cardiomyocytes.

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List of Abbreviations

Arg541Cys	R541C
Arg541Gly	R541G
Atrial Fibrillation	AT
Atrio-ventricular block	AVB
Barrier to autointegration factor	BAF
Bradycardia	Br
Cyclin dependent kinase	CDK
Dilated cardiomyopathy and conduction disease	DCM-CD
Dulbecco's Modified Eagle's Medium	DMEM
Emery-Dreifuss muscular dystrophy	EDMD
Fetal Bovine Serum	FBS
Fluorescence resonance energy transfer	FRET
Heart failure	HF
Inner nuclear membrane	INM
Intermediate filament	IF
Intra-ventricular block	IVB
Lamina-associated polypeptide 2	LAP2
Lamina-associated polypeptide 2 isoform alpha	LAP2α
Left anterior fascicular block	LAFB
Left bundle branch block	LBBB
Left ventricular exosystoly	LVE
Limb Girdle Muscular Dystrophy Type 1 B	LGMD Type 1B

Mitogen activating protein kinase	MAPK
Mouse embryonic fibroblasts	MEF
Mouse myoblastic cell line	C2C12
Nuclear envelope	NE
Nuclear localization signal	NLS
Nuclear pore complex	NPC
Outer nuclear membrane	ONM
Pacemaker implantation	PI
Paraformaldehyde	PFA
PKC alpha	PKCα
Protein kinase A	PKA
Protein kinase C	PKC
Retinoblastoma protein	pRb
Right bundle branch block	RBBB
Supraventricular tachycardia	SVT
Thr528Met	T528M
Tyr481Stop	Y481X
Universal Mutation Database -LMNA database	UMD-LMNA database
Ventricular extra systoles	VST
Ventricular tachycardia	VT
Wild type	WT

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Chapter One: Introduction

Chapter 1 – Introduction

1.1 Structure of the nuclear envelope

In eukaryotes, the nucleus is composed of two main compartments: the nucleoplasm and the nuclear envelope (NE). The major components of the NE are the inner nuclear membrane (INM), the outer nuclear membrane (ONM), the nuclear pore complexes (NPCs), and the nuclear lamina. The lamins are subdivided into A- and B-types, both of which belong to type V intermediate filament (IF) proteins that form large paracrystalline arrays. They are a major element of the nuclear lamina, a complex and intricate meshwork of proteins that underlie the INM. The A-type lamins are encoded by the gene *LMNA* (McKeon et al., 1986). The B-type lamins are B1 encoded by *LMNB1,* and B2-B3 encoded by *LMNB2* (Moir et al., 2000). B3 is a splice variant of lamin B2, which is expressed in germ cells of mammals (Biamonti et al., 1992; Furukawa and Hotta, 1993; Pollard et al., 1990).

1.1.1 The LMNA gene, structure and expression

The human LMNA gene is found at chromosome 1q21.2 and spans about 57.6 kb with 12 exons (Figure 1.1). Exon 1 codes for the N-terminal head domain and the first part of the central rod domain. Exons 2 to 6 code for the remainder of the central rod domain and exons 7 through 9 code for the carboxyl-terminal tail domain. Exons 11 and 12 are lamin A specific (Lin and Worman, 1993). The major A-type lamins, lamins A and C are produced through alternative splicing within exon 10 of LMNA gene (Fisher et al., 1986; McKeon et al., 1986).

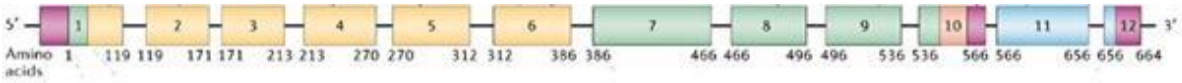


Figure 1.1 Schematic of LMNA gene and its' introns and exons. The LMNA gene is 57.6 kb long and consists of 12 exons. Adapted with permission from Macmillan Publishers Ltd:[Nature Reviews Genetics], (Capell and Collins, 2006),copyright©(2006).

Lamin A and C proteins are expressed in all terminally differentiated somatic cells with the exception of erythrocytes (Rober et al., 1990). There are also two other minor A-type lamin isoforms: lamin Adelta10 (AΔ10) (Machiels et al., 1996) and C2 (Furukawa et al., 1994). Lamin AΔ10 isoform is expressed in variety of tissues and in lung cancer cells higher concentration was observed (Machiels et al., 1996); whereas lamin C2 is expressed in spermatocytes (Furukawa et al., 1994). The specific expressions of the A-type isoforms suggest that lamins have cell and tissue specific functions (Goldman et al., 2002).

Lamin A and C are identical for the first 566 amino acids but differ at the C-terminus where lamin A has 98 unique amino acids and lamin C consists of 6 unique amino acids (Fisher et al., 1986; McKeon et al., 1986). Lamin C and lamin AΔ10 proteins differ in their C-terminal tail domains from each other and from lamin A. Lamin C does not have the C-terminal CAAX sequence whereas lamin AΔ10 contains the CAAX box as well as the site where proteolytic processing of lamin A's C-terminal end takes place (Machiels et al., 1996; Weber et al., 1989). Lamin C2 isoform has an identical sequence to lamin C except the N-terminal segment (non-α-helical head and α-helical 1A domain) is replaced by a short non-α-helical segment (Furukawa et al., 1994).

Lamin A protein is synthesized as precursor, prelamin A, which is composed of amino acids Cys-Ser-Ile-Met (CSIM) / (CAAX motif) at its C-terminus. CAAX motif of the

prelamin A undergoes a multistep posttranslational processing (Figure 1.2) which involves three enzymes. First, a farnesyl moiety is added to the cysteine by the enzyme farnesyltransferase. This allows the endoproteolytic cleavage of the last 3 amino acids (SIM) by zinc metalloprotease related to Step24p (ZmpSte24) (Rusiñol and Sinensky, 2006). After which, the C-terminal cysteine is carboxymethylated by an isoprenylcysteine carboxyl methyltransferase (Icmt) to generate farnesylated/carboxymethylated prelamin A. Finally, ZmpSte24 cleaves the last 15 C-terminal amino acids of prelamin A to produce a mature lamin A (Young et al., 2006). The tetrapeptide CAAX motif post-translational modifications is essential for mature lamin A targeting to the INM (Rusiñol and Sinensky, 2006) and establishment of protein-protein interactions (Rusiñol and Sinensky, 2006). The lamin C-specific amino acids are translated from contiguous DNA after amino acid 566. However, prelamin A processing appears to be dispensable in mice as the direct synthesis of mature lamin A, bypasses prelamin A synthesis and processing, and does not lead to detectable disease phenotype. Thus, prelamin A processing has minimal importance for the targeting of mature lamin A to the nuclear rim in mouse tissue (Coffinier et al., 2010).

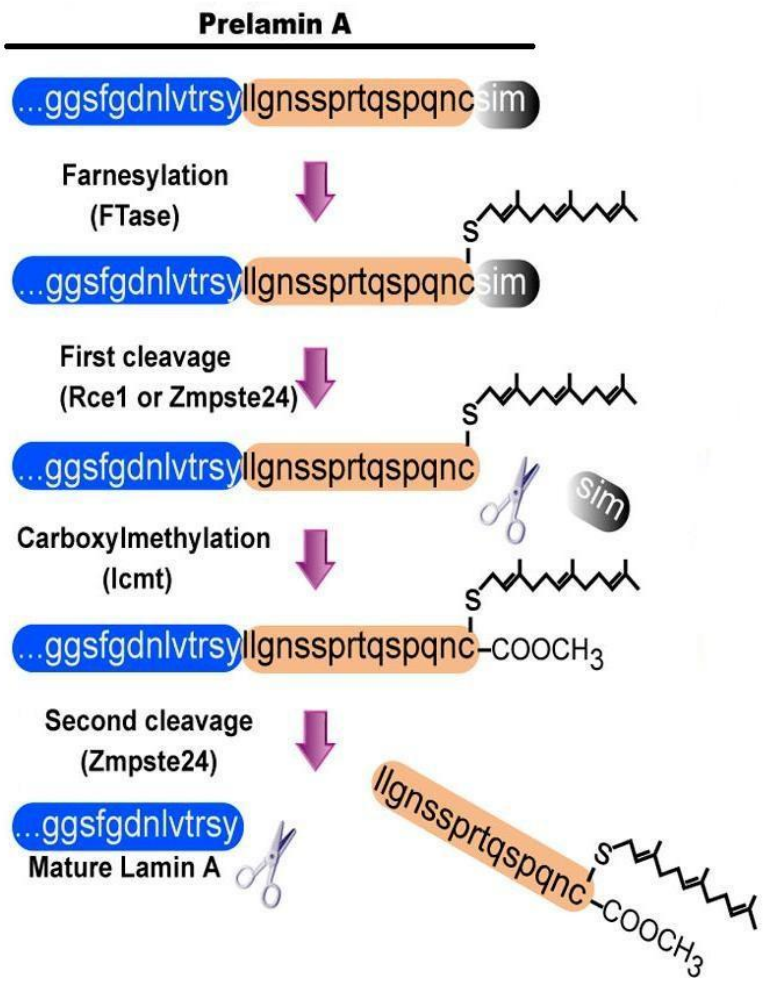


Figure 1.2 Schematic of post-translational processing of pre-lamin A into mature lamin A. The CAAX motif in pre-lamin A is CISM. In the first step a farnesyl moiety is attached to the cysteine residue by farnesyltransferase. Next, the last 3 amino acid residues (SIM) are cleaved by an endopeptidase ZmpSte24. The lone cysteine is then carboxymethylated by an isoprenylcysteine carboxyl methyltransferase (Icmt) to generate carboxymethylated prelamin A. Lastly; ZmpSte24 cleaves the last 15 amino acids upstream of the CSIM motif to produce a mature lamin A. Adapted with permission from The Rockefeller University Press:[Journal of Cell Biology], (Meshorer and Gruenbaum, 2008)),copyright ©(2008).

Metazoan cells contain a variety of lamins. *Caenorhabditis elegans* have only one lamin gene (*lmn-1*) encoded as Ce-lamin, which is closely associated with B-type lamins and it is expressed in all cells except for the mature sperm (Liu et al., 2000). *Drosophila melanogaster* has two lamin genes (*Dm0* and *C*). Lamin *Dm0* is expressed in all cells throughout development, whereas lamin C is initially expressed late in embryonic development and in differentiated cells (Bossie and Sanders, 1993; Gruenbaum et al., 1988).

Lamin A and C are found predominantly at the inner nuclear lamina which is the nuclear rim. They are also found within the nucleoplasm, which is distinct from the peripheral lamin A and C (Hozak et al., 1995). Lamin A and C in the nucleoplasm are more dynamic and less tightly bound to the nucleoplasm than those associated with the lamina (Broers et al., 1999; Moir et al., 2000; Muralikrishna and Parnaik, 2001). Transfection studies demonstrate that wild type lamin A always localizes to the inner nuclear lamina with little nucleoplasmic localization whereas, lamin C forms intranuclear aggregate in the inner nuclear laminar (Fong et al., 2006; Raharjo et al., 2001) (Figure 1.3). Lamin C foci exhibit a close connection with the inner nuclear envelope (Sylvius et al., 2008) and intranuclear lamin C-GFP have shown to be more mobile than intranuclear lamin A-GFP (Broers et al., 2005). Similarly, the lamin C only mouse model expressed lamin C at the inner nuclear lamina. This was established in wild type cells by Fong, Ng et al. (2006) thus indicating the existence of compensatory mechanisms. A study by Pugh et al (1997) also showed that the integration of lamin C into the lamina is facilitated by lamin A in Swiss 3T3 cells.

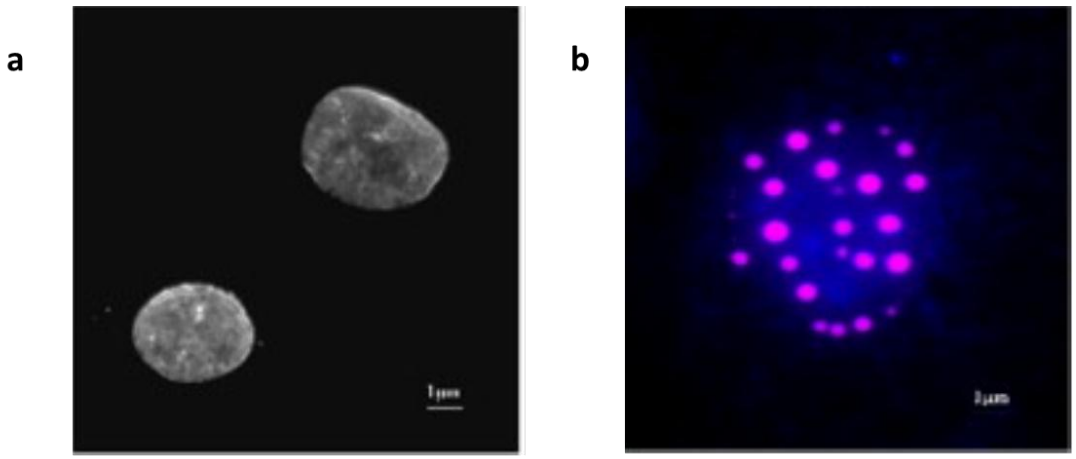


Figure 1.3 Nuclear distribution of lamin A and lamin C. (a) Lamin A is found distributed homogenously at the inner nuclear lamina while (b) lamin C is found as small intranuclear aggregates distributed evenly within the nucleoplasm. Cos7 cells were transiently transfected with (a) lamin A or (b) lamin C in pECFP and pEYFP fluorescent expression vectors, respectively. Cells were visualized using fluorescent confocal microscopy. Adapted with permission from Elsevier: [Journal of Experimental Cell Research], (Sylvius et al., 2008), copyright © (2008).

1.1.2 Structure and Assembly

Intermediate filament (IF) are so named as average diameter of assembled intermediate fibers (10 to 12 nm), are between that of actin microfilaments (7 to 10nm) and that of microtubules (25nm). IF consists of six subtypes and they are characterized on the basis of genomic structure and nucleotide sequence. Nuclear lamins are type V intermediate filament proteins which are only found in metazoan cells.

1.1.2.1 Characteristic Structural Features

Nuclear lamins are comprised of three major domains, a long α-helical domain flanked by globular amino-terminal (head) and carboxy-terminal (tail) domains. The central α-helical or rod domain spans approximately half of the lamin molecule (about 350 residues)

and comprises four α-helical segments termed 1A, 1B, 2A and 2B (Figure 1.4). Each α-helical segment has heptad-repeat periodicity, characteristic of coiled-coil proteins, and is connected by short intervening subdomains denoted L1, L12, and L2. The carboxy-terminal tail domain contains a nuclear localization signal, an immunoglobulin-like domain (Ig) and a CAAX box. The structure of the human lamin A and C Ig domain contains a characteristic Ig fold and forms a compact β sandwich comprising two β sheets formed from nine β strands connected by short loops (Dhe-Paganon et al., 2002; Krimm et al., 2002).

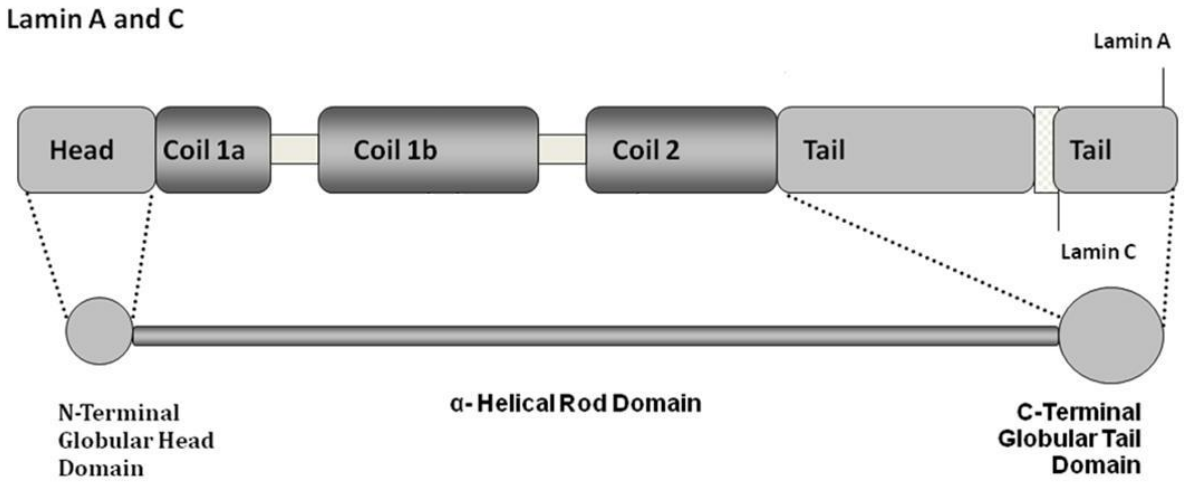


Figure 1.4 Schematic of lamin A and C protein. Checkered portion represents lamin C specific amino acids. Grey bars are linker regions.

1.1.2.2 Assembly of the nuclear lamins

Lamin formation is a three step process: (1) lamins α-helical rod domain, coils around the other to form the lamin dimer. (2) Two or more lamin dimers arrange in parallel in a polar head-to-tail manner which then (3) associate laterally to form an intermediate filament (Figure 1.5). Lamin dimerization is dependent on the presence of the rod domain (Moir et al., 1991). The last 20 residues of the N-terminal head domain are essential for proper head-to-tail polymer formation (Heitlinger et al.; Isobe et al., 2007). The involvement of C-terminal

tail domain in mature lamin formation is more unclear. Although, C-terminal tail domain deletion enhances head to tail polymerization (Heitlinger et al.; Moir et al., 1991)., however they are also seen to associate latterly, staggered or antiparallel (Sasse et al., 1998).On the other hand, high-resolution microscopy of endogenous lamins A and C (Shimi et al., 2008) and fluorescence resonance energy transfer (FRET) analysis of exogenous lamin A (Delbarre et al., 2006) support the existence of separate lamin A and C homopolymers in close contact with each other. Recent biochemical analysis also suggests that lamin A and C form homodimers and homopolymers *in vivo*, via unknown mechanisms (Kolb et al., 2011).

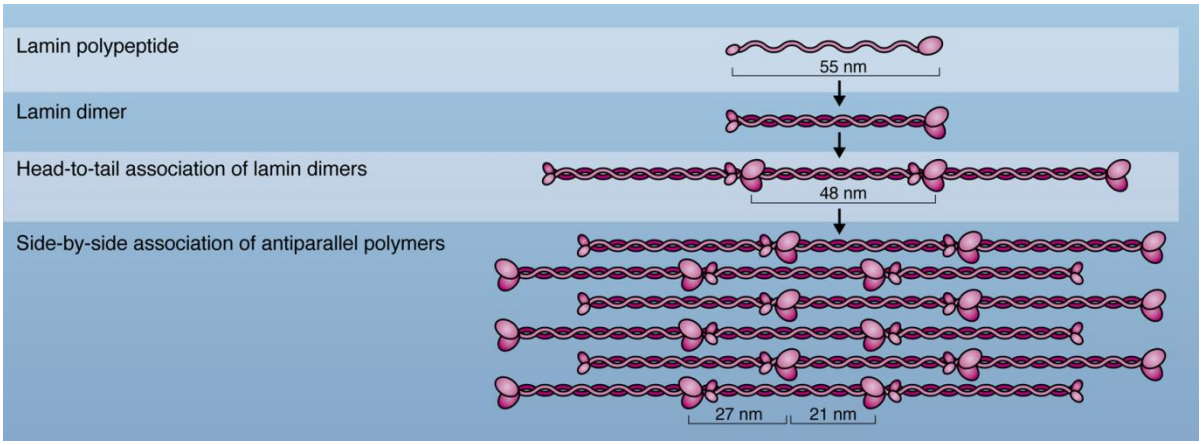


Figure 1.5 Schematic of lamin assembly into filaments. Adapted with permission from The Company of Biologists Ltd: [Journal of Science], (Ho and Lammerding, 2012), copyright © (2012).

1.2 Lamin A and C functions and binding partners

Lamin A and C provide a multitude of functions, including providing structural support to the nucleus, facilitation of chromatin organization; gene regulation, cellular signaling and DNA repair (Dechat et al., 2010; Dechat et al., 2008).

The lamin A and C proteins have over 30 known binding partners (Table 1.1), including proteins of the inner nuclear membrane, transcription factors, enzymes, and chromatin (Heessen and Fornerod, 2007; Ho et al., 2011; Simon and Wilson, 2013). Furthermore, it is important to make note that the C-terminal tail domain of lamin A and C, contains the binding sites for majority of the lamin-binding proteins (Goldman et al., 2002; Schirmer and Foisner, 2007; Simon and Wilson, 2013; Zastrow et al., 2004).

1.2.1 Lamins and chromatin

Lamin A and C interact with chromatin either directly or through histones and other lamin associated proteins, such as lamin B receptor (LBR), heterochromatin protein 1 (HP1), barrier to autointegration factor (BAF), emerin, inner nuclear membrane protein (MAN1), and several lamina-associated polypeptide 2 (LAP2) isoforms (Wilson and Foisner, 2010). These interactions can occur at the nuclear periphery and in the inner nuclear membrane. A direct role of lamins in the regulation of chromatin was demonstrated in studies of cardiomyocytes and mouse embryonic fibroblasts (MEF) derived from *Lmna*$^{-/-}$ mice, which showed a partial loss of peripheral heterochromatin, ectopic chromosome condensation and mid-positioning of centromeric heterochromatin (Dittmer and Misteli, 2011; Nikolova et al., 2004; Sullivan et al., 1999). Moreover, lamins together with emerin, nuclear actin and myosin have been proposed to form intranuclear complexes that are responsible for moving

chromosome segments or genes to transcription sites (Chuang et al., 2006; Dundr et al., 2007; Ho and Lammerding, 2012).

1.2.2 Lamins and nuclear architecture

Lamin A and C are also important for the incorporation and spacing of nuclear pore complexes (Al-Haboubi et al., 2011; Goldberg et al., 1995; Osouda et al., 2005; Smythe et al., 2000), regulation of nuclear size (Levy and Heald, 2010) and in providing shape and mechanical properties of the nucleus (Broers et al., 2004). Cells lacking lamins A and C have fragile nuclei and under mechanical strain, deform easily and thus, display altered mechanotransduction signaling (Broers et al., 2004; Lammerding et al., 2004). In *Drosophila melanogaster,* mutation in lamin *Dm0* showed reduced lamin expression which resulted in severe mutant phenotype affecting viability, fertility and locomotion. The observed cellular changes included NPC clusters, cytoplasmic annulate lamellae, and incomplete nuclear envelopes. This demonstrated that lamin *Dm0* is required for normal nuclear envelope assembly and nuclear pore distribution (Lenz-Bohme et al., 1997).Moreover, RNA interference experiment showed that mutant lamin expression in *C. elegans*, induced abnormalities in the nuclear shape, clustering of nuclear pores and improper segregation of chromosomes during mitosis (Liu et al., 2000).

Table 1.1 Table of lamin A and C interacting proteins including characteristics, domain of interaction and reference

Protein	Characteristics	Reference	Domain of Interaction
Actin	Major constituent of microfilaments and plays an important role in cell shape, movement and structure	Sasseville and Langelier 1998	Carboxy Terminal Domain
Arachidonate 12-lipoxygenase (12R-LOX, ALOX12B)	Oxyreductase converts arachidonic acid to 12R-hydroperoxyeixosateraenoic acid	Tang et al 2000	Residues 463-664
Barrier-To-Autointegration Factor 1 (BAF)	Plays a role in nuclear reassembly by interacting with both DNA and nuclear membrane proteins to recruit chromatin to the nuclear periphery.	Holaska et al 2003	Unknown
c-Fos	Involved in transcriptional and cell cycle control in mammalian cells. Once component of the dimeric AP-1 transcription factor complex in the regulation of a variety of cellular processes (cell differentiation, cell proliferation, neoplasmic transformation and apoptosis).	Ivorra et al 2006	Residues 81-219, 243-388 and 453-571
Core histones, H2A, H2B, H3, H4	Histones play important roles in the maintenance of chromosome integrity, DNA recombination, and DNA replication. Core histones are responsible for creating a protein core around which 146bp of DNA are wrapped in approximately 2 left-handed super helical turns giving rise to the nucleosome core particle.	Taniura et al 1995	Residues 396-430
Cyclin D3 (CCND3)	Forms a complex with CDK4 or CDK6 which are involved in the G1/S transition of the cell cycle.	Mariappan et al 2007	Residues 383-474
E1B 19K	Inhibitor of apoptosis both during viral infection and transformation	Rao et al 1997	Residues 252-390
Emerin (EMD)	Protein of inner nuclear membrane associated with X-lined Emery Dreifuss muscular dystrophy. It mediates membrane anchorage to the cytoskeleton.	Clements et al 2000	Residues 384-566
Epidermal Growth Factor (EGF)	Promotes growth and differentiation. Has profound effect on differentiation of specific cells in vivo and is a potent mitogenic factor for a variety of cultured cells of both ectodermal and mesodermal origin.	Zhong et al 2005	Unknown
hnRNP E1	Involved in several RNA-related biological processes (transcription, pre-mRNA processing, mature mRNA transport to the cytoplasm and translation). Regulates initiation of RNA replication and translation.	Zhong et al 2005	Unknown
Lamin B1	Intermediate filament protein. Component of the nuclear lamina.	Ye and Worman 1995	Unknown
Lamin Companion 1 (Lco1)	Involved in organizing internal lamin network.	Vlcek et al Aug 2004	Residues 394-572
Lamina-associated polypeptides 1A and 1B (LAP1A, 1B)	Integral membrane proteins of the nuclear envelope. May be involved in the organization of synaptic cell-cell contact.	Foisner and Gerace 1993	Unknown
Lem domain-containing (LEMD3, MAN1)	Bind directly to BAF, Has 2 transmembrane domains.	Mansharamani and Wilson 2005	Globular Tail Domain
LEM2	MAN1 related protein. Potential function is structural organization of a subset of NE components.	Brachner et al 2005	Globular Tail Domain Residues 319-566
LUMA	A novel ER protein enriched at the nuclear envelope. It is highly conserved with wide tissue distribution	Bengtsson and Otto 2008	Unknown
Mel18	zinc finger protein localized in the nucleus. A sequence-specific DNA-binding protein and is a negative transcriptional regulator.	Zhong et al 2005	Unknown
Mesenchymal Lim Interacting Protein	Novel lamin interacting partner. Expressed primarily in cardiac muscle, skeletal muscle and brain.	Ahmady et al 2011	Rod 1 Domain

(MLIP)	Essential for muscle differentiation.		
Nuclear Prelamin A Recognition Factor (NARF, IOP2)	Component of the endoprotease complex likely involved in the posttranslational maturation of prelamin A to lamin A.	Barton et al 1999	Carboxy Terminal Domain of prelamin A
Nuclear Titin (TTN, Connectin)	In non-muscle cells, nuclear isoforms are essential for chromosome condensation and chromosome segregation during mitosis.	Zastrow et al 2006	Residues 461-536
Nucleoporin Nup88	Nucleoporin Nup88 resides on the cytoplasmic face of the NPC and is found in a biochemically defined complex with the nucleoporins Nup214 and Nup358 as well as the nuclear export factor CRM1.It is crucial for CRM1 mediated nuclear export.	Lussi et al 2011	Tail domain of lamin A, residues 243-664
Nucleoporin Nup153	Nup153, which is a highly dynamic glycoprotein being involved in multiple nuclear processes, such as nuclear protein import, RNA export, nuclear assembly/disassembly and mitosis	Al-Haboubi et al 2011	Ig fold, Residues 436-544
PCNA (Proliferating Cell Nuclear Antigen)	Nuclear protein. Co-factor for DNA polymerase delta. Helps increase processivity of leading strand synthesis during DNA replication.	Shumaker et al 2008	Residues 436-544
Protein Kinase C Alpha (PKCA, PRKCA)	Calcium activated and phospholipid-dependent kinase involved in signal transduction and cell communication. Normally expressed in cytosol but lots of evidence points to its localization in the nucleus. Reports on isolated nuclei say that PKCA can be found constitutively in the nucleus. Confocal microscopy experiments show that it is found in cytoplasm and nucleus in resting cells. PKCs have been known to phosphorylate nuclear proteins (Buchner 1995 Eur J Biochem).	Martelli et al 2000	Residues 500 - 664
Retinoblastoma 1 (Rb1, Rb)	Regulator of cell proliferation and differentiation. Tumor suppression factor.	M.A. Mancini et al 1994	Residues 247-355
Sterol Regulatory Element Binding Transcription Factor 1 (SREBF1, SREBP1) a and c	Acts mainly to stimulate cholesterol biosynthesis	Lloyd et al 2002, Capanni et al 2005	Carboxy terminal Domain, Residues 389-664
SUN1	Role in nuclear positioning by connecting nuclear envelope to cytoplasm. NTD positions it at INM and CTD with Nesprin isoforms in NE.	Haque et al 2006	Residues 389-664
Synaptic Nuclear Envelope Protein 1 (SYNE1, Nuclear Envelope Spectrin Repeat Protein 1, Nesprin-1, MYNE-1)	Transmembrane protein of the inner nuclear membrane. Connects the nucleus to the cytoskeleton by interacting with the nuclear envelope and F-actin in the cytoplasm. Expressed primarily in cardiac, skeletal and smooth muscle tissue.	Mislow et al 2002	Unknown
SYNE-2, Nesprin-2, NUANCE	Localizes more to the outer nuclear membrane than Nesprin-1 and is more abundant in cardiac and skeletal muscle than its cousin	Zhang et al 2005 Libotte et al 2005	Residues 243-387 and 384 -566
Thymopoietin (TMPO, TP, Lamina-associated polypeptide 2A LAP2)	Non-membrane isoform of LAP family. Identified as part of nucleoskeleton and is implicated in nuclear structure organization during cell cycle.	Dechat et al 2000	Residues 319-572
Ubiquitin Conjugating Enzyme E21 (Ubc9, UBE1)	E2 conjugating enzyme of SUMO pathway. Functions in transfer of ubiquitin or SUMO to an active cysteine residue of a substrate protein.	Zhong et al 2005	Unknown
Zinc Finger Protein 239 (ZNF239, MOK2)	Transcription factors able to recognize DNA and RNA and nuclear ribonucleoproteins.	Dreuillet et al 2002	Residues 243-387

1.2.3 Lamins and the cytoskeleton

Nuclei are linked to the cytoskeleton through various structural proteins, including B-type lamins, NUP153, lamina-associated polypeptide 2 isoform alpha (LAP2α), nesprins, SUN domain-containing proteins, nuclear actin, and protein 4.1R (Ho and Lammerding, 2012). These interactions form structural network that could enhance nuclear stability. The interaction of lamins with SUN proteins and nesprins (Méjat, 2010) is known as linker of nucleoskeleton and cytoskeleton (LINC) complex (Crisp et al., 2006). The LINC complex is important for the intracellular force transmission, cell migration and cell polarization.

1.2.4 Transcription

Lamin A and C play a large role in the regulation of gene transcription and are associated with numerous transcription regulators, that affect cellular proliferation, differentiation and apoptosis (Wilson and Foisner, 2010). For example, the loss of lamins A and C lead to the reduction of retinoblastoma protein pRb (RB1), due to proteolytic degradation, which altered cell cycle dynamics (Johnson et al., 2004; Markiewicz et al., 2002; Moiseeva et al., 2011; Nitta et al., 2007).

1.2.5 Cellular Signaling

Lamins through protein-protein interactions are thought to regulate the activity and availability of proteins within signaling cascades including the retinoblastoma (Rb), Wnt/β-catenin, transforming growth factor beta (TGFβ) and mitogen activating protein kinase (MAPK) pathways.

1.2.6 Post-translational Modification

Lamins are extensively post-translationally modified. Mature lamin A can be acetylated (Schreiber and Kennedy, 2013) *O*-GlcNAcylated (Bolte and Cordelières, 2006; Hübner et al., 2006) or phosphorylated (Dundr et al., 2007; Haas and Jost, 1993; Hübner et al., 2006) at various positions or SUMOylated by SUMO1 (Simon et al., 2013) SUMO2 in the rod domain (Zhang and Sarge 2008) or SUMO3 in the tail domain (Shimi et al., 2008).

1.2.6. 1 Phosphorylation

A-type lamins contain 61 known phosphorylation sites in which more than 30 are conserved. These sites are phosphorylated by multiple kinases (Eggert et al., 1991; Simon and Wilson, 2013). However, two phosphorylation sites Ser-568 and Ser-570 are unique to the lamin C isoform (Simon and Wilson, 2013). Lamins are activated, modified and modulated by three kinases: mitotic cyclin dependent kinase (CDK1), protein kinase C (PKC) and protein kinase A (PKA). Phosphorylation site at Ser-22 in human lamin A and C located in the head domain is evolutionarily conserved and is phosphorylated by CDK1 (Peter and Reimer, 2012) along with Ser-392 which are important for head-to-tail association of lamin A and C dimers (Dechat et al., 2008). At the beginning of mitosis A-type lamins disassemble in a phosphorylation-depended manner by mitotic kinase CDK1 (Fields and Thompson, 1995).Phosphorylation sites at Ser-404- and Ser-406 are required for depolymerisation of lamin A and C filaments during mitosis by CDK1 (Haas and Jost, 1993; Heald and McKeon, 1990; Molloy and Little, 1992). Phosphorylation of the A-type lamin at Ser-37 in *Drosophila*, (homologous to human lamin A and C Ser 22) increased the solubility of the lamin protein and eliminated its ability to interact with chromatin *in vitro* (Delbarre et al., 2006).

During mitosis, lamins are also regulated by the protein kinase C (PKC) family (Hookana et al., 2012; Tikkanen et al., 2012). In zebrafish, lamins are phosphorylated by PKC prior to CDK1 (Simon et al., 2013), thus suggesting PKC phosphorylation could 'unmask' sites for CDK1 phosphorylation (Buendia et al., 2001). Many mitotic phosphorylation sites are clustered in the head domain and near the nuclear localization signal (NLS). However, PKC family members are also known to regulate non-mitotic functions of lamins (Simon and Wilson, 2013). Ser-525 which is located in the Ig-fold domain in lamin A and C is reported to be phosphorylated only during interphase (Eggert et al., 1993). In addition, PKC alpha (PKCα), a PKC isozyme was identified as a lamin A and C binding partner during insulin-like growth factor 1 (IGF-1) treatment (Martelli et al., 2000).PKCα will be addressed later in this chapter.

PKA is another kinase that phosphorylates lamin A and C. Human lamin A and C are phosphorylated by S6-kinase II at Ser-404 and the functional significance is currently unknown (Simon and Wilson, 2013; Ward and Kirschner, 1990), however, it has been detected under a variety of cellular conditions. For example, following insulin treatment, the Akt kinase phosphorylates lamin A and C at Ser-404 in HEK 293 T cells (Cenni et al., 2008). Furthermore, cells expressing lamin A bearing the mutations Ser404Ala or Arg401Cys have disorganised lamin networks and nuclear blebbing (Cenni et al., 2008).

1.2.6.2 Sumoylation

Sumoylation is a post-translational modification that involves the covalent binding of the SUMO (small ubiquitin-like modifier) protein to the lysine residue of its substrate (Gareau and Lima, 2010). SUMO modifications regulate localization, function and interaction of target proteins, and influence pathways including nuclear import/export,

transcription, apoptosis, cell cycle regulation, and protein stability (Geiss-Friedlander and Melchior, 2007). Human lamin A and C are shown covalently modified by SUMO2 at Lys-201, both in vivo and in vitro. This modification is important for lamin A and C localization and filament assembly; both activities are disrupted by p.K210R and cardiomyopathy-causing mutations p.E203G and p.E203K (Zhang and Sarge, 2008). In addition, Ubc9, the E2 conjugating enzyme of the SUMO pathway was identified to bind to lamin A and C.

Sumo1 and Ubc9 were found to be mislocalized in C2C12 and Cos7 cells transfected with DCM and EDMD associated lamin A and C mutations *in vitro* and *in vivo*, in primary myoblasts and myopathic muscle tissues from *Lmna* $^{H222P/H222P}$ mice (Boudreau et al., 2012; Sylvius et al., 2005).In cell models, trapping of Sumo1 correlated with an increased steady-state level of sumoylation (Boudreau et al., 2012). The disruption of a critical post-translational modifying process has the potential to affect the post-translational regulation of tissue-specific sumoylated proteins which may lead to the tissue-specific symptoms observed in patients with various laminopathies (Boudreau et al., 2012). Furthermore, lamin A tail domain was shown to be modified, both *in vitro* and in Cos-7 cells by Sumo1. SUMO1 modification of the lamin A tail targeted two residues K420 and K486 (Simon et al., 2013). Lamin C could also be modified by SUMO1 since residues 1-566 of lamin A are identical to lamin C (Simon et al., 2013).

