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Mitogen Activated Protein Kinase Inhibitors Improve Heart Function and Prevent Fibrosis in Cardiomyopathy Caused by Mutation in Lamin A/C Gene

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Abstract

Background—Mutations in the lamin A/C gene, *LMNA*, can cause dilated cardiomyopathy. We have shown abnormal activation of the extracellular signal-regulated kinase (ERK) and the c-jun N-terminal kinase (JNK) branches of the mitogen-activated protein kinase (MAPK) signaling cascade in hearts from *Lmna*$^{H222P/H222P}$ mice that develop dilated cardiomyopathy. We recently showed that partial inhibition of ERK and JNK signaling prior to the onset of cardiomyopathy in *Lmna*$^{H222P/H222P}$ mice prevented the development of left ventricle dilatation and decreased cardiac ejection fraction at a time when these occurred in untreated mice.

Methods and Results—To determine if pharmacological inhibitors of ERK and JNK signaling could be clinically useful to treat cardiomyopathy caused by *LMNA* mutation, we administered them to *Lmna*$^{H222P/H222P}$ mice after they developed left ventricular dilatation and decreased ejection fraction. *Lmna*$^{H222P/H222P}$ mice were treated with ERK and JNK signaling inhibitors from 16 to 20 or, in pilot experiments, 19 to 24 weeks of age. The inhibitors blocked increased

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Disclosures

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expression of RNAs encoding natriuretic peptide precursors and proteins involved in sarcomere architecture that occurred in placebo-treated mice. Echocardiography and histological analysis demonstrated that treatment prevented left ventricular end systolic dilatation, increased ejection fraction and decreased myocardial fibrosis.

Conclusions—Inhibitors of ERK and JNK signaling could potentially be used to treat humans with cardiomyopathy caused by *LMNA* mutations.

Keywords

Cardiomyopathy; pharmacology; *LMNA*; mitogen-activated protein kinase

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Dilated cardiomyopathy is characterized by ventricular dilatation and impaired systolic function with 20% to 48% of cases familial (1). Mutations in *LMNA* encoding A-type nuclear lamins have been shown to cause a several human diseases (2) with at least 3 having dilated cardiomyopathy as a predominant feature: autosomal Emery-Dreifuss muscular dystrophy (3), limb girdle muscular dystrophy type 1B (4) and dilated cardiomyopathy type 1A (5). Given the phenotypic overlap of these disorders, they can be described as *LMNA* dilated cardiomyopathy with variable skeletal muscle involvement (6). *LMNA* mutations appear to be responsible for approximately 8% of familial cardiomyopathies (7–10). The onset of symptoms in *LMNA* cardiomyopathy is variable, ranging from the first to sixth decade of life and occurring most frequently in the third decade (7–11). It has a natural history more aggressive than most other familial cardiomyopathies, with high rates of arrhythmias leading to sudden death and advanced heart failure necessitating cardiac transplantation (7,11,12).

To identify potential targets to treat cardiomyopathy caused by *LMNA* mutation, we have been examining cellular signaling pathways in hearts of *Lmna* H222P knock in mice, a model of the human disease. Male $Lmna^{H222P/H222P}$ mice develop left ventricular (LV) dilatation and depressed contractile function starting at approximately 8–10 weeks of age and invariably develop LV dilatation and decreased cardiac contractility at 16 weeks (13). We have shown abnormal activation of the extracellular signal-regulated kinase (ERK) and the c-Jun N-terminal kinase (JNK) branches of the mitogen-activated protein kinase (MAPK) signaling cascade in hearts of *Lmna* H222P knock in mice prior to the onset of clinically detectable cardiomyopathy (14). We have also shown that lamin A variants that cause cardiomyopathy activate ERK and JNK when expressed in cultured cells (14). Based on these results, we hypothesized that activation of ERK and JNK plays a primary pathogenic role in the development of cardiomyopathy. Our recent work has shown that small molecule inhibitors of ERK and JNK signaling administered to male $Lmna^{H222P/H222P}$ mice prior to the onset of detectable cardiomyopathy prevented LV dilatation and decreases in cardiac ejection fraction (EF) at an age when placebo-treated mice had significant abnormalities in these parameters (15,16).

A critical question relevant to potential treatment of human subjects with ERK and JNK inhibitors regards their effectiveness after the onset of cardiac dysfunction. It would be impractical to use such drugs as prophylactic treatment in asymptomatic humans with *LMNA* mutations, especially given the variable age of onset, usually in adulthood. To help answer this question, we initiated the present study to determine if inhibitors of ERK and JNK signaling would be beneficial in $Lmna^{H222P/H222P}$ mice after LV dilatation and decreased cardiac EF have already occurred.

