# Frontiers in iPS Technology

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### Generation of iPSC-based cardiomyocytes for investigating mechanisms of dilated cardiomyopathy due to Lamin A/C mutations

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**Purpose:** Dilated cardiomyopathy (DCM) is a primary disease of the heart muscle. It represents a major cause of heart failure, ultimately leading to heart transplantation. Approximately 30-50% of cases are familial. Several genes have been linked to DCM and, among these, mutations of Lamin A/C are the most common. Lamins are expressed in all somatic cells and control many cellular processes such as nuclear integrity, nucleo-cytoskeletal coupling, transcription and chromatin remodelling. However, the role of Lamin A in the heart and the consequences of its mutations in DCM need to be further elucidated.

Induced pluripotent stem cells (iPSC) allow the studying of the molecular mechanisms of diseases in cardiomyocytes (CMs) derived from them.

**Methods:** We generated human CMs from iPSC derived from a Lamin-defective DCM family carrying the mutation K219T in the Lamin A/C gene. A family sub-group with a more severe phenotype and carrying an additional variant (L4855F) in the titin gene was also included. Our major aim was to understand how nuclear lamina defects lead to functional and molecular dysfunctions in DCM patients: in addition, we aimed to determine whether the titin mutation has a role in the disease development.

**Results:** As expected, patients' fibroblasts and CMs exhibit disorganized Lamin A/C expression, nuclei enlargement and envelope invaginations. CMs derived from DCM-iPSC also showed an abnormal sarcomeric organization, with a higher percentage of abnormal cells in the double mutant than in the single mutant cells (70% vs 50%). Functional analysis of the calcium handling also revealed an increased susceptibility of mutant cells to β-adrenergic stimulation. A global transcriptional profile of DCM-CMs was carried out using Illumina microarray technology. Gene ontology analysis of the dysregulated genes revealed the involvement of molecular functions relative to transcriptional regulation and DNA synthesis, indicating the key role of Lamin A in chromatin remodeling and regulation of transcription also in human CMs. We also found a strong co-localization of the genomic loci of these genes with lamin associated domains (LAD), regions of interactions between the genome and the lamina indicative of a repressive chromatin environment. How the mutation in lamin may interfere with these mechanisms is under investigation.

**Conclusions:** Our data indicate that iPSC—derived CMs may represent a good model to investigate functional and molecular phenotypes of lamin-dependent DCM. Results shed light on the function of the nuclear lamina in heart pathophysiology and regulation of CM chromatin architecture.