1.3 Lamin A and C mutations and associated diseases

1.3.1 Laminopathies

Mutations in the *LMNA* gene are associated with numerous heritable human diseases. Diseases associated with *LMNA* mutations are collectively termed as laminopathies, which include systemic disorders and tissue restricted diseases (Capell and Collins, 2006;

Verstraeten et al., 2006). Currently laminopathies comprise 17 distinct diseases which include cardiomyopathy, muscular dystrophy, lipodystrophy and premature aging syndromes (Figure 1.6). Manifestation of laminopathies is predominantly associated with mesenchymal tissues, such as skeletal muscle, cardiac muscle, adipose tissue, connective tissue and bone. To date, 458 different mutations from 2,206 individuals have been reported Universal Mutation Database -*LMNA* database (UMD-*LMNA* database) (*http://www.umd.be/LMNA/*). The phenotype-genotype relationship between the location of the mutation and the associated disease phenotype remains obscure. Thus, this raises the question as to why there is such variation in disease phenotype and tissue specificity.

Currently three main hypotheses have been proposed to explain phenotypes resulting from the *LMNA* mutations: 1. the structural hypothesis - the mutations lead to abnormalities in the nuclear lamina resulting in a fragile nuclear architecture thus increased sensitivity to physical stressors and; 2. the gene regulatory hypothesis – *LMNA* mutations alter gene expression profiles either by directly interacting with chromatin or indirectly by disrupting protein-protein interactions (Lammerding et al., 2004); 3. the cell toxicity hypothesis - mutated prelamin A may accumulate within patients' nuclei producing cytotoxicity and disease (Tesson F et al., 2013). It is unlikely the three hypotheses are mutually exclusive and thus, a combination most likely explains these varied phenotypes.

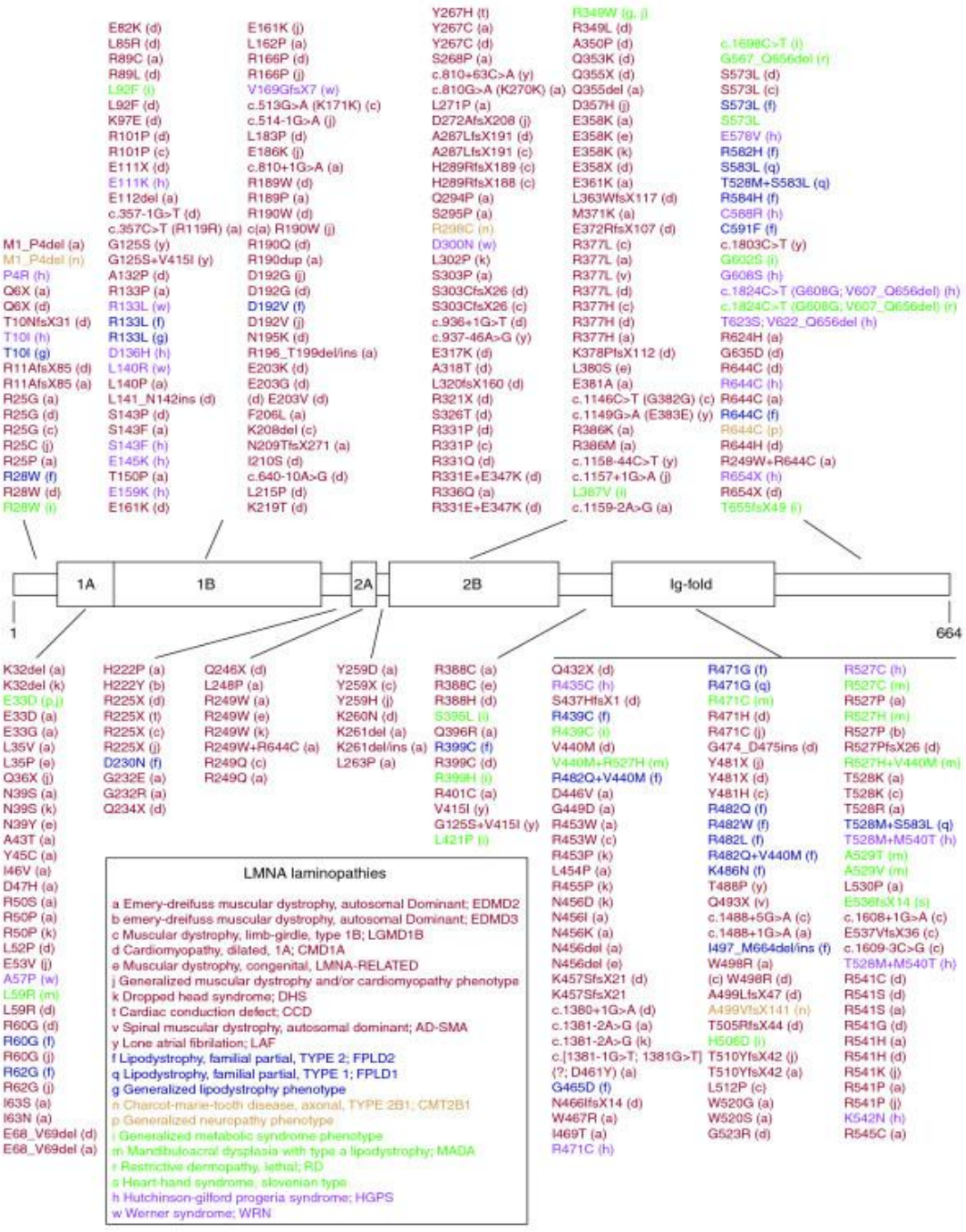


Figure 1.6 Summary of disease-associated LMNA mutations mapped onto the human lamin A/C protein. Colours indicate the class of disease. Mutations affecting amino acids 1 to 566 affect both lamin A and C isoforms, whereas mutations found in the C-terminal 566-664 amino acids are specific to the lamin A isoform. fs, frameshift; del, deletion; ins, insertion; c, coding. Adapted with permission from BioMed Central Ltd: [Genome Biology], (Dittmer and Misteli, 2011), copyright©2011.

1.4 Pathogenesis of *LMNA* related dilated cardiomyopathy

1.4.1 Genotype /Phenotype Correlation

A significant number of the lamin mutations are associated with striated muscle-specific laminopathies. Phenotypic clustering in relation to tissue involvement and age at the onset of disease for striated muscle laminopathies have been observed (Benedetti et al., 2007; Hegele, 2005). The analysis of UMD-*LMNA (http://www.umd.be/LMNA)* database by Bertrand *et al* (2011) showed that nonsense and frameshift mutations are frequently associated with dilated cardiomyopathy and conduction disease (DCM-CD) in patients. These two categories of mutations putatively decrease lamin A and C levels either by nonsense mediated mRNA decay or degradation of the truncated proteins. Decreased lamin A and C levels are associated with DCM-CD patients thus suggesting that the myocardium is more sensitive to decreased lamin A and C content than skeletal muscle (Bertrand et al., 2011; Cattin et al., 2013). Furthermore, Gupta et al (2010) showed that a heterozygous deletion encompassing exons 3-12 of the *LMNA* gene led to a decreased in lamin A and C expression in cardiomyocytes. Moreover this has been confirmed by Narula *et al* (2012) in a recent study in *LMNA* DCM-CD patients in which reduced *LMNA* mRNA levels in blood and myocardium correlated with relative loss of lamin A and C in cardiomyocytes.

Benedetti et al (2007) studied two groups of *LMNA*-mutated patients presenting with cardiac and/or neuromuscular symptoms either with onset in the first decade of life or patients with onset within or after the third decade of life. Eighty-nine percent of early-onset patients carried missense substitutions and in-frame deletions maintaining the reading frame of the putative protein, whereas 37% of late-onset patients, and remarkably 60% of those with cardiac disease, harbored frameshift mutations presumably leading to a truncated

protein (Benedetti et al., 2007). In addition, missense variants associated with late-onset DCM-CD were predominantly found in coil 2B, while Ig-like domain and coil 2A were associated with early-onset (Benedetti et al., 2007). This suggests that late-onset phenotypes could emerge due to the loss of function of lamin protein in addition to haploinsufficiency such that residual lamin may mask clinical symptoms. In contrast, early-onset patients mostly harbour in-frame mutations which produce full-length protein, that is located in the Ig-fold domain involved in protein interactions. Therefore missense mutations could derail molecular interactions that are critical for proper cellular signaling (Cattin et al., 2013).

1.4.1.1 Cardiac-specific Laminopathies

A-type lamins are expressed in all differentiated cell types. Dilated Cardiomyopathy (DCM) and Atrial Fibrillation (AF) are the two cardiac-specific diseases associated with mutations in the *LMNA* gene. Majority of *LMNA* mutation carriers with various forms of muscular dystrophy develop cardiac complications such as conduction defects, arrhythmias, and left ventricular dilatation (Sylvius and Tesson, 2006).

Dilated Cardiomyopathy

Dilated cardiomyopathy (DCM), one of the most common forms of heart muscle disease is characterized by the dilatation of one or both ventricles, impaired systolic contraction, myocytes death and myocardial fibrosis (Luk et al., 2009). It often leads to heart failure and is one of the two most common indications for heart transplantation. DCM is clinically heterogeneous as patients may be symptomless but can also have very severe heart failure. This disease usually manifests in middle age but incidence in pediatric populations is 1.13 cases per 100,000 individuals (Karkkainen and Peuhkurinen, 2007; Wilkinson et al.,

2010). Idiopathic DCM (IDC) accounts for 10,000 deaths annually in the US (Dec and Fuster, 1994). About 30-50% of idiopathic DCM cases are estimated to be of genetic origin (Grunig et al., 1998). Although familial DCM is generally inherited following an autosomal dominant pattern (about 70%), autosomal recessive, X-linked, and mitochondrial transmission also exist (Grunig et al., 1998). Inherited DCM shows an age-dependant penetrance. This has been substantiated for DCM caused by *LMNA* mutations; a meta-analysis by Van Berlo *et al* (2005) revealed that cardiac conduction disease/dysrhythmias usually develop below the age of 30 years, followed by heart failure up to 20 years later. The genetics of familial DCM is complex and includes over 50 disease-causing genes. DCM associated genes include those encoding sarcomere, cytoskeletal, nuclear envelope, the Z-disk, the mitochondria, the sarcoplasmic reticulum, and RNA binding proteins, as well as proteins that are involved in the regulation of calcium metabolism (Hershberger and Siegfried, 2011; Karkkainen and Peuhkurinen, 2007; Parvari and Levitas, 2012). *LMNA* is mutation are most frequently associated with DCM in approximately 6% patients and 33% of patients with *LMNA*-DCM are associated with conduction disease (Cattin et al., 2013; Sylvius and Tesson, 2006). Most patients with *LMNA* mutations present with conduction defects in addition to typical DCM symptoms and present with a worse prognosis than other forms of DCM (Sylvius et al., 2005; Sylvius and Tesson, 2006). The disease is characterized by malignant ventricular tachycardia, extreme bradycardia (Br) due to high degree atrioventricular block (AVB), atrial standstill and end-stage heart failure. Even if sudden death from arrhythmias is prevented by implanting defibrillator, the progressive heart failure eventually becomes resistant to treatment leading to heart transplantation (Meune et al., 2006).

Atrial Fibrillation

Atrial fibrillation (AF) is the most common form of cardiac arrhythmia involving the atria. AF is characterized by severe contractile dysfunction that is caused by rapid and irregular atrial activity at the beginning of each heartbeat. AF is typically identified by the absence of a P wave on an ECG. The prevalence of AF rises with increasing age, ranging from <1% in young adults to 2.3% in people over 40 years and > 5% in those older than 65 years (Feinberg et al., 1995). In addition, the prevalence is strongly influenced by the presence of underlying diseases such as hypertension, valve disease, myocardial infarction and ischemic heart disease (Kannel et al., 1982; Krahn et al., 1995). However, it can be present without discernible cardiovascular disease – lone AF and this is found in 15% of all AF cases (Darbar et al., 2003).

Recently our collaborators have identified a patient with AF without the DCM component at Thr528Met. Overexpression study of the AF-associated *LMNA* variant in C2C12 mouse myoblasts results in the formation of sickle-shaped lamin A/C aggregates within the nucleus of C2C12 cells, a phenotype that has never been reported to be associated with *LMNA* mutations (Malek et al., 2011) and is further explored in this thesis.

Protein Kinase C alpha

Previous studies have shown that cytosolic PKCα rapidly translocates to the plasma membrane upon activation. PKCα has also been observed at the nuclear lamina and within the nucleoplasm (Leach et al., 1989; Neri et al., 1994). PKCα is a known binding partner of lamin A and C. PKC phosphorylates lamin A on Ser 403 and 404 (Leukel and Jost, 1995), however the characterization of the interaction indicates that PKCα interacts with lamin A and C at the C-terminal tail which is the last 166 amino acids (Martelli et al., 2002; Martelli

et al., 2000). Thus this binding is independent to lamin A and C phosphorylation by PKC at Ser403, and Ser404, Lamin A and C act as a scaffold bringing PKCα to its downstream nuclear substrates such as C23/nucleolin (Martelli et al., 2002), PTB-associated splicing factor, p68 RNA helicase, and hnRNP A3 and L (Rosenberger et al 2002). PKCα is found at perinuclear regions and to a very small extent within the nucleus (Leach et al., 1989; Neri et al., 1994) PKCα functions as a nodal integrator of cardiac contractility by sensing intracellular Ca^{2+} and signal transduction. As such PKCα has been implicated in heart failure (Braz et al., 2004) and cardiac hypertrophy via phorbol myristate acetate (PMA) mediated induction (Vijayan et al., 2004). Furthermore, abnormal PKCα function results in irregular potassium ion channel (I_{Kach}) activity associated with chronic AF (Voigt et al., 2007). Thus, the deregulation of PKCα signaling can contribute to the development of DCM and AF.

Mammalian PKCα is a serine/threonine kinase and member of the classical PKCs, which has four conserved (C1 to C4) regions. PKCα consists of 672 amino acids and is distributed in all tissues, in contrast to other PKC isotypes whose expression is restricted in the particular tissues. PKCα plays important roles in the control of major cellular functions: proliferation, apoptosis, differentiation, motility. PKCα's distinct cellular responses are due to regulated dynamic interactions with other factors. PKCα is activated by the release of a pseudo-substrate from the active site when increase in diacylglycerol (DG) and Ca^{2+} ions in the cell upon stimulation occurs (Dutil and Newton, 2000) . Also, kinase activity of PKCα is regulated by phosphorylation of three conserved residues in its kinase domain: the activation-loop site p.Thr-497, the autophosphorylation site phospho-Thr-638 and the hydrophobic c-terminal site phosphor-Ser-657 (Bornancin and Parker, 1997). Two forms of PKCα have been characterized: PKCα1 and PKCα2 with different substrate specificities in

skeletal muscle. PKCα1 phosphorylates histone IIIS and peptides derived from epidermal growth factor (EGF) receptor and glycogen synthase to a greater extent than PKCα2. Furthermore, incubation of crude muscle extracts with PKCα1 and/or α2 produce different protein phosphorylation patterns (Schmitz-Peiffer et al., 1996) .

1.4.1.2 Skeletal Muscle-Specific Laminopathies

Autosomal Dominant Emery Dreifuss Muscular Dystrophy (AD-EDMD), and Limb Girdle Muscular Dystrophy Type 1 B (LGMD1B) are the two striated-muscle-specific laminopathies that are associated with *LMNA* gene (Broers et al., 2006). In 1999, Bonne *et al* found a family of AD-EDMD patients with *LMNA* mutations. AD-EDMD is characterised by early joint contractures of Achilles tendons, elbows and rigid spine with slow progressive weakening and atrophy of muscles of the upper arms and lower legs which later extends to the muscles of the shoulders and hips (Emery, 2002). Usually after the onset of muscle disease, cardiac symptoms are observed which are characterized by conduction system defects with atrial paralysis and DCM that can result in stroke and sudden death (Sanna et al., 2003).

1.4.2 Intra-Familial Variability

In some laminopathies there is no clear distinction between phenotypes. Different members in the same family had DCM with conduction defects, DCM with EDMD-like skeletal muscle abnormalities, and DCM with LGMD-like skeletal muscle dystrophy (Brodsky et al., 2000). Similarly, a deletion in exon 6 causing a frameshift at codon 320 have presented with classical DCM, mild AD-EDMD and LGMD1B (Brodsky et al., 2000). The Arg644Cys mutation can cause DCM-CD (Genschel and Schmidt, 2000), FPLD (Rankin and Ellard, 2006), skeletal myopathy (Mercuri et al., 2005) and atypical Hutchinson Guildford

Progeria Syndrome (HGPS) (Csoka et al., 2004), as well as motor neuropathies (Rankin and Ellard, 2006) indicating a role of modifier genes and/or gene-environment interactions in the phenotypic variability of *LMNA* variants.

1.5. Modeling of Laminopathies

1.5.1 Mouse models

Over the years, various mouse models either expressing knock-in or knockout lamin A and C expressions recapitulating the cardiac symptoms observed in patients have been created in order to elucidate the pathogenesis of *LMNA* related DCM with conduction defects (DCM-CD) (Arimura et al., 2005; Bertrand et al., 2012; Jahn et al., 2012; Mounkes et al., 2005; Sullivan et al., 1999). It is also important to note that the human laminopathies are autosomal dominant whereas mice with laminopathy symptoms have homozygous mutations in all studies except one. Indeed, Wolf and colleagues showed that heterozygous *Lmna*-null mutation reduced lamin A and C protein levels in hearts and caused early-onset cardiac conduction system disease and late onset DCM in mice. These phenotypes modeled cardiac manifestations caused by dominant human *LMNA* mutations.

Mice lacking lamin A and C *Lmna* $^{-/-}$ were the first model to be generated. These mice developed muscular dystrophy with DCM, axonal neuropathy, reduced adipose tissue with death by 8 weeks of age. More than 80% of embryonic fibroblasts (MEF$^{-/-}$) from these mice had irregularly shaped nuclei with herniations lacking NPCs, lamin B and lap2 (Nikolova et al., 2004; Sullivan et al., 1999). Mice with heterozygous *Lmna* $^{+/-}$ genotype, expressed 50% of lamin A and C and by 10 weeks of age displayed conduction system defects that led to the apoptosis of the conduction tissue and the development of mild ventricular dilation by 1 year of age. Approximately 20% of mice died after 8 months of age

(Chandar et al., 2010; Wolf et al., 2008). Both *Lmna* $^{-/-}$ and *Lmna*$^{+/-}$ mice exhibited an abnormal mechano-transduction pathway (Cupesi et al., 2010; Nikolova et al., 2004). In null mice *Lmna* $^{-/-}$ partial rescue was possible using cardiomyocytes-specific lamin A (Frock et al., 2012).

1.5.2 Cellular Models

Skin fibroblasts isolated from patients with cardiac-or-skeletal-specific laminopathies most often had abnormal nuclear shape, including blebs, herniation, lack of B-type lamins (Muchir et al., 2004), clustering of nuclear pores, large aggregates of highly condensed heterochromatin, micronucleus formation, and accumulation of mitochondria around the nuclear envelope (Chung-Wah Siu, 2012). Patient tissue samples and skin fibroblasts provided a method to visualize the pathophysiology of disease-associated mutations. However, they are not easily acquired and the development of mouse model is a lengthy and expensive process. This has led to the use of established cellular models in an effort to elucidate the mechanisms leading to particular disease phenotype. Commonly used models are based in C2C12 cells and Cos7 cells for the study of laminopathies.

Cos7 cells are immortalized CV-1 cells derived from African Green Monkey's kidney cells which were infected with SC40 monkey virus genome that results in a defective genomic replication. This is a continuous cell line that has fast *in vitro* growth rates and high transfection rates.

C2C12 is a mouse myoblastic cell line isolated from thigh muscle of C3H mice. C2C12 cells differentiate rapidly and form contractile myotubes. They also express muscle-specific proteins. C2C12 cells are used to model striated muscle laminopathies including

DCM because there is no reliable cardiac cell line that expresses cardiac-specific proteins. HL-1 cells are mouse cardiomyocyte cell lines. They have been shown to beat in cultures (Claycomb et al., 1998).However HL-1 cells did not have the proper lamin A and C phenotype when stained for endogenous lamins using antibodies. P19 cells are embryonic mouse stem cells. In the presence of Dimethyl sulfoxide (DMSO), P19 cells differentiate into cardiac cells (Jamali et al., 2001) and are observed to beat in culture when differentiated (van der Heyden et al., 2003) However, only 10% of the P19 cells differentiate into cardiac cells (Gupta, 2010) (M.Sc. thesis), hence very difficult to measure the effect of lamin mutations on endogenous PKCα since it would be masked by the presence of other cell types in the culture. H9C2 rat cardiomyoblast cell line has been widely used as a cardiac cell model. However, these cells do not beat in culture (Watkins et al., 2011). This cell line using lipofectamine and lipofectamine®LTX, gave a transfection efficiency of 10-15%. In addition, H9C2 cells have low expression of several GATA-4 regulated genes (Gupta, 2010). Primary cardiomyocytes were also tried in the lab and they proved to be difficult to transfect with commercially available reagents (Gupta, 2010). Also creating viral vectors carrying wild-type or mutant lamins to infect cell lines is difficult since lamin A and C are required to be transfected in 1:1 stoichiometric ratio. Although, beating cardiomyocytes can be generated from pluripotent stem cells, early attempts at differentiation were poorly reproducible, inefficient, and the yields were relatively low (Yang et al., 2013).

Recently Ho et al. (2011) generated induced pluripotent stem cells (iPSC) from one patient with inherited DCM and two patients with accelerated forms of aging (atypical Werner syndrome and Hutchinson Gilford Progeria). These iPSC lines were pluripotent and in the undifferentiated state displayed normal nuclear membrane morphology relative to

donor fibroblasts. The differentiated iPSC progeny, however, recapitulated the disease phenotypes. These cell lines thus represent a new model system for the study of lamin A/C mutations in vitro and should provide valuable insights into their role in normal physiology and in the development of cardiomyopathic disease syndromes.

Point mutations associated with cardiac and skeletal muscles disease, present with variable phenotypes such as mutant lamin A and C aggregates (Ostlund et al., 2001; Raharjo et al., 2001; Sylvius et al., 2005; Sylvius et al., 2008) as well as honeycomb pattern of lamin A and C (Kandert et al., 2009; Muchir et al., 2004), however other mutations appear to have no effect on lamin A and C localization and assembly (Sylvius et al., 2008). Moreover, when the mutant of each lamin isoform is expressed individually, it presents with a different phenotype compared to the other isoform (Hübner et al., 2006; Ostlund et al., 2001; Sylvius et al., 2005; Sylvius et al., 2008). This suggests that although unable to properly organize in the nuclear rim and form the lamina, lamin A mutants are still able to associate with the nuclear envelope. Lamin C is intranuclear when expressed alone and is re-incorporated into the nuclear lamina when expressed with lamin A (Sylvius et al., 2008). The nuclear lamins play an important role in nuclear stability by providing nuclear architecture. The formation of mutant aggregates indicates the disruption of normal lamina assembly, and hence reduced ability to cope with mechanical stress. Research has shown that mutant lamins have decreased ability to form connections with the inner nuclear membrane (Broers et al., 2005; Sylvius et al., 2008). These studies can lead to the inference that the aggregates are diminishing the lamina stability.

Investigations into the molecular and cellular mechanisms by which specific mutation lead to DCM have shown that point mutations in the *LMNA* gene can affect lamina, filament,

and protein complex assembly in a mutation-dependent manner (Muchir et al., 2004; Sylvius et al., 2008; Wiesel et al., 2008). *LMNA* mutations result in the clustering of nuclear pore complexes, nuclear blebs, loss of peripheral heterochromatin and irregularly shaped nuclei in patient cardiomyocytes (Arbustini et al., 2002; Bilinska et al., 2006; Sylvius et al., 2005; Verga et al., 2003). Furthermore, in cell cultures when overexpressed, the mutant lamins A and C form aggregates within the nucleus and result in the mislocalization of other structural proteins such as emerin, retinoblastoma, RNA splicing factors, and Mok2 (Arimura et al., 2005; Makhnevych et al., 2009; Rodriguez et al., 2001; Sylvius et al., 2008; Zhang and Sarge, 2008).

1.6 Rationale for the Study of DCM and AF-Associated *LMNA* variants

Lamin A and C mutations can lead to altered activation of major signal transduction pathways in cells. Although the mechanisms that connect nuclear envelope to cell signaling is unclear, it is important to elucidate this because (1) altered signaling can be linked to pathological progression, and (2) specific cell signaling therapies could be used to correct signaling defects attributed via *LMNA* mutations (Schreiber and Kennedy, 2013).

Human heart tissue biopsy of patients with *LMNA* DCM-CD showed elevated p38α phosphorylation (Choi et al., 2012; Muchir et al., 2012). Gene expression studies using microarray has revealed the $Lmna^{H222P}$ knock-in mice and humans with *LMNA* cardiomyopathy have abnormal activation of ERK1/2, Jun N-terminal kinase (JNK), p38α signaling pathways, which are branches of the mitogen activated protein kinase (MAPK) cascade and of AKT/mammalian target of rapamycin (mTOR) signaling pathway (Choi et al., 2012; Muchir et al., 2007; Muchir et al., 2009). This abnormal activation has been

detected ahead of cardiac dysfunction. Furthermore, recent work showed that both MAPK and mTOR pathways are also dysregulated in hearts of null mice *Lmna*$^{-/-}$ (Ramos et al., 2012).

How *LMNA* mutation lead to the activation of the MAPK pathway remains to be determined. However, MAP kinases are known to be activated by mechanical stress, and reduced A-type lamin function is associated with impaired activation of mechanosensitive genes in cardiomyocytes (Schreiber and Kennedy, 2013). Interestingly, PKCα can directly or indirectly activate signaling cascade involving all three different branches of the MAP-kinase-signaling pathways described above. Thus, this thesis will determine how PKCα, could mediate the pathological progression of DCM and/or AF in lamin A and C variants by observing the cellular distribution and activity of PKCα. The following variants were chosen as these variants were known to either progress into DCM (Arg541Cys and Arg541Gly), AF (Thr528Met), or DCM/AF (Tyr481Stop) in patients.

The Thr528Met variant (AF) was found in a 72-year-old Caucasian male, with a 7-year history of paroxysmal AF. The individual also suffered from first-degree AV block with a family history of sudden cardiac death (Saj et al., 2012) . *In vitro* expression in C2C12 mouse myoblasts gave rise to characteristic aggregates indicating an uneven distribution of lamin protein within the nucleus. Additionally, in 25% of the cells, the aggregates were not circular, but sickle-shaped. The aggregates appeared to form extensions of lamin protein with nearby aggregates and thus illustrating the sickle-form. Furthermore, approximately 10% of cells exhibited leaking of lamin A/C into the cytoplasm (Labib, S M.Sc. thesis).

The arginine located at amino acid (a. a) position 541 of LMNA is a "hotspot" that has been reported to mutate to serine, histidine, glycine and cysteine. Arg541Ser variant associated with DCM has been reported twice by Sylvius *et al* (2005) and Karkkainen *et al* (2006), whereas Vytopil *et al* (2003) reported a Arg541His variant associated with Emery-Dreifuss muscular dystrophy (EDMD) with cardiac arrhythmia. Our collaborators also found a variant at the same position but with arginine to glycine substitution in a 23-year-old male with a family history of DCM and sudden cardiac death. Cellular transfection of fluorescently labeled lamin A/C with Arg541Gly led to the development of abnormal lamin aggregates in about 80% of the cells. In 60% of these aggregated cells, the aggregates were sickle-shaped as opposed to the common circular aggregates (Malek et al., 2011). Moreover, Saj *et al* (2010) reported a 19-year-old patient with the LMNA Arg541Cys variant. However cellular model of this variant is yet to be elucidated.

A 36 year old patient with severe heart failure and AF was found to have a point mutation in the form of a stop codon, Tyr481Stop. He was given a heart transplant at 40 and has a family history of the disease. The given mutation result in the premature termination of the protein within the tail region. Cellular transfection of fluorescently labeled variant Tyr481Stop lamin A and C led to the development of large aggregates which were between 1 and 10 per cell. These aggregates were found in about 85% of the cells. The majority of cells with aggregates, however, contained between 2 and 4 aggregates per cell.

All four variants used in the course of this study are located in the C-terminal tail region of the lamin A/C protein. The tail appears to promote secondary structural organization of lamin dimers in the formation of longitudinal polar head-to-tail polymers (Sasse et al., 1998). The novel point mutation in the variants studied could cause polarity

change that could cause misassembly of lamin dimers. This misassembly has the potential to make the nuclear envelope more fragile especially in mechanically stressed tissues, such as cardiac muscle. This can possibly explain the clinical phenotypes that are observed in some DCM patients related to *LMNA* mutations. Furthermore, the particular nature and function of lamin A/C and PKCα interaction is unknown. Hence, determining whether the *LMNA* mutations are impairing the interaction between lamin A/C and PKCα and thereby downstream targets is a potential avenue for studying the pathological progression of laminopathies.

1.7 Hypotheses and Objectives of Proposed Study

Hypothesis: the AF (Thr528Met), DCM/AF (Tyr481Stop), and DCM-associated (Arg541Cys and Arg541Gly) *LMNA* variants will disrupt the cellular distribution of PKCα therefore resulting in impaired PKCα function.

In order to study this, three objectives have to be met:

1. Determine the effect of the DCM-associated variant, Arg541Cys, on lamin A and C nuclear distribution compared to the previously described phenotypes of Thr528Met, Tyr481Stop, and Arg541Gly variants.
2. Determine the effect of the AF and DCM-associated variants on cellular distribution of PKCα.
3. Determine the effect of the AF and DCM-associated variants on the activity of PKCα.

Chapter Two: Experimental Procedures

Chapter 2 – Experimental Procedure

2.1 Cloning lamin A into pEYFP vectors

Wild type and mutant lamin A were cloned into pEYFP vectors (Figures 2.1). Previously cloned lamin A-pECFP was subjected to restriction enzyme digestion using *BamH*1 and *Xba*I. The restriction enzyme digestion products were run on 0.6% agarose gel and band of interest was cut for gel extraction (QIAquick Gel Extraction KiQiagen, USA). Empty pEYFP vectors' *Xba*I site coincided with DNA adenine methylation (*dam*). To remove *dam* methylation, empty pEYFP vector DNA was transformed into One Shot INV110 (Invitrogen, New York, USA) competent cells. Empty pEYFP vector DNA was cut with restriction enzyme *BamH*I and *Xba*I in double digestion overnight at 37°C. Digests were run on 0.6% agarose gel and digested bands were cut out for gel extraction. Vector and insert DNA were ligated in a 1:7 ratio respectively, using T4 ligase for 1 hour at room temperature. Samples were transformed into DH5*alpha* (DH5α) Super Competent *E. coli* strain (Invitrogen, New York, USA) and grown in lysogeny broth (LB) supplemented with kanamycin. Direct sequencing was performed to confirm fidelity of the clones.

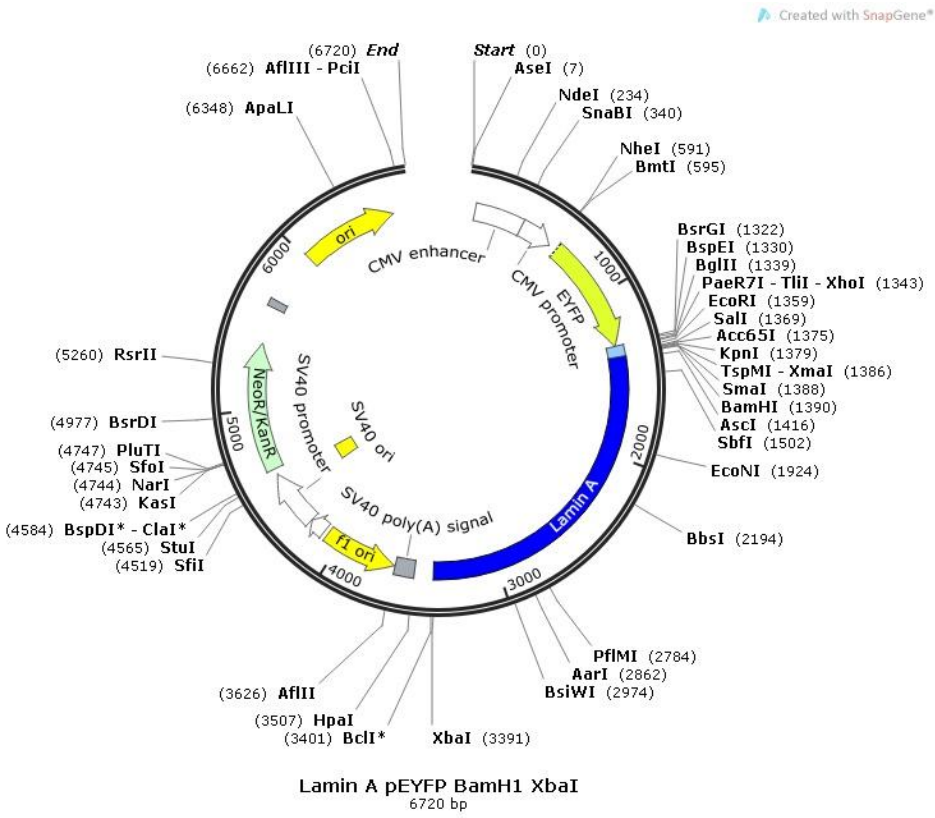


Figure 2.1 Vector map of wild type lamin A in pEYFP vector

2.2 Site-directed Mutagenesis

Site-directed mutagenesis was performed using Stratagene's site-directed mutagenesis kit on previously cloned wild type lamin A-CFP and lamin C-YFP to introduce Arg541Cyt point mutation. The following primers were used: a) Gly541Cyst Forward 5″ GAA GAA GTG GCC ATG TGC AAG CTG GTG CGC TC3″ and b) Reverse 5″GAG CGC ACC AGC TTG CAC ATG GCC ACT TCT TC3″. Briefly, DNA was amplified using primers and High Fidelity PfuUltra Taq Polymerase (Invitrogen, New York, USA). PCR products were digested with *Dpn1* restriction enzyme for 1 hour at 37°C to break down the product to facilitate transformation. DNA was transformed into XL10 Gold *E Coli* (Stratagene, USA) and grown in LB supplemented with kanamycin. Direct sequencing was

performed to confirm presence of mutations. For general upkeep of all clones used, constructs were cloned into DH5α competent cells (Invitrogen, New York, USA). Mini and Maxi preparations of the DNA were performed according to manufacturer's protocols (Qiagen and Invitrogen, respectively USA). All constructs were sequenced prior to use.

2.3 Cell Model and Culture

C2C12 embryonic mouse myoblast cell line was obtained from American Type Culture Collection (ATCC) (Virginia, USA) were used as a muscle model system. The C2C12 cells were grown and maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, New York, USA) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco®, USA) and 1:100 L-glutamine at 37°C and 5% CO_2. Cells were kept below 80% confluence to prevent differentiation.

2.4 Transfection

For protein harvesting, C2C12 cells were transfected with Metafectene-Pro (Biontex Laboratories, San Diego, California, USA) as it provides better transfection efficiency. Cells for microscopy can be transfected with Metafectene-Pro however, it is expensive to do so hence lipofectamine-2000 (Invitrogen, New York, USA) was used. Both methods are based on the properties of lipofection which uses liposomes to inject genetic material. Cells were transfected when they are between 70 to 80% confluence and harvested after 18-23 hours. Briefly, plasmic DNA was incubated with lipofection reagent to allow for DNA-liposome complexes to form. These complexes were then incubated with the cells for 4-6 hours to allow for efficient transfer. The following clones were used: wild type (WT) or mutant lamin A in pECFP (previously cloned, Sylvius et al 2005)/pEYFP, WT or mutant lamin C in

pEYFP (Sylvius et al 2005). All vectors contained CMV promoter for constitutively active expression.

2.5 Cell Fixation and Staining

C2C12 cells grown on coverslips were washed with 1 X Phosphate Buffered Saline (PBS) (Gibco®, USA) three times and fixed with ice-cold methanol for five minutes at -20°C. Methanol was washed off in three washes with 1 X PBS for five minutes each. For PKCα staining, cells were fixed with 4% paraformaldehyde (PFA) for 20 minutes at room temperature, washed three times in 1 X PBS. Following fixation, cells were blocked and permeablilized with 0.1% TritonX100 in 5% FBS for 20 minutes at room temperature. Cells were hybridized with primary antibody anti-PKCα rabbit polyclonal IgG antibody (sc-208) from Santa Cruz Biotechnology (Texas, USA) in 1 X PBS and 1.5% FBS for 1.5 hours at 37°C, washed three times with 1 X PBS then incubated with fluorescently labeled secondary antibody Alexa Fluor 647 goat anti-rabbit IgG (Invitrogen, New York, USA) diluted in 1 X PBS and 1.5% FBS. Cells were washed with 1 X PBS three times. Then coverslips were mounted on glass slides with mounting medium ProLong® Gold Antifade Reagent from Life Technologies (New York, USA). Coverslips made for each transfection are used to estimate transfection efficiency and to assay protein localization. They are visualised using Carl Zeiss Axio Imager Z2 research microscope and Zeiss LSM 510 META AxioVert confocal microscope.

2.6 Colocalization Analysis

Quantitive colocalization analysis of PKCα and lamin A and C was performed using the Image J -1.46r software and JACoP v2.0 plug-in. Threshold Mander's coefficients tM1 and tM2 and the percentage of pixel intensity above threshold colocalised in each of the two

channels (Ch1 for lamin A and C (green) and Ch2 for PKCα (red) were calculated using the threshold algorithm of Costes et al (2004) (Bolte and Cordelières, 2006).