Methods

An expanded Methods section is available in the Online Data Supplement.

Lmna$^{H222P/H222P}$ mice were generated and genotyped using polymerase chain reaction (PCR) primers as described (13). Drugs were dissolved in dimethyl sulfoxide (DMSO) are delivered into the peritoneal cavity by injection at 3 mg/kg/day for 5 days a week. Equal volumes of DMSO were administered as placebo. Cardiac structure and contractility were assessed by echocardiography. Representative stained cardiac sections were photographed using a Microphot SA (Nikon) light microscope attached to a Spot RT Slide camera (Diagnostic Instruments) with a 10x objective. Images were processed using Adobe Photoshop CS (Adobe Systems). RNA transcripts measured using real-time reverse transcription-polymerase chain reaction (RT-PCR) were quantified using iQ SYBR green super mix (Bio-Rad). Statistical comparisons were made using an unpaired Student's *t*-test or a one-way analysis of variance with the Tukey *post hoc* test to evaluate the significance of differences between means.

Results

Rationale for Treatment of *Lmna*$^{H222P/H222P}$ Mice

Our hypothesis was that treatment with a MAPK/ERK kinase (MEK) 1/2 inhibitor, which inhibits activation of ERK, or a JNK inhibitor would improve cardiac structure and function in *Lmna*$^{H222P/H222P}$ mice when the compounds are administered after these parameters are significantly abnormal. Because the animal care facility at Columbia University Medical Center prohibits removal and re-entry of mice from its barrier facility, we could not obtain echocardiograms on individual subjects before and after treatment. To test our hypothesis, we therefore assigned male *Lmna*$^{H222P/H222P}$ mice 16 weeks of age to 3 different treatment arms (placebo DMSO, n=28; MEK1/2 inhibitor PD98059, n=22; JNK inhibitor SP600125, n=29) and examined parameters of cardiac structure and function at 20 weeks of age, after 4 weeks treatment. At 16 weeks, male *Lmna*$^{H222P/H222P}$ mice are known to have markedly increased LV end diastolic diameter (LVEDD) and LV end systolic diameter (LVESD) compared to *Lmna*$^{+/+}$ mice (13,15,16). *Lmna*$^{H222P/H222P}$ mice also have depressed cardiac contractility, with fractional shortening (FS) decreased by 20%–40% compared to *Lmna*$^{+/+}$ mice (13,15). Myocardial fibrosis occurs in *Lmna*$^{H222P/H222P}$ mice at 16 weeks of age (16). At 20 weeks, LVEDD and LVESD increase further in *Lmna*$^{H222P/H222P}$ mice and cardiac contractility also progressively deteriorates (16). During the 4-week treatment protocol, 6 mice in the DMSO group, 3 in the PD98059 group and 3 in the SP600125 group died prior reaching 20 weeks of age for evaluation.

Effect of PD98059 and SP600125 on ERK and JNK Signaling

Systemic administration of the MEK1/2 inhibitor, PD98059, and the JNK inhibitor, SP600125, to *Lmna*$^{H222P/H222P}$ mice from 16 to 20 weeks of age partially blocked the phosphorylation of ERK1/2 (Figure 1A) and JNK (Figure 1B), respectively, in hearts. At 3 mg/kg/day, PD98059 was highly selective for blocking ERK signaling, as phosphorylation of JNK was not significantly inhibited (Figure 1A). At 3 mg/kg/day, SP600125 was specific of the JNK signaling, as phosphorylation of ERK1/2 was not significantly inhibited (Figure 1B).

Effect of the PD98059 and SP600125 on Cardiac Expression of Natriuretic Peptides and Myosin Light Chain

One of the features of dilated cardiomyopathy is the upregulation of cardiac hormones such as natriuretic peptides as a compensatory mechanism to maintain cardiac output (17,18). Upregulation of genes involved in sarcomere organization also occurs (19,20). We therefore assayed expression of *Mlc-2a* mRNA, encoding a cardiac isoform of myosin light chain, and *NppA* and *NppB* mRNAs, encoding natriuretic peptides precursors in hearts from *Lmna*$^{+/+}$ mice, DMSO-treated *Lmna*$^{H222P/H222P}$ mice and inhibitor-treated *Lmna*$^{H222P/H222P}$ mice

(Figure 2). In hearts from DMSO-treated *Lmna*$^{H222P/H222P}$ mice, expression of *Mlc-2a* mRNA was significantly increased approximately 30-fold compared to hearts of *Lmna*$^{+/+}$ mice (Figure 2). Similarly, in hearts from *Lmna*$^{H222P/H222P}$ mice, *NppA* and *NppB* mRNA levels showed significant 36-fold and 17-fold increases in expression compared to hearts of *Lmna*$^{+/+}$ mice (Figure 2). Treatment of *Lmna*$^{H222P/H222P}$ mice with PD98059 or SP600125 significantly decreased the expression of *Mlc-2a*, *NppA* and *NppB* mRNAs at 20 weeks of age (Figure 2). Hence, pharmacological inhibition of ERK or JNK signaling reversed molecular compensatory processes that occur in *Lmna*$^{H222P/H222P}$ mice with cardiomyopathy.

