# 2015 PhD proposal

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| **Project / Research program:** | CIFRE Grant SANOFI – Centre de Recherche en Myologie<br>"Altered calcium homeostasis and *LMNA*-cardiomyopathy" |
| **Project Leader:** | **Antoine Muchir, PhD, CR1 Inserm & Gisèle Bonne, PhD-HDR, DR2 Inserm.** |
| **Department/Unit:** | Centre de Recherche en Myologie / Institut de Myologie<br>UPMC - Inserm UMRS 974 / CNRS FRE 3617 / AIM |
| **PhD scientific project title:** | Exploration of altered calcium homeostasis in *LMNA*-cardiomyopathy |
| **Site:** | Institut de myologie, G.H. Pitié-Salpêtrière ; 47, boulevard de l'Hôpital.<br>F-75 651 Paris Cedex 13 - France |
| **Proposed starting date** | Fall 2015 |
| **Contact** | Gisèle Bonne (g.bonne@institut-myologie.org)<br>Antoine Muchir (a.muchir@institut-myologie.org) |

**Detail project Milestones & Objective**

***Background***

Dilated cardiomyopathy (DCM), one of the leading causes of heart failure in Europe, is characterized by an increase in both myocardial mass and volume resulting in poor left ventricular function. In 1999, we and other identified that *LMNA* mutations cause cardiomyopathy with ventricular arrhythmias and conduction defects (*LMNA*-cardiomyopathy), associated or not with striated muscle disease, the Emery-Dreifuss muscular dystrophy (EDMD) *(Bonne et al. 1999; Fatkin et al. 1999)*. *LMNA* encodes nuclear A-type lamins, which are the main constituents of the nuclear lamina, a meshwork underlying the inner nuclear membrane of metazoan cells *(Fisher et al. 1986; McKeon et al. 1986; Aebi et al. 1986)*. Lamins have been implicated in structural support to the nucleus and in processes such as chromatin organization, gene regulation, DNA replication and RNA splicing *(Dechat et al. 2008)*. The inner nuclear membrane contains specific integral and associated proteins that connect the A-type lamins, which are proposed to form a mechanical link, called the LINC (LInker of Nucleoskeleton and Cytoskeleton) complex *(Stewart et al. 2007)*, tethering the nucleo- and cyto-skeleton. Thus, the nucleus is considered as a mechanical sensor, which could partially explain the cardiac muscle disease, considering that heart is constantly subjected to mechanical forces *(Lombardi et al. 2011)*. However, the mechanism through which mutated lamins cause cardiac dysfunction and arrhythmias remains obscure.

Our previous work has focused on investigation of the contractile dysfunction, and we demonstrated an early abnormal activation of the stress activated MAP kinases signaling pathways in the heart of *Lmna*$^{H222P}$ knock-in mice (*Lmna*$^{H222P}$), a mouse model of *LMNA*-cardiomyopathy we created *(Arimura et al. 2005, Muchir et al. 2007)*. Pharmacological or genetic blockade of signaling in the MAPK cascade in *Lmna*$^{H222P}$ mice prevents left ventricular dilatation and deterioration in cardiac contractility, if administered prior or after the onset of detectable heart disease *(Muchir et al. 2009; Wu et al. 2010, Wu et al. 2011; Muchir et al. 2011; Wu et al. 2014)*.

Ca2+ is one of the major actors of the excitation-contraction coupling in cardiac muscle. The release of Ca2+ from the sarcoplasmic reticulum is triggered via the Ryanodin receptor type 2 (RYR2) and promotes cardiac contraction. A recent study demonstrated the relationship among increased S-nitrosylation of RyR2, diastolic sarcoplasmic reticulum $Ca^{2+}$ leak and the development of a cardiac phenotype (*Fauconnier et al. 2010*). We initiated quantification of RyR2 S-nitrosylation in the hearts of *Lmna*$^{H222P}$ knock-in mice, as well as patch-clamp analysis and calcium release experiments from *Lmna*$^{H222P}$ isolated cardiomyocytes *(Collaboration with AM Gomez, Chatenay-Malabry)*. Our preliminary data on RYR2 S-nitrosylation, calcium sparks and NCX expression strongly underline a potential role of calcium homeostasis in the cardiomyopathy linked to mutations in *LMNA*.