2.7 Cytoplasmic and Nuclear Protein Extraction

Nuclear proteins were extracted using the Active Motif Nuclear Extract kit (AM40010) (Active Motif, California, USA) as described in user manual. Cells were gently scraped in 1 X PBS with phosphatase inhibitors, centrifuged for 5 minutes at 500 rpm in a centrifuge pre-cooled at 4°C. The cells pellet were kept on ice and resuspended in a 1X Hypotonic Buffer to lyse cell membranes. Cell suspension was spun down for 30 seconds at 14,000 x g in a microcentrifuge pre-cooled at 4ºC.The supernatant (cytoplasmic fraction) was then collected into a pre-chilled microcentrifuge tubes. The nuclear pellet was suspended with lysis buffer supplemented with 10mM Dithiothreitol (DTT) and protease inhibitors and incubated for 30 minutes on ice on a rocking platform set at 150 rpm. The supernatant (nuclear fraction) was collected into a pre-chilled microcentrifuge tube after centrifugation for 10 minutes at 14,000 x gravity.

2.8 Western Blot

Samples were run on 4-20% gradient Tris-Glycine (Novex®, Life Technologies, New York, USA) SDS-PAGE gel at constant voltage (120V) for 2.0 hours in a Tris-Glycine Running Buffer (18.8 g glycine, 3.3 g Tris, 1.0 g SDS, 1000 ml ddH_2O).Proteins were transferred onto nitrocellulose membrane overnight at constant current (120mA) in transfer buffer (14.2 g glycine, 3.0 g Tris, 200 ml methanol, 800 ml ddH_2O). The membrane was stained with Ponceau Red staining to visualize protein loading/transfer and blocked with 5-10% skim milk powder in 1X PBS-Tween20 (0.05% Tween20, 1000 ml 1 X PBS) (PBST-milk), and incubated overnight with primary antibody (Table 2.1) or as directed by the

manufacture. The membrane was washed three times in PBST- for five minutes each, incubated with secondary antibody for 1 hour at room temperature, and washed two times with PBST for ten minutes each. Amersham ECL Western Blotting Detection Reagent was added for 2 minutes (#RPN2109) and the protein was visualized using an electrochemiluminescence detecting machine (Bio-Rad VersaDoc Imaging System).

Table 2.1 List of primary and secondary antibodies with company name and country of origin.

Primary Antibody	**Corresponding Secondary Antibody**
Anti- PKCα rabbit polyclonal IgG (sc-208) (Santa Cruz Biotechnology, USA)	Goat anti-rabbit IgG-HRP (sc-2004) (Santa Cruz Biotechnology, USA)
Anti-phospho-PKCα rabbit polyclonal IgG (Ser657) (Millipore, USA)	Goat anti-rabbit IgG-HRP (sc-2004)
Anti-Beta Actin (C4)(sc-47778) mouse monoclonal IgG_1 (Santa Cruz Biotechnology, USA) (housekeeping protein)	Goat anti-mouse IgG-HRP (sc-2005) (Santa Cruz Biotechnology, USA)
Anti GAPDH mouse monoclonal IgG (G041) (abm inc, Canada) (housekeeping protein)	Goat anti-mouse IgG-HRP (sc-2005) (Santa Cruz Biotechnology, USA)
Anti-Alpha 6-Tubulin (Ser-165) phospho-specific rabbit polyclonal (TP4131) (ECM Biosciences, USA)	Goat anti-rabbit IgG-HRP (sc-2004)
Alpha 6 Tubulin (a.a.160-169) (TP4281) rabbit polyclonal (ECM Biosciences, USA)	Goat anti-rabbit IgG-HRP (sc-2004)

2.9 Western Blot Analysis

QuantityOne basic 4.6.3 (Bio-Rad Laboratories, Hercules, CA, USA) program was used for the analysis of Western blot images. Samples were normalized to the respective housekeeping protein to account for protein loading differences. As absolute densitometry values vary greatly between blots, wild-type values were set at 100% and lamin A/C variant values were compared against the wild-type as a ratio. As these calculations have not been previously shown to follow a normal distribution, a non-parametric, unpaired t-test using Mann-Witney Multiple comparison with Bonferroni correction was used with statistical significance set at p, 0.01.

Chapter Three: Results

Chapter 3 - Results

Objective 3.1: Determine the effect of the DCM-associated variant, Arg541Cys, on lamin A and C nuclear distribution compared to the previously described phenotypes of Thr528Met, Tyr481Stop, and Arg541Gly variants.

Initially Arg541Cys *LMNA* variant was described by Forrisier *et al* (2003) in two members of the French family with left ventricular (LV) apical aneurysm, severe ventricular rhythm (SVR) disturbance, left bundle branch block (LBBB). Later, Hookana *et al* (2003) reported in a Finnish family with akinesis of the posterior LV wall, severe ventricular arrhythmias and sudden cardiac death and more recently by our collaborators Malek *et al* (2010).

Arg541Cys has been characterised as an arginine to cysteine mutation. Cysteine is usually classified as a hydrophilic amino acid due to the polarity of its thiol side chain. The thiol side chain often participates in enzymatic reactions, serving as a nucleophile. The thiol is susceptible to oxidation to give the disulphide derivative cysteine, which serves an important structural role in many proteins.

Cellular expression of DCM-associated Arg541Ser presented with no aberrant phenotype. *In vitro* studies have not been performed using Arg541Cys variant. The Arg541Gly and Arg541Cys variants have been shown to occur in DCM associated laminopathies. Hence, it would be interesting to observe if the Arg541Gly and Arg541Cys mutations will have different effects on the cellular distribution of PKCα.

Briefly, point mutations were introduced into lamin A and C fluorescent expression vectors via site-directed mutagenesis. Mutations were confirmed using direct sequencing. The following experiment has been confirmed in three trials.

Cellular transfection of Arg541Cys variant lamin A and C into C2C12 cells were performed. Approximately 50% of the cells developed aggregates of lamin A and C. In 60% of these aggregated cells, the aggregates formed were sickle-shaped as opposed to the common circular aggregates (Figure 3.1). They were similar to the sickles observed in the Thr528Met and Arg541Gly variants. Furthermore, approximately 10% of the cells exhibited leaking of the lamins into the cytoplasm.

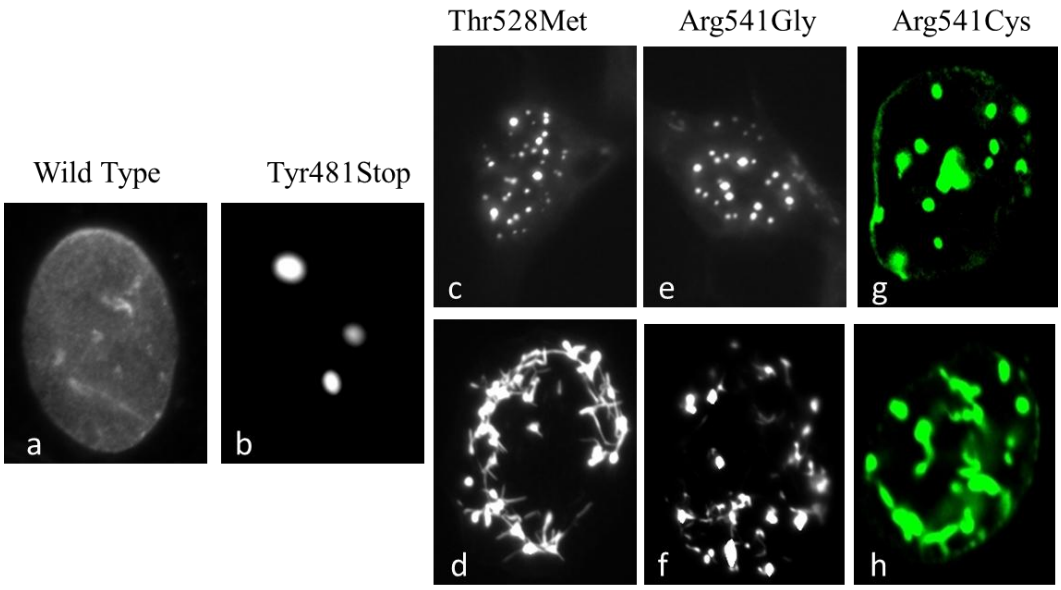


Figure 3.1 The phenotypic characteristic of Arg541Cys variant in comparison to the previously described phenotypes of Thr528Met, Tyr481Stop and Arg541Gly variants. C2C12 mouse myoblast cells were transiently transfected with wild type or variant lamin A and C expressed in fluorescent expression vectors pECFP and pEYFP, respectively. The cells were fixed and visualized by fluorescence microscopy. (a) wild type (Malek et al., 2011), (b) Tyr481Stop, (c-d) Thr528Met showing the cell nucleus with aggregates and sickle-shaped aggregates respectively (Labib, Sarah, MSc., Thesis), (e-f) Arg541Gly showing the cell nucleus with aggregates and sickle-shaped aggregates respectively (Malek et al., 2011), (g-h) Arg541Cys showing the cell nucleus with aggregates and sickle-shaped aggregates respectively. The cells were visualized by fluorescent microscopy.

Objective 3.2: Determine the effect of the DCM and AF variant on cellular distribution of PKCα.

Aim 3.2.1: Identify the cellular distribution of PKCα in C2C12 cells transfected with wild type or variant lamin A-pECFP/pEYFP and lamin C-pEYFP.

C2C12 cells transfected between 18 – 23 hours with wild type or variant lamin A-pECFP and lamin C-pEYFP were fixed and stained for PKCα using primary and secondary antibodies. Lamin A and C, co-localize in all cells. Only three colours can be used for imaging colocalization. This is because overlapping colours are often indiscernible. Thus, creating lamin A and C contained in the same expression vector (pEYFP) would provide a better observation on the cellular distribution of PKCα. Hence, using three different colours, one on lamin A and C, PKCα and the nucleus; a better visualization and quantification of the recruitment of PKCα into the nucleus in the presence of variant LMNA can be achieved. Hence, full-length wild type and variant lamin A cDNAs were inserted into pEYFP (Clontech Laboratories Inc, Mountain View, California, USA).Subsequently C2C12 cells were transfected with wild type or variant lamin A and C in pEYFP (alone or together) using lipofectamine, fixed and stained for PKCα and nucleus using primary PKCα rabbit polyclonal antibody and secondary antibody Alexa Fluor 647 goat anti-rabbit polyclonal and Hoechst 33258 (Invitrogen, New York, USA) respectively.

Result:

When C2C12 cells were transfected with wild type lamin A and lamin C, PKCα was found as expected in the cytoplasm with light staining in the nucleus (Figure 3.2). In cells transfected with Thr528Met (AF) variant lamin A and C, PKCα's presence was increased in the nucleus, while considerable staining was observed within the cytoplasm. Similar phenotype was observed in Arg541Cys and Arg541Gly (DCM) variants. However, no change in the cellular re-distribution of PKCα was observed in cells transfected with Tyr481Stop (DCM/AF) variant. PKCα recruitment is cyclic in nature, hence these experiments were performed at time intervals of 18, 20, 22, 24 hours post-transfection in order to elucidate if PKCα recruitment into the nucleus changes with time. The recruitment of PKCα into the nucleus of wild type, AF, DCM/AF, and DCM variants did not change significantly at different time intervals post-transfection.

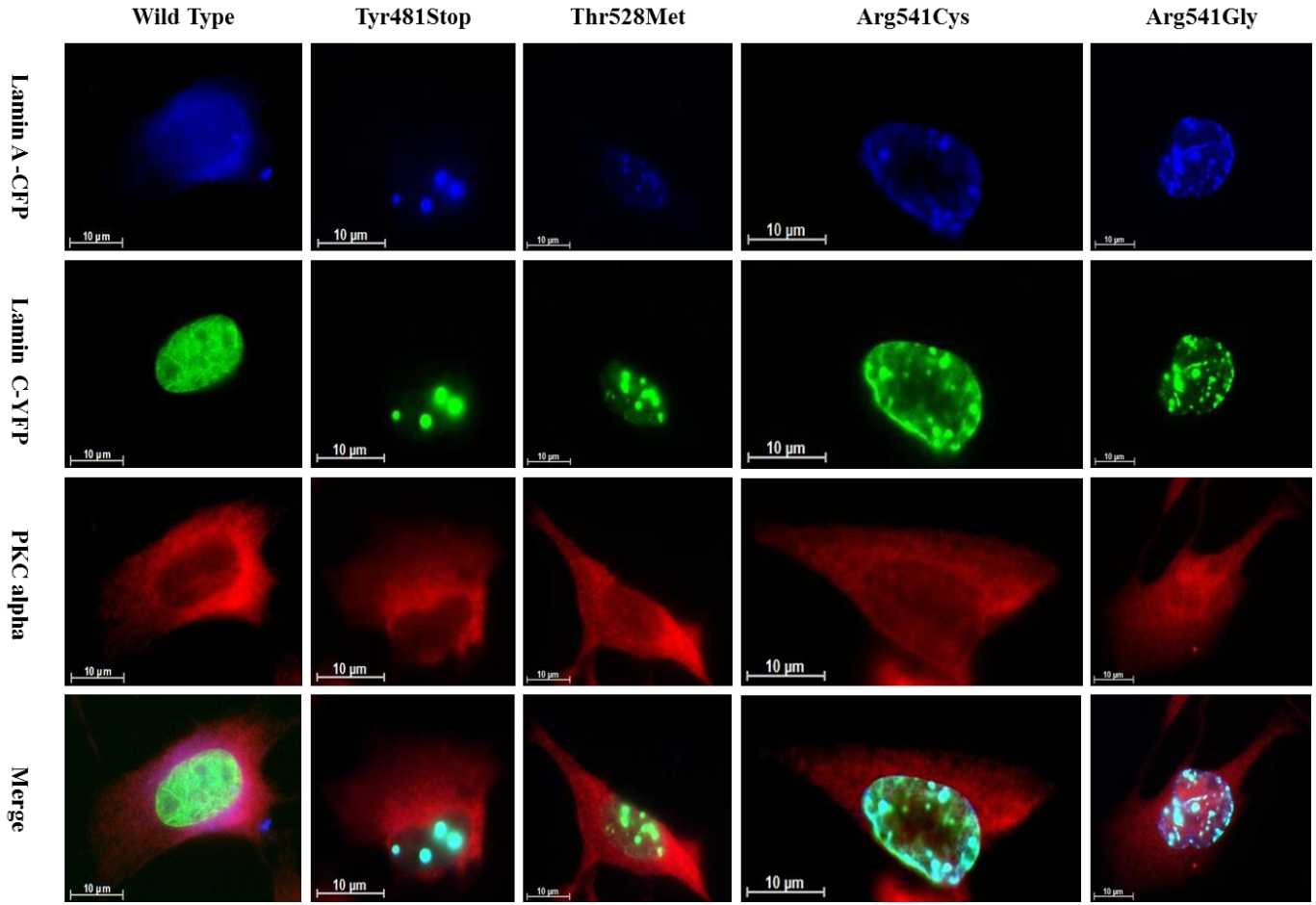


Figure 3.2 Relocalization of PKCα within the nucleoplasm in the presence of Thr528Met and Arg541Gly LMNA variants. PKCα localization is distributed by lamin A/C in a variant-dependent manner. Fluorescent microscopy images of C2C12 cells expressing wild type and variant lamin A-CFP (blue), lamin C-YFP (green) and PKCα (red) (n=3).

Result: Nucleus of cells expressing wild type and variant lamin A/C and PKCα

To illustrate further that PKCα was present in the nucleus; the nucleus was stained with Hoechst (Figure 3.3).The variants Thr528Met and Arg541Cys resulted in increased distribution of PKCα in the nucleus. Wild type and variant lamin A/C Arg541Gly and Tyr481Stop showed PKCα distribution was predominantly in the cytoplasm

Result: Wild type and variant lamin A and C in pEYFP and PKCα

When C2C12 cells were transfected with wild type lamin A and C, PKCα was found as expected in the cytoplasm with light staining in the nucleus (Figure 3.4). In cells transfected with Thr528Met (AF) variant lamin A and C, PKCα's presence was increased in the nucleus, while staining was still evident within the cytoplasm. Similar phenotype was observed in DCM-associated variant Arg541Cys. However, no change in the cellular distribution of PKCα was observed in cells transfected with Arg541Gly (DCM), Tyr481Stop (DCM/AF) variants and wild type lamin A and C. Quantitative colocalization analysis of PKCα and lamin A and C was performed using the ImageJ-1.46r software and JACoP v2.0 plug-in. Thresholded Manders coefficients tM1 and tM2 were used to characterize the degree of overlap between the two channels tM1 for lamin A and C and tM2 for PKCα from the confocal images. Two-sample t-test and Mann-Whitney test were used to perform statistical analysis on Mander's coefficient (tM2) which was obtained from the colocalization analysis of the confocal images (Figure 3.5) (Tables 3.1 and 3.2). Two-sample t-test assumes parametric distribution whereas Mann-Whitney test assumes non-parametric distribution. Therefore, a more robust statistical analysis can be produced when both of these tests are utilized. Statistical ($p \leq 0.05$) significance was observed in the colocalization of PKCα and lamin A/C in the nucleus of AF (Thr528Met) and DCM-associated variant (Arg541Cys).

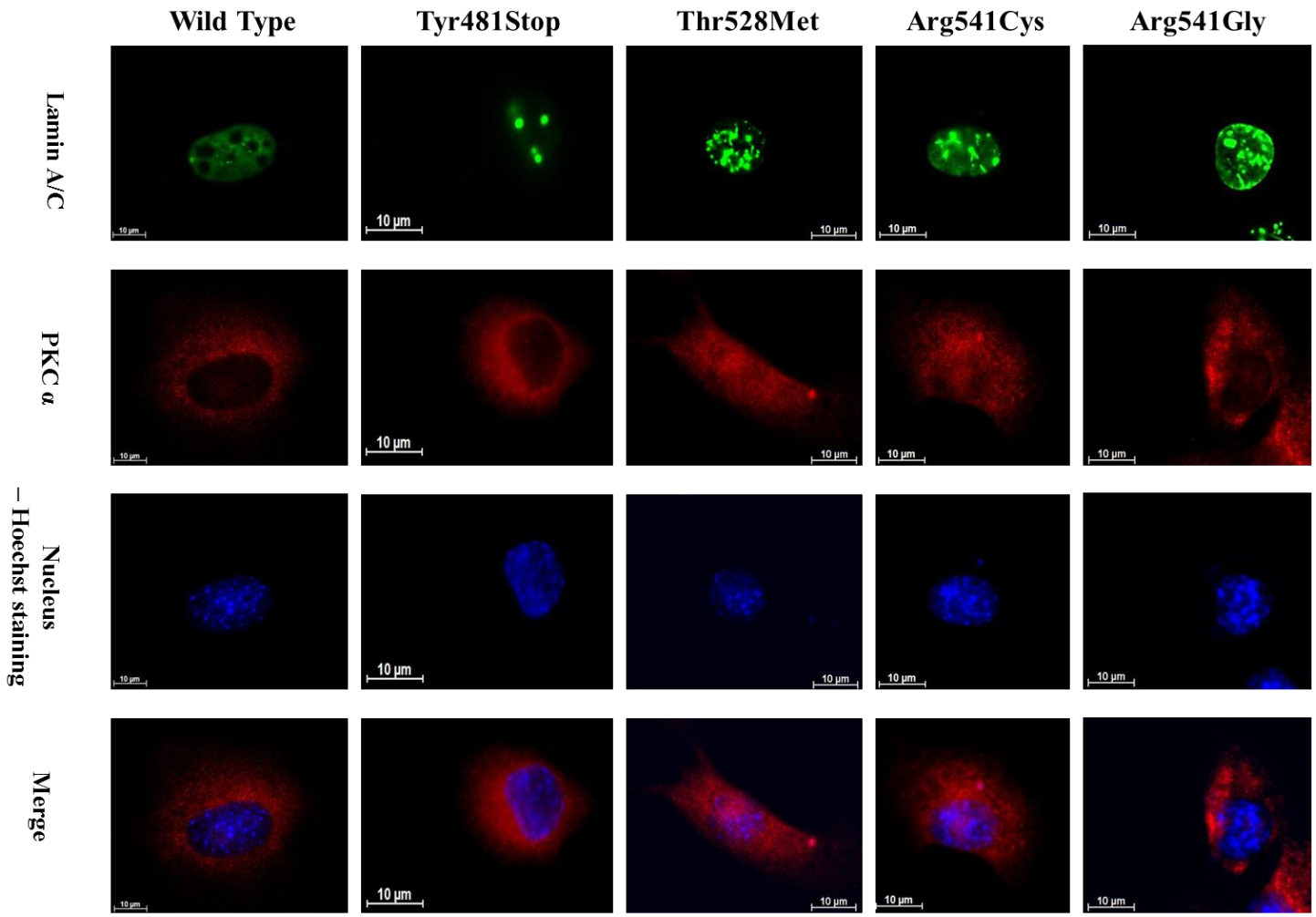


Figure 3.3 Relocalization of PKCα within the nucleoplasm in the presence of Thr528Met and Arg541Cys variants. Fluorescent microscopy images of C2C12 cells expressing wild type and mutant lamin A-YFP, lamin C-YFP (shown in green), PKCα (red), nucleus (blue) (n=3).

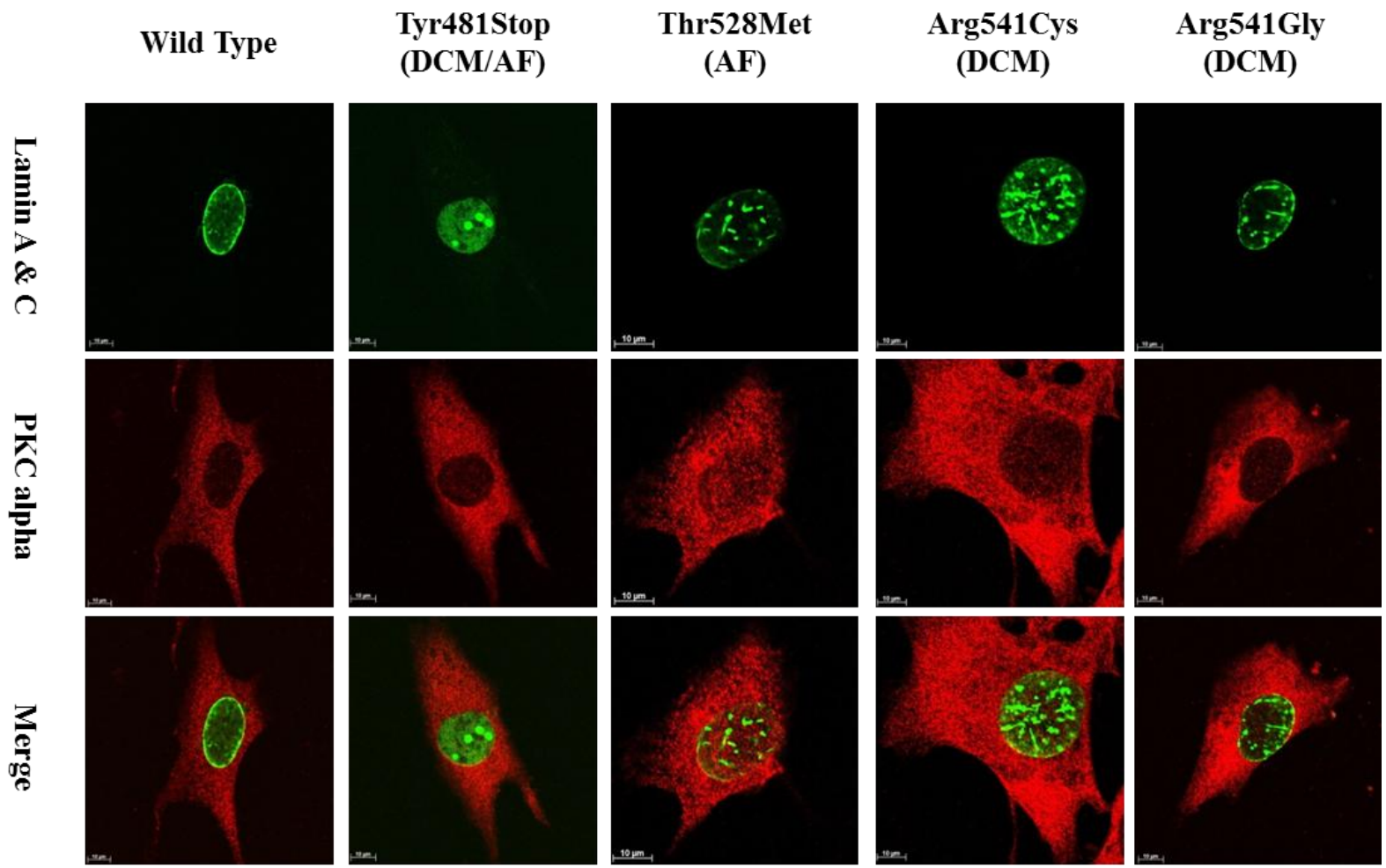


Figure 3.4 Relocalization of PKCα within the nucleoplasm in the presence of Thr528Met and Arg541Cys variants. PKCα localization is distributed by lamin A/C in a variants-dependent manner. Confocal microscopy images of C2C12 cells expressing wild type and variant lamin A-YFP, lamin C-YFP (shown in green) and PKCα (red) (n=3).

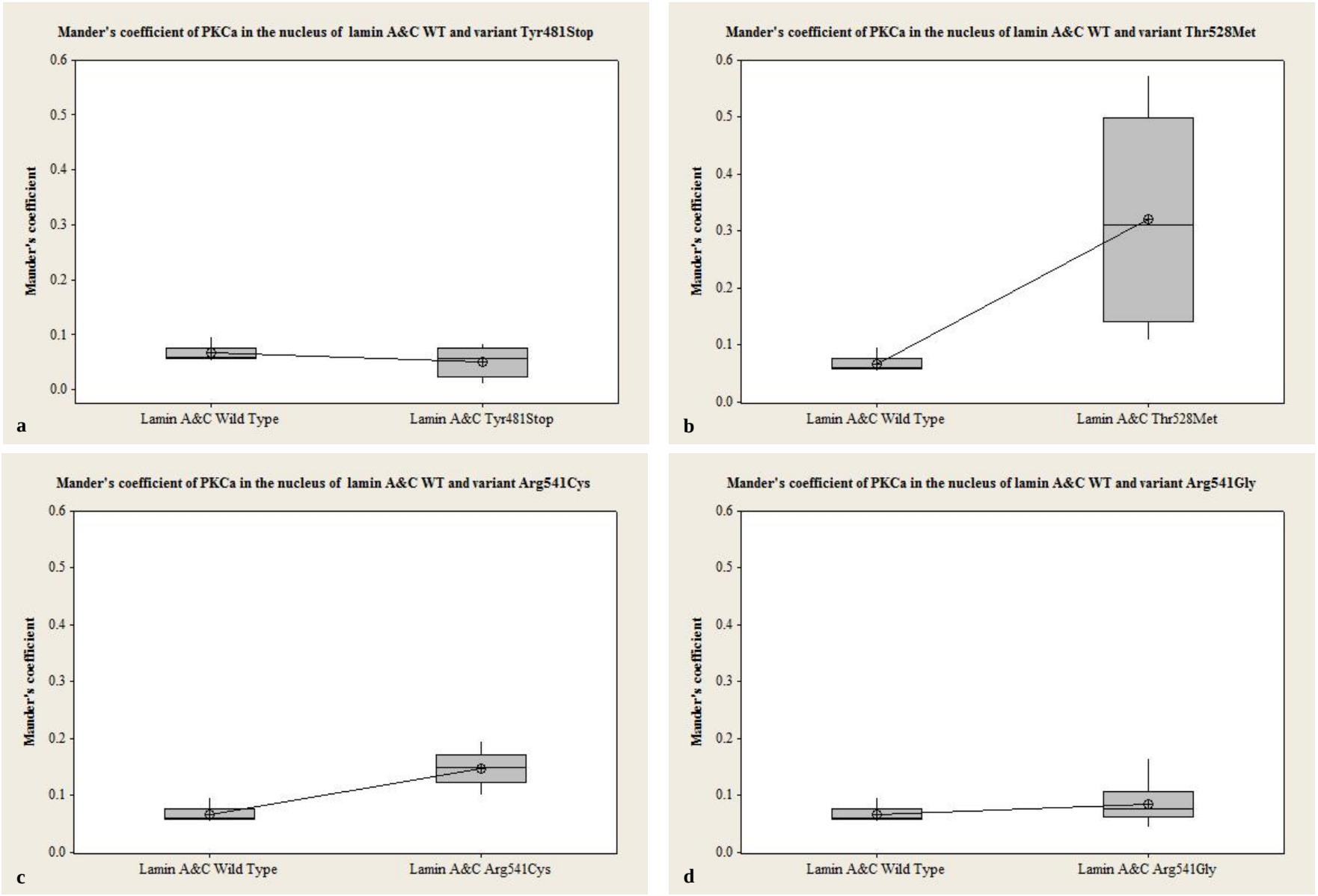


Figure 3.5 Boxplots representing Mander's coefficient of PKCα in the nucleus of lamin A and C in wild type and variants (a) Tyr481Stop, (b) Thr528Met, (c) Arg541Cys and (d) Arg541Gly.

Table 3.1 Quantitive analysis of colocalization of PKCα and lamin A/C using two sample t-test.

Two sample t-test	N	Mean	Standard Deviation	Standard Mean	P-value (p≤0.05)
Wild Type	11	0.0669	0.0135	0.0041	N/A
Tyr481Stop	12	0.0517	0.0270	0.0078	0.102
Thr528Met	9	0.3210	0.1730	0.0580	0.002*
Arg541Cys	19	0.1475	0.0272	0.0062	0.000**
Arg541Gly	11	0.0848	0.0366	0.0110	0.154

Table 3.2 Quantitive analysis of colocalization of PKCα and lamin A/C using Mann-Whitney test.

Mann-Whitney test	N	Median	P-value (p≤0.05)
Wild Type	11	0.0600	N/A
Tyr481Stop	12	0.0575	0.3248
Thr528Met	9	0.3110	0.0002*
Arg541Cys	19	0.14900	0.0000**
Arg541Gly	11	0.07600	0.1891

Aim 3.2.2: Investigate if the cellular distribution of PKCα in C2C12 cells is influenced by lamin A in the variants Thr528Met and Arg541Cys.

C2C12 cells were transfected with wild type or variant lamin A in pEYFP alone using lipofectamine, fixed and stained for PKCα and nucleus using primary PKCα antibody (rabbit polyclonal) and secondary antibody Alexa Fluor 647 (goat anti-rabbit) and Hoechst 33258 respectively.

Result: wild type and variant lamin A and PKCα

We next investigated if the distribution of PKCα was influenced by lamin A alone in the presence of wild type and variants. When C2C12 cells were transfected with wild type lamin A, PKCα was found as expected in the cytoplasm with light staining in the nucleus (Figure 3.6). The presence of PKCα increased in the nucleus, while staining was still evident within the cytoplasm in in Arg541Cys (DCM) variant. However, there was little or no change in cellular distribution of PKCα in cells transfected with wild type and Thr528Met (AF). Also, quantitative colocalization analysis of PKCα and lamin A was performed using the ImageJ-1.46r software and JACoP v2.0 plug-in. Thresholded Manders coefficients tM1 and tM2 were used to characterize the degree of overlap between the two channels tM1 for lamin A and tM2 for PKCα from the confocal images. Two-sample t-test and Mann-Whitney test were used to perform statistical analysis on Mander's coefficient (tM2) which was obtained from the colocalization analysis of the confocal images (Figure 3.6 and 3.7) (Tables 3.3 and 3.4). There was no statistical ($p \leq 0.05$) significance in the colocalization of PKCα and lamin A in the nucleus of DCM-associated variant (Arg541Cys) and AF variant (Thr528Met) (Tables 3.3 and 3.4).

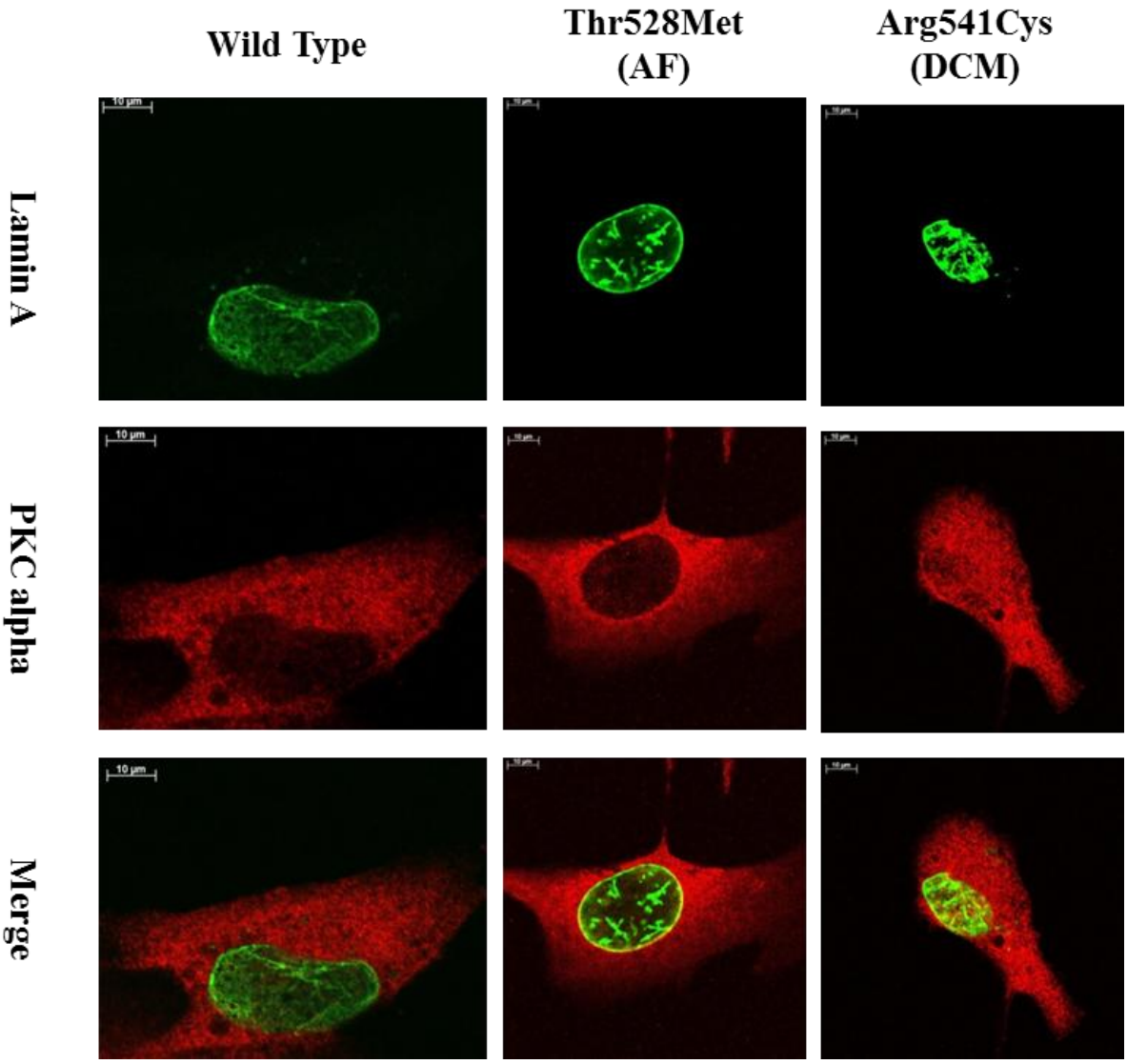
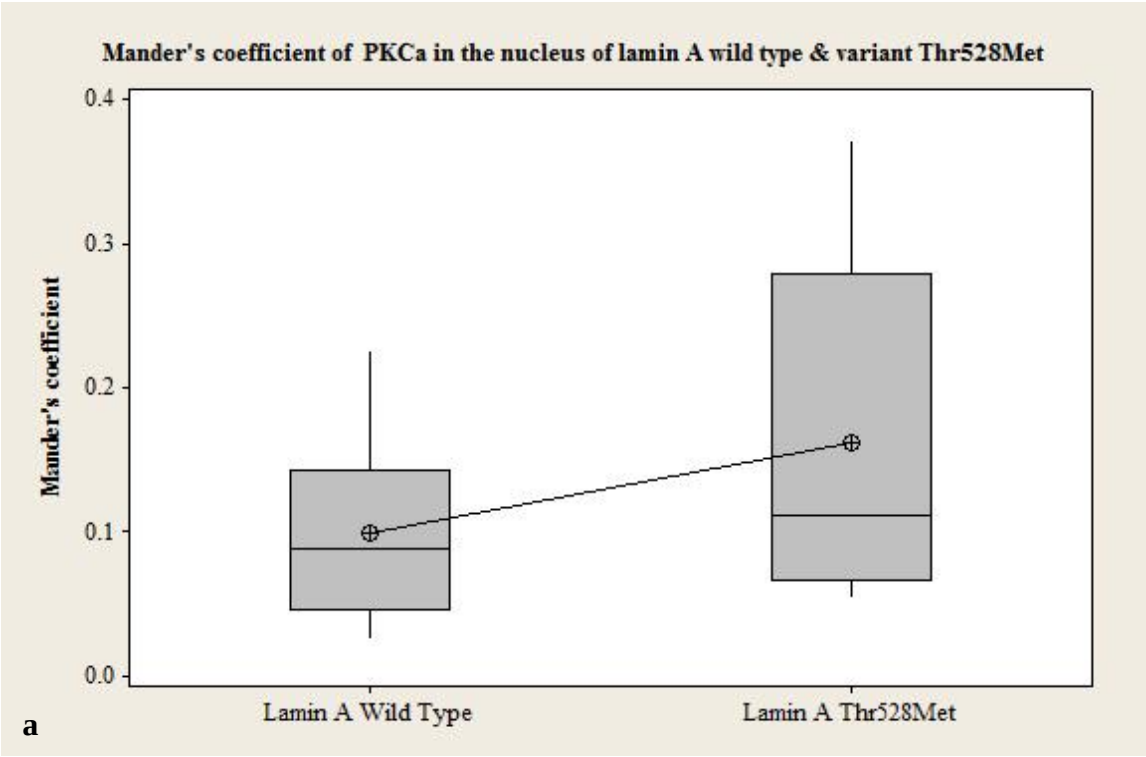


Figure 3.6 Relocalization of PKCα within the nucleoplasm in the presence of lamin A Arg541Cys variant. Confocal microscopy images of C2C12 cells expressing wild type and variant lamin A-pEYFP (shown in green) and PKCα (red) (n=3).



