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Pharmacological inhibition of c-Jun N-terminal kinase signaling prevents cardiomyopathy caused by mutation in *LMNA* gene

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ABSTRACT

Mutations in *LMNA*, which encodes A-type nuclear lamins, cause disorders of striated muscle that have as a common feature dilated cardiomyopathy. We have demonstrated an abnormal activation of both 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 from $Lmna^{H222P/H222P}$ mice that develop dilated cardiomyopathy. We previously showed that pharmacological inhibition of cardiac ERK signaling in these mice delayed the development of left ventricle dilatation and deterioration in ejection fraction. In the present study, we treated $Lmna^{H222P/H222P}$ mice with SP600125, an inhibitor of JNK signalling. Systemic treatment with SP600125 inhibited JNK phosphorylation, with no detectable effect on ERK. It also blocked increased expression of RNAs encoding natriuretic peptide precursors and proteins involved in the architecture of the sarcomere that occurred in placebo-treated mice. Furthermore, treatment with SP600125 significantly delayed the development of left ventricular dilatation and prevented decreases in cardiac ejection fraction and fibrosis. These results demonstrate a role for JNK activation