Effect of PD98059 and SP600125 on LV Dilatation and Contractility in *Lmna*$^{H222P/H222P}$ Mice

After 4 weeks of treatment with DMSO, PD98059 or SP600125, *Lmna*$^{H222P/H222P}$ mice were anesthetized and cardiac dimensions and function measured by echocardiography. M-mode transthoracic echocardiography showed increased LVEDD and LVESD in *Lmna*$^{H222P/H222P}$ mice treated with DMSO compared to *Lmna*$^{+/+}$ mice (Figure 3). *Lmna*$^{H222P/H222P}$ mice treated with PD98059 and SP600125 had significantly smaller LVESD compared to the DMSO-treated mice (Figure 3). FS and EF were reduced in *Lmna*$^{H222P/H222P}$ mice compared to *Lmna*$^{+/+}$ mice but increased in the *Lmna*$^{H222P/H222P}$ mice treated with PD98059 or SP600125.

Table 1 shows the composite echocardiographic data for the 3 treatment arms for *Lmna*$^{H222P/H222P}$ mice and *Lmna*$^{+/+}$ mice for comparison. Compared to *Lmna*$^{+/+}$ mice, *Lmna*$^{H222P/H222P}$ mice treated with DMSO had significantly increased LVEDD and LVESD. The EF of DMSO-treated male *Lmna*$^{H222P/H222P}$ mice at 20 weeks was 53.87% ± 2.58%, which was decreased by 28% compared to *Lmna*$^{+/+}$ mice. *Lmna*$^{H222P/H222P}$ mice treated with PD98059 or SP600125 had a statistically significant reduction in the LVESD compared to mice treated with DMSO; however, LVEDD was not significantly different. *Lmna*$^{H222P/H222P}$ mice treated with PD98059 had an EF of 65.46% ± 2.64%, an increase of approximately 22% ($P<0.005$) compared to the DMSO-treated group. EF of *Lmna*$^{H222P/H222P}$ mice treated with SP600125 was 61.88% ± 1.66%, an increase of approximately 15% ($P<0.005$) compared to the DMSO-treated group. Overall, these results showed that PD98059 and SP600125 have positive effects on cardiac contractility when administered after cardiac dysfunction occurs in *Lmna*$^{H222P/H222P}$ mice.

Effect of PD98059 and SP600125 on Myocardial Fibrosis in *Lmna*$^{H222P/H222P}$ Mice

Later-stage cardiomyopathy caused by *LMNA* mutations is characterized by myocardial fibrosis (21,22). Sirius Red and Gomori's trichrome staining of hearts from *Lmna*$^{H222P/H222P}$ mice 20 weeks of age treated with DMSOhad a significant increase in fibrosis compared to hearts from *Lmna*$^{+/+}$ mice (Figure 4A,B). In contrast, *Lmna*$^{H222P/2PH222P}$ mice treated with PD98059 or SP600125 had a lower degree of cardiac fibrosis than DMSO-treated mice (Figure 4A,B). We quantified the myocardial fibrotic area of each animal by determining the ratio of fibrotic tissue (blue stained with Gomori's trichrome) to the total tissue area in each micrograph (Figure 4C). Hearts from DMSO-treated *Lmna*$^{H222P/H222P}$ mice had 15.01 ± 0.9% fibrotic tissue per total surface examined (Figure 4D). Systemic treatment with PD98059 or SP600125 significantly lowered the area of fibrotic tissue to 4.48% ± 1% ($P<0.0005$) and 5.86% ± 0.4% ($P<0.0005$), respectively (Figure 4D).

Excessive extracellular matrix, predominantly collagen proteins, defines fibrotic tissue. We therefore determined expression of genes encoding a protein of the extracellular matrix (*Fn1* encoding fibronectin) and genes encoding type I collagen (*Col1a1* and *Co1a2*) using real-

time RT-PCR. At 20 weeks of age, hearts from *Lmna*$^{H222P/H222P}$ mice treated with DMSO had a 5-fold increase of *Col1a1*, a 4-fold increase of *Co1a2* and a 4-fold increase of *Fn1* mRNAs compared to hearts from *Lmna*$^{+/+}$ mice (Figure 5). Treatment with PD98059 and SP600125 significantly lowered the expression of *Col1a1*, *Col1a2* and *Fn1* (Figure 5). These results demonstrated that *Lmna*$^{H222P/H222P}$ mice treated with either MEK1/2 or JNK inhibitors had decreased progression of myocardial fibrosis.