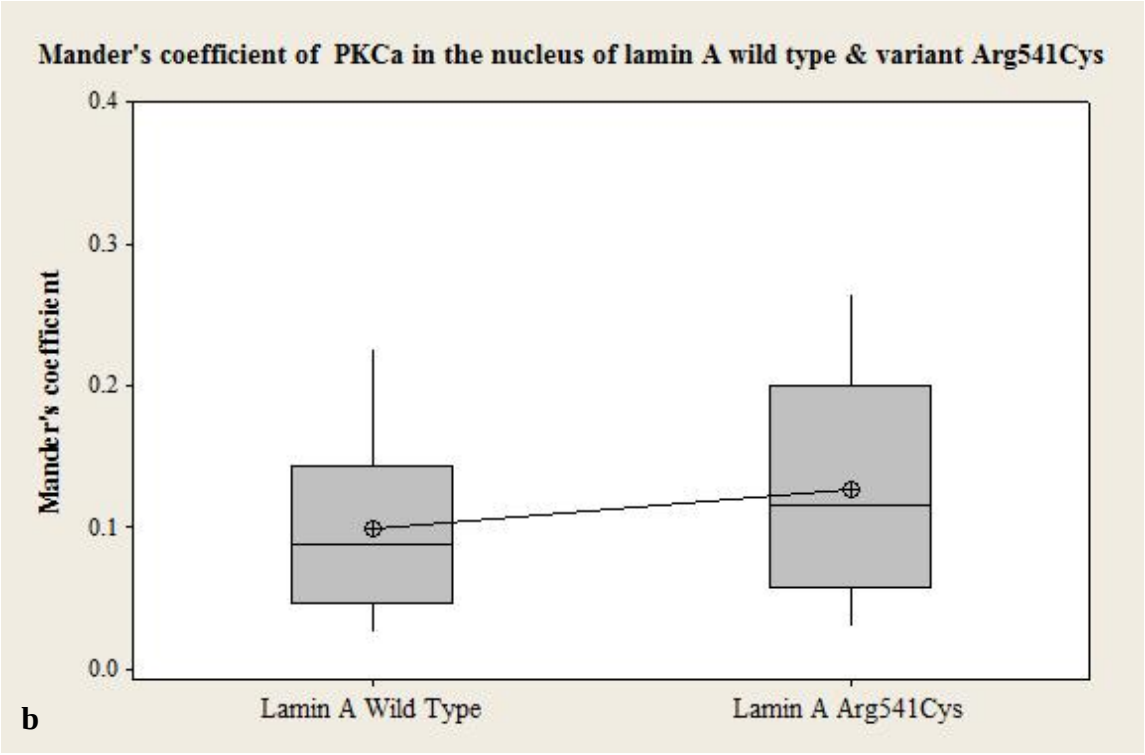


Figure 3.7 Boxplots representing Mander's coefficient of PKCα in the nucleus of lamin A in wild type and variant (a) Thr528Met and (b) Arg541Cys.

Table 3.3 Quantitive analysis of colocalization of PKCα and lamin A using two- sample t-test.

Two sample t-test	N	Mean	Standard Deviation	Standard Mean	P-value (p≤0.05)
Wild Type	14	0.0989	0.0628	0.017	N/A
Thr528Met	13	0.1616	0.112	0.031	0.093
Arg541Cys	10	0.1270	0.0776	0.025	0.359

Table 3.4 Quantitive analysis of colocalization of PKCα and lamin A using Mann-Whitney test.

Mann-Whitney test	N	Median	P-value (p≤0.05)
Wild Type	14	0.0885	N/A
Thr528Met	13	0.1110	0.1389
Arg541Cys	10	0.1160	0.3641

Aim 3.2.3: Investigate if the cellular distribution of PKCα in C2C12 cells is influenced by lamin C in the Thr528Met and Arg541Cys variants.

C2C12 cells were transfected with wild type or variant lamin C in pEYFP alone using lipofectamine, fixed and stained for PKCα and nucleus using primary PKCα antibody (rabbit polyclonal) and secondary antibody Alexa Fluor 647 goat anti-rabbit and Hoechst 33258 respectively.

Result: wild type and variant lamin C and PKCα

We next investigated if the distribution of PKCα was influenced by lamin C alone in the presence of wild type and variants. When C2C12 cells were transfected with wild type lamin C, PKCα was found as expected in the cytoplasm with light staining in the nucleus (Figure 3.8). The presence of PKCα increased in the nucleus, while staining was still evident within the cytoplasm in in Thr528Met (AF) variant. However, there was little or no change in cellular distribution of PKCα in cells transfected with wild type and Arg541Cys (DCM) variant. Also, quantitative colocalization analysis of PKCα and lamin C was performed using the ImageJ-1.46r software and JACoP v2.0 plug-in. Thresholded Manders coefficients tM1 and tM2 were used to characterize the degree of overlap between the two channels tM1 for lamin C and tM2 for PKCα from the confocal images. Two-sample t-test and Mann-Whitney test were used to perform statistical analysis on Mander's coefficient (tM2) which was obtained from the colocalization analysis of the confocal images (Figure 3. 10) (Tables 3.5 and 3.6). There was no statistical ($p \leq 0.05$) significance in the colocalization of PKCα and lamin C in the nucleus of DCM-associated variant (Arg541Cys) and AF variant (Thr528Met) (Tables 3.5 and 3.6).

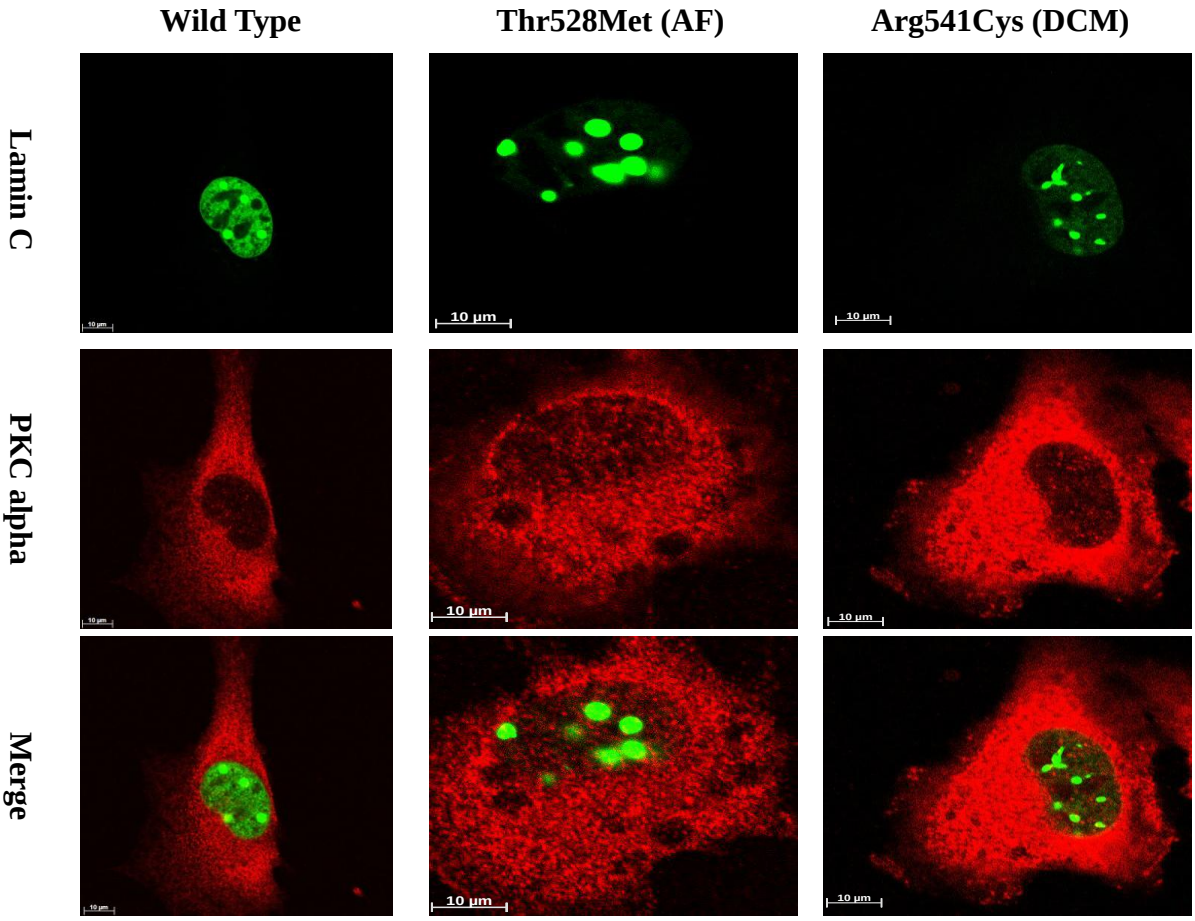


Figure 3.8 Relocalization of PKCα within the nucleoplasm in the presence of Thr528Met variant. Confocal microscopy images of C2C12 cells expressing wild type and variant lamin C-pEYFP (shown in green) and PKCα (red) (n=3).

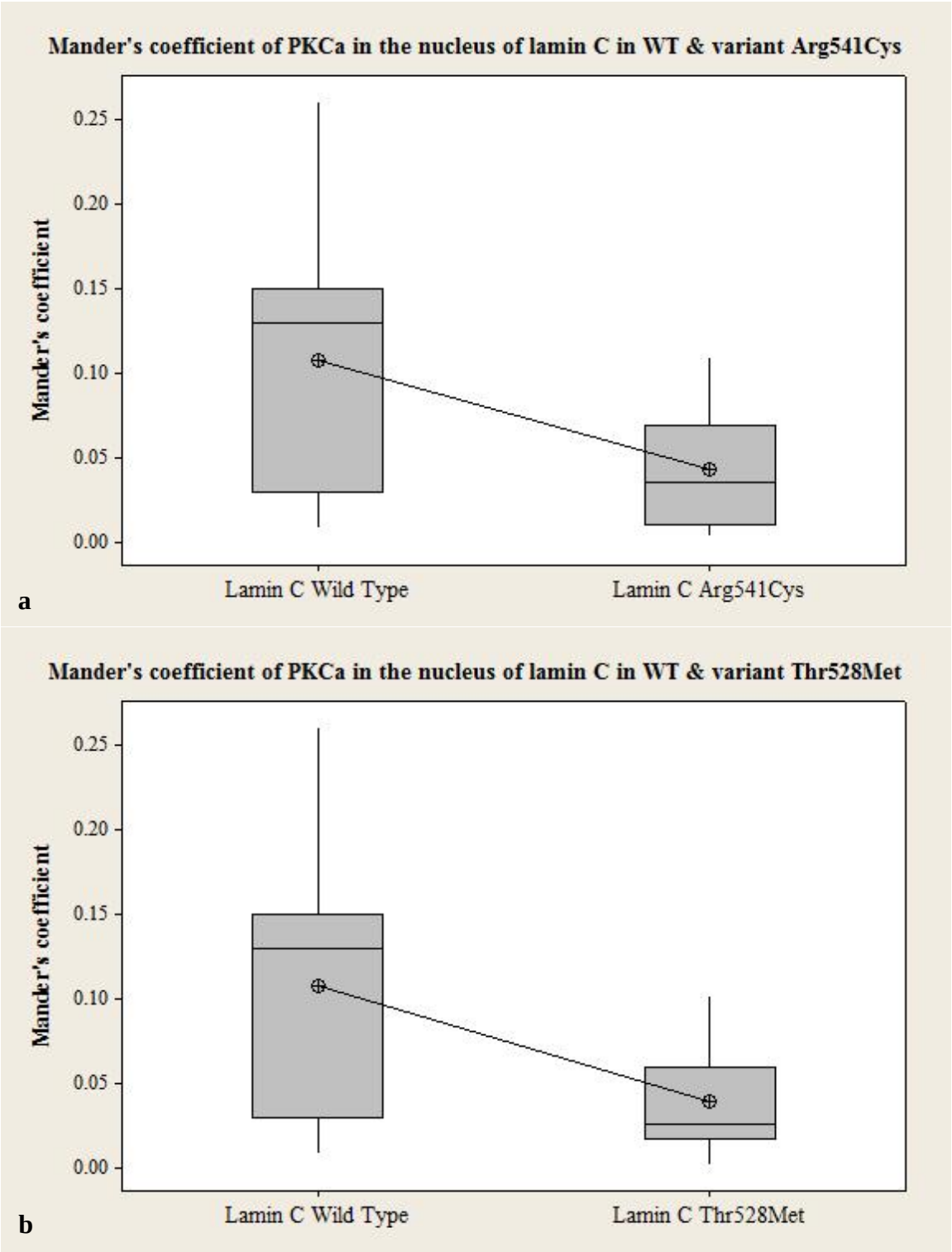


Figure 3.9 Boxplots representing Mander's coefficient of PKCα in the nucleus of lamin C in wild type and variant (a) Thr528Met and (b) Arg541Cys.

Table 3.5 Quantitive analysis of colocalization of PKCα and lamin C using two sample t-test.

Two sample t-test	N	Mean	Standard Deviation	Standard Mean	P-value (p≤0.05)
Wild Type	7	0.1077	0.0877	0.033	N/A
Thr528Met	9	0.0391	0.0312	0.010	0.089
Arg541Cys	10	0.0436	0.0346	0.011	0.109

Table 3.6 Quantitive analysis of colocalization of PKCα and lamin C using Mann-Whitney test.

Mann-Whitney test	N	Median	P-value (p≤0.05)
Wild Type	7	0.1300	N/A
Thr528Met	9	0.0260	0.1123
Arg541Cys	10	0.0360	0.1073

Objective 3.3: Determine the effect of the AF and DCM-associated variants on PKCα activity.

Aim 3.3.1: To measure the ratio of phosphorylated and non-phosphorylated PKCα in the cytoplasm and nucleus in wild type or variant lamin A and C transfected C2C12 cells.

This was performed to determine the effect of the AF and DCM-associated variants on the activity of PKCα. C2C12 cells that were transfected with wild type or variant lamin A and C using metafectene were subjected to nuclear and cytoplasmic protein extraction. Proteins were run on SDS-PAGE gels, and Western Blotting was performed with antibodies against phosphorylated (anti-phospho-PKCα rabbit polyclonal Ser-657) and non-phosphorylated PKCα (anti-PKCα rabbit polyclonal) to measure the ratio of phosphorylated and non-phosphorylated PKCα in the cytoplasm to nucleus in wild type and variant lamin A and C containing C2C12 cells. Phosphorylation of Ser-657 is currently used as a marker for PKCα activation. Hence, phospho-PKCα Ser-657 was used to measure the phosphorylation of PKCα present in the extracts of cells transfected with wild type and variant lamin A/C.

Results: Nuclear Protein

Western blotting was performed on nuclear extracts from C2C12 cells transfected with wild-type (WT) or variant lamin A and C for phosphorylated and non-phosphorylated PKCα (Figure 3.10). Densitometry analysis was performed on the bands corresponding to phosphorylated PKCα (81kDa) and non-phosphorylated PKCα (76kDa). There was a statistically significant ($p \leq 0.0079$) increase in the phosphorylated levels of PKCα in the cells expressing Arg541Cys, as compared to cells expressing wild-type lamin A and C (Figure

3.11). There was also an increase in the levels of non-phosphorylated PKCα in cells expressing the Thr528Met variant lamin A and C ($p \leq 0.0048$) (Figure 3.12).

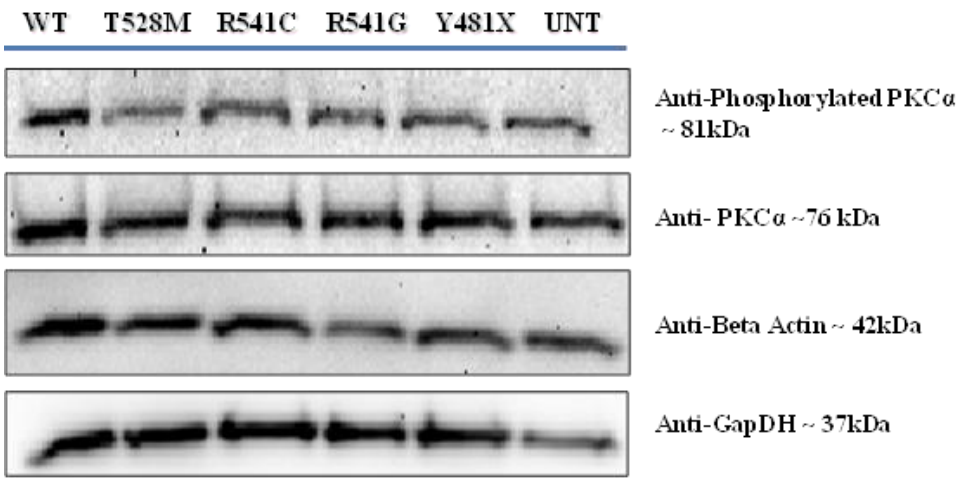


Figure 3.10 Lamin A and C expression of Arg541Cys variant shows increase levels of phosphorylated PKCα. Western blot analysis of phosphorylated and non-phosphorylated PKCα in nuclear protein harvested from C2C12 untransfected (UNT) cells or transfected with wild-type(WT) or variant lamin A-pECFP and C-pEYFP (n=6) was performed. Blots were re-probed for Beta Actin and GapDH as loading control.

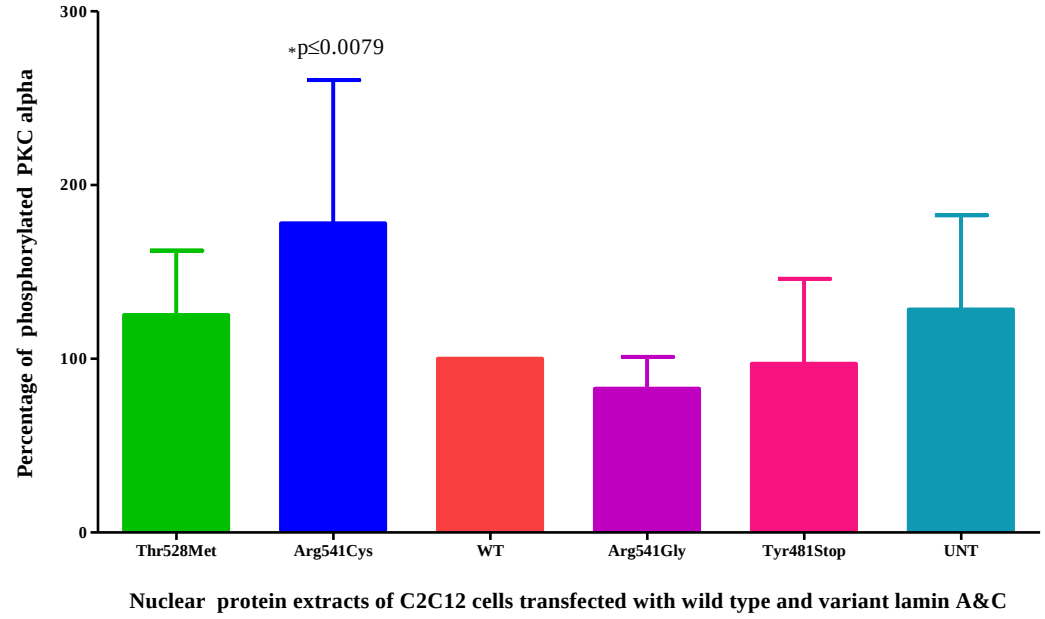


Figure 3.11 Percentage of phosphorylated PKCα in the nuclear protein harvested from C2C12 untransfected (UNT) or transfected with wild-type (WT) or variant lamin A in pECFP and C in pEYFP vectors.

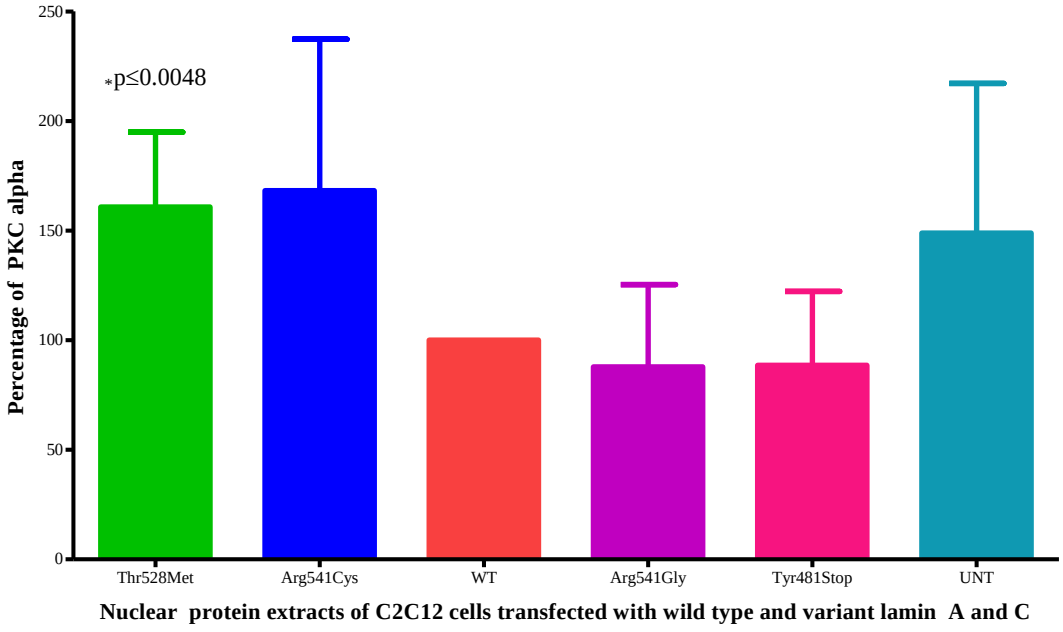


Figure 3.12 Percentage of PKCα in the nuclear protein harvested from C2C12 untransfected (UNT) or transfected with wild-type (WT) or variant lamin A in pECFP and C in pEYFP vectors.

Results: Cytoplasmic Protein

Following Western blotting, densitometry analysis was performed on the bands corresponding to phosphorylated PKCα (81kDa) and non-phosphorylated PKCα (76kDa) in the cytoplasmic protein harvested from C2C12 untransfected (UNT) cells or transfected with wild-type (WT) or variant lamin A-pECFP and C-pEYFP (n=6) (Figure 3.13). There was statistically significant ($p \leq 0.0022$) increase in the phosphorylated levels of PKCα in the cells expressing Arg541Cys, as compared to cells expressing wild-type lamin A and C (Figure 3.14). However, no statistical significance was found in the level of non-phosphorylated PKCα in the cells expressing lamin A and C variants (Figure 3.15).

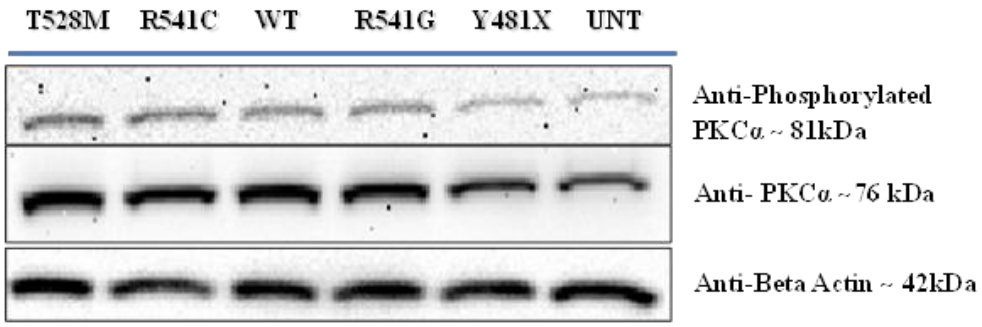


Figure 3.13 Lamin A and C expression of Arg541Cys variant shows increase levels of phosphorylated PKCα. Western blot analysis of phosphorylated and non-phosphorylated PKCα in cytoplasmic protein harvested from C2C12 untransfected (UNT) cells or transfected with wild-type(WT) or variant lamin A-pECFP and C-pEYFP (n=6) was performed. Blots were re-probed for Beta Actin as loading control.

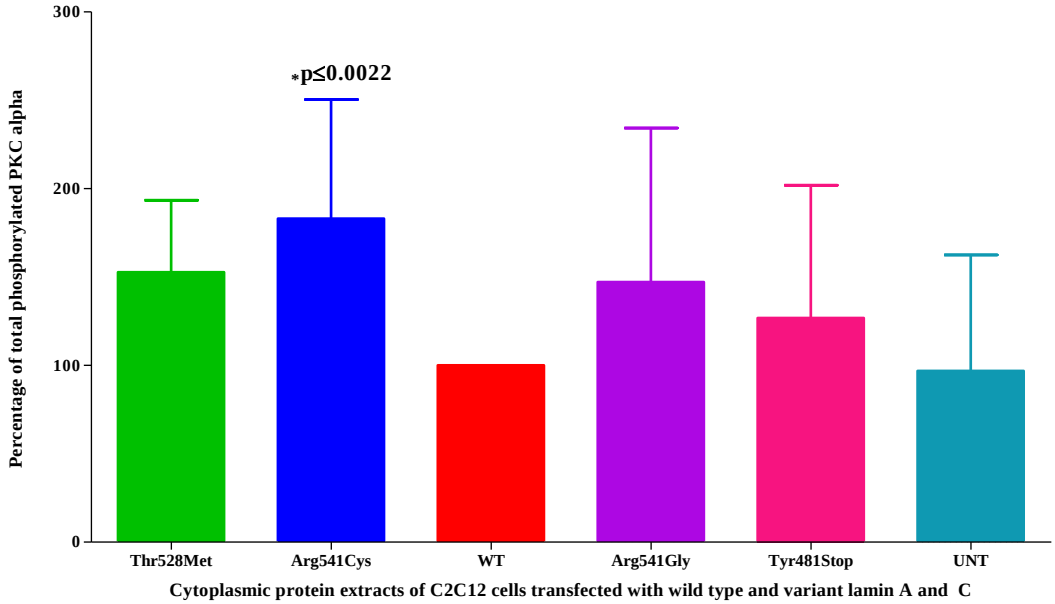


Figure 3.14 Percentage of phosphorylated PKCα in the cytoplasmic protein harvested from C2C12 cells untransfected (UNT) or transfected with wild type (WT) or variant lamin A in pECFP and C in pEYFP vectors.

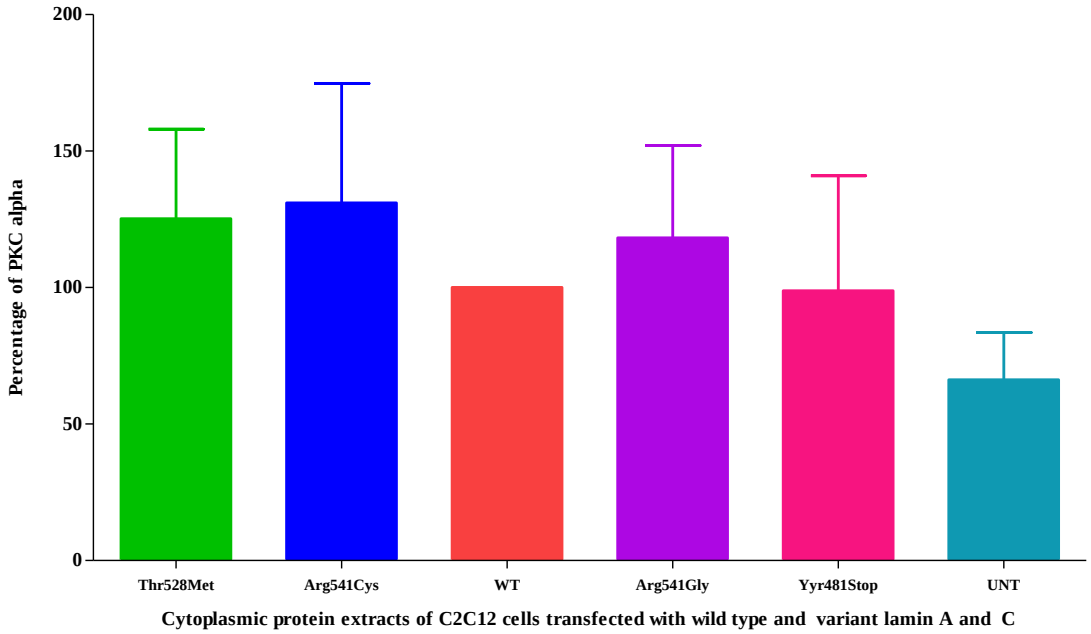


Figure 3.15 Percentage of PKCα in the cytoplasmic protein harvested from C2C12 untransfected (UNT) or transfected with wild-type (WT) or variant lamin A in pECFP and C in pEYFP vectors.

Aim 3.3.2: To quantify PKCα phosphorylation state

PKCα activity was quantified by measuring the phosphorylation of known phosphorylated PKCα substrate in the nuclear and cytoplasmic protein extracts, harvested from C2C12 mouse myoblast cells, transfected with wild type and variant lamin A and C. Proteins were run on SDS-PAGE gels, and Western blotting was performed with an antibody against a known PKCα substrate, phospho specific alpha-6-tubulin (Ser165) rabbit polyclonal antibody. Ser165 of alpha-6-tubulin is only phosphorylated by phosphorylated PKCα. Thus phosphorylation level of alpha-6-tubulin (Ser165) could be used to quantify the level of phosphorylation of PKCα.

Following Western blotting, densitometry analysis was performed on the bands corresponding to alpha-6-tubulin phosphorylated at serine 165 (50kDa) and alpha-6-tubulin (a.a 160-169) (50kDa) in the nuclear and cytoplasmic protein extracts harvested from C2C12 untransfected cells (UNT) or transfected with wild-type (WT) or variant lamin A-pECFP and C-pEYFP (Figure 3.16) and (Figure 3.19). In both nuclear and cytoplasmic extracts no statistically significant increase was observed in phosphorylated alpha-6-tubulin at serine 165 and alpha-6-tubulin in the cells expressing lamin A and C variant as compared to cells expressing wild-type lamin A and C (Figures 3.17-3.18) and (Figures 3.20-3.21). In the nuclear and cytoplasmic extracts of cells expressing the Arg541Cys variant, there was a significant increase in the phosphorylation level of PKCα. However, there was no corresponding increase in the phosphorylated alpha-6-tubulin (Ser165) level in the nuclear and cytoplasmic extracts of Arg541Cys variant. PKCα is known to phosphorylate various substrates; hence other substrates present in the cellular extracts as well as the cyclic

nature of the phosphorylation state of PKCα could have contributed to the discrepancy that was observed.

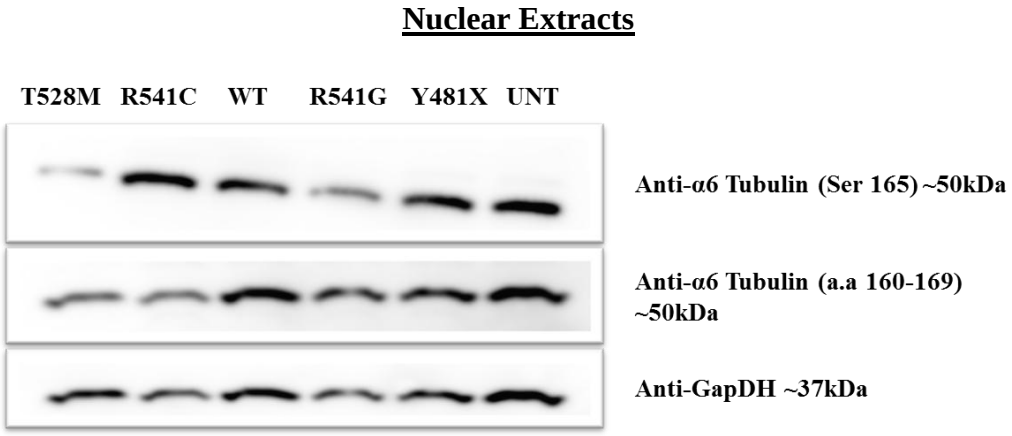


Figure 3.16 Lamin A and C variants expressing phosphorylated anti-alpha-6-tubulin (Ser165). Western blot analysis of phosphorylated alpha-6-tubulin (Ser165) and alpha-6-tubulin (a.a. 160-169) in nuclear protein harvested from C2C12 untransfected (UNT) cells or transfected with wild-type (WT) or variant lamin A-pECFP and C-pEYFP (n=3) was performed. Blots were re-probed for GapDH as loading control.

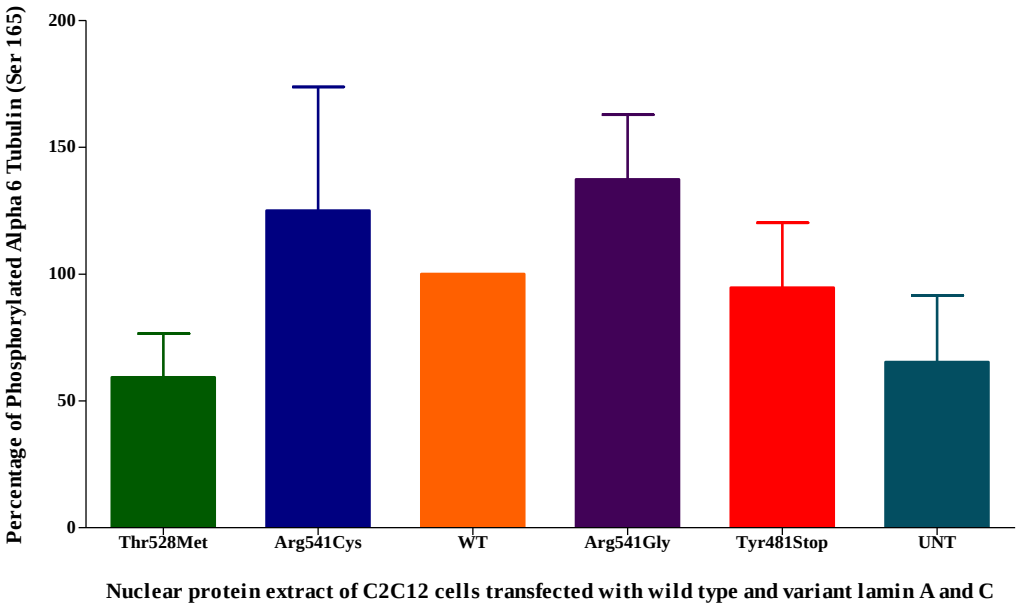


Figure 3.17 Percentage of phosphorylated alpha-6-tubulin (Ser165) in the nuclear protein harvested from C2C12 untransfected (UNT) or transfected with wild-type (WT) or variant lamin A in pECFP and C in pEYFP vectors.

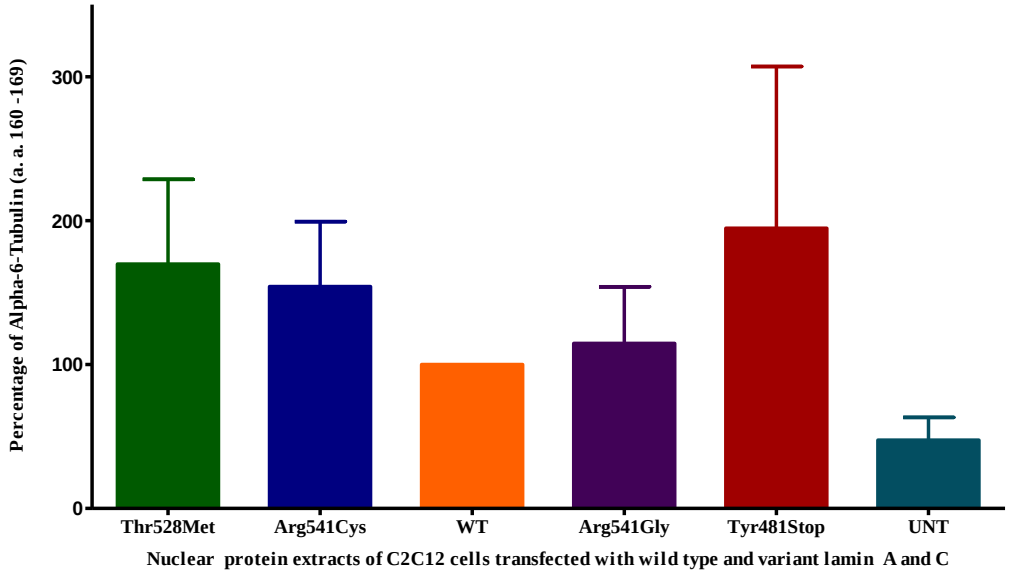


Figure 3.18 Percentage of alpha-6-tubulin (a. a. 160 - 169) in the nuclear protein harvested from C2C12 untransfected (UNT) or transfected with wild-type (WT) or variant lamin A in pECFP and C in pEYFP vectors.