***Objectives***

**Aim 1. Assess the expression of the components of the excitation-contraction coupling in *LMNA*-cardiomyopathy.**

We have access to two mouse models of *LMNA*-cardiomyopathy (*Lmna* H222P and delK32 mouse models) (*Arimura et al. 2005; Cattin et al. 2013*). Heart muscle samples from patients will be collected from explanted hearts during cardiac transplantations. Through our network of clinicians and surgeons, we already collected cardiac biopsies (left ventricle) from 4 patients carrying different *LMNA* mutations. All tissue samples have been obtained with appropriate ethical approvals from the Institut de Myologie and provided without patient identifiers. We will i/ study the RyR2 S-nitrosylation and determine expression level of calstabin 2 (western blot, qPCR), and ii/ investigate the major actors of the $Ca^{2+}$ homeostasis: NCX, Calmodulin, CamKII and its targets phospholamban/RyR2 and phosphorylated phospholamban/RyR2, Serca2, Ip3, Ip3 receptor and DHPR (qPCR, western blot, immunohistochemistry).

**Aim 2. Develop iPSC-derived cardiomyocyte models that recapitulates the molecular and/or cellular abnormalities of *LMNA*-cardiomyopathy.**

We have access to one line of iPSC derived from fibroblasts of patients carrying *LMNA* mutations. We aim at generating additional lines as we currently have access to primary skin fibroblasts from 8 patients with *LMNA*-cardiomyopathy (if time and resources allow, in collaboration with I-Stem). Cardiomyocytes will be derived from these IPSC lines. We will assess the expression, post-translational modifications and localization of the main components involve in the calcium homeostasis, as described in Aim 1 (S-nitrosylation RyR2, NCX…). We will also study, in collaboration with AM Gomez, the ion channels activity (patch-clamp analysis, calcium

transient) on iPSC-derived cardiomyocytes. All this analysis will provide new insights on the pathophysiology and also define standard read-outs for drug screening.

**Aim 3. Analyze the role played by oxidative stress in the alteration of excitation-contraction in *LMNA*-cardiomyopathy.**

In the heart, mechanical stretch during diastolic filling activates mechanotransduction signaling pathways that have broad implications for cardiac health and disease. A small stretch of a ventricular myocyte causes a burst of $Ca^{2+}$ sparks, the elementary events corresponding to the release of free $Ca^{2+}$ from intracellular stores. The mechanism by which this occurs has remained elusive. Recent work from others showed that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (NOX2) is overactive in a pathological model of cardiomyopathy, and this produces reactive oxygen species (ROS) in a process dependent on microtubules (X-ROS signaling), which triggers arrhythmogenic calcium waves in isolated cardiomyocytes (*Proser et al. 2011*). X-ROS signaling thus provides a mechanistic explanation for the mechanotransduction of $Ca^{2+}$ release in the heart and offers new therapeutic possibilities. We will assess the level of oxidative stress in the heart from *Lmna* H222P / delK32 mouse models and humans using the fluorescent ROS sensor 2',7'-dichlorofluorescein diacetate with or without application of the antioxidant N-acetylcysteine. We will also assess the impact of ROS on the activation of CaMKII (by quantification of oxidized CAMKII) (Luczak et al. 2014) and the phosphorylation of RYR2 on the CaMKII specific site (ser2814) whose excessive phosphorylation has been linked to atrial fibrillation, arrhythmias and sudden death trough alteration of Ca2+ handling. Additional functional readouts (contractility, Ca2+ transients, Ca2+ sparks…) will be used on iPSC-CM derived from patients and adult cardiomyocytes from *LMNA*-cardiomyopathy mouse models with or without NAC, Nox2 inhibitors or CaMKII inhibitors to assess the causal role of oxidative stress in the alteration of excitation-contraction in *LMNA*-cardiomyopathy. Given the predominant role of oxidative stress (Terentyev et al. 2008; Murdoch et al. 2006) and cytoskeletal dysfunction (Heins et al. 2000) in the progression of cardiomyopathy, X-ROS signaling may have important pathophysiological effects in the context of *LMNA*-cardiomyopathy.