Effect of PD98059 and SP600125 on Nuclear Shape in Cardiomyocytes in *Lmna*$^{H222P/H222P}$ Mice

We have reported abnormal elongation of nuclei in cardiomyocytes of *Lmna*$^{H222P/H222P}$ mice (15,16). Nuclei in cardiomyocytes in hearts from *Lmna*$^{H222P/H222P}$ mice treated with DMSO were elongated compared to those in *Lmna*$^{+/+}$ mice (Figure 6A). Nuclei of cardiomyocytes in hearts of *Lmna*$^{H222P/H222P}$ mice treated with PD98059 or SP600125 *Lmna*$^{H222P/H222P}$ mice had an overall shape that was more “rounded” than those in hearts of mice treated with DMSO (Figure 6A). Mean length of cardiomyocyte nuclei in hearts of *Lmna*$^{H222P/H222P}$ mice treated with DMSO was significantly longer than in hearts from *Lmna*$^{+/+}$ mice ($P<0.0005$) (Figure 6B). The mean lengths of nuclei in cardiomyocytes in hearts from *Lmna*$^{H222P/H222P}$ mice treated with PD98059 or SP600125 were significantly shorter than the in hearts of mice in the DMSO-treated group ($P<0.0005$) (Figure 6B). Similar nuclear elongation has also been reported in *Lmna* knockout mice, suggesting a role of lamins in determining nuclear shape in cardiomyocytes (23,24). While other abnormalities in nuclear morphology have been observed in hearts of *Lmna*$^{H222P/H222P}$ mice when cardiac tissue is examined by electron microscopy (13), we could not assess these ultrastructural alterations with the light microscopic methods we used.

Pilot Study of PD98059 and SP600125 to Treat More Advanced Heart Disease in *Lmna*$^{H222P/H222P}$ mice

In a pilot study, we assessed treatment of *Lmna*$^{H222P/H222P}$ mice with PD98059 and SP600125 at a more advanced stage of disease and for a longer time. We assigned male *Lmna*$^{H222P/H222P}$ mice at 19 weeks of age to 3 different treatment arms (placebo DMSO, n=4; MEK1/2 inhibitor PD98059, n=3; JNK inhibitor SP600125, n=3) and examined parameters of cardiac structure and function. Systemic administration of PD98059 and SP600125 to *Lmna*$^{H222P/H222P}$ mice partially blocked phosphorylation of ERK1/2 and JNK in hearts from 24 week-old mice (Figure IA in the online-only Data Supplement). At 24 weeks, *Lmna*$^{H222P/H222P}$ treated with PD98059 had decreased LV dilatation and increased FS compared to DMSO-treated mice (Figure IB in the online-only Data Supplement). There was also a trend toward decreased LV dilatation and increased FS in the *Lmna*$^{H222P/H222P}$ mice treated with SP600125 (Figure IB in the online-only Data Supplement). Cardiac expression of *Mlc-2a, NppA, NppB, Col1a1 and Col1a2* mRNAs was also significantly reduced in the inhibitor-treated *Lmna*$^{H222P/H222P}$ mice at 24 weeks, except for NppB in those treated with SP600125 (Figure IC in the online-only Data Supplement).

Discussion

Our previous work has documented the effectiveness of inhibiting ERK and JNK signaling in preventing or delaying the onset of cardiomyopathy in *Lmna*$^{H222P/H222P}$ mice (15,16). In those studies, MEK and JNK inhibitors were administered prior the onset of any detectable structural or functional cardiac abnormalities. A critical remaining question was if MEK and JNK inhibitors would be effective in improving heart function in *Lmna*$^{H222P/H222P}$ mice when initiated after the onset of cardiac disease, which would be more analogous to potential treatment in human patients. In this study, we therefore tested the extent to which a treatment course starting after the onset of cardiac disease in *Lmna*$^{H222P/H222P}$ mice would

be beneficial. Our results showed that pharmacological inhibitors of ERK and JNK signaling blocked increased expression of RNAs encoding natriuretic peptide precursors and proteins involved in sarcomere architecture, prevented LV end systolic dilatation, increased cardiac ejection fraction and decreased myocardial fibrosis. Two recent studies showed that either a calcium-sensitizing agent (25) or a β-blocker (24) also improved cardiac function in mouse models of *Lmna*-associated cardiomyopathy. Our work provides support for the possibility that MEK or JNK inhibitors could overcome the lack of definitive treatments for human patients suffering for cardiac disease caused by *LMNA* mutations.