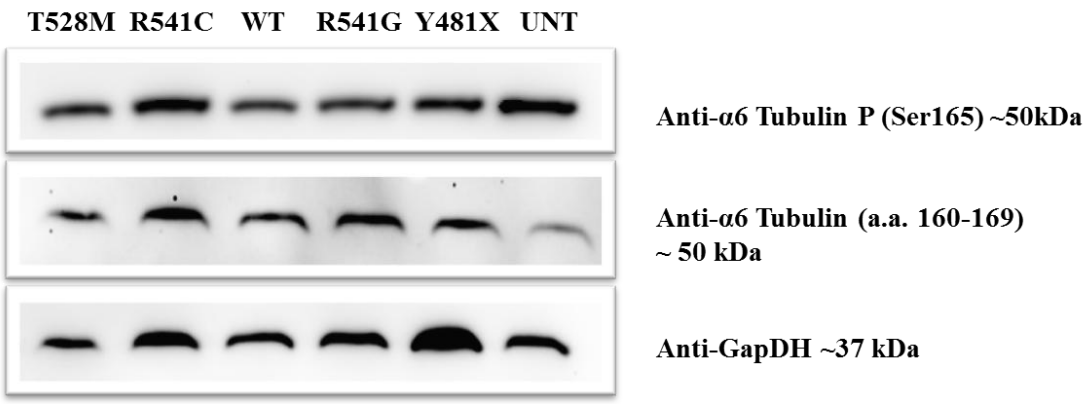


Figure 3.19 Lamin A and C variants expressing phosphorylated anti-alpha-6-tubulin (Ser165). Western blot analysis of phosphorylated alpha-6-tubulin (Ser165) and alpha-6-tubulin (a. a. 160-169) in cytoplasmic protein harvested from C2C12 untransfected (UNT) cells or transfected with wild-type (WT) or variant lamin A-pECFP and C-pEYFP (n=4) was performed. Blots were re-probed for GapDH as loading control.

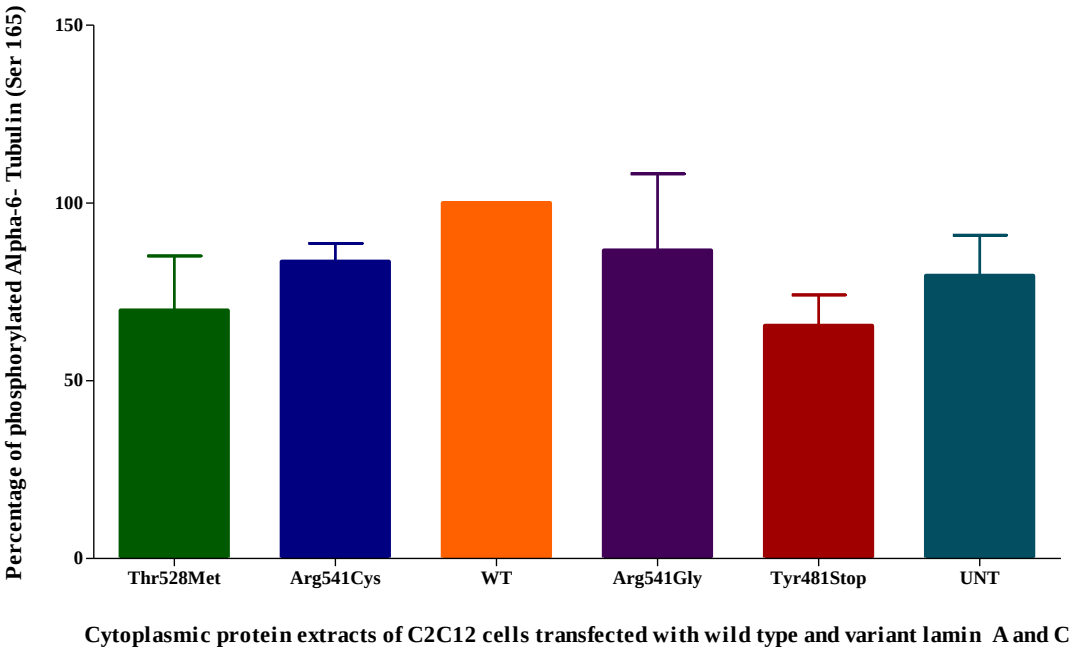


Figure 3.20 Percentage of phosphorylated alpha-6-tubulin (Ser165) in the cytoplasmic protein harvested from C2C12 untransfected (UNT) or transfected with wild-type (WT) or variant lamin A in pECFP and C in pEYFP vectors.

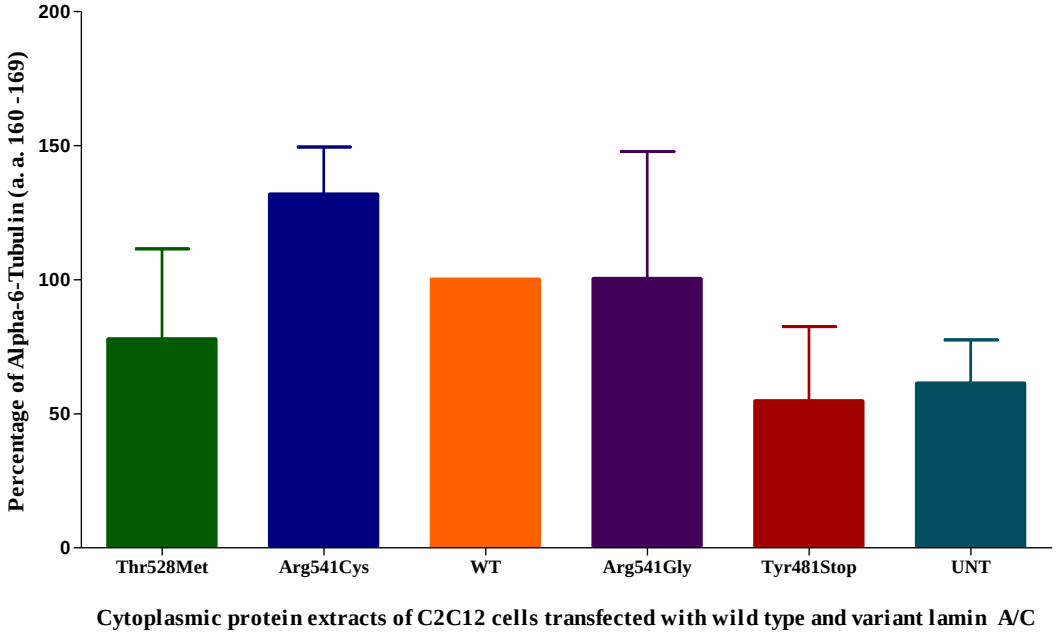


Figure 3.21 Percentage of alpha-6-tubulin (a. a. 160-169) in the cytoplasmic protein harvested from C2C12 untransfected (UNT) or transfected with wild-type (WT) or variant lamin A in pECFP and C in pEYFP vectors.

Chapter Four: Discussion

Chapter 4 - Discussion

This thesis was set out to determine how *LMNA* variants exert their tissue-specific effects by investigating perturbations in lamin A and C interaction with its binding partner PKCα via assessment of its cellular distribution and activity. The clinical history, genetic evidence and functional evidence which were found with regards to the variants studied in the course of this research have been summarized in Table 4.1.

Table 4.1 Summarising the clinical history, genetic and functional evidence of the variants studied

Lamin A and C Variants					
		Tyr481Stop (DCM/AF)	**Thr528Met (AF)**	**Arg541Cys(DCM)**	**Arg541Gly (DCM)**
Clinical History		A 36 year old patient with RBBB, a variety of arrhythmias (SVT, AF, VT), 2° AVB, and HF requiring heart transplantation at age 40. Three children affected aged (11, 13 and 18). 13 year old son - 2° AVB treated with pacemaker implantation. Others have VA Family history of sudden death; patient`s father died at the age of 47 years of unknown cardiac disease.	A 72 year old male with 7 year history of paroxymal AF, 1°-3° AVB, & significant conduction disease (Br and SVA); family history of sudden death; Father died suddenly at the age of 66 years and his brother died suddenly at the age of 70 years. Underwent pacemaker implantation. Variant found in two of his daughters. Older daughter had mild LV dysfunction. Younger daughter aged 40 diagnosed with Br, paroxymal 2° AVB. Older sister aged 47 demonstrated Br, mild LV hypokinesis.	A 19 year old male with LBBB, VT, VF, PVB, fibrosis, SWMA, SCD; a family history of sudden death. Mother died shortly after giving birth at age 20, her brother died suddenly at age of 18 years and grandmother from mother`s side died at age of 25 years. Currently, patient is NYHA class II heart failure, receiving an ACE-inhibitor, beta-blocker and spironolactone.	A 23-year old man with IVB, LBBB, Br, SVA, PVB, VA, TC. Has a family history of DCM. The father of the patient had an angiographically proven DCM, severe ventricular arrhythmias and died while on waiting list for heart transplantation at age of 30 years. Father`s sister with apical LV aneurysm and ventricular arrhythmias. Died suddenly at age of 39 years.
Genetic Evidence		c.1443C>G Blinkska et al 2006 reported in the above described patient.	c.1583C>T Twice reported in a compound heterozygous state Savage et al (2004) reported patient with T528M /S583L Verstraeten et al (2006) reported T528M/M540T in a patient with HGPS ~6% fibroblast from patient`s father had abnormal nuclei Saj et al (2012) reported in the above described patient.	c.1621C>T Reported three times. Forissier et al (2003) reported in two members (disease revealed early- father aged 22; daughter aged 20) of a French family with a history of ventricular disturbances and uncommon form of DCM with LVA without AVB. Hookana et al (2008) reported cardiac arrest and distinctly focal left ventricular fibrosis localized to inferior wall of LV, and sudden cardiac death in a Finnish family. Saj et al (2010) reported in the above described patient.	c.1621C>T Malek et al (2011) reported in the above described patient.
		All four variants were queried against Exome Variant Server (EVS) which contains SNP information from 6000 individuals. Thus far, the variants studied were not found in the non-affected population.			
Functional Evidence	**Localization of PKCα staining**	cytoplasm	nucleus	nucleus	cytoplasm
	Non-phosphorylated PKCα	No significant increase in the levels of non-phosphorylated PKCα in the nucleus and/or cytoplasm	**Significant increase in the level of non-phosphorylated PKCα in the nucleus**	No significant increase in the level of non-phosphorylated PKCα in the nucleus and/or cytoplasm	No significant increase in the level of non-phosphorylated PKCα in the nucleus and/or cytoplasm
	Phosphorylated PKCα	No significant increase in the levels of phosphorylated PKCα in the nucleus and/or cytoplasm	No significant increase in the levels of phosphorylated PKCα in the nucleus and/or cytoplasm	**Significant increase in the level of phosphorylated PKCα in the nucleus and cytoplasm**	No significant increase in the level of non-phosphorylated PKCα in the nucleus and/or cytoplasm
Predictive Algorithm using PolyPhen-2		-	Variant is predicted to be probably damaging with a score 1.000.	Variant is predicted to be probably damaging with a score 1.000.	Variant is predicted to be probably damaging with a score 1.000.

4.1 Presence of both lamins A and C required for PKCα translocation into nucleus of C2C12 cells.

The confocal images showed recruitment of PKCα into the nucleus of cells transfected with lamin A and C variants that are associated with AF (Thr528Met) and DCM (Arg541Cys). Statistical analysis showed significant ($p \leq 0.05$) increase in PKCα colocalization with lamin A and C in the nucleus of cells transfected with Thr528Met and Arg541Cys variants. Furthermore, when Thr528Met and Arg541Cys lamin A variants were transfected alone, only Arg541Cys variant showed recruitment of PKCα into the nucleus of C2C12 cells. However, there was no recruitment of PKCα into the nucleus of cells that were transfected alone with lamin C of Thr528Met and Arg541Cys variants. Moreover, no significant colocalization of lamin A or C with PKCα was observed in the nucleus of cells that were either transfected with Thr528Met and Arg541Cys variants. Thus, the presence of both lamins A and C was needed for the translocation of PKCα into the nucleus of cells that were transfected with lamin A and C Thr528Met and Arg541Cys variants. This was further validated by Western blotting in which nuclear extracts of cells transfected with lamin A and C Thr528Met and Arg541Cys variants were subjected to quantification of phosphorylated and non-phosphorylated PKCα. Nuclear and cytoplasmic extracts from Arg541Cys indicated a significant increase in the level of phosphorylation of PKCα. However, in the case of Thr528Met variant, the nuclear extract showed significant increase in the level of non-phosphorylated PKCα. Unfortunately, quantification of phosphorylated state of PKCα using anti-alpha-6-tubulin (Ser165) did not show statistical significance in the nuclear and cytoplasmic extracts of wild-type or variant lamin A and C expressing cells.

We expected correlation between increased phosphorylation of PKCα substrates and increased expression and phosphorylation of PKCα, however, this was not the case. PKCα is implicated in a variety of biological processes and it is known to phosphorylate plethora of substrates. As such, it is possible that other PKCα substrates could have been phosphorylated by the increased PKCα activity and thus accurate measurement of PKCα activity could have been hindered in the process. Although PKCα is expressed in C2C12 mouse myoblasts, not all PKCα substrates are expressed and coupled with the difficulty in obtaining of phosphospecific antibodies against mouse cell line contributed to our choice of alpha-6-tubulin (Ser165) antibody. At position Serine 165, alpha-6-tubulin is known to be phosphorylated only by PKCα. Also, phosphorylation of PKCα is cyclic in nature and this could have impacted the phosphorylation state of the chosen substrate in this study. Therefore the results obtained should be revalidated using another method.

The discrepancies observed in the confocal images in regards to the redistribution of PKCα into the nucleus in the various lamin A and C variants can be explained further. As previously mentioned, the PKCα interacts within last 166 residues of lamin A, which means from position 499 amino acids. The Tyr481Stop is a nonsense variant and thus the variant follows the wild type lamin A and C behaviour with regards to cellular distribution of PKCα.

The variant Thr528Met in heterozygous state is not only associated with AF but non-valvular AF with conduction disease (Saj et al., 2012). This variant identified in a 72 year old patient with a 7-year history of paroxysmal AF with significant conduction disease which lead to pacemaker implantation. Furthermore, the left ventricle was not enlarged and had preserved LVEF of 61%. Thus having a normal LVEF at the age of 72 years shows that the

increased presence of PKCα within the nucleus in its non-phosphorylated states may play a part in halting the progression of the disease.

The Arg541Cys and Arg541Gly are two pathogenic variants that do not present with the classic form of cardiolaminopathy. The discrepancies observed in the confocal images in regards to the redistribution of PKCα into the nucleus of C2C12 cells that were transfected with either Arg541Cys or Arg541Gly variant can be explained further by the 3-D structural analysis. The section 4.2 discusses how the Adamed group carried out the structural analysis of the native Arg541 and Gly541 variant.

4.2 The structural analysis of Arg541Gly lamin A and C protein by Adamed Group

X-ray crystallography and nuclear magnetic resonance (NMR) are two methods which are capable of producing a spatial model of a protein structure, thereby allowing the study and analysis of the effects of point mutations on protein function. Determination of protein structure is difficult and expensive. To facilitate this, publicly accessible online database known as Protein Data Bank (PDB) has been established.

The PDB contains lamin A and C protein fragments corresponding two regions: 2β-coil and the globular domain of the Ig fold (Figure 4.1).

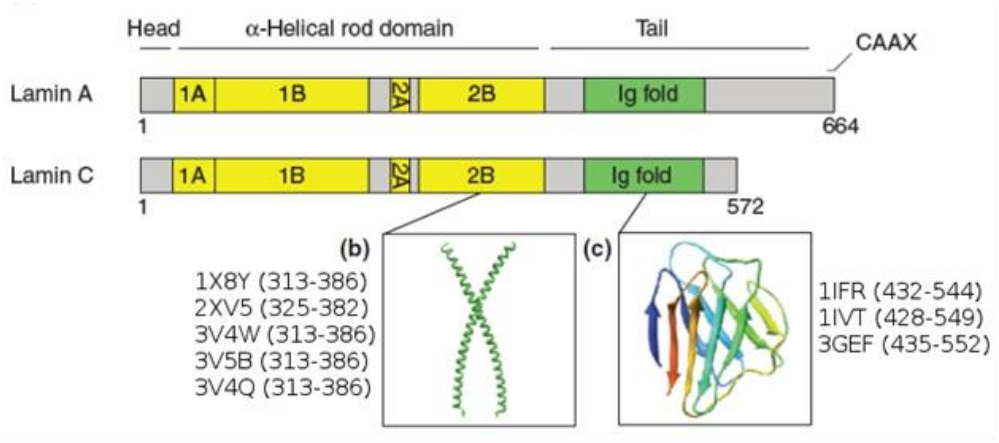


Figure 4.1 The schematic diagram of the available PDB structures corresponding to each domain of *LMNA*. In parentheses are the ranges of amino acids included in each of the structures. Adapted with permission from BioMed Central Ltd: [Genome Biology], (Dittmer and Misteli, 2011), copyright©2011

The spatial structure of the Ig fold is dominated by two-β strands which include 12 β-sheets composed of 54 amino acids. The amino acid Arg 541 is in the last part of the β ribbon, which interacts with the first β-ribbon to stabilize the N-and C-terminal of the Ig fold. The guanidine group of arginine is directed into the space between the β-sheets and is involved in numerous hydrogen interaction found in the water molecules as well as with

His433. Thus, it was assumed that the variation Arg541Gly will lead to changes in the local interactions in the region, and thereby reducing the stability of the entire domain via destabilizing the interactions between the ends of the polypeptide chain. In order to verify this hypothesis, a molecular dynamic simulation (MD) of native and mutant Arg541Gly were tested and then compared for variability of this protein region.

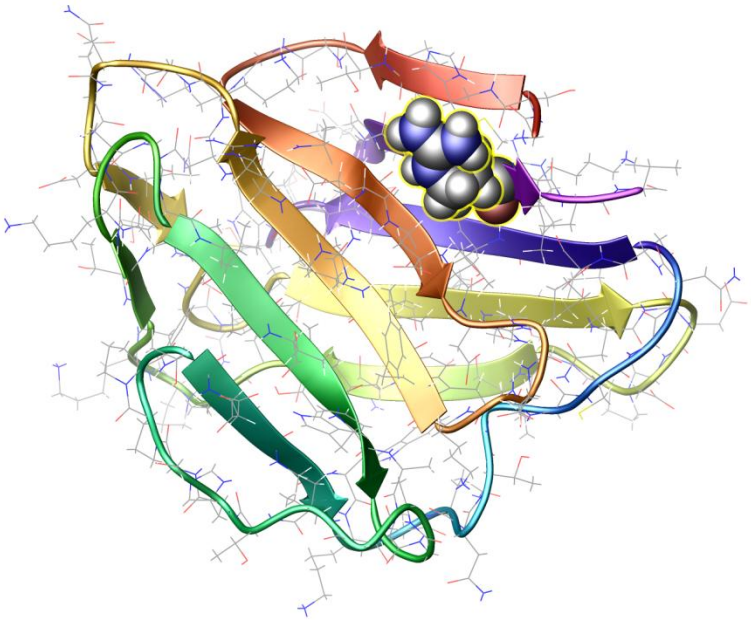


Figure 4.2 Structure of PDB 1IFR. Color scheme corresponds to the position in the protein chain from the red N-terminus to C-terminus blue. Arg541 is shown in CPK representation and additionally decorated in yellow.

Molecular dynamic simulation was carried out between native structure of the model and its' variant 1IFR Arg541Gly (Figure 4.2). The C-terminal β-strand of Arg541Gly variation behaved similarly to the native structure. That is, native structure in this region was observed to slightly shift its' β-strand position. Despite this minor change all hydrogen bonds of the β-strands were retained and all of its side chains in the regions fluctuated similar to those of the crystal structure. However, major changes were observed to occur from the N-terminus β-strand (amino acids 433-437). The fluctuation in this region was relatively large.

Interestingly, during the half-way mark of the stimulation, the crystal structure was subjected towards the solvent, rotational change of His434 occurred thereby directly filling the inner space of the protein usually inhabited by native Arg541 (Figure 4.3). This conformational change appears to partially compensate for the loss of the guanidine group as a result of the mutation Arg to Gly. Therefore, no major conformational changes in the C-terminus β-strand were observed which would lead to significant changes in the overall domain structure (Figure 4.4).

Arginine has polar positive side chain which has the ability to form multiple hydrogen bonds. Furthermore, arginine binds negatively charged group and is thus found on the outside of the proteins where it can interact with the polar environment. The Arg541Gly results in the change of arginine into a glycine which is non-polar neural side chain. It is the smallest of the 20 amino acids. Due to its hydrogen side chain, it is ambivalent, meaning it is comfortable in both hydrophobic and hydrophilic environment. Cysteine is considered to be a hydrophilic amino acid. The cysteine side chain has been shown to stabilize hydrophobic interactions in micelles to a greater degree than the side chain in the non-polar amino acid glycine (Heitmann, 1968). Cysteine participates in numerous posttranslational modifications. The nucleophilic thiol group allows cysteine to conjugate to other groups.

The overall domain structure when the arginine at position 541 of the *LMNA* gets mutated to cysteine, could lead to significant changes that are not observed in Arg to Gly mutation. Perhaps there is no rotational change of His434 to compensate when Arg541Cys occurs. This could possibly explain the significant increase in the PKCα in the nucleus of the Arg541Cys variants.

The arginine at position 541 has also been reported to mutate to other amino acids other than glycine and cysteine. Sylvius *et al* (2006) and Karkkainen *et al* (2004) described

Arg541Ser variation, whereas Vytopil *et al* (2003) reported Arg541His variation. Interestingly, these variations were not associated with distinct localized pathological changes similar to those reported for Arg541Cys (Saj et al., 2010). The possibility of PKCα activity could play a role in disease progression with Arg541His and Arg541Ser variants should also be investigated in the future.

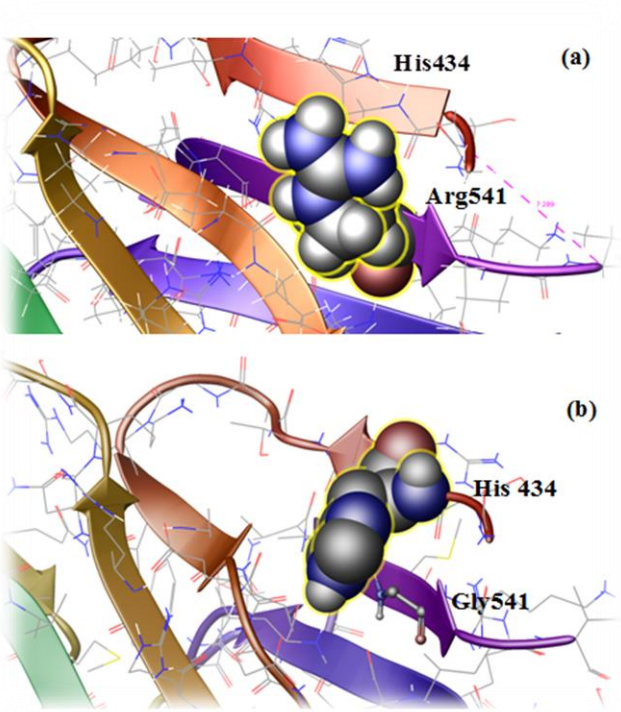


Figure 4.3 (a) The magnified crystal structures of 1IFR with Arg 541 represented by CPK. (b) The fragment corresponding to mutant Arg541Gly simulation with His434 filling the space inhabited by Arg541.

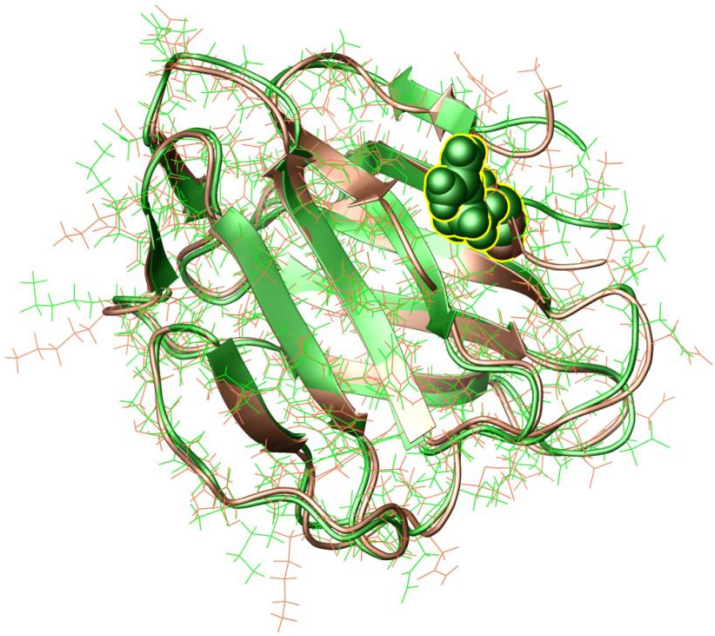


Figure 4.4 Structure of the MD simulation for the non-mutated (green) and the mutant (bronze). Arg541 and Gly541 represented by CPK respectively for the structure without modification and alteration.

4.3 The functional consequence of PKCα translocation in laminopathies

The functional consequence of nuclear translocation of PKCα with respect to laminopathies is unknown. Typically PKCα is found throughout the cytoplasm and at perinulcear regions, and to a very small extent within the nucleus (Leach et al., 1989; Neri et al., 1994). PKCα translocates to the nucleus upon stimulation by phorbol-12-myristate-13-acetate (PMA) and insulin-like growth factor 1 (IGF-1) (Divecha et al., 1991; Leach et al., 1989; Neri et al., 1998; Zini et al., 1995). The mode and function of nuclear translocation is not well understood. Approximately 50 percent of nuclear PKCα are associated with the nuclear matrix compared to its interaction with cytoplasmic filaments, thus implicating PKCα's presence in the nuclear structure (Zini et al., 1995). Furthermore, PKCα translocation to the nucleus appears to be dependent on cytoplasmic integrity. PKCα nuclear translocation cannot be induced when the cytoskeleton is disrupted and it is not associated

with classical methods of nuclear transport such as via NLS and NPC (Schmalz et al., 1998; Schmalz et al., 1996).

PKCα is expressed ubiquitously in tissues and has been implicated in oocyte development and fertilization (Fan et al., 2002). Furthermore, overexpression of PKCα promoted proliferation in some cell types but caused cell cycle arrest and differentiation in other types (Nakashima, 2002). Moreover, expression of PKCα in MCF-7 human breast cancer cells, exhibited an enhanced proliferation and lead to the development of aggressive phenotype (Ways et al., 1995). The proliferation effect appears to be mediated via activation of ERK/MAPK cascade initiated by Raf-1 and/or up regulated by p21Waf/Cip1 (Besson and Yong, 2000), which facilitates the formation of complex between cyclin and CDK.

The C2C12 cells expressing the DCM variant Arg541Cys showed statistically significant level of colocalization of PKCα and lamin A and C and increased level of phosphorylated PKCα in the cytoplasm and nucleus. In 2007, Muchir *et al* showed that abnormal activation of ERK1/2 signalling in hearts from *Lmna*$^{H222P/+}$ and *Lmna*$^{H222P/H222P}$ mice developed cardiomyopathy. Similarly hearts of human patients with LMNA cardiomyopathy show abnormally activated ERK1/2 signalling (Muchir et al., 2012). Knock-in mice *Lmna* ^{H222P} showed abnormal activation of c-Jun N-terminal kinase and p38α signaling. These activations were detected ahead of cardiac dysfunction (Choi et al., 2012; Muchir et al., 2007; Muchir et al., 2009). Furthermore, hearts of *Lmna* null mice have dysregulated MAPK and mTOR signaling (Ramos et al., 2012).Inhibition of ERK1/2 signalling using MEK1/2 inhibitors have shown to prevent the development of cardiomyopathy in *Lmna*$^{H222P/H222P}$ mice and improve the cardiac function even after impaired contractility (Muchir et al., 2009; Muchir et al., 2012). As previously mentioned, PKCα is a fundamental regulator of cardiac contractility and Ca^{2+} ion handling in myocytes. Thus, PKCα functions as a nodal integrator of cardiac contractility by sensing intracellular Ca^{2+} ions and signal transduction events,

which can profoundly affect propensity towards heart failure (Braz et al., 2007). Moreover, *PKCα*$^{-/-}$ mice are hypercontractile, while hearts of mice overexpressing *PKCα* are hypocontractile. Enhanced cardiac contractility protected hearts of *PKCα*$^{-/-}$ mice against pressure overload-induced heart failure and dilated cardiomyopathy. PKCα has also been shown to induce MAPK activation via ERK1/2 phosphorylation in cells induced for PKCα nuclear translocation. Furthermore, PKCα can directly or indirectly activate signaling cascade involving all three different branches of the MAP-kinase-signaling pathways described above (Figure 4.5). In addition, PKCα was able to induce hypertrophy via enhanced sarcomeric organization, increased cell surface area, ANF expression and H3-leucine incorporation (Braz et al., 2002). It is possible that the destabilization of the nuclear lamina may induce PKCα nuclear translocation via nucleo-cytoskeletal disruption which then activates the MAPK signaling cascade, thus leading to the development of the cardiac phenotype in laminopathies. Therefore, the dramatic course of the disease with Arg541Cys variant, especially in regards to early LV dilation and remodelling might be explained by increased presence of phosphorylated PKCα in the nucleus in C2C12 cells.

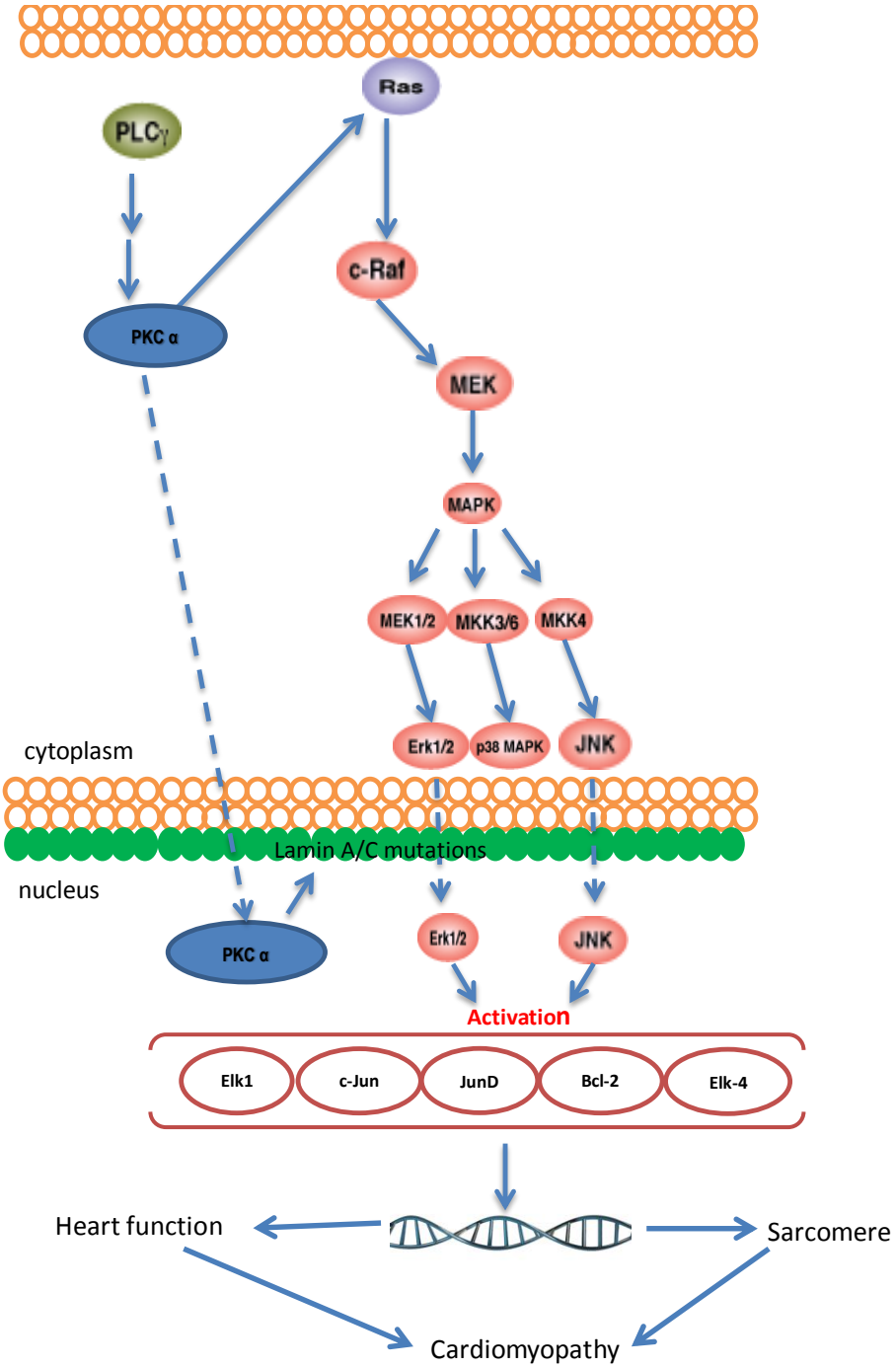


Figure 4.5 Model showing how lamin A/C variants in the nuclear lamina may lead to cardiomyopathy. Lamin A/C variants in the nuclear lamina activate MAPK cascades, via PKCα. This leads to the activation of G-protein (Ras), protein kinase (c-Raf) and subsequent enhanced phosphorylation of Erk1/2 and JNK, resulting in nuclear translocation. In the nucleus, pERK1/2 and pJNK activate transcription factors such as Elk1, bcl-2, JunD, c-Jun and Elk 4, leading to increased synthesis of these proteins. Increased amount and activity of transcription factors alter expression of other genes encoding muscle fibers and sarcomeres. Aberrant expression of these proteins leads to development of cardiomyopathy.

4.4 Conclusion

Discovery that PKCα recruitment into the nucleus may play a role in some *LMNA* variants thereby causing the development of DCM and AF is highly promising. However, more work is needed to delineate how laminopathy-causing variants trigger cell- and tissue-specific phenotypes through perturbations in cell signaling and gene transcription. Firstly, re-quantification of the phosphorylation state of PKCα using a different method such as radiometric based filtration binding assay. This assay is considered the 'gold standard' to which non-radiometric methods are compared. In this assay, the kinase reaction is performed in the presence of 32 P-γ-ATP or 33 P-γ-ATP, followed by binding of the final radioisotope labeled products to filters after which unreacted phosphate can be washed away without interfering with the detection of real phosphorylated products. Secondly, the position Arg541 is a "hot spot" for mutations and investigating the cellular distribution and activity of PKCα in Arg541His and Arg541Ser variants would provide insights into PKCα distribution and activity. Lastly, the above experiments should be carried out again *in vivo* using zebrafish as a model for laminopathies.

References

Al-Haboubi, T., D.K. Shumaker, J. Koser, M. Wehnert, and B. Fahrenkrog. 2011. Distinct association of the nuclear pore protein Nup153 with A- and B-type lamins. *Nucleus*. 2:500-509.

Arbustini, E., A. Pilotto, A. Repetto, M. Grasso, A. Negri, M. Diegoli, C. Campana, L. Scelsi, E. Baldini, A. Gavazzi, and L. Tavazzi. 2002. Autosomal dominant dilated cardiomyopathy with atrioventricular block: a lamin A/C defect-related disease. *Journal of the American College of Cardiology*. 39:981-990.

Arimura, T., A. Helbling-Leclerc, C. Massart, S. Varnous, F. Niel, E. Lacene, Y. Fromes, M. Toussaint, A.M. Mura, D.I. Keller, H. Amthor, R. Isnard, M. Malissen, K. Schwartz, and G. Bonne. 2005. Mouse model carrying H222P-Lmna mutation develops muscular dystrophy and dilated cardiomyopathy similar to human striated muscle laminopathies. *Human molecular genetics*. 14:155-169.

Benedetti, S., I. Menditto, M. Degano, C. Rodolico, L. Merlini, A. D'Amico, L. Palmucci, A. Berardinelli, E. Pegoraro, C.P. Trevisan, L. Morandi, I. Moroni, G. Galluzzi, E. Bertini, A. Toscano, M. Olive, G. Bonne, F. Mari, R. Caldara, R. Fazio, I. Mammi, P. Carrera, D. Toniolo, G. Comi, A. Quattrini, M. Ferrari, and S.C. Previtali. 2007. Phenotypic clustering of lamin A/C mutations in neuromuscular patients. *Neurology*. 69:1285-1292.