**Aim 4. Test molecules with potential therapeutic effect on IPSC-derived cardiomyocytes and on two mouse models of *LMNA*-cardiomyopathy.**

Anti-arrhythmic agents are a group of pharmaceuticals that are used to suppress abnormal rhythms of the heart. Among the different classes of anti-arrhythmic, we will focus on class II and IV. Class II, β-adrenergic blockers are routinely used in cardiac laminopathic patients. Class IV which slow calcium channel blockers in particular the DHPR, are rarely used in laminopathy context. These two classes of anti-arrhythmic and new classes of molecules (in collaboration with partner, to be determined) will be tested on cardiomyocytes derived from patient iPSC, and will be further functionally validate (ECG, echocardiography) on mouse models ($Lmna^{H222P}$ and $Lmna^{delK32}$ mice; *Cattin et al, 2013*).

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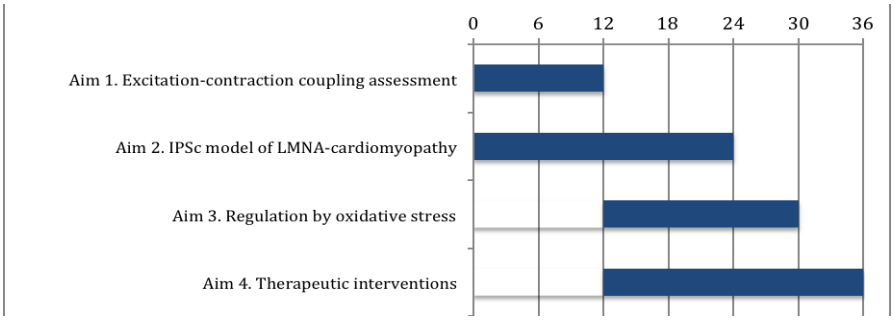
| | ***Milestones***<br>  |
| **Required skills:** | • Cellular and molecular biology, biochemistry (expertise available in the team).<br>• Patch-clamp, calcium transient (expertise available in collaboration with Ana-Maria Gomez, Chatenay-Malabry).<br>• IPSC platform: not available in the team.<br>• Echocardiography, ECG (expertise available in the laboratory). |
| **Qualification and / or training desired:** | • Student should have a strong background on cellular and molecular biology. An experience in the other skills will be an added value. |
| **Experience:** | • Master 2 |
| **Additional information (if needed)** | **Bibliography**<br>Aebi U, Cohn J, Buhle L, Gerace L. The nuclear lamina is a meshwork of intermediate-type filaments. ***Nature*** 1986, 323, 560-564.<br>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.<br>Bonne G, Di Barletta MR, Varnous S, et al. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. ***Nat Genet.*** 1999, 21:285-288.<br>Cattin ME, Bertrand AT, Schlossarek S, Le Bihan MC, Skov Jensen S, Neuber C, Crocini C, Maron S, Laine J, Mougenot N, Varnous S, Fromes Y, Hansen A, Eschenhagen T, Decostre V, Carrier L, Bonne G. Heterozygous LmnadelK32 mice develop dilated cardiomyopathy through a combined pathomechanism of haploinsufficiency and peptide toxicity. ***Hum Mol Genet*** 2013;22:3152-3164.<br>Fatkin D, MacRae C, Sasaki T, et al. 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.<br>Fauconnier J, Thireau J, Reiken S, Cassan C, Richard S, Matecki S, Marks AR, Lacampagne A. Leaky RyR2 trigger ventricular arrhythmias in Duchenne muscular dystrophy. ***Proc Natl Acad Sci USA*** 2010;107:1559-1564<br>Fisher DZ, Chaudhary N, Blobel G. cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. ***Proc Natl Acad Sci USA*** 1986;83:6450-6454. |

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