Changes in myocardial structure and function in response to injury and proliferation of the non-myocyte cell populations of the heart, referred to as myocardial remodelling (26), alter cardiac performance over the long term. Part of such remodelling includes fibrosis, which results in exaggerated mechanical stiffness and causes systolic dysfunction (27). Established therapies for heart failure may also drive a significant part of their benefit from actions on cardiac fibroblasts. A beneficial effect on cardiac fibrosis has been reported for angiotensin converting enzyme inhibitors (28–30), angiotensin receptor blockers (31,32), diuretics (33) and aldosterone antagonists (34–36). Treatment of $Lmna^{H222P/H222P}$ mice with MEK or JNK inhibitors had a profound beneficial effect on myocardial fibrosis, a characteristic of later-staged cardiomyopathy caused by *LMNA* mutations (21,22). Activation of ERK and JNK signaling pathways by various stimuli have been correlated to several cellular processes such as cell proliferation and remodelling of extra-cellular matrix (37). Inhibition of ERK and JNK signaling pathways could therefore have a beneficial effect on cardiac function by also acting directly to decrease the proliferation of myocardial fibroblasts. Such a hypothesis needs to be tested. It also remains to be determined if simultaneous inhibition of both ERK and JNK signaling has additive effects in cardiomyopathy caused by *Lmna* mutation.

Our study in $Lmna^{H222P/H222P}$ mice was designed similar to a human clinical trial. It assessed primary endpoints (LV dilatation, EF) and “surrogate” secondary endpoints (expression of natriuretic peptide precursors) that are used in many human clinical heart failure trials. While mortality is a reasonable endpoint in phase III clinical trial for advanced heart failure, it is rarely if ever used in the initial drug assessment phase or in treatment of subjects with heart disease that is not end stage (38), as were both the case in our study. Furthermore, $Lmna^{H222P/H222P}$ mice have diaphragmatic muscle involvement (not reported in humans with *LMNA* mutations) and significant skeletal muscle pathology as they age, which may be non-cardiac causes of mortality (13). Nonetheless, the measurements of LV function we used correlate with prognosis in many human clinical trials and their behaviour parallels changes in mortality with treatment (38). For example, LV end-systolic volume, which is determined by measuring LVESD, is the major determinant of survival in human subjects after recovery from myocardial infarction and after coronary artery bypass grafting for impaired LV function (39,40). A study by Heywood et al. (41) also showed in human subjects with an EF less than 40% treated with angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers that an increase of more than 15% in EF resulted in mortality of only about 2% per year. In our study, PD98059 and SP600125 improved the EF of $Lmna^{H222P/H222P}$ mice approximately 22% and 15%, respectively, compared to placebo. Taking into account that EF improvement is an important predictor for survival in human subjects with systolic dysfunction, we speculate that small molecules inhibitors of the ERK and JNK signaling pathways could have a positive effect on survival of patients with *LMNA* mutations. While not an endpoint or our study, during the 4-week treatment protocol starting 16 weeks of age, 6 mice in the DMSO group, 3 in the PD98059 group and 3 in the SP600125 group died prior to reaching 20 weeks of age, suggesting that treatment with MEK1/2 or JNK inhibitors trended towards improved survival. Furthermore, our pilot study treating $Lmna^{H222P/H222P}$ mice up to 24 weeks of age, when they have a mortality rate of

approximately 25% (13), showed improvements in echocardiographic and cardiac biochemical parameters.

The choice of therapeutic agents in clinical trials is predicated, at least in part, on the efficacy of drugs studied in murine models of disease (42–44). Both PD98059 and SP600125, which we used in this study to respectively inhibit ERK and JNK signaling, are tool compounds and are not suitable for use in humans secondary to problems with bioavailability and toxicity (45). Therefore, any future clinical trial of MEK or JNK inhibitor in human subjects with cardiomyopathy caused by *LMNA* mutations would require the use of superior drugs, including possibly those that are already entered the pipeline of pharmaceutical companies for other indications. For example, a second-generation oral MEK inhibitor, PD0325901 (Pfizer), has markedly improved properties, including better potency against MEK, better bioavailability, increased metabolic stability and a longer time of MEK suppression (46). PD0325901 has been administered to humans and has entered a phase II clinical trial to treat advanced non-small cell lung cancer (47,48). Similarly, AZD6244/ARRY-142886 (AstraZeneca/Array Biopharma) is in phase II clinical trials for patients with cancers (49). Superior JNK inhibitors are also in preclinical development for use in humans (50). Hence, our results in $Lmna^{H222P/H222P}$ mice with cardiac dysfunction could lay the foundation for clinical trials of MEK and JNK inhibitors, currently being developed for cancer and inflammatory conditions in human subjects with cardiomyopathy caused by *LMNA* mutations.