Bertrand, A.T., K. Chikhaoui, R.B. Yaou, and G. Bonne. 2011. Clinical and genetic heterogeneity in laminopathies. *Biochemical Society transactions*. 39:1687-1692.

Bertrand, A.T., L. Renou, A. Papadopoulos, M. Beuvin, E. Lacene, C. Massart, C. Ottolenghi, V. Decostre, S. Maron, S. Schlossarek, M.E. Cattin, L. Carrier, M. Malissen, T. Arimura, and G. Bonne. 2012. DelK32-lamin A/C has abnormal location and induces incomplete tissue maturation and severe metabolic defects leading to premature death. *Human molecular genetics*. 21:1037-1048.

Besson, A., and V.W. Yong. 2000. Involvement of p21(Waf1/Cip1) in protein kinase C alpha-induced cell cycle progression. *Molecular and cellular biology*. 20:4580-4590.

Biamonti, G., M. Giacca, G. Perini, G. Contreas, L. Zentilin, F. Weighardt, M. Guerra, G. Della Valle, S. Saccone, S. Riva, and et al. 1992. The gene for a novel human lamin maps at a highly transcribed locus of chromosome 19 which replicates at the onset of S-phase. *Molecular and cellular biology*. 12:3499-3506.

Bilinska, Z.T., N. Sylvius, J. Grzybowski, A. Fidzianska, E. Michalak, E. Walczak, M. Walski, K. Bieganowska, E. Szymaniak, B. Kusmierczyk-Droszcz, B. Lubiszewska, T. Wagner, F. Tesson, and W. Ruzyllo. 2006. Dilated cardiomyopathy caused by LMNA mutations. Clinical and morphological studies. *Kardiologia polska*. 64:812-819; discussion 820-811.

Bolte, S., and F.P. Cordelières. 2006. A guided tour into subcellular colocalization analysis in light microscopy. *Journal of Microscopy*. 224:213-232.

Bonne, G., M.R. Di Barletta, S. Varnous, H.M. Becane, E.H. Hammouda, L. Merlini, F. Muntoni, C.R. Greenberg, F. Gary, J.A. Urtizberea, D. Duboc, M. Fardeau, D. Toniolo, and K. Schwartz. 1999. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nature genetics*. 21:285-288.

Bornancin, F., and P.J. Parker. 1997. Phosphorylation of protein kinase C-alpha on serine 657 controls the accumulation of active enzyme and contributes to its phosphatase-resistant state. *The Journal of biological chemistry*. 272:3544-3549.

Bossie, C.A., and M.M. Sanders. 1993. A cDNA from Drosophila melanogaster encodes a lamin C-like intermediate filament protein. *Journal of cell science*. 104 (Pt 4):1263-1272.

Boudreau, E., S. Labib, A.T. Bertrand, V. Decostre, P.M. Bolongo, N. Sylvius, G. Bonne, and F. Tesson. 2012. Lamin A/C mutants disturb sumo1 localization and sumoylation in vitro and in vivo. *PloS one*. 7:e45918.

Braz, J.C., O.F. Bueno, L.J. De Windt, and J.D. Molkentin. 2002. PKC alpha regulates the hypertrophic growth of cardiomyocytes through extracellular signal-regulated kinase1/2 (ERK1/2). *The Journal of cell biology*. 156:905-919.

Braz, J.C., K. Gregory, A. Pathak, W. Zhao, B. Sahin, R. Klevitsky, T.F. Kimball, J.N. Lorenz, A.C. Nairn, S.B. Liggett, I. Bodi, S. Wang, A. Schwartz, E.G. Lakatta, A.A. DePaoli-Roach, J. Robbins, T.E. Hewett, J.A. Bibb, M.V. Westfall, E.G. Kranias, and J.D. Molkentin. 2004. PKC-alpha regulates cardiac contractility and propensity toward heart failure. *Nature medicine*. 10:248-254.

Brodsky, G.L., F. Muntoni, S. Miocic, G. Sinagra, C. Sewry, and L. Mestroni. 2000. Lamin A/C Gene Mutation Associated With Dilated Cardiomyopathy With Variable Skeletal Muscle Involvement. *Circulation*. 101:473-476.

Broers, J.L., B.M. Machiels, G.J. van Eys, H.J. Kuijpers, E.M. Manders, R. van Driel, and F.C. Ramaekers. 1999. Dynamics of the nuclear lamina as monitored by GFP-tagged A-type lamins. *Journal of cell science*. 112:3463-3475.

Broers, J.L.V., C.J. Hutchison, and F.C.S. Ramaekers. 2004. Laminopathies. *The Journal of Pathology*. 204:478-488.

Broers, J.L.V., H.J.H. Kuijpers, C. Östlund, H.J. Worman, J. Endert, and F.C.S. Ramaekers. 2005. Both lamin A and lamin C mutations cause lamina instability as well as loss of internal nuclear lamin organization. *Experimental cell research*. 304:582-592.

Broers, J.L.V., F.C.S. Ramaekers, G. Bonne, R.B. Yaou, and C.J. Hutchison. 2006. Nuclear Lamins: Laminopathies and Their Role in Premature Ageing. *Physiological Reviews*. 86:967-1008.

Buendia, B., J.C. Courvalin, and P. Collas. 2001. Dynamics of the nuclear envelope at mitosis and during apoptosis. *Cell Mol Life Sci*. 58:1781-1789.

Capell, B.C., and F.S. Collins. 2006. Human laminopathies: nuclei gone genetically awry. *Nat Rev Genet*. 7:940-952.

Cattin, M.E., A. Muchir, and G. Bonne. 2013. 'State-of-the-heart' of cardiac laminopathies. *Current opinion in cardiology*. 28:297-304.

Cenni, V., J. Bertacchini, F. Beretti, G. Lattanzi, A. Bavelloni, M. Riccio, M. Ruzzene, O. Marin, G. Arrigoni, V. Parnaik, M. Wehnert, N.M. Maraldi, A. de Pol, L. Cocco, and S. Marmiroli. 2008. Lamin A Ser404 is a nuclear target of Akt phosphorylation in C2C12 cells. *J Proteome Res*. 7:4727-4735.

Chandar, S., L.S. Yeo, C. Leimena, J.C. Tan, X.H. Xiao, V. Nikolova-Krstevski, Y. Yasuoka, M. Gardiner-Garden, J. Wu, S. Kesteven, L. Karlsdotter, S. Natarajan, A. Carlton, S. Rainer, M.P. Feneley, and D. Fatkin. 2010. Effects of mechanical stress and carvedilol in lamin A/C-deficient dilated cardiomyopathy. *Circulation research*. 106:573-582.

Choi, J.C., A. Muchir, W. Wu, S. Iwata, S. Homma, J.P. Morrow, and H.J. Worman. 2012. Temsirolimus activates autophagy and ameliorates cardiomyopathy caused by lamin A/C gene mutation. *Science translational medicine*. 4:144ra102.

Chuang, C.H., A.E. Carpenter, B. Fuchsova, T. Johnson, P. de Lanerolle, and A.S. Belmont. 2006. Long-range directional movement of an interphase chromosome site. *Current biology : CB*. 16:825-831.

Chung-Wah Siu, Y.-K.L., Jenny Chung-Yee Ho, Wing-Hon Lai1, Yau-Chi Chan , Kwong-Man Ng, Lai-Yung Wong, Ka-Wing Au, YeeMan Lau, Jinqiu Zhang, Kenneth Weijian Lay, Alan Colman, and Hung-Fat Ts. 2012. Modeling of lamin A/C mutation premature cardiac aging using patient-specific induced pluripotent stem cells. *Aging* 4.

Claycomb, W.C., N.A. Lanson, Jr., B.S. Stallworth, D.B. Egeland, J.B. Delcarpio, A. Bahinski, and N.J. Izzo, Jr. 1998. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. *Proceedings of the National Academy of Sciences of the United States of America*. 95:2979-2984.

Coffinier, C., H.J. Jung, Z. Li, C. Nobumori, U.J. Yun, E.A. Farber, B.S. Davies, M.M. Weinstein, S.H. Yang, J. Lammerding, J.N. Farahani, L.A. Bentolila, L.G. Fong, and S.G. Young. 2010. Direct synthesis of lamin A, bypassing prelamin a processing, causes misshapen nuclei in fibroblasts but no detectable pathology in mice. *The Journal of biological chemistry*. 285:20818-20826.

Crisp, M., Q. Liu, K. Roux, J.B. Rattner, C. Shanahan, B. Burke, P.D. Stahl, and D. Hodzic. 2006. Coupling of the nucleus and cytoplasm: role of the LINC complex. *The Journal of cell biology*. 172:41-53.

Csoka, A.B., H. Cao, P.J. Sammak, D. Constantinescu, G.P. Schatten, and R.A. Hegele. 2004. Novel lamin A/C gene (LMNA) mutations in atypical progeroid syndromes. *Journal of medical genetics*. 41:304-308.

Cupesi, M., J. Yoshioka, J. Gannon, A. Kudinova, C.L. Stewart, and J. Lammerding. 2010. Attenuated hypertrophic response to pressure overload in a lamin A/C haploinsufficiency mouse. *Journal of molecular and cellular cardiology*. 48:1290-1297.

Darbar, D., K.J. Herron, J.D. Ballew, A. Jahangir, B.J. Gersh, W.-K. Shen, S.C. Hammill, D.L. Packer, and T.M. Olson. 2003. Familial atrial fibrillation is a genetically heterogeneous disorder. *J Am Coll Cardiol*. 41:2185-2192.

Dec, G.W., and V. Fuster. 1994. Idiopathic Dilated Cardiomyopathy. *New England Journal of Medicine*. 331:1564-1575.

Dechat, T., S.A. Adam, P. Taimen, T. Shimi, and R.D. Goldman. 2010. Nuclear lamins. *Cold Spring Harbor perspectives in biology*. 2:a000547.

Dechat, T., K. Pfleghaar, K. Sengupta, T. Shimi, D.K. Shumaker, L. Solimando, and R.D. Goldman. 2008. Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. *Genes & development*. 22:832-853.

Delbarre, E., M. Tramier, M. Coppey-Moisan, C. Gaillard, J.C. Courvalin, and B. Buendia. 2006. The truncated prelamin A in Hutchinson-Gilford progeria syndrome alters segregation of A-type and B-type lamin homopolymers. *Human molecular genetics*. 15:1113-1122.

Dhe-Paganon, S., E.D. Werner, Y.I. Chi, and S.E. Shoelson. 2002. Structure of the globular tail of nuclear lamin. *The Journal of biological chemistry*. 277:17381-17384.

Dittmer, T.A., and T. Misteli. 2011. The lamin protein family. *Genome biology*. 12:222.

Divecha, N., H. Banfic, and R.F. Irvine. 1991. The polyphosphoinositide cycle exists in the nuclei of Swiss 3T3 cells under the control of a receptor (for IGF-I) in the plasma membrane, and stimulation of the cycle increases nuclear diacylglycerol and apparently induces translocation of protein kinase C to the nucleus. *The EMBO journal*. 10:3207-3214.

Dundr, M., J.K. Ospina, M.H. Sung, S. John, M. Upender, T. Ried, G.L. Hager, and A.G. Matera. 2007. Actin-dependent intranuclear repositioning of an active gene locus in vivo. *The Journal of cell biology*. 179:1095-1103.

Dutil, E.M., and A.C. Newton. 2000. Dual role of pseudosubstrate in the coordinated regulation of protein kinase C by phosphorylation and diacylglycerol. *The Journal of biological chemistry*. 275:10697-10701.

Eggert, M., N. Radomski, D. Linder, D. Tripier, P. Traub, and E. Jost. 1993. Identification of novel phosphorylation sites in murine A-type lamins. *Eur J Biochem*. 213:659-671.

Eggert, M., N. Radomski, D. Tripier, P. Traub, and E. Jost. 1991. Identification of phosphorylation sites on murine nuclear lamin C by RP-HPLC and microsequencing. *FEBS letters*. 292:205-209.

Emery, A.E. 2002. The muscular dystrophies. *Lancet*. 359:687-695.

Fan, H.Y., C. Tong, M.Y. Li, L. Lian, D.Y. Chen, H. Schatten, and Q.Y. Sun. 2002. Translocation of the classic protein kinase C isoforms in porcine oocytes: implications of protein kinase C involvement in the regulation of nuclear activity and cortical granule exocytosis. *Experimental cell research*. 277:183-191.

Feinberg, W.M., J.L. Blackshear, A. Laupacis, R. Kronmal, and R.G. Hart. 1995. Prevalence, age distribution, and gender of patients with atrial fibrillation. Analysis and implications. *Archives of internal medicine*. 155:469-473.

Fields, A.P., and L.J. Thompson. 1995. The regulation of mitotic nuclear envelope breakdown: a role for multiple lamin kinases. *Progress in cell cycle research*. 1:271-286.

Fisher, D.Z., N. Chaudhary, and G. Blobel. 1986. cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. *Proceedings of the National Academy of Sciences*. 83:6450-6454.

Fong, L.G., J.K. Ng, J. Lammerding, T.A. Vickers, M. Meta, N. Cote, B. Gavino, X. Qiao, S.Y. Chang, S.R. Young, S.H. Yang, C.L. Stewart, R.T. Lee, C.F. Bennett, M.O. Bergo, and S.G. Young. 2006. Prelamin A and lamin A appear to be dispensable in the nuclear lamina. *The Journal of clinical investigation*. 116:743-752.

Forissier, J.F., G. Bonne, C. Bouchier, L. Duboscq-Bidot, P. Richard, C. Wisnewski, S. Briault, C. Moraine, O. Dubourg, K. Schwartz, and M. Komajda. 2003. Apical left ventricular aneurysm without atrio-ventricular block due to a lamin A/C gene mutation. *Eur J Heart Fail*. 5:821-825.

Frock, R.L., S.C. Chen, D.F. Da, E. Frett, C. Lau, C. Brown, D.N. Pak, Y. Wang, A. Muchir, H.J. Worman, L.F. Santana, W.C. Ladiges, P.S. Rabinovitch, and B.K. Kennedy. 2012. Cardiomyocyte-specific expression of lamin a improves cardiac function in Lmna-/- mice. *PloS one*. 7:e42918.

Furukawa, K., and Y. Hotta. 1993. cDNA cloning of a germ cell specific lamin B3 from mouse spermatocytes and analysis of its function by ectopic expression in somatic cells. *The EMBO journal*. 12:97-106.

Furukawa, K., H. Inagaki, and Y. Hotta. 1994. Identification and cloning of an mRNA coding for a germ cell-specific A-type lamin in mice. *Experimental cell research*. 212:426-430.

Gareau, J.R., and C.D. Lima. 2010. The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition. *Nat Rev Mol Cell Biol*. 11:861-871.

Geiss-Friedlander, R., and F. Melchior. 2007. Concepts in sumoylation: a decade on. *Nat Rev Mol Cell Biol*. 8:947-956.

Genschel, J., and H.H. Schmidt. 2000. Mutations in the LMNA gene encoding lamin A/C. *Human mutation*. 16:451-459.

Goldberg, M., H. Jenkins, T. Allen, W.G. Whitfield, and C.J. Hutchison. 1995. Xenopus lamin B3 has a direct role in the assembly of a replication competent nucleus: evidence from cell-free egg extracts. *J Cell Sci*. 108 (Pt 11):3451-3461.

Goldman, R.D., Y. Gruenbaum, R.D. Moir, D.K. Shumaker, and T.P. Spann. 2002. Nuclear lamins: building blocks of nuclear architecture. *Genes & development*. 16:533-547.

Gruenbaum, Y., Y. Landesman, B. Drees, J.W. Bare, H. Saumweber, M.R. Paddy, J.W. Sedat, D.E. Smith, B.M. Benton, and P.A. Fisher. 1988. Drosophila nuclear lamin precursor Dm0 is translated from either of two developmentally regulated mRNA species apparently encoded by a single gene. *The Journal of cell biology*. 106:585-596.

Grunig, E., J. Tasman, H. Kucherer, W. Franz, W. Kubler, and H. Katus. 1998. Frequency and phenotypes of familial dilated cardiomyopathy. *Journal of the American College of Cardiology*. 31:186-194.

Gupta, P. 2010. Lamin AC mutations and heart [microform] : nuclear envelope damage or disruption of transcription?
Haas, M., and E. Jost. 1993. Functional analysis of phosphorylation sites in human lamin A controlling lamin disassembly, nuclear transport and assembly. *European journal of cell biology*. 62:237-247.

Heald, R., and F. McKeon. 1990. Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. *Cell*. 61:579-589.

Heessen, S., and M. Fornerod. 2007. The inner nuclear envelope as a transcription factor resting place. *EMBO Rep*. 8:914-919.

Hegele, R. 2005. LMNA mutation position predicts organ system involvement in laminopathies. *Clinical genetics*. 68:31-34.

Heitlinger, E., M. Peter, A. Lustig, W. Villiger, E.A. Nigg, and U. Aebi. The role of the head and tail domain in lamin structure and assembly: Analysis of bacterially expressed chicken Lamin A and truncated B2 lamins. *Journal of Structural Biology*. 108:74-91.

Heitmann, P. 1968. A model for sulfhydryl groups in proteins. Hydrophobic interactions of the cystein side chain in micelles. *European journal of biochemistry / FEBS*. 3:346-350.

Hershberger, R.E., and J.D. Siegfried. 2011. Update 2011: clinical and genetic issues in familial dilated cardiomyopathy. *J Am Coll Cardiol*. 57:1641-1649.

Ho, C.Y., and J. Lammerding. 2012. Lamins at a glance. *Journal of cell science*. 125:2087-2093.

Ho, J.C., T. Zhou, W.H. Lai, Y. Huang, Y.C. Chan, X. Li, N.L. Wong, Y. Li, K.W. Au, D. Guo, J. Xu, C.W. Siu, D. Pei, H.F. Tse, and M.A. Esteban. 2011. Generation of induced pluripotent stem cell lines from 3 distinct laminopathies bearing heterogeneous mutations in lamin A/C. *Aging*. 3:380-390.

Hookana, E., M.J. Junttila, K.S. Kaikkonen, O. Ukkola, Y.A. Kesaniemi, M.L. Kortelainen, and H.V. Huikuri. 2012. Comparison of family history of sudden cardiac death in nonischemic and ischemic heart disease. *Circ Arrhythm Electrophysiol*. 5:757-761.

Hozak, P., A.M. Sasseville, Y. Raymond, and P.R. Cook. 1995. Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. *Journal of cell science*. 108:635-644.

Hübner, S., J.E. Eam, K.M. Wagstaff, and D.A. Jans. 2006. Quantitative analysis of localization and nuclear aggregate formation induced by GFP-lamin A mutant proteins in living HeLa cells. *Journal of Cellular Biochemistry*. 98:810-826.

Isobe, K., R. Gohara, T. Ueda, Y. Takasaki, and S. Ando. 2007. The last twenty residues in the head domain of mouse lamin A contain important structural elements for formation of head-to-tail polymers in vitro. *Bioscience, biotechnology, and biochemistry*. 71:1252-1259.

Jahn, D., S. Schramm, M. Schnolzer, C.J. Heilmann, C.G. de Koster, W. Schutz, R. Benavente, and M. Alsheimer. 2012. A truncated lamin A in the Lmna -/- mouse line: implications for the understanding of laminopathies. *Nucleus*. 3:463-474.

Jamali, M., P.J. Rogerson, S. Wilton, and I.S. Skerjanc. 2001. Nkx2-5 activity is essential for cardiomyogenesis. *The Journal of biological chemistry*. 276:42252-42258.

Johnson, B.R., R.T. Nitta, R.L. Frock, L. Mounkes, D.A. Barbie, C.L. Stewart, E. Harlow, and B.K. Kennedy. 2004. A-type lamins regulate retinoblastoma protein function by promoting subnuclear localization and preventing proteasomal degradation. *Proceedings of the National Academy of Sciences of the United States of America*. 101:9677-9682.

Kandert, S., M. Wehnert, C.R. Muller, B. Buendia, and M.C. Dabauvalle. 2009. Impaired nuclear functions lead to increased senescence and inefficient differentiation in human myoblasts with a dominant p.R545C mutation in the LMNA gene. *European journal of cell biology*. 88:593-608.

Kannel, W.B., R.D. Abbott, D.D. Savage, and P.M. McNamara. 1982. Epidemiologic Features of Chronic Atrial Fibrillation. *New England Journal of Medicine*. 306:1018-1022.

Karkkainen, S., T. Helio, R. Miettinen, P. Tuomainen, P. Peltola, J. Rummukainen, K. Ylitalo, M. Kaartinen, J. Kuusisto, L. Toivonen, M.S. Nieminen, M. Laakso, and K. Peuhkurinen. 2004. A novel mutation, Ser143Pro, in the lamin A/C gene is common in finnish patients with familial dilated cardiomyopathy. *European heart journal*. 25:885-893.

Karkkainen, S., and K. Peuhkurinen. 2007. Genetics of dilated cardiomyopathy. *Annals of medicine*. 39:91-107.

Kärkkäinen, S., E. Reissell, T. Heliö, M. Kaartinen, P. Tuomainen, L. Toivonen, J. Kuusisto, M. Kupari, M.S. Nieminen, M. Laakso, and K. Peuhkurinen. 2006. Novel mutations in the lamin A/C gene in heart transplant recipients with end stage dilated cardiomyopathy. *Heart*. 92:524-526.

Kolb, T., K. Maass, M. Hergt, U. Aebi, and H. Herrmann. 2011. Lamin A and lamin C form homodimers and coexist in higher complex forms both in the nucleoplasmic fraction and in the lamina of cultured human cells. *Nucleus*. 2:425-433.

Krahn, A.D., J. Manfreda, R.B. Tate, F.A. Mathewson, and T.E. Cuddy. 1995. The natural history of atrial fibrillation: incidence, risk factors, and prognosis in the Manitoba Follow-Up Study. *The American journal of medicine*. 98:476-484.

Krimm, I., C. Ostlund, B. Gilquin, J. Couprie, P. Hossenlopp, J.P. Mornon, G. Bonne, J.C. Courvalin, H.J. Worman, and S. Zinn-Justin. 2002. The Ig-like structure of the C-terminal domain of lamin A/C, mutated in muscular dystrophies, cardiomyopathy, and partial lipodystrophy. *Structure*. 10:811-823.

Lammerding, J., P.C. Schulze, T. Takahashi, S. Kozlov, T. Sullivan, R.D. Kamm, C.L. Stewart, and R.T. Lee. 2004. Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. *The Journal of clinical investigation*. 113:370-378.

Leach, K.L., E.A. Powers, V.A. Ruff, S. Jaken, and S. Kaufmann. 1989. Type 3 protein kinase C localization to the nuclear envelope of phorbol ester-treated NIH 3T3 cells. *The Journal of cell biology*. 109:685-695.

Lenz-Bohme, B., J. Wismar, S. Fuchs, R. Reifegerste, E. Buchner, H. Betz, and B. Schmitt. 1997. Insertional mutation of the Drosophila nuclear lamin Dm0 gene results in defective nuclear envelopes, clustering of nuclear pore complexes, and accumulation of annulate lamellae. *The Journal of cell biology*. 137:1001-1016.

Leukel, M., and E. Jost. 1995. Two conserved serines in the nuclear localization signal flanking region are involved in the nuclear targeting of human lamin A. *European journal of cell biology*. 68:133-142.

Levy, D.L., and R. Heald. 2010. Nuclear size is regulated by importin alpha and Ntf2 in Xenopus. *Cell*. 143:288-298.

Lin, F., and H.J. Worman. 1993. Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. *The Journal of biological chemistry*. 268:16321-16326.

Liu, J., T. Rolef Ben-Shahar, D. Riemer, M. Treinin, P. Spann, K. Weber, A. Fire, and Y. Gruenbaum. 2000. Essential roles for Caenorhabditis elegans lamin gene in nuclear organization, cell cycle progression, and spatial organization of nuclear pore complexes. *Molecular biology of the cell*. 11:3937-3947.

Luk, A., E. Ahn, G.S. Soor, and J. Butany. 2009. Dilated cardiomyopathy: a review. *Journal of clinical pathology*. 62:219-225.

Machiels, B.M., A.H. Zorenc, J.M. Endert, H.J. Kuijpers, G.J. van Eys, F.C. Ramaekers, and J.L. Broers. 1996. An alternative splicing product of the lamin A/C gene lacks exon 10. *The Journal of biological chemistry*. 271:9249-9253.

Makhnevych, T., Y. Sydorskyy, X. Xin, T. Srikumar, F.J. Vizeacoumar, S.M. Jeram, Z. Li, S. Bahr, B.J. Andrews, C. Boone, and B. Raught. 2009. Global map of SUMO function revealed by protein-protein interaction and genetic networks. *Molecular cell*. 33:124-135.

Malek, L.A., S. Labib, L. Mazurkiewicz, M. Saj, R. Ploski, F. Tesson, and Z.T. Bilinska. 2011. A new c.1621 C > G, p.R541G lamin A/C mutation in a family with DCM and regional wall motion abnormalities (akinesis/dyskinesis): genotype-phenotype correlation. *J Hum Genet*. 56:83-86.

Markiewicz, E., T. Dechat, R. Foisner, R.A. Quinlan, and C.J. Hutchison. 2002. Lamin A/C Binding Protein LAP2α Is Required for Nuclear Anchorage of Retinoblastoma Protein. *Molecular Biology of the Cell*. 13:4401-4413.

Martelli, A.M., R. Bortul, G. Tabellini, I. Faenza, A. Cappellini, R. Bareggi, L. Manzoli, and L. Cocco. 2002. Molecular characterization of protein kinase C-alpha binding to lamin A. *Journal of cellular biochemistry*. 86:320-330.

Martelli, A.M., G. Tabellini, R. Bortul, L. Manzoli, R. Bareggi, G. Baldini, V. Grill, M. Zweyer, P. Narducci, and L. Cocco. 2000. Enhanced nuclear diacylglycerol kinase activity in response to a mitogenic stimulation of quiescent Swiss 3T3 cells with insulin-like growth factor I. *Cancer research*. 60:815-821.

McKeon, F.D., M.W. Kirschner, and D. Caput. 1986. Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins. *Nature*. 319:463-468.

Méjat, A. 2010. LINC complexes in health and disease. *Nucleus*. 1:40-52.

Mercuri, E., S.C. Brown, P. Nihoyannopoulos, J. Poulton, M. Kinali, P. Richard, R.J. Piercy, S. Messina, C. Sewry, M.M. Burke, W. McKenna, G. Bonne, and F. Muntoni. 2005.

Extreme variability of skeletal and cardiac muscle involvement in patients with mutations in exon 11 of the lamin A/C gene. *Muscle Nerve*. 31:602-609.

Meshorer, E., and Y. Gruenbaum. 2008. Gone with the Wnt/Notch: stem cells in laminopathies, progeria, and aging. *The Journal of cell biology*. 181:9-13.

Meune, C., J.H. Van Berlo, F. Anselme, G. Bonne, Y.M. Pinto, and D. Duboc. 2006. Primary prevention of sudden death in patients with lamin A/C gene mutations. *The New England journal of medicine*. 354:209-210.

Moir, R.D., A.D. Donaldson, and M. Stewart. 1991. Expression in Escherichia coli of human lamins A and C: influence of head and tail domains on assembly properties and paracrystal formation. *Journal of cell science*. 99 (Pt 2):363-372.

Moir, R.D., T.P. Spann, R.I. Lopez-Soler, M. Yoon, A.E. Goldman, S. Khuon, and R.D. Goldman. 2000. Review: The Dynamics of the Nuclear Lamins during the Cell Cycle--Relationship between Structure and Function. *Journal of Structural Biology*. 129:324-334.

Moiseeva, O., V. Bourdeau, M. Vernier, M.-C. Dabauvalle, and G. Ferbeyre. 2011. Retinoblastoma-independent regulation of cell proliferation and senescence by the p53–p21 axis in lamin A/C-depleted cells. *Aging cell*. 10:789-797.

Molloy, S., and M. Little. 1992. p34cdc2 kinase-mediated release of lamins from nuclear ghosts is inhibited by cAMP-dependent protein kinase. *Experimental cell research*. 201:494-499.

Mounkes, L.C., S.V. Kozlov, J.N. Rottman, and C.L. Stewart. 2005. Expression of an LMNA-N195K variant of A-type lamins results in cardiac conduction defects and death in mice. *Human molecular genetics*. 14:2167-2180.

Muchir, A., J. Medioni, M. Laluc, C. Massart, T. Arimura, A.J. van der Kooi, I. Desguerre, M. Mayer, X. Ferrer, S. Briault, M. Hirano, H.J. Worman, A. Mallet, M. Wehnert, K. Schwartz, and G. Bonne. 2004. Nuclear envelope alterations in fibroblasts from patients with muscular dystrophy, cardiomyopathy, and partial lipodystrophy carrying lamin A/C gene mutations. *Muscle & nerve*. 30:444-450.

Muchir, A., P. Pavlidis, V. Decostre, A.J. Herron, T. Arimura, G. Bonne, and H.J. Worman. 2007. Activation of MAPK pathways links LMNA mutations to cardiomyopathy in Emery-Dreifuss muscular dystrophy. *The Journal of clinical investigation*. 117:1282-1293.

Muchir, A., S.A. Reilly, W. Wu, S. Iwata, S. Homma, G. Bonne, and H.J. Worman. 2012. Treatment with selumetinib preserves cardiac function and improves survival in cardiomyopathy caused by mutation in the lamin A/C gene. *Cardiovascular research*. 93:311-319.

Muchir, A., W. Wu, and H.J. Worman. 2009. Reduced expression of A-type lamins and emerin activates extracellular signal-regulated kinase in cultured cells. *Biochimica et biophysica acta*. 1792:75-81.

Muralikrishna, B., and V.K. Parnaik. 2001. SP3 and AP-1 mediate transcriptional activation of the lamin A proximal promoter. *European journal of biochemistry / FEBS*. 268:3736-3743.

Nakashima, S. 2002. Protein kinase C alpha (PKC alpha): regulation and biological function. *Journal of biochemistry*. 132:669-675.

Narula, N., V. Favalli, P. Tarantino, M. Grasso, A. Pilotto, R. Bellazzi, A. Serio, F.I. Gambarin, P. Charron, B. Meder, Y. Pinto, P.M. Elliott, J. Mogensen, M. Bolognesi, M. Bollati, and E. Arbustini. 2012. Quantitative expression of the mutated lamin A/C gene in patients with cardiolaminopathy. *Journal of the American College of Cardiology*. 60:1916-1920.

Neri, L.M., A.M. Billi, L. Manzoli, S. Rubbini, R.S. Gilmour, L. Cocco, and A.M. Martelli. 1994. Selective nuclear translocation of protein kinase C alpha in Swiss 3T3 cells treated with IGF-I, PDGF and EGF. *FEBS letters*. 347:63-68.

Neri, L.M., P. Borgatti, S. Capitani, and A.M. Martelli. 1998. Nuclear diacylglycerol produced by phosphoinositide-specific phospholipase C is responsible for nuclear translocation of protein kinase C-alpha. *The Journal of biological chemistry*. 273:29738-29744.

Nikolova, V., C. Leimena, A.C. McMahon, J.C. Tan, S. Chandar, D. Jogia, S.H. Kesteven, J. Michalicek, R. Otway, F. Verheyen, S. Rainer, C.L. Stewart, D. Martin, M.P. Feneley, and D. Fatkin. 2004. Defects in nuclear structure and function promote dilated cardiomyopathy in lamin A/C-deficient mice. *The Journal of clinical investigation*. 113:357-369.

Nitta, R.T., C.L. Smith, and B.K. Kennedy. 2007. Evidence that Proteasome-Dependent Degradation of the Retinoblastoma Protein in Cells Lacking A-Type Lamins Occurs Independently of Gankyrin and MDM2. *PloS one*. 2:e963.

Osouda, S., Y. Nakamura, B. de Saint Phalle, M. McConnell, T. Horigome, S. Sugiyama, P.A. Fisher, and K. Furukawa. 2005. Null mutants of Drosophila B-type lamin Dm(0) show aberrant tissue differentiation rather than obvious nuclear shape distortion or specific defects during cell proliferation. *Dev Biol*. 284:219-232.

Ostlund, C., G. Bonne, K. Schwartz, and H.J. Worman. 2001. Properties of lamin A mutants found in Emery-Dreifuss muscular dystrophy, cardiomyopathy and Dunnigan-type partial lipodystrophy. *Journal of cell science*. 114:4435-4445.

Parvari, R., and A. Levitas. 2012. The mutations associated with dilated cardiomyopathy. *Biochemistry research international*. 2012:639250.

Peter, A., and S. Reimer. 2012. Evolution of the lamin protein family: what introns can tell. *Nucleus*. 3:44-59.

Pollard, K.M., E.K. Chan, B.J. Grant, K.F. Sullivan, E.M. Tan, and C.A. Glass. 1990. In vitro posttranslational modification of lamin B cloned from a human T-cell line. *Molecular and cellular biology*. 10:2164-2175.

Pugh, G.E., P.J. Coates, E.B. Lane, Y. Raymond, and R.A. Quinlan. 1997. Distinct nuclear assembly pathways for lamins A and C lead to their increase during quiescence in Swiss 3T3 cells. *Journal of cell science*. 110 (Pt 19):2483-2493.

Raharjo, W.H., P. Enarson, T. Sullivan, C.L. Stewart, and B. Burke. 2001. Nuclear envelope defects associated with LMNA mutations cause dilated cardiomyopathy and Emery-Dreifuss muscular dystrophy. *Journal of cell science*. 114:4447-4457.

Ramos, F.J., S.C. Chen, M.G. Garelick, D.F. Dai, C.Y. Liao, K.H. Schreiber, V.L. MacKay, E.H. An, R. Strong, W.C. Ladiges, P.S. Rabinovitch, M. Kaeberlein, and B.K. Kennedy. 2012. Rapamycin reverses elevated mTORC1 signaling in lamin A/C-deficient mice, rescues cardiac and skeletal muscle function, and extends survival. *Science translational medicine*. 4:144ra103.

Rankin, J., and S. Ellard. 2006. The laminopathies: a clinical review. *Clin Genet*. 70:261-274.

Rober, R.A., H. Sauter, K. Weber, and M. Osborn. 1990. Cells of the cellular immune and hemopoietic system of the mouse lack lamins A/C: distinction versus other somatic cells. *Journal of cell science*. 95 (Pt 4):587-598.

Rodriguez, M.S., C. Dargemont, and R.T. Hay. 2001. SUMO-1 conjugation in vivo requires both a consensus modification motif and nuclear targeting. *The Journal of biological chemistry*. 276:12654-12659.

Rusiñol, A.E., and M.S. Sinensky. 2006. Farnesylated lamins, progeroid syndromes and farnesyl transferase inhibitors. *Journal of cell science*. 119:3265-3272.

Saj, M., R. Dabrowski, S. Labib, A. Jankowska, M. Szperl, G. Broda, H. Szwed, F. Tesson, Z.T. Bilinska, and R. Ploski. 2012. Variants of the Lamin A/C (LMNA) Gene in Non-Valvular Atrial Fibrillation Patients: A Possible Pathogenic Role of the Thr528Met Mutation. *Molecular diagnosis & therapy*. 16:99-107.

Saj, M., A. Jankowska, M. Lewandowski, H. Szwed, M. Szperl, R. Ploski, and Z.T. Bilinska. 2010. Dilated cardiomyopathy with profound segmental wall motion abnormalities and ventricular arrhythmia caused by the R541C mutation in the LMNA gene. *International journal of cardiology*. 144:e51-e53.

Sanna, T., A. Dello Russo, D. Toniolo, M. Vytopil, G. Pelargonio, G. De Martino, E. Ricci, G. Silvestri, V. Giglio, L. Messano, E. Zachara, and F. Bellocci. 2003. Cardiac features of Emery-Dreifuss muscular dystrophy caused by lamin A/C gene mutations. *European heart journal*. 24:2227-2236.

Sasse, B., U. Aebi, and N. Stuurman. 1998. A TaillessDrosophilaLamin Dm0Fragment Reveals Lateral Associations of Dimers. *Journal of Structural Biology*. 123:56-66.
Schirmer, E.C., and R. Foisner. 2007. Proteins that associate with lamins: Many faces, many functions. *Experimental cell research*. 313:2167-2179.

Schmalz, D., F. Hucho, and K. Buchner. 1998. Nuclear import of protein kinase C occurs by a mechanism distinct from the mechanism used by proteins with a classical nuclear localization signal. *Journal of cell science*. 111 (Pt 13):1823-1830.

Schmalz, D., F. Kalkbrenner, F. Hucho, and K. Buchner. 1996. Transport of protein kinase C alpha into the nucleus requires intact cytoskeleton while the transport of a protein containing a canonical nuclear localization signal does not. *Journal of cell science*. 109 (Pt 9):2401-2406.

Schmitz-Peiffer, C., C.L. Browne, and T.J. Biden. 1996. Characterization of two forms of protein kinase C alpha, with different substrate specificities, from skeletal muscle. *The Biochemical journal*. 320 (Pt 1):207-214.

Schreiber, K.H., and B.K. Kennedy. 2013. When lamins go bad: nuclear structure and disease. *Cell*. 152:1365-1375.

Serirom, S., W.H. Raharjo, K. Chotivanich, S. Loareesuwan, P. Kubes, and M. Ho. 2003. Anti-adhesive effect of nitric oxide on Plasmodium falciparum cytoadherence under flow. *The American journal of pathology*. 162:1651-1660.

Shimi, T., K. Pfleghaar, S. Kojima, C.G. Pack, I. Solovei, A.E. Goldman, S.A. Adam, D.K. Shumaker, M. Kinjo, T. Cremer, and R.D. Goldman. 2008. The A- and B-type nuclear lamin networks: microdomains involved in chromatin organization and transcription. *Genes & development*. 22:3409-3421.

Simon, D.N., T. Domaradzki, W.A. Hofmann, and K.L. Wilson. 2013. Lamin A tail modification by SUMO1 is disrupted by familial partial lipodystrophy-causing mutations. *Molecular biology of the cell*. 24:342-350.

Simon, D.N., and K.L. Wilson. 2013. Partners and post-translational modifications of nuclear lamins. *Chromosoma*. 122:13-31.

Smythe, C., H.E. Jenkins, and C.J. Hutchison. 2000. Incorporation of the nuclear pore basket protein nup153 into nuclear pore structures is dependent upon lamina assembly: evidence from cell-free extracts of Xenopus eggs. *Embo J*. 19:3918-3931.

Sullivan, T., D. Escalante-Alcalde, H. Bhatt, M. Anver, N. Bhat, K. Nagashima, C.L. Stewart, and B. Burke. 1999. Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. *The Journal of cell biology*. 147:913-920.

Sylvius, N., Z.T. Bilinska, J.P. Veinot, A. Fidzianska, P.M. Bolongo, S. Poon, P. McKeown, R.A. Davies, K.L. Chan, A.S. Tang, S. Dyack, J. Grzybowski, W. Ruzyllo, H. McBride, and F. Tesson. 2005. In vivo and in vitro examination of the functional significances of novel lamin gene mutations in heart failure patients. *Journal of medical genetics*. 42:639-647.

Sylvius, N., A. Hathaway, E. Boudreau, P. Gupta, S. Labib, P.M. Bolongo, P. Rippstein, H. McBride, Z.T. Bilinska, and F. Tesson. 2008. Specific contribution of lamin A and lamin C in the development of laminopathies. *Experimental cell research*. 314:2362-2375.

Sylvius, N., and F. Tesson. 2006. Lamin A/C and cardiac diseases. *Current opinion in cardiology*. 21:159-165.

Tesson F, Saj M, Mohamed-Uvaize M, Nicola H, Płoski R, and B.Z. T. 2013. Dilated Cardiomyopath- A clinical problem.(Manuscript submitted)

thesis, S.L.-M.S. 2010. The effect of *LMNA* mutations on lamin A/C and binding partner interactions and cellular distribution. *In* Department of Biochemistry, Microbiology and Immunology. Vol. M.Sc. University of Ottawa, Ottawa.

Tikkanen, J.T., V. Wichmann, M.J. Junttila, M. Rainio, E. Hookana, O.P. Lappi, M.L. Kortelainen, O. Anttonen, and H.V. Huikuri. 2012. Association of early repolarization and sudden cardiac death during an acute coronary event. *Circ Arrhythm Electrophysiol*. 5:714-718.

van Berlo, J.H., W.G. de Voogt, A.J. van der Kooi, J.P. van Tintelen, G. Bonne, R.B. Yaou, D. Duboc, T. Rossenbacker, H. Heidbuchel, M. de Visser, H.J. Crijns, and Y.M. Pinto. 2005. Meta-analysis of clinical characteristics of 299 carriers of LMNA gene mutations: do lamin A/C mutations portend a high risk of sudden death? *Journal of molecular medicine*. 83:79-83.

van der Heyden, M.A., M.J. van Kempen, Y. Tsuji, M.B. Rook, H.J. Jongsma, and T. Opthof. 2003. P19 embryonal carcinoma cells: a suitable model system for cardiac electrophysiological differentiation at the molecular and functional level. *Cardiovascular research*. 58:410-422.

Verga, L., M. Concardi, A. Pilotto, O. Bellini, M. Pasotti, A. Repetto, L. Tavazzi, and E. Arbustini. 2003. Loss of lamin A/C expression revealed by immuno-electron microscopy in dilated cardiomyopathy with atrioventricular block caused by LMNA gene defects. *Virchows Archiv : an international journal of pathology*. 443:664-671.

Verstraeten, V.L.R.M., J.L.V. Broers, M.A.M. van Steensel, S. Zinn-Justin, F.C.S. Ramaekers, P.M. Steijlen, M. Kamps, H.J.H. Kuijpers, D. Merckx, H.J.M. Smeets, R.C.M. Hennekam, C.L.M. Marcelis, and A. van den Wijngaard. 2006. Compound heterozygosity for mutations in LMNA causes a progeria syndrome without prelamin A accumulation. *Human molecular genetics*. 15:2509-2522.

Vijayan, K., E.L. Szotek, J.L. Martin, and A.M. Samarel. 2004. Protein kinase C-alpha-induced hypertrophy of neonatal rat ventricular myocytes. *American journal of physiology. Heart and circulatory physiology*. 287:H2777-2789.

Voigt, N., A. Friedrich, M. Bock, E. Wettwer, T. Christ, M. Knaut, R.H. Strasser, U. Ravens, and D. Dobrev. 2007. Differential phosphorylation-dependent regulation of constitutively active and muscarinic receptor-activated IK,ACh channels in patients with chronic atrial fibrillation. *Cardiovascular research*. 74:426-437.

Vytopil, M., S. Benedetti, E. Ricci, G. Galluzzi, A. Dello Russo, L. Merlini, G. Boriani, M. Gallina, L. Morandi, L. Politano, M. Moggio, L. Chiveri, I. Hausmanova-Petrusewicz, R. Ricotti, S. Vohanka, J. Toman, and D. Toniolo. 2003. Mutation analysis of the lamin A/C gene (LMNA) among patients with different cardiomuscular phenotypes. *Journal of medical genetics*. 40:e132.

Ward, G.E., and M.W. Kirschner. 1990. Identification of cell cycle-regulated phosphorylation sites on nuclear lamin C. *Cell*. 61:561-577.

Watkins, S., G. Borthwick, and H. Arthur. 2011. The H9C2 cell line and primary neonatal cardiomyocyte cells show similar hypertrophic responses in vitro. *In Vitro Cell.Dev.Biol.-Animal*. 47:125-131.

Ways, D.K., C.A. Kukoly, J. deVente, J.L. Hooker, W.O. Bryant, K.J. Posekany, D.J. Fletcher, P.P. Cook, and P.J. Parker. 1995. MCF-7 breast cancer cells transfected with protein kinase C-alpha exhibit altered expression of other protein kinase C isoforms and display a more aggressive neoplastic phenotype. *The Journal of clinical investigation*. 95:1906-1915.

Weber, K., U. Plessmann, and P. Traub. 1989. Maturation of nuclear lamin A involves a specific carboxy-terminal trimming, which removes the polyisoprenylation site from the precursor; implications for the structure of the nuclear lamina. *FEBS letters*. 257:411-414.

Wiesel, N., A. Mattout, S. Melcer, N. Melamed-Book, H. Herrmann, O. Medalia, U. Aebi, and Y. Gruenbaum. 2008. Laminopathic mutations interfere with the assembly, localization, and dynamics of nuclear lamins. *Proceedings of the National Academy of Sciences of the United States of America*. 105:180-185.

Wilkinson, J.D., D.C. Landy, S.D. Colan, J.A. Towbin, L.A. Sleeper, E.J. Orav, G.F. Cox, C.E. Canter, D.T. Hsu, S.A. Webber, and S.E. Lipshultz. 2010. The pediatric cardiomyopathy registry and heart failure: key results from the first 15 years. *Heart failure clinics*. 6:401-413, vii.

Wilson, K.L., and R. Foisner. 2010. Lamin-binding Proteins. *Cold Spring Harbor perspectives in biology*. 2.

Wolf, C.M., L. Wang, R. Alcalai, A. Pizard, P.G. Burgon, F. Ahmad, M. Sherwood, D.M. Branco, H. Wakimoto, G.I. Fishman, V. See, C.L. Stewart, D.A. Conner, C.I. Berul, C.E. Seidman, and J.G. Seidman. 2008. Lamin A/C haploinsufficiency causes dilated cardiomyopathy and apoptosis-triggered cardiac conduction system disease. *Journal of molecular and cellular cardiology*. 44:293-303.

Yang, H.-T., M. Zhang, J. Huang, H. Liang, P. Zhang, and K.R. Boheler. 2013. Cardiomyocytes derived from pluripotent stem cells: Progress and prospects from China. *Experimental cell research*. 319:120-125.

Young, S.G., M. Meta, S.H. Yang, and L.G. Fong. 2006. Prelamin A farnesylation and progeroid syndromes. *The Journal of biological chemistry*. 281:39741-39745.
Zastrow, M.S., S. Vlcek, and K.L. Wilson. 2004. Proteins that bind A-type lamins: integrating isolated clues. *Journal of cell science*. 117:979-987.

Zhang, Y.Q., and K.D. Sarge. 2008. Sumoylation regulates lamin A function and is lost in lamin A mutants associated with familial cardiomyopathies. *The Journal of cell biology*. 182:35-39.

Zini, N., A.M. Martelli, L.M. Neri, A. Bavelloni, P. Sabatelli, S. Santi, and N.M. Maraldi. 1995. Immunocytochemical evaluation of protein kinase C translocation to the inner nuclear matrix in 3T3 mouse fibroblasts after IGF-I treatment. *Histochemistry and cell biology*. 103:447-457.

APPENDIX I

Review – Submitted to the Cardiology Journal

Title: Dilated Cardiomyopathy – A Clinical Problem

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Abstract:

Dilated cardiomyopathy (DCM) is one of the leading causes of heart failure (HF) and heart transplant. Mutations in 60 genes have been associated with DCM. Approximately 6% of all DCM cases are caused by mutations in lamin A/C (LMNA). LMNA codes for type-V intermediate filaments that support the structure of the nuclear membrane and are involved in chromatin structure and gene expression. Most LMNA mutations result in striated muscle diseases while the rest affects the adipose tissue, peripheral nervous system, multiple tissues or lead to progeroid syndromes/overlapping syndromes. Patients with LMNA mutations exhibit a variety of cellular and physiological phenotypes. This paper explores the current phenotypes observed in LMNA-caused DCM, the results and implications of the cellular and animal models of DCM and the prevailing theories on the pathogenesis of laminopathies.

Key Words: Genetics, Dilated Cardiomyopathy, LMNA

Introduction

Dilated cardiomyopathy (DCM) is a disease of the heart muscle characterized by the dilatation of left or both ventricles and reduced systolic function in the absence of abnormal loading conditions (hypertension, valve disease) or coronary artery disease sufficient to cause global systolic impairment[1]. The incidence of idiopathic dilated cardiomyopathy (IDC) is estimated to be between 5 and 8 new cases per 100,000 individuals annually (depending on the diagnostic criteria used). After age adjustment, the prevalence averaged 36 annual cases per 100,000[2]. However, these figures may be underestimated as many individuals with DCM are asymptomatic. A study by Devereux et al. [3] estimates that 14% of middle-aged and elderly American population has asymptomatic left ventricular systolic dysfunction (LVSD), while another study marked this condition as prevalent in six percent of all asymptomatic individuals[4]. A proportion of this population may develop DCM in the future. The prevalence of DCM among children under 18 years of age is also significant occurring at a rate of 0.57 cases per 100,000 children, but in infants under one year of age this proportion reaches 4.4 cases per 100,000[5].

DCM is a significant health concern. It is the third most frequent cause of heart failure in the United States after coronary artery disease and hypertension. Furthermore, DCM is also a primary indication for heart transplantation [6] and is marked by considerable morbidity as well as mortality. During the last two decades, evident improvement in treating heart failure has been achieved through earlier diagnosis, availability of ACE-inhibitors, beta-blockers and aldosterone inhibitors as well as implantation of internal cardiac defibrillators and cardiac resynchronization therapy. However, much has still to be accomplished to decrease DCM prevalence especially concerning the early diagnosis of asymptomatic individuals.

It is believed that 20% to 50% of IDC cases have in fact familial causation[7-8]. In hypertrophic cardiomyopathy (HCM), genetic variations can be identified in 50% to 75% of patients[9]. The mutations linked to HCM are found mostly in one of two genes encoding the myosin heavy chain 7 (*MYH7*) and the myosin-binding protein C (*MYBPC3*), accounting for approximately 80% of all cases of the disease[10]. Conversely, thorough screening revealed that the genetic variation can be found in only 30% to 35% of familial DCM cases, *LMNA* mutations present in approximately six percent of all DCM cases[9]. However, a recent work employing next-generation sequencing to study *TTN*, encoding the largest human protein - titin, revealed truncations in 27% of DCM patients. Thus, *TTN* truncations are the most common known genetic cause of DCM[11].

So far, more than 60 genes have been associated with DCM. The pattern of the disease inheritance is mostly autosomal dominant although X-linked and recessive modes of transmission are also observed[8]. Mutations in genes encoding proteins of various cellular components and pathways are involved. The first major group consists of genes encoding sarcomeric proteins, notably *MYH6*, *MYH7*, *TNNT2*, *MYBPC3*, *ACTC* and *TTN*, with a subgroup including Z-disk genes such as *MYPN*, *TCAP, BAG3* and *ACTN2*. These genes were also associated with HCM. Another group contains genes encoding cytoskeletal proteins such as laminin alpha 4, metavinculin, desmin and dystrophin. Genes that encode mitochondrial protein (*TAZ*), RNA binding protein (*RBM20*), transcription factor (*EYA4*), ion channels (*SCN5A*, *ABCC*) and genes encoding nuclear envelope proteins, including *LMNA* and *TMPO*, were also associated with DCM[9]. Table 1 presents all genes that are associated with non-syndromic and syndromic DCM.

Syndromic DCM is a multi-faceted condition either with skeletal muscle involvement, such as muscular dystrophies due to mutations in lamin A/C (Emery-Dreifuss muscular dystrophy - EDMD, limb girdle muscular dystrophy - LGMD) and dystrophin

(Duchenne and Becker muscular dystrophies), or with the involvement of other organs, such as Barth Syndrome, hemochromatosis, hearing loss-associated DCM due to mutation in *EYA4* gene, and conditions resulting from mutations in mitochondrial DNA (Kearns-Sayre syndrome)[12]. Non-syndromic DCM, on the other hand, pertains solely to heart conditions (in vast majority DCM with or without conduction disease), which are usually associated with mutations in sarcomeric, desmosomal and ion channel genes, like *SCN5A*-encoding the sodium channel subunit protein[8].

Up to now, most efforts of geneticists focused on finding the mutations within one or few of the above mentioned genes. Mendelian model of disease inheritance was found to be prevalent when attempting to explain the development of DCM by the presence of a rare variant, usually non-synonymous, and in one gene. For such variant to be considered as a pathogenic disease-causing mutation, several factors have to be met. Firstly, one needs to verify the segregation of the variant with the disease phenotype in at least one large, multi-generational family, with both affected and unaffected members to estimate the penetrance. Secondly, the presence of the candidate mutation had to be excluded in at least 100 chromosomes from unaffected individuals. Thirdly, the demonstration of the evolutionary conservation of the mutated amino acid strengthens the hypothesis [9]. Lastly, functional consequences of the mutation have to be demonstrated. Even when these criteria are fulfilled, genetic background of DCM is difficult to diagnose, since incomplete penetrance is often observed, thus questioning the pathogenicity of identified rare variant. In majority of cases incomplete penetrance is age-related. For instance, it was reported that seven percent of *LMNA* mutation carriers exhibit cardiac-related phenotypes if under 20 years of age, 66% when carriers are between 20 and 39, 86% when carriers are between 40 and 59 years, and 100% when carriers are over 60 years of age[13]. Another complicating factor that clouds the genetic diagnosis is the variability of expression within one phenotype. While some

mutation carriers may develop all the symptoms of the disease, other family members carrying the mutation exhibit only some aspects of it and may remain with a subclinical form of the disease. The onset of DCM can vary greatly, as can the severity and the rate of progression of the disease. The variability may also pertain to the range of phenotypes as in the case of 960delT mutation, which presented with three differing phenotypes within one family: pure DCM, DCM with EDMD-like symptoms and DCM with LGMD-like symptoms[14]. Thus, until now, genotype-phenotype correlation has only been established with DCM with prominent conduction disease and mutations in genes coding largely for lamins A/C (one-third of cases), desmin and SCN5A. In addition, genetic influence of other factors modifying the phenotype of DCM, environmental-related factors such as diet or physical condition, and other more frequent cardiac diseases with overlapping symptoms, or all of these, have to be taken into consideration.

Despite recent technological progress that makes gene screening both less time-consuming and cost-efficient, genetic screening currently reveals that only 30 to 35% of familial DCM follow the Mendelian model of disease inheritance[9], while the remaining have a more complex multi-variant origin, which also encompasses the non-rare variants.

LMNA mutations in DCM

Mutations in *LMNA* were at first identified in a family with EDMD in 1999[15]. In the same year, the association between *LMNA* mutations and DCM was reported[16]. Since then an ever-growing number of mutations in *LMNA* have been identified. They can be divided according to the observed phenotype. Most *LMNA* mutations have been associated with striated muscle diseases (79.1%), followed by adipose tissue (8.6%) and peripheral nervous tissue disorders (0.3%). 9.3% of *LMNA* mutations lead to progeroid syndromes, while 10.9% cause overlapping syndromes, with multiple tissue involvement[17].

Table 2 encompasses the most current list of *LMNA* mutations that lead to DCM, either isolated, with sole cardiac features, or as a part of diagnosis of other, more complex conditions commonly affecting skeletal muscle, such as EDMD or LGMD, but also encompassing other tissues and, for instance, leading to Charcot-Marie-Tooth disease, familial partial lipodystrophy, general lipodystrophy,_hypogonadism, Hutchinson-Gilford progeria syndrome or diabetes mellitus. Table 2 also summarizes the span of phenotypic traits reported to be associated with a given mutation. It was created by combining information from four main *LMNA* mutation databases: the Human Intermediate Filament Database[18], the Leiden Muscular Dystrophy website (www.dmd.nl), the HGMD® Professional 2012.4 and the Universal Mutation Database (www.umd.be/LMNA/), as well as by adding mutations reported in the literature. We were able to find 161 *LMNA* mutations leading to DCM (Figure 1).

LMNA gene encompasses twelve exons and encodes lamin A and lamin C, as well as two other isoforms. Lamin A is longer (664 amino acids) than lamin C (572 amino acids). Both lamin isoforms are identical for the first 566 amino acids, after which lamin C contains a unique sequence of five basic amino acids. Lamin C lacks a part of exon 10, and exons 11 and 12. Amino acids from 567 to 664 are in turn unique to lamin A. In addition, prelamin A contains a CaaX motif at the COOH-terminal, which is the site that undergoes posttranslational modifications. Lamins are divided into three domains: a short globular head, a predominant α-helical rod and a globular tail. The rod domain comprises several coiled-coil domains, separated by linker regions, which are evolutionarily highly conserved (Figure 1)[19].

Most lamin mutations leading to DCM are found in the head and rod domains encompassing more than half of lamin A and two-thirds of lamin C. DCM mutations are rarely found in the tail domain, which includes many phosphorylation sites, as compared to

mutations linked to EDMD, familial partial lipodystrophy and Hutchinson-Gilford progeria syndrome[18]; Human Genome Mutation Database). However, hot spot(s) for DCM or other diseases affecting the striated muscle cannot be identified. Conversely, in adipose tissue defects approximately 80% of cases carry a substitution of the Arg482 residue, while 85% of mandibuloacral dysplasia cases are caused by a homozygous mutation at the p.527 residue and 77% of HGPS patients carry the c.1827C>T substitution within exon 11[19].

Studies of Cellular Phenotypes Associated with *LMNA* mutations

DCM patients with *LMNA* mutations display highly variable cardiomyocyte phenotype. A DCM patient encompassing 3-12 exon deletion showed diminished lamin A and C staining in the endomyocardial biopsy, with discontinuous nuclear envelopes and invasion of mitochondria into the nuclear space[20]. Another DCM patient carrying a *LMNA* mutation displayed dramatic morphological alterations in approximately 30% of the cardiomyocyte's nuclei, including a complete loss of the nuclear envelope[21]. However, other mutation carriers did not present with such dramatic abnormalities[20, 21]. Nevertheless, cardiomyocytes from DCM patients with *LMNA* mutations usually display reduced lamin A and C in the nuclei with nuclear membrane damage such as focal disruptions, blebs and nuclear pore clustering[22,23].

Skin fibroblasts isolated from patients with cardiac-or-skeletal-specific laminopathies most often had abnormal nuclear shape, including blebs and herniation[24]. Lamin A and C distribution were affected in these cells, and were either present in a honeycomb pattern [24] or distributed unevenly along the inner nuclear lamina[25]. Some fibroblasts had lamin A and C aggregates close to the lamina which did not interact with emerin, DNA or RNA[26]. Patient tissue heart samples and skin fibroblasts provide a method to visualize the pathophysiology of disease-associated mutations, however, they are

not easy to acquire. Currently no specific therapy exists for patients with *LMNA*-related DCM. This has encouraged researchers to establish both mice and cellular models in an effort to elucidate the mechanisms leading to the disease phenotypes. Unraveling the molecular mechanisms might provide insights into the pathophysiology of this disease which could be translated into novel therapy in the future.

Lamin A and C deficient mice developed dilated cardiomyopathy. Furthermore the cardiac tissue of these mice had fragmented nuclear architecture with the centromeric heterochromatin relocated from the periphery to the interior of the nucleus[27]. Studies on knock-out animals have shown that *Lmna*$^{-/-}$ mice are indistinguishable from their litter mates at birth but these mice rapidly exhibited growth retardation with clinical and histological features of EDMD, followed by severe DCM with conduction abnormalities and death by eight weeks of age[27,28]. A study by Wolf et al [29] showed that 50 percent of *Lmna*$^{+/-}$ mice had normal cardiac lamin A and C expression levels but displayed cardiac abnormalities. The *Lmna*$^{H222P/H222P}$ mice harbouring the EDMD mutation developed muscular dystrophy and DCM with atrio-ventricular conduction defect at adulthood and died by 13 months of age[30]. Male *Lmna*$^{H222P/H222P}$ mice developed significant left ventricular dilatation and by 16 weeks of age had decreased ejection fraction[30]. In another study, *Lmna*$^{N195K/N195K}$ mice, harboring a DCM with conduction system disease mutation, died at an early age due to arrhythmia. Surprisingly, both *Lmna*$^{H222P/+}$ and *Lmna*$^{N195K/+}$ mice were found to have a phenotype and life expectancy similar to the wild-type[30,31]. Cells derived from both *Lmna*$^{-/-}$ and *Lmna*$^{N195K/N195K}$ mice were observed to have damaged and misshapen nuclei, showed increased fragility under mechanical strain and impaired gene transcription[27,31-33].

Cellular models have shown that lamin A and C proteins are found distributed together in a homogeneous meshwork. However, wild type lamin A transfected alone has

consistently shown to localize to the inner nuclear lamina with some nucleoplasmic localization. Conversely, lamin C, has been shown to localize as intranuclear aggregate[21,34-38]. Intranuclear lamin C has shown to be more mobile than intranuclear lamin A[38,39]. Likewise, the lamin C only mouse model expressed lamin C at the inner nuclear lamina as established in wild type cells [37] thus indicating the existence of compensatory mechanisms. Pugh et al. [34]studied the incorporation of the lamin A and C in Swiss 3T3 cells and found that the incorporation of lamin C into the lamina was made possible by lamin A.

In an attempt to identify deregulation in striated muscle specific laminopathies, including DCM and EDMD, researches have been focused on skeletal muscle differentiations. Lamin A and C play a pivotal role in myoblast differentiation. *In vitro,* cells expressing disease-associated *LMNA* mutations displayed an inhibition of myoblast differentiation [40] (F. Tesson personal communication) and myoblasts lacking lamin A and C expression showed decreased differentiation potential with a downregulation of MyoD and pRb and an upregulation of Myf5[41]. These studies suggest that disruption of lamin A and C may weaken contractile tissues such as skeletal and cardiac muscle.

Lamin A and C also play a role in the regulation of signaling cascades such as the Sumo pathway. Sumo pathway regulates a wide range of cellular processes through the attachment of small ubiquitin-related modifier (sumo) to various substrates. Sumo1 was found to be mislocalized in presence of lamin A and C mutants both *in vitro* (C2C12 and Cos7 cells) and *in vivo* (primary myoblasts and myopathic muscle tissue from the *Lmna*$^{H222P/H222P}$ mice)[21,42]. In cell models, trapping of sumo1 correlated with an increased steady-state level of sumoylation. Ubc9, the E2 conjugating enzyme of the Sumo pathway was also mislocalized to the mutant aggregates[42]. Lamin A has been shown to be covalently modified by Sumo 2 and 3[43]. The disruption of a critical post-translational

modifying process has the potential to affect the post-translational regulation of tissue-specific sumoylated proteins which may lead to the tissue-specific symptoms observed in patients with various laminopathies[42].

Recent studies using induced pluripotent stem cells derived cardiomyocytes (iPSCS-CMs) from DCM patients with *LMNA* mutations showed accelerated nuclear senescence and apoptosis under electrical stimulation. This study also showed that activation of stress response MEK1/ERK1/2 pathway contributes to increase apoptosis in $LMNA^{R225X/WT}$dermal fibroblasts after electrical stimulation[44]. Moreover, this apoptotic effect could be attenuated by pharmacological blockade of the MEK1/ERK1/2 pathway. Study of gene expression profile showed that mouse models of laminopathies also displayed ERK pathway activation in heart muscle[45,46]. Importantly, the pharmacological blockade of the ERK1/2 pathway prevented the development of DCM in this model[47]. These studies have shed new light on MEK1 pathway as a potential therapeutic target in *LMNA*-associated DCM.

Conclusion: The mechanistic hypotheses

Until now, three main hypotheses have been proposed to explain the mechanism of pathogenesis of laminopathies: the structural, the gene expression and the toxicity hypotheses. The structural hypothesis states that mutations within lamin A/C lead to disorganization of the proteineous meshwork, instability of the nuclear envelope and disorganization of chromatin, which in turn leads to the overall inability of the cell to properly function in contracting tissue environment, such as striated muscles[48,49]. Building on this hypothesis, recent studies identified repetitive disruptions of the nuclear envelope in presence of lamin A/C mutations[20,50]. This disruption impaired protein distribution into cell compartments. Translocations of large amounts of protein into the cytoplasm could trigger aggresome formation or even induce cell apoptosis[50]. On the

other hand, translocation of transcription factors into the cytoplasm might impair gene expression. The gene expression hypothesis is based on the regulatory role of lamin A/C in chromatin organization and DNA transcription. Mutated lamins might disrupt the protein meshwork through their interaction with other proteins of the nuclear envelope, which may lead to epigenetic changes in the chromatin, which may in turn disrupt various complex signaling pathways[51]. Lastly, the cell toxicity hypothesis proposes that mutated prelamin A may accumulate within patients' nuclei to the point that they might become toxic to the cell and lead to development of the disease[52]. These hypotheses are likely to be not mutually exclusive and combining them might allow describing the mechanisms underlying the initiation and/or the development of laminopathies. Ultimatly, a better understanding of the pathogenesis of the disease may suggest novel strategies targeting the underlying molecular defects.

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List of abbreviations

DCM: Dilated cardiomyopathy

IDC: Idiopathic dilated cardiomyopathy

IPSC-CMs: Induced pluripotent stem cells derived cardiomyocytes

LVSD: Left ventricular systolic dysfunction

HCM: Hypertrophic cardiomyopathy

EDMD: Emery-Dreifuss muscular dystrophy

LGMD: Limb girdle muscular dystrophy

Reference List

1. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, Dubourg O, Kuhl U, Maisch B, McKenna WJ, Monserrat L, Pankuwelt S, Rapezzi C, Seferovic P, Tavazzi L, Keren A: **[Classification of the cardiomyopathies].** *Kardiol Pol* 2008, **66:**533-40, discussion.

2. Dec GW, Fuster V: **Idiopathic dilated cardiomyopathy.** *N Engl J Med* 1994, **331:**1564-1575.

3. Devereux RB, Roman MJ, Paranicas M, Lee ET, Welty TK, Fabsitz RR, Robbins D, Rhoades ER, Rodeheffer RJ, Cowan LD, Howard BV: **A population-based assessment of left ventricular systolic dysfunction in middle-aged and older adults: the Strong Heart Study.** *Am Heart J* 2001, **141:**439-446.

4. Redfield MM, Jacobsen SJ, Burnett JC, Jr., Mahoney DW, Bailey KR, Rodeheffer RJ: **Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic.** *JAMA* 2003, **289:**194-202.

5. Towbin JA, Lowe AM, Colan SD, Sleeper LA, Orav EJ, Clunie S, Messere J, Cox GF, Lurie PR, Hsu D, Canter C, Wilkinson JD, Lipshultz SE: **Incidence, causes, and outcomes of dilated cardiomyopathy in children.** *JAMA* 2006, **296:**1867-1876.

6. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, Moss AJ, Seidman CE, Young JB: **Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention.** *Circulation* 2006, **113:**1807-1816.

7. Ghosh N, Haddad H: **Recent progress in the genetics of cardiomyopathy and its role in the clinical evaluation of patients with cardiomyopathy.** *Curr Opin Cardiol* 2011, **26:**155-164.

8. Taylor MR, Carniel E, Mestroni L: **Cardiomyopathy, familial dilated.** *Orphanet J Rare Dis* 2006, **1:**27.

9. Hershberger RE, Siegfried JD: **Update 2011: clinical and genetic issues in familial dilated cardiomyopathy.** *J Am Coll Cardiol* 2011, **57:**1641-1649.

10. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, Benaiche A, Isnard R, Dubourg O, Burban M, Gueffet JP, Millaire A, Desnos M, Schwartz K, Hainque B, Komajda M: **Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy.** *Circulation* 2003, **107:**2227-2232.

11. Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, Christodoulou D, Conner L, DePalma SR, McDonough B, Sparks E, Teodorescu DL, Cirino AL, Banner NR,

Pennell DJ, Graw S, Merlo M, Di Lenarda A, Sinagra G, Bos JM, Ackerman MJ, Mitchell RN, Murry CE, Lakdawala NK, Ho CY, Barton PJ, Cook SA, Mestroni L, Seidman JG, Seidman CE: **Truncations of titin causing dilated cardiomyopathy.** *N Engl J Med* 2012, **366:**619-628.

12. Hershberger RE, Morales A, Siegfried JD: **Clinical and genetic issues in dilated cardiomyopathy: a review for genetics professionals.** *Genet Med* 2010, **12:**655-667.

13. Pasotti M, Klersy C, Pilotto A, Marziliano N, Rapezzi C, Serio A, Mannarino S, Gambarin F, Favalli V, Grasso M, Agozzino M, Campana C, Gavazzi A, Febo O, Marini M, Landolina M, Mortara A, Piccolo G, Vigano M, Tavazzi L, Arbustini E: **Long-term outcome and risk stratification in dilated cardiolaminopathies.** *J Am Coll Cardiol* 2008, **52:**1250-1260.

14. Brodsky GL, Muntoni F, Miocic S, Sinagra G, Sewry C, Mestroni L: **Lamin A/C gene mutation associated with dilated cardiomyopathy with variable skeletal muscle involvement.** *Circulation* 2000, **101:**473-476.

15. Bonne G, Di Barletta MR, Varnous S, Becane HM, Hammouda EH, Merlini L, Muntoni F, Greenberg CR, Gary F, Urtizberea JA, Duboc D, Fardeau M, Toniolo D, Schwartz K: **Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy.** *Nat Genet* 1999, **21:**285-288.

16. Fatkin D, MacRae C, Sasaki T, Wolff MR, Porcu M, Frenneaux M, Atherton J, Vidaillet HJ, Jr., Spudich S, De Girolami U, Seidman JG, Seidman C, Muntoni F, Muehle G, Johnson W, McDonough B: **Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease.** *N Engl J Med* 1999, **341:**1715-1724.

17. Bertrand AT, Chikhaoui K, Yaou RB, Bonne G: **Clinical and genetic heterogeneity in laminopathies.** *Biochem Soc Trans* 2011, **39:**1687-1692.

18. Szeverenyi I, Cassidy AJ, Chung CW, Lee BT, Common JE, Ogg SC, Chen H, Sim SY, Goh WL, Ng KW, Simpson JA, Chee LL, Eng GH, Li B, Lunny DP, Chuon D, Venkatesh A, Khoo KH, McLean WH, Lim YP, Lane EB: **The Human Intermediate Filament Database: comprehensive information on a gene family involved in many human diseases.** *Hum Mutat* 2008, **29:**351-360.

19. Bertrand AT, Chikhaoui K, Yaou RB, Bonne G: **Clinical and genetic heterogeneity in laminopathies.** *Biochem Soc Trans* 2011, **39:**1687-1692.

20. Gupta P, Bilinska ZT, Sylvius N, Boudreau E, Veinot JP, Labib S, Bolongo PM, Hamza A, Jackson T, Ploski R, Walski M, Grzybowski J, Walczak E, Religa G, Fidzianska A, Tesson F: **Genetic and ultrastructural studies in dilated cardiomyopathy patients: a large deletion in the lamin A/C gene is associated with cardiomyocyte nuclear envelope disruption.** *Basic Res Cardiol* 2010, **105:**365-377.

21. Sylvius N, Bilinska ZT, Veinot JP, Fidzianska A, Bolongo PM, Poon S, McKeown P, Davies RA, Chan KL, Tang AS, Dyack S, Grzybowski J, Ruzyllo W, McBride H, Tesson F: **In vivo and in vitro examination of the functional significances of**

novel lamin gene mutations in heart failure patients. *J Med Genet* 2005, **42:**639-647.

22. Arbustini E, Pilotto A, Repetto A, Grasso M, Negri A, Diegoli M, Campana C, Scelsi L, Baldini E, Gavazzi A, Tavazzi L: **Autosomal dominant dilated cardiomyopathy with atrioventricular block: a lamin A/C defect-related disease.** *J Am Coll Cardiol* 2002, **39:**981-990.

23. Fidzianska A, Bilinska ZT, Tesson F, Wagner T, Walski M, Grzybowski J, Ruzyllo W, Hausmanowa-Petrusewicz I: **Obliteration of cardiomyocyte nuclear architecture in a patient with LMNA gene mutation.** *J Neurol Sci* 2008, **271:**91-96.

24. Muchir A, Worman HJ: **The nuclear envelope and human disease.** *Physiology (Bethesda)* 2004, **19:**309-314.

25. Favreau C, Dubosclard E, Ostlund C, Vigouroux C, Capeau J, Wehnert M, Higuet D, Worman HJ, Courvalin JC, Buendia B: **Expression of lamin A mutated in the carboxyl-terminal tail generates an aberrant nuclear phenotype similar to that observed in cells from patients with Dunnigan-type partial lipodystrophy and Emery-Dreifuss muscular dystrophy.** *Exp Cell Res* 2003, **282:**14-23.

26. Muchir A, Medioni J, Laluc M, Massart C, Arimura T, van der Kooi AJ, Desguerre I, Mayer M, Ferrer X, Briault S, Hirano M, Worman HJ, Mallet A, Wehnert M, Schwartz K, Bonne G: **Nuclear envelope alterations in fibroblasts from patients with muscular dystrophy, cardiomyopathy, and partial lipodystrophy carrying lamin A/C gene mutations.** *Muscle Nerve* 2004, **30:**444-450.

27. Nikolova V, Leimena C, McMahon AC, Tan JC, Chandar S, Jogia D, Kesteven SH, Michalicek J, Otway R, Verheyen F, Rainer S, Stewart CL, Martin D, Feneley MP, Fatkin D: **Defects in nuclear structure and function promote dilated cardiomyopathy in lamin A/C-deficient mice.** *J Clin Invest* 2004, **113:**357-369.

28. Sullivan T, Escalante-Alcalde D, Bhatt H, Anver M, Bhat N, Nagashima K, Stewart CL, Burke B: **Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy.** *J Cell Biol* 1999, **147:**913-920.

29. Wolf CM, Wang L, Alcalai R, Pizard A, Burgon PG, Ahmad F, Sherwood M, Branco DM, Wakimoto H, Fishman GI, See V, Stewart CL, Conner DA, Berul CI, Seidman CE, Seidman JG: **Lamin A/C haploinsufficiency causes dilated cardiomyopathy and apoptosis-triggered cardiac conduction system disease.** *J Mol Cell Cardiol* 2008, **44:**293-303.

30. Arimura T, Helbling-Leclerc A, Massart C, Varnous S, Niel F, Lacene E, Fromes Y, Toussaint M, Mura AM, Keller DI, Amthor H, Isnard R, Malissen M, Schwartz K, Bonne G: **Mouse model carrying H222P-Lmna mutation develops muscular dystrophy and dilated cardiomyopathy similar to human striated muscle laminopathies.** *Hum Mol Genet* 2005, **14:**155-169.

31. Mounkes LC, Kozlov SV, Rottman JN, Stewart CL: **Expression of an LMNA-N195K variant of A-type lamins results in cardiac conduction defects and death in mice.** *Hum Mol Genet* 2005, **14:**2167-2180.

32. Sullivan T, Escalante-Alcalde D, Bhatt H, Anver M, Bhat N, Nagashima K, Stewart CL, Burke B: **Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy.** *J Cell Biol* 1999, **147:**913-920.