CLINICAL PERSPECTIVE

Heart failure is responsible for considerable morbidity and mortality and dilated cardiomyopathy (DCM) is a major cause. Molecular genetic studies have revealed mutations in various genes in patients with familial DCM but the precise mechanisms of how they lead to heart muscle damage remain largely unknown. Mutations in *LMNA* encoding A-type nuclear lamins appear to be responsible for approximately 8% of cases of familial DCM and patients with*LMNA* mutations have a poorer prognosis than those with DCM caused by mutations in most other genes. We have previously shown an abnormal activation of the extracellular signal-regulated kinase (ERK) and the c-jun N-terminal kinase (JNK) branches of the mitogen-activated protein kinase signaling cascade in hearts of mice with DCM caused by a mutation in *Lmna*. We now establish that treating these mice with chemical inhibitors of ERK and JNK after the onset of left ventricular dilatation and decreased cardiac ejection fraction, a time when human patients would be considered for therapy, improves cardiac function and significantly decreases myocardial fibrosis. These results provide proof of concept that pharmacological inhibitors of ERK and JNK signaling, some of which are currently in clinical development for other indications, could be studied in human clinical trials of patients with DCM caused by *LMNA* mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Non-standard Abbreviations

LV	left ventricular
ERK	extracellular signal-regulated kinase
JNK	c-jun N-terminal kinase
MAPK	mitogen-activated protein kinase
DMSO	dimethyl sulfoxide
MEK	MAPK/ERK kinase
SEM	standard error of mean
PCR	polymerase chain reaction
RT-PCR	reverse transcription-polymerase chain reaction
LVEDD	left ventricular end diastolic diameter
LVESD	left ventricular end systolic diameter

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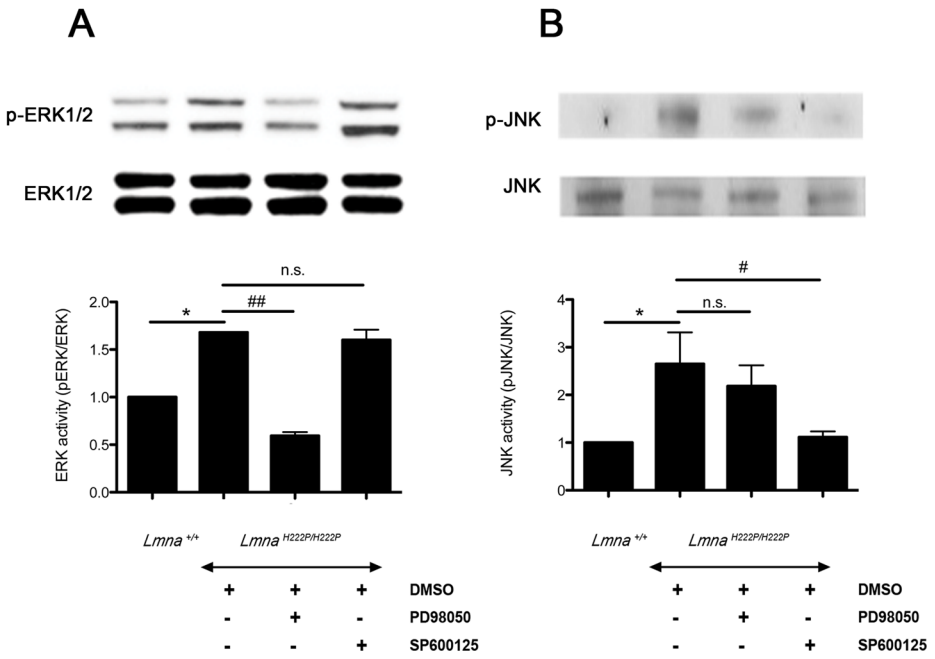

Figure 1.

(A) Representative immunoblots using antibodies against phophorylated ERK1/2 (p-ERK) and total ERK1/2 (ERK) and **(B)** against phophorylated JNK (p-JNK) and total JNK to probe proteins extracted from hearts from *Lmna*$^{H222P/H222P}$ mice treated with PD98059, SP600125 or DMSO. Blots of proteins extracted from hearts of *Lmna*$^{+/+}$ mice are shown for comparison. The graphs show quantification of **(A)** pERK/total ERK and **(B)** pJNK/total JNK. n=4 in each group. Comparison between DMSO-treated *Lmna*$^{H222P/H222P}$ mice and *Lmna*$^{+/+}$ mice; **P*<0.05. Comparison between PD98059-treated and SP600125-treated and DMSO-treated *Lmna*$^{H222P/H222P}$ mice; $^{\#}P$<0.05, $^{\#\#}P$<0.005, n.s.: not significant.

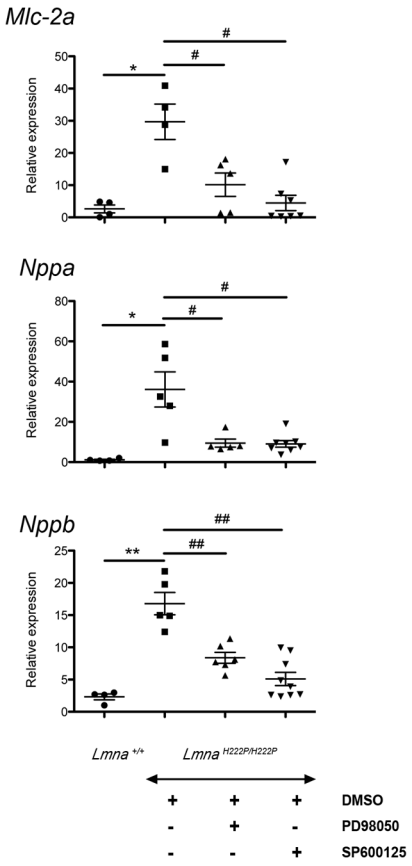

Figure 2.

Effect of PD98059 and SP600125 on cardiac expression of natriuretic peptides and myosin light chain in *Lmna*$^{H222P/H222P}$ mice. Dot diagrams indicate the expression levels of *Mlc-2a* mRNA encoding the cardiac isoform of myosin light chain, *Nppa* mRNA encoding the atrial natriuretic factor and *Nppb* encoding the brain natriuretic peptide in hearts from *Lmna*$^{+/+}$ mice and *Lmna*$^{H222P/H222P}$ mice treated with PD98059, SP600125 or DMSO. n=4 in each group. Values were obtained using the ΔΔCT method using *Gapdh* as housekeeping gene (see Full Materials and Methods). **P*<0.05, ***P*<0.005, $^{\#}P$<0.05, $^{\#\#}P$<0.005.

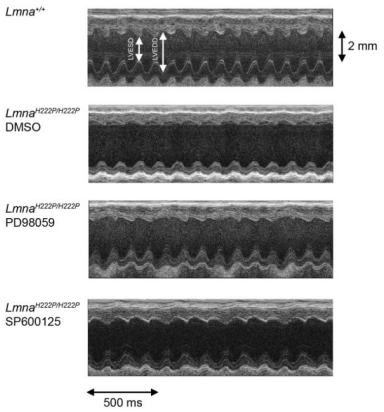

Figure 3.

Representative transthoracic M-mode echocardiographic tracings from *Lmna*$^{H222P/H222P}$ mice treated with PD98059, SP600125 or DMSO. Tracings from *Lmna*$^{+/+}$ mice are shown for comparison. LVESD and LVEDD are indicated.

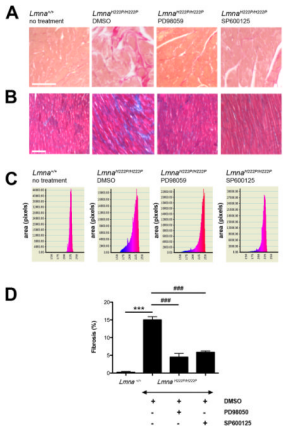

Figure 4.

(A) Sirius red and **(B)** Gomori's trichrome staining of cross-sections of hearts from *Lmna*$^{H222P/H222P}$ mice treated with PD98059, SP600125 or DMSO. A cross-section of heart from a *Lmna*$^{+/+}$ mouse is shown for comparison. Scale bar: 50 μm. **(C)** Quantification of fibrotic area in hearts from mice. n=3 in each group. Y-axis corresponds to the area (pixels) and X-axis represents the color spectrum (red corresponds to the muscle tissue and blue corresponds to the connective tissue). **(D)** Bars indicate the percentage of fibrosis per surface area of myocardium examined in hearts from *Lmna*$^{+/+}$ mice and *Lmna*$^{H222P/H222P}$ mice treated with PD98059, SP600125 or DMSO. n=3 in each group. $^{***}P<0.0005$, $^{###}P<0.0005$.