33. Lammerding J, Schulze PC, Takahashi T, Kozlov S, Sullivan T, Kamm RD, Stewart CL, Lee RT: **Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction.** *J Clin Invest* 2004, **113:**370-378.

34. Pugh GE, Coates PJ, Lane EB, Raymond Y, Quinlan RA: **Distinct nuclear assembly pathways for lamins A and C lead to their increase during quiescence in Swiss 3T3 cells.** *J Cell Sci* 1997, **110 (Pt 19):**2483-2493.

35. Raharjo WH, Enarson P, Sullivan T, Stewart CL, Burke B: **Nuclear envelope defects associated with LMNA mutations cause dilated cardiomyopathy and Emery-Dreifuss muscular dystrophy.** *J Cell Sci* 2001, **114:**4447-4457.

36. Vaughan A, Alvarez-Reyes M, Bridger JM, Broers JL, Ramaekers FC, Wehnert M, Morris GE, Whitfield WGF, Hutchison CJ: **Both emerin and lamin C depend on lamin A for localization at the nuclear envelope.** *J Cell Sci* 2001, **114:**2577-2590.

37. Fong LG, Ng JK, Lammerding J, Vickers TA, Meta M, Cote N, Gavino B, Qiao X, Chang SY, Young SR, Yang SH, Stewart CL, Lee RT, Bennett CF, Bergo MO, Young SG: **Prelamin A and lamin A appear to be dispensable in the nuclear lamina.** *J Clin Invest* 2006, **116:**743-752.

38. Sylvius N, Hathaway A, Boudreau E, Gupta P, Labib S, Bolongo PM, Rippstein P, McBride H, Bilinska ZT, Tesson F: **Specific contribution of lamin A and lamin C in the development of laminopathies.** *Exp Cell Res* 2008, **314:**2362-2375.

39. Broers JL, Kuijpers HJ, Ostlund C, Worman HJ, Endert J, Ramaekers FC: **Both lamin A and lamin C mutations cause lamina instability as well as loss of internal nuclear lamin organization.** *Exp Cell Res* 2005, **304:**582-592.

40. Favreau C, Higuet D, Courvalin JC, Buendia B: **Expression of a mutant lamin A that causes Emery-Dreifuss muscular dystrophy inhibits in vitro differentiation of C2C12 myoblasts.** *Mol Cell Biol* 2004, **24:**1481-1492.

41. Frock RL, Kudlow BA, Evans AM, Jameson SA, Hauschka SD, Kennedy BK: **Lamin A/C and emerin are critical for skeletal muscle satellite cell differentiation.** *Genes Dev* 2006, **20:**486-500.

42. Boudreau E, Labib S, Bertrand AT, Decostre V, Bolongo PM, Sylvius N, Bonne G, Tesson F: **Lamin A/C mutants disturb sumo1 localization and sumoylation in vitro and in vivo.** *PLoS One* 2012, **7:**e45918.

43. Zhang YQ, Sarge KD: **Sumoylation regulates lamin A function and is lost in lamin A mutants associated with familial cardiomyopathies.** *J Cell Biol* 2008, **182:**35-39.

44. Siu CW, Lee YK, Ho JC, Lai WH, Chan YC, Ng KM, Wong LY, Au KW, Lau YM, Zhang J, Lay KW, Colman A, Tse HF: **Modeling of lamin A/C mutation**

premature cardiac aging using patient-specific induced pluripotent stem cells. *Aging (Albany NY)* 2012, **4:**803-822.

45. Muchir A, Pavlidis P, Decostre V, Herron AJ, Arimura T, Bonne G, Worman HJ: **Activation of MAPK pathways links LMNA mutations to cardiomyopathy in Emery-Dreifuss muscular dystrophy.** *J Clin Invest* 2007, **117:**1282-1293.

46. Muchir A, Pavlidis P, Bonne G, Hayashi YK, Worman HJ: **Activation of MAPK in hearts of EMD null mice: similarities between mouse models of X-linked and autosomal dominant Emery Dreifuss muscular dystrophy.** *Hum Mol Genet* 2007, **16:**1884-1895.

47. Muchir A, Shan J, Bonne G, Lehnart SE, Worman HJ: **Inhibition of extracellular signal-regulated kinase signaling to prevent cardiomyopathy caused by mutation in the gene encoding A-type lamins.** *Hum Mol Genet* 2009, **18:**241-247.

48. Broers JL, Peeters EA, Kuijpers HJ, Endert J, Bouten CV, Oomens CW, Baaijens FP, Ramaekers FC: **Decreased mechanical stiffness in LMNA-/- cells is caused by defective nucleo-cytoskeletal integrity: implications for the development of laminopathies.** *Hum Mol Genet* 2004, **13:**2567-2580.

49. Fidzianska A, Hausmanowa-Petrusewicz I: **Architectural abnormalities in muscle nuclei. Ultrastructural differences between X-linked and autosomal dominant forms of EDMD.** *J Neurol Sci* 2003, **210:**47-51.

50. De Vos WH, Houben F, Kamps M, Malhas A, Verheyen F, Cox J, Manders EM, Verstraeten VL, van Steensel MA, Marcelis CL, van den WA, Vaux DJ, Ramaekers FC, Broers JL: **Repetitive disruptions of the nuclear envelope invoke temporary loss of cellular compartmentalization in laminopathies.** *Hum Mol Genet* 2011, **20:**4175-4186.

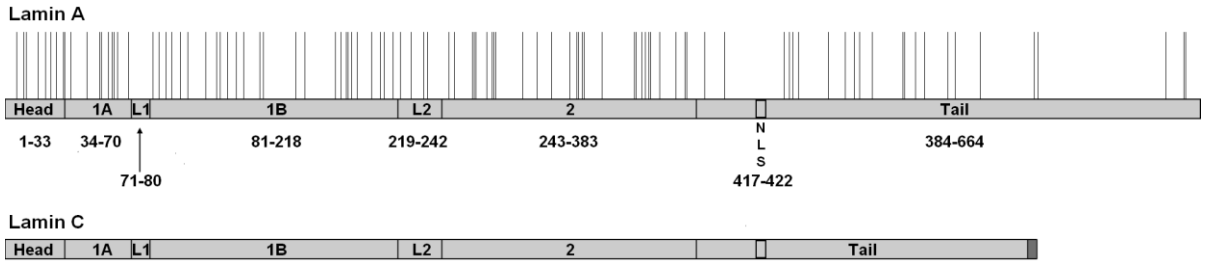
51. Maraldi NM, Capanni C, Cenni V, Fini M, Lattanzi G: **Laminopathies and lamin-associated signaling pathways.** *J Cell Biochem* 2011, **112:**979-992.

52. Navarro CL, Cau P, Levy N: **Molecular bases of progeroid syndromes.** *Hum Mol Genet* 2006, **15 Spec No 2:**R151-R161.

Figure legends

Figure 1. Protein map of lamin A and lamin C with currently known mutations of both transcripts plotted onto lamin A protein. NLS - nuclear localization signal. Dark shaded area at the C-terminal of lamin C represents lamin C-unique sequence.

Figure1


Lamin A
Head
1A
L1
1B
L2
2
Tail
1-33
34-70
71-80
81-218
219-242
243-383
N
L
S
417-422
384-664
Lamin C
Head
1A
L1
1B
L2
2
Tail

Tables

Gene	Protein	Location	Reference *
ABCC9	Sulfonylurea receptor 2A	12p12	[1]
ACTC1	Cardiac actin	15q14	[2,3]
ACTN2	α-Actinin 2	1q42-q43	[4,5]
ANKRD1	Ankyrin repeat domain-containing protein 1	10q23	[6,7]
BAG3	BCL2-associated athanogene 3	10q25-q26	[8,9]
BMP10	Bone morphogenetic protein 10	2p14	[10]
CHRM2	Cholinergic receptor, muscarinic 2	7q31-q35	[11]
CRYAB	αB-Crystallin	11q22	[12,13]
CSRP3	Cysteine and glycine-rich protein 3 (cardiac LIM protein)	11p15	[4,5,14]
CTF1	Cardiotrophin 1	16p11	[15]
DES	Desmin	2q35	[16-18]
DMD	Dystrophin	Xp21	[19-21]
DNAJC19	Dnaj (hsp40) homologue, subfamily c, member 19	3q26	[22]
DOLK	Dolichol kinase	9q34	[23]
DSC2	Desmocollin 2	18q12	[24]
DSG2	Desmoglein 2	18q12	[24]
DSP	Desmoplakin	6q24	[24,25]
EMD	Emerin	Xq28	[26]
EYA4	Eyes absent (drosophila) homologue 4	6q23	[27]
FHL2	Four-and-α-half LIM protein 2	2q12-q14	[28]
FKTN	Fukutin	9q31	[31]
FLT1	fms-related tyrosine kinase 1	13q12	[32]
FOXD4	Forkhead box D4	9p11	[33]
GATAD1	GATA zinc finger domain containing 1	7q21-q22	[34]
HSPB7	Heat shock protein beta-7	1p36-p34	[35]
ILK	Integrin-linked kinase	11p15	[36]
LAMA2	Laminin alpha 2 (merosin)	6q22-q23	[37]
LAMA4	Laminin-α4	6q21	[36]
LAMP2	Lysosome-associated membrane protein 2	Xq24	[38]
LDB3	LIM domain binding 3 (Z1 and Z4 isoforms)	10q22-q23	[39-41]
LMNA	Lamin A/C	1p1-1q1	[42-45]
MURC	Muscle-related coiled-coil protein	9q31	[46]
MYBPC3	Myosin-binding protein C	11p11	[32,47,48]
MYH6	α-Myosin heavy chain	14q12	[49,50]
MYH7	β-Myosin heavy chain	14q12	[41,47,51-53]
MYPN	Myopallidin	10q21	[54]
NEBL	Nebulette	10p12	[55]
NEXN	Nexilin (F actin binding protein)	1p31	[56]
PDLIM3	PDZ LIM domain protein 3	4q35	[57]
PLN	Phospholamban	6q22	[58-61]
POLG	Polymerase (DNA directed), gamma	15q25	[62]
PSEN1	Presenilin 1	14q24	[63,64]
PSEN2	Presenilin2	1q31-q42	[63]
RBM20	Ribonucleic acid-binding protein	10q25-26	[65-67]
SCN5A	Sodium channel, voltage-gated, type V, alpha subunit	3p21	[68,69]
SDHA	Succinate dehydrogenase complex, subunit a, flavoprotein	5p15.33	[70]
SGCB	β-Sarcoglycan	4q12	[71,72]
SGCD	δ-Sarcoglycan	5q33	[73,74]
SYNE1	Nesprin-1	6q25	[75]
SYNM	Synemin	15q26	[32]
TAZ	Tafazzin	Xq28	[76,77]

TCAP	Telethonin	17q12	[78]
TCF21	Transcription factor 21	6pter-qter	[79]
TMPO	Thymopoietin	12q22	[80]
TNNC1	Troponin C, slow	3p21-p14	[50,81,82]
TNNI3	Cardiac troponin I	19q13	[83-85]
TNNT2	Cardiac troponin T	1q32	[51,81,86,87]
TPM1	α-Tropomyosin	15q22	[50,88,89]
TTN	Titin	2q24	[90-93]
TXNRD2	Thioredoxin reductase 2	22q11	[94]
VCL	Vinculin/metavinculin	10q23	[5,95]
VPS13A	Vacuolar protein sorting 13A (yeast), CHAC	9q21	[96]

Table 1. Genes reported in association with dilated cardiomyopathy[97,98,Human Genome Mutation Database].
*: References are listed in "Supplementary file

Nucleotide change	Protein change	DCM phenotype range	Domain	Reference *
674bp deletion incl. start codon	deletion of 5' part	1° AVB, LBBB, AF, PVB, VT, HF, SCD	Head	[1]
c.16C>T	p.Gln6X	1°-2° AVB, AF, AFLu, PAB, PVB, VT, HF, SCD	Head	[2]
c.28_29insA	p.Thr10AsnfsX31	1°-2° AVB, AF, PVB, MD	Head	[3]
c.31delC	p.Arg11AlafsX85	(2°) AVB	Head	[4,5]
c.46_49dup	p.Ser18GlnfsX24	AVB	Head	[6]
c.48_51dupCAGC	p.Ser18GlnfsX23	n/a	Head	[6]
c.65C>T	p.Ser22Leu	PVB, HF	Head	[7]
c.73C>T	p.Arg25Cys	CA, AF, PVB, MD, HF	Head	[8]
c.73C>G	p.Arg25Gly	1°/3° AVB, TC, AF, PVB, PAB, LGMD, HF	Head	[9]
c.78C>T	p.Ile26Ile	HF, LBBB, AF	Head	[10]
c.82C>T	p.Arg28Trp	AVB, AF, PM, FPLD, HF	Head	[4,11]
c.94_96delAAG	p.Lys32del	LAFB, CA, AF, EDMD	Head	[12,13]
c.99G>T	p.Glu33Asp	1° AVB, Br, AF, CA, CMT2, MD, leuconychia	Head	[14]
c.106C>T	p.Gln36X	1°-2° AVB, VT	Coil 1A	[15]
c.134A>G	p.Tyr45Cys	AF, AFL, MD	Coil 1A	[16]
c.154C>G	p.Leu52Val	n/a	Coil 1A	[17]
c.155T>C	p.Leu52Pro	AVB, LBBB, RBBB, AF, PVB, HF	Coil 1A	[18]
c.158A>T	p.Glu53Val	AVB, AF, HF	Coil 1A	[19]
c.169G>C	p.Ala57Pro	atypical WS, hypogonadism, sloping shoulders	Coil 1A	[20]
c.176T>G	p.Leu59Arg	CCD, hypogonadism, ovarian failure, MAD	Coil 1A	[21,22]
c.178C>G	p.Arg60Gly	1°/3° AVB, LBBB, Br, AF, VA, DM, FPLD, PN, HF, SCD	Coil 1A	[23-26]
c.184C>G	p.Arg62Gly	CCD, 1° AVB, AF, PM, FPLD, HF	Coil 1A	[11,26]
c.203_208delAGGTGG	p.Glu68_Val69del	3° AVB, EDMD2	Coil 1A	[4,27]
c.215G>T	p.Arg72Leu	n/a	Linker 1	[17]
c.244G>A	p.Glu82Lys	1°-3° AVB, LBBB, AF, VF, SVT, HF	Coil 1B	[28,29]
c.254T>G	p.Leu85Arg	CCD, PM, AF, HF, SCD	Coil 1B	[23]
c.266G>T	p.Arg89Leu	1°-3° AVB, AF, VT, MA, HF / AVB, AF, VT, EDMD, HF	Coil 1B	[4,5,30-32]
c.274C>T	p.Leu92Phe	LBBB, AF, PVB, HF	Coil 1B	[33,34]
c.289A>G	p.Lys97Glu	1°/3° AVB, LBBB, PVB, HF	Coil 1B	[4,5,35]
c.302G>C	p.Arg101Pro	AF, HF / AVB, AF, PVB, VT, LGMD	Coil 1B	[31]/ [94]
c.331G>T	p.Glu111X	3° AVB, PVB, HF	Coil 1B	[4,5,35]
c.348_349insG	p.Lys117GlufsX10	AVB, AF, SCD	Coil 1B	[36]
c.356+1G>T	n/a	n/a	Coil 1B	[37]
c.357-1G>T	n/a	LBBB, AF, PVB, VT, VF, HF	Coil 1B	[4,31,34]
c.357C>T	p.Arg119Arg	CCD, PM, LGMD, HF	Coil 1B	[16]
c.367_369del	p.Lys123del	1°-3° AVB, LBBB, PVB, VT, VF, MP, SCD	Coil 1B	[38]

c.380_381ins24bp	p.Ile128_Ala129insArgValThrLeuIleSerSerArg	CCD	Coil 1B	[34]
c.384ins24	p.Ile128delinsIleSer	n/a	Coil 1B	[33]
c.394G>C	p.Ala132Pro	1^{o} AVB, Br, AF, HF	Coil 1B	[39]
c.398G>T	p.Arg133Leu	CCD, LD	Head	[40]
c.405_425dup	p.Asn142delinsLysLys	1^{o} AVB, AF, VT, PVB	Coil 1B	[41]
c.425_426insGGCACTGGAGGCTCTGCTGAA	p.Leu141_Asn142insLysAspLeuAspAlaLeuLeu	1^{o} AVB, AF, PVB, VT, HF	Coil 1B	[41]
c.427T>C	p.Ser143Pro	1^{o}-3^{o} AVB, LBBB, LAFB, SSS, Br, AF, PVB, VF, VT, HF	Coil 1B	[39,42]
c.481G>A	p.Glu161Lys	AVB, LBBB, Br, AF, PVB, LAFB, VT, HF	Coil 1B	[4,3,19,34,43]
c.497G>C	p.Arg166Pro	AVB, LBBB, AF, VT, HF	Coil 1B	[31,44]
c.514-1G>A	n/a	VT, VF	Coil 1B	[4]
c.548T>C	p.Leu183Pro	AVB	Coil 1B	[4]
c.556G>A	p.Glu186Lys	CCD, HF	Coil 1B	[19]
c.565C>T	p.Arg189Trp	PVB	Coil 1B	[45]
c.568C>T	p.Arg190Trp	1^{o} AVB, RBBB, Br, LAFB, AF, AFL, PVB, HF	Coil 1B	[4,5,7,19,35,39,46,47]
c.569G>A	p.Arg190Gln	1^{o}-3^{o} AVB, Br, AF, AFL, VT, HF	Coil 1B	[31,43]
c.575A>G	p.Asp192Gly	1^{o} AVB, LAFB, HF	Coil 1B	[46,48,49]
c.575A>T	p.Asp192Val	LBBB, FLPD, HF	Coil 1B	[26]
c.585C>G	p.Asn195Lys	1^{o}-3^{o} AVB, Br, AF, HF, SCD	Coil 1B	[8,23]
c.585C>A	p.Asn195Lys	1^{o}, 3^{o} AVB, AF, HF, SCD	Coil 1B	[8]
c.607G>A	p.Glu203Lys	1^{o} AVB, LBBB, RBBB, HF	Coil 1B	[50]
c.608A>G	p.Glu203Gly	1^{o}-2^{o} AVB, AF, HF, SCD	Coil 1B	[23]
c.608A>T	p.Glu203Val	2^{o} AVB, Br, SWMA, HF	Coil 1B	[23,43]
c.622_624delAAG	p.Lys208del	1^{o} AVB, PVB, VT, LGMD	Coil 1B	[8]
c.629T>G	p.Ile210Ser	AF, HF	Coil 1B	[31,51]
c.640-10A>G	n/a	1^{o}-3^{o} AVB, LBBB, RBBB, AF, VT, HF	Coil 1B	[52]
c.644T>C	p.Leu215Pro	1^{o}-2^{o} AVB, SSS, Br, LBBB, AF, AFL, SVT, VT, PVB, SCD, HF	Coil 1B	[53]
c.656A>C	p.Lys219Thr	(3^{o}) AVB	Linker 2	[4,43]
c.657G>C	p.Lys219Asn	1^{o} AVB, VT, PM	Linker 2	[54]
c.673C>T	p.Arg225X	(1^{o}) AVB, Br, AF, LVE, PVB, SCD	Linker 2	[8,44,50]
c.676C>G	p.Leu226Val	n/a	Linker 2	[37]
c.694G>C	p.Gly232Arg	AVB, RBBB, PVB, EDMD	Linker 2	[18]
c.700C>T	p.Gln234X	2^{o} AVB, Br, HF	Linker 2	[31]

c.736C>T	p.Gln246X	AVB	Coil 2	[4]
c.746G>A	p.Arg249Gln	AVB, PVB, EDMD or LGMD1B	Coil 2	[18]
c.767T>G	p.Val256lGy	AVB	Coil 2	[55]
c.775T>C	p.Tyr259His	AVB, AF, VT	Coil 2	[44]
c.780G>C	p.Lys260Asn	AVB, SSS, ASS, HF	Coil 2	[4,56]
c.781_783del3ins18	p.Lys261delins6	AVB, LBBB, AF, PVC, EDMD2	Coil 2	[18]
c.799T>C	p.Tyr267His	CCD, SCD	Coil 2	[57]
c.800A>G	p.Tyr267Cys	AVB, TC, EDMD2	Coil 2	[4,58]
c.810G>A	p.Lys270Lys	CCD, AF, PM, EDMD, LGMD	Coil 2	[16]
c.811-3C>T	n/a	n/a	Coil 2	[37]
c.812T>C	p.Leu271Pro	AF, Br, SVT, VT, EDMD, HF	Coil 2	[16,59]
c.815_818delACAAinsCCAGAC	p.Asp272AlafsX208	AVB, AF, VT	Coil 2	[44]
c.832G>A	p. Ala278hTr	AVB, AF, VF, SVT, PM, PVB	Coil 2	[60]
c.855delG	p.Ala287LeufsX191	AF, ASS, LGMD	Coil 2	[58]
c.859insC	p.Ala287fs	AF	Coil 2	[34]
c.883T>C	p.Ser295Pro	CCD, MD	Coil 2	[16]
c.908_909delCT	p.Ser303CysfsX26	1^{o} AVB, Br, SSS, AF / 1^{o}-3^{o} AVB, AF, PVB, VT, LGMD, HF	Coil 2	[61,62]
c.936G>C	p.Gln312His	AVB, AF, CCD, HF	Coil 2	[63]
c.IVS5+1G>T (c.936+1G>T)	n/a	1^{o}-2^{o} AVB, VT, PM	Coil 2	[4, 56, 64]
c.937-11C>G	p.Leu313GlyfsX31	1^{o}-3^{o} AVB, LGMD1B, VT, AF	Coil 2	[65]
c.949G>A	p.Glu317Lys	1^{o}/3^{o} AVB, AFL, LBBB, HF	Coil 2	[4,5,35,34]
c.952G>A	p.Ala318Thr	TC, PVB, HF	Coil 2	[31]
c.958delC	p.Leu320fs	n/a	Coil 2	[17]
c.959delT	p.Leu320fsX160	2^{o}-3^{o} AVB, LBBB, AF, VT, SVT, PVB, LGMD, EDMD, HF, SCD	Coil 2	[66,67]
c.961C>T	p.Arg321X	1^{o} AVB, RBBB, AF, VT, HF	Coil 2	[68,69]
c.[992G>A; =]+[=; 1039G>A]	p.[Arg331Glu; =]+[=; Glu347Lys]	AF, PM	Coil 2	[58]
c.992G>C	p.Arg331Pro	AVB, PM, LGMD	Coil 2	[58]
c.992G>A	p.Arg331Gln	1^{o} AVB, AFL, VT, HF	Coil 2	[69]
c.1003C>T	p.Arg335Trp	3^{o} AVB, RBBB, SSS, AF, VT	Coil 2	[17,70]
c.1004G>A	p.Arg335Gln	n/a	Coil 2	[37]
c.1039G>A	p.Glu347Lys	AF, PM, SCD	Coil 2	[13,71]
c.1044G>T	p.Met348Ile	EDMD, PM, CCD	Coil 2	[72]
c.1045C>T	p.Arg349Trp	CCD, SVA, MD, HF, SCD	Coil 2	[8]
c.1046G>T	p.Arg349Leu	AF, HF	Coil 2	[73]
c.1048G>C	p.Ala350Pro	AVB, LBBB, AF, PVB	Coil 2	[18]
c.1057C>A	p.Gln353Lys	CA, MP, HF	Coil 2	[48]

c.1063C>T	p.Gln355X	2° AVB, AF, VT, HF	Coil 2	[7,69]
c.1069G>C	p.Asp357His	AVB, VT	Coil 2	[74]
c.1070A>C	p.Asp357Ala	2° AVB, Br, AF, VES, VT, VF, HF, SCD	Coil 2	[70]
c.1072G>T	p.Glu358X	1°-2° AVB, VT	Coil 2	[75]
c.1085_1085delT	p.Leu363TrpfsX117	1°-2° AVB, AF, VT, HF	Coil 2	[39]
c.1102_1130dupGCCCTGGACATGGAGATCCACGCCTACCG	p.Lys378ProfsX112	LBBB, VT, LGMD, HF	Coil 2	[76]
c.1111_1125del15	p.Met371_Ala375del	n/a	Coil 2	[17]
c.1114delG	p.Glu372ArgfsX107	1° AVB, Br, AF, HF	Coil 2	[31]
c.1129C>T	p.Arg377Cys	LGMD, HF	Coil 2	[77]
c.1130G>A	p.Arg377His	1°-3° AVB, LBBB, RBBB, AF, PVB, VT, VF, LGMD, EDMD, HF, SCD	Coil 2	[3,8,18,30, 41,78]
c.1130G>T	p.Arg377Leu	(2°) AVB, Br, SSS, AF, AFL, ASS, ATC, VT, LGMD1B, EDMD, HF, SCD	Coil 2	[4,8,79,80]
c.1157G>C	p.Arg386Thr	FPLD, CCD	Tail	[81]
c.1157+1G>A	n/a	VT, VF	Tail	[4]
c.1157+1G>T	p.Arg386SerfsX21	VES, VF, HF	Tail	[70]
c.1163G>A	p.Arg388His	Br, PVB, HF, SCD	Tail	[31]
c.1189C>T	p.Arg397Cys	n/a	Tail	[37]
c.1195C>T	p.Arg399Cys	HF	Tail	[31]
c.1197_1240del44	p.Gly400Argfs*11	AVB, SSS	Tail	[55]
c.1292C>G	p.Ser431*_	AVB	Tail	[55]
c.1294C>T	p.Gln432X	3° AVB, LBBB, RBBB, AF, VT, HF	Tail	[69]
c.1303C>T	p.Arg435Cys	HF	Tail	[13]
c.1307_1308insGCAC	p.Ser437HisfsX1	2° AVB, Br, TC, AF, PVB, HF, SCD	Tail	[31]
c.1318G>A	p.Val440Met	LAFB, VES	Tail	[69]
c.1370delA	p.Lys457SerfsX21	AF, LGMD	Tail	[58]
c.1380+1G>A	n/a	(1°) AVB, AF, VF, HF	Tail	[8,34]
c.1397_1397delA	p.Asn466IlefsX14	1° AVB, LBBB, Br, AF, VF, VT, HF, SCD	Tail	[41,82]
c.1412G>A	p.Arg471His	AF, VT, HF	Tail	[31]
c.1424_1425insAGA	p.Gly474_Asp475insGlu	HF	Tail	[31]
c.1443C>G	p.Tyr481X	2° AVB, RBBB, SVT, VT, HF	Tail	[46,48,49]
c.1489-1G>T	p. Ile497-Glu536del	2° AVB, Br, AF, AFL, VT, EDMD	Tail	[70]
c.1492T>A	p.Trp498Arg	AVB, EDMD2	Tail	[4]
c.1493_1493delG	p.Ala499LeufsX47	AF, VT, HF	Tail	[39]
c.1496delC	p.Ala499Val	1°-2° AVB, RBBB, Br, ATC, ASS, AN	Tail	[83]

c.1512_1513insAG	p.Thr505ArgfsX44	1° AVB, AF, PVB, HF, SCD	Tail	[8]
c.1526_1527insC	p.Thr510TyrfsX42	1° AVB / AVB, EDMD2	Tail	[4] /[84]
c.1549C>T	p.Gln517X	3° AVB , AF, EDMD, VF, SCD	Tail	[70]
c.1560G>A	p.Trp520X	2° AVB, LBBB, Br	Tail	[70]
c.1567G>A	p.Gly523Arg	LBBB	Tail	[33,34]
c.1579_1580insCTGC	p.Arg527ProfsX26	1°-2° AVB, LBBB, LAFB, HF	Tail	[4,5,35]
c.1583C>T	p.Thr528Met	1°-3° AVB, Br, AF, SVA	Tail	[10]
c.1608+1G>T	n/a	n/a	Tail	[37]
c.IVS9-3C>G (c.1609-3C>G)	loss of exon 10	3° AVB, Br, PM, LGMD1B	Tail	[85]
c.1621C>T	p.Arg541Cys	LBBB, VT, VF, PVB, fibrosis, SWMA, SCD	Tail	[86-88]
c.1621C>A	p.Arg541Ser	VT, HF / LGMD1B, HF	Tail	[16]/ [46,39,48]
c.1621C>G	p.Arg541Gly	IVB, LBBB, Br, SVA, PVB, VA, TC	Tail	[89]
c.1622G>A	p.Arg541His	AVB, AF, PVB	Tail	[18]
c.1622G>C	p.Arg541Pro	PVB, VT, HF	Tail	[8]
c.1711C>A	p.Arg571Ser	1°-3° AVB, Br, AF	Tail	[23]
c.1713C>A	p.Ser571Arg	2°, 3° AVB, PM, HF, AF	Tail	[23]
c.1714insCTGC	p.Ser572LeufsX8	1°, 2° AVB, PM, LBBB	Tail	[35]
c.1718C>T	p.Ser573Leu	AVB, VT	Tail	[4,30]
c.1904G>A	p.Gly645Asp	HF	Tail	[90]
c.1930C>T	p.Arg644Cys	AVB, AF, VT, HF / LGMD1B, HF, SCD	Tail	[4,43,69]/ [91]
c.1960C>T	p.Arg654X	AVB, LBBB, SSS, AF, VT, HF, SCD	Tail	[31]
c.1964_1965insG	p.Thr655fsX49	PM, VT, MH, POS, HF, SCD	Tail	[92]
c.[1699 to 183_1699–160inv24; 568_1699–184del; 1699 to 159_1995+6997del]	double deletion with break points in exon 3, intron 10, downstream of gene	1°-3° AVB, AF, AFL, Br, VA, SCD	Coil/Tail	[93]
deletion exons 3-12	deletion > 4,704 bp	VT, HF	Coil/Tail	[48]

Table 2. LMNA mutations associated with dilated cardiomyopathy comprising most current information from four databases: Human Intermediate Filament Database [18] (database updated 2012-11-28), Leiden Muscular Dystrophy website (www.dmd.nl), HGMD® Professional 2012.4 (database updated 2012-12-14) and Universal Mutation Database (www.umd.be/LMNA/). 1°, 2°, 3° - atrio-ventricular block degree, in parenthesis when degree specified only in some studies, AF - atrial fibrillation, AFL - atrial flutter, AN - axonal neuropathy, ASS - atrial standstill, ATC - atrial tachycardia, AVB - atrio-ventricular block, Br - bradycardia, CA - cardiac abnormalities, CCD - cardiac conduction disease, CMT2 - Charcot-Marie-Tooth disease, DM - diabetes mellitus, EDMD(2) - Emery-Dreifuss muscular dystrophy (type 2), FPLD - familial partial lipodystrophy, HF - heart failure, IVB - intra-ventricular block, LAFB - left anterior fascicular block, LBBB - left bundle branch block, LGMD(1B) - limb girdle muscular dystrophy (type 1B), LVE - left ventricular exosystoly, MA - muscular atrophy, MAD - mandibuloacral dysplasia, MD - muscular dystrophy, MH - muscular hypertrophy, MP - myopathy, PAB - premature atrial beats, PM - pacemaker implantation, PN - peripheral neuropathy, POS - polycystic ovary syndrome, PVB - premature ventricular beats, RBBB - right bundle branch block, SCD - sudden cardiac death, SSS - sick sinus syndrome, SVA - supraventricular arrhythmia, SVT - supraventricular tachycardia, SWMA - segmental wall motion abnormalities, TC - tachycardia, VA- ventricular arrhythmia, VES - ventricular extra systoles, VF - ventricular fibrillation, VT - ventricular tachycardia, LD - lipodystrophy, WS - Werner syndrome, "/" used to separate differing phenotypes

*: References are listed in "Supplementary file"

Curriculum Vitae

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EDUCATION

Master of Science, **Biochemistry- Specialization Human Molecular Genetics,** Expected June 2013
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Bachelor of Science with Honours in Biomedical Science, 2004
School of Veterinary and Life Sciences, Molecular and Biomedical Sciences, Murdoch University, Perth, Australia
Thesis: The assessment of HOX11 function *in vivo* using *Caenorhabditis elegans.*
Advisors: Wayne Greene, PhD and Andrew Mounsey, PhD

Bachelor of Science, 2003
School of Veterinary and Life Sciences, Molecular and Biomedical Sciences, Murdoch University, Perth, Australia
Majoring in Molecular Biology and Biomedical Science
Minoring in Forensic Chemistry

RELATED EXPERIENCE

Research

MSc Research, Department of Biochemistry, University of Ottawa
September 2010 – present

- Characterized the altered nuclear localization of a novel disease associated LMNA mutation. Confirmed the cellular distribution of PKC alpha during lamin A/C mutation
- Established that lamin A/C binding partner PKC alpha localization is affected by lone atrial fibrillation associated LMNA mutations and dilated cardiomyopathy associated LMNA mutations.
- Designed and conducted experiments for the activity and functional study of PKC alpha redistribution by certain lamin A/C mutations
- Provided training to new students joining the laboratory

BSc Research, School of Veterinary and Life Sciences, Molecular and Biomedical Sciences, Murdoch University, Perth, Australia
January 2004 – December 2004

- Sought to use *Caenorhabditis elegans* as model system to help understand HOX11 function in vivo
- HOX11 and HOX11 binding mutant cDNA fragments were cloned into the *C. elegans* expression vector using a two-step cloning approach

Teaching

Teaching Assistant, Interdisciplinary School of Health Science, University of Ottawa,
September 2010 – Present

- Advised students during office hours, proctored and corrected assignments, quizzes, and midterm for the undergraduates in the Health Science program.
    - Fall 2013 – History of Health Care HSS2121
    - Winter 2013 - History of Health Care HSS2121
    - Fall 2012 - Global Citizenship and Health
    - Winter 2012 - Human Activity and Occupation HSS2104
    - Fall 2011 - Health Problems HSS2101B
    - Winter 2011 - Health Problems HSS2101A
    - Fall 2010 - Biological Basis of Diseases HSS3301A

Teaching Assistant, School of Veterinary and Life Sciences, Molecular and Biomedical Sciences, Murdoch University, Perth, Australia
January 2004 – June 2005

- Instructed and assisted students with their fetal pig and specimen dissections
- Clarified and explained further from lecture materials
- Answered laboratory class questions, proctored exams, marked laboratory report books, assignments and exams
- Advised students studying the undergraduate course - Introduction to the Human Body during office hours.
- Encouraged and guided students with their essay writing skills for their assignments

EMPLOYMENT HISTORY

Summer Research Student in Dr. Frédérique Tesson's Genetics Laboratory,
University of Ottawa, Ottawa, Canada
May 2010 – Aug 2010

High School Teacher, Ottawa Islamic School, Ottawa, Canada
Sep 2009 –April 2010

- Lectured grade 10 and 11 students in science and biology respectively
- During this time acquired various skills to deal with troubled youth along with numerous problem solving techniques

Private Tutor, Sri Lanka.
Jan 2006 – Jan 2009

- Taught biology and chemistry for London Advance and Ordinary level students

Laboratory Scientist, Polyclinic Pvt. Ltd, Sri Lanka
Aug 2005 –July 2007

- Performed molecular diagnostics using molecular techniques
- Also provided administrative support to a team of health care providers by registering and scheduling appointments for patients, answering phone calls, filing and billing in an efficient and productive manner

Teaching Assistant – Tutor/Demonstrator, Murdoch University, Perth, Australia
Jan 2004 – June 2005

Honours Scholar, Murdoch University, Perth, Australia
Jan 2004 – Dec 2004

- Prepared competent cells and tested the competency of pre-made competent cells
- Carried out general maintenance and storage of materials and laboratory equipment
- Experienced in preparation of solutions and reagents and record keeping of all experiments
- Also organized social, sporting and environmental events as a Student Village Residents Club member – Village Green Member

Project Assistant, Amrad Pharmaceuticals, Victoria, Australia
July 2003 - Nov 2003
Assisted with their research project on "Street Drugs and Narcotics"

Research Attachment, Royal Perth Hospital, Perth, Australia
Jan 2003 – April 2003

- Developed yeast two hybrid system
- Implemented construction and screening of plasmid DNA libraries
- Employed the yeast-two-hybrid system to identify the role of FcγRIIα and explored the protein interaction between FcγRIIα receptor and its possible binding partners alone or in combination with CRP and/or IgG

Laboratory Attachment, Royal Brunei Hospital (RIPAS), Brunei Darussalam
Nov 2002 – Jan 2003

- Followed standard procedures of blood sugar, lipid profile, and liver function tests including sample processing, priming the machines and releasing the final results
- Conducted routine gross examination of tissue, fine needle aspiration biopsy and cell processing
- Observed microscopic morphology of cells, blood grouping and blood cross-matching procedures

AWARDS AND GRANTS

May 2013 - 2nd Place at Annual BMI Poster Day
Jan 2011 - University of Ottawa Faculty of Graduate and Postdoctoral Studies Travel Grant
Jan 2011 - University of Ottawa Faculty of Medicine Biochemistry Program Travel Grant
Sep 2010 - University of Ottawa Bursary

CONFERENCE & SYMPOSIA PRESENTATIONS

The Fourth Annual Canadian Human Genetics Conference, Banff, Alberta, Canada - April 2011
University of Ottawa Heart Institute Cardiovascular Symposium, Ottawa, Ontario, Canada – May 2011

References and portfolio available upon request