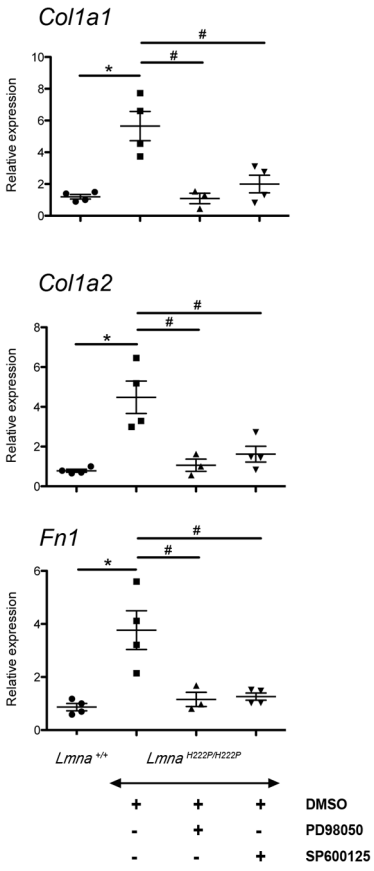

Figure 5.

Effect of PD98059 and SP600125 on cardiac expression of genes encoding collagen and fibronectin in *Lmna*$^{H222P/H222P}$ mice. Dot diagrams indicate the expression of *Col1a1*, *Col1a2* and *Fn1* in heart from *Lmna*$^{+/+}$ mice and *Lmna*$^{H222P/H222P}$ mice treated with PD98059, SP600125 or DMSO. n=3 in each group. Values were obtained using the ΔΔCT method using *Gapdh* as housekeeping gene (see Full Materials and Methods).
**P*<0.05, $^{\#}$*P*<0.05.

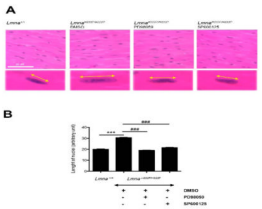

Figure 6.

(A) Histological analysis of cross-sections of hearts from *Lmna*$^{H222P/H222P}$ mice treated with PD98059, SP600125 or DMSO. Heart from a *Lmna*$^{+/+}$ mouse is shown for comparison. Sections are stained with hematoxylin and eosin. Yellow lines with arrowheads demonstrate the measurement of nuclear length. Scale bar: 25 μm. **(B)** Quantification of nuclear elongation in cardiomyocytes from mice. Cardiomyocyte nuclei were measured along the yellow lines with arrowheads. Bars indicate the length of cardiomyocyte nuclei in the indicated hearts. Values are means ± SEM for n=150, 290, 690 and 575 cardiomyocytes from *Lmna*$^{+/+}$ mice, DMSO-treated *Lmna*$^{H222P/H222P}$ mice, PD98059-treated *Lmna*$^{H222P/H222P}$ mice, and SP600125-treated *Lmna*$^{H222P/H222P}$ mice, respectively. ***P<0.0005, $^{###}P$<0.0005.

Table 1

Echocardiographic data at 20 weeks of age for *Lmna*$^{+/+}$ mice and *Lmna*$^{H222P/H222P}$ mice treated with DMSO placebo or treated with SP600125 or PD98059

Genotype (Treatment Group)	*n*	HR	LVEDD (mm)	LVESD (mm)	EF (%)	FS (%)
Lmna$^{+/+}$	12	400	3.50 ± 0.06	2.07 ± 0.08	73.21 ± 1.17	41.71 ± 1.01
Lmna$^{H222P/H222P}$ (DMSO)	22	372	3.87 ± 0.11 *	3.00 ± 0.13 ***	53.87 ± 2.58 ***	27.86 ± 1.54 ***
Lmna$^{H222P/H222P}$ (PD98059)	19	350	3.55 ± 0.11	2.41 ± 0.11 ‡‡‡	65.46 ± 2.64 ‡‡	35.91 ± 1.88 ‡‡
Lmna$^{H222P/H222P}$ (SP600125)	26	363	3.73 ± 0.08	2.67 ± 0.10 ‡	61.88 ± 1.66 ‡‡	33.11 ± 1.16 ‡‡

Values are means ± SEM.

HR indicates heart rate in beats per minute.

Comparison between DMSO-treated *Lmna*$^{H222P/H222P}$ and *Lmna*$^{+/+}$ mice:

*P<0.05,

***P<0.0005.

Comparison between SP600125-treated and DMSO-treated *Lmna*$^{H222P/H222P}$:

‡P<0.05,

‡‡P<0.005,

‡‡‡P<0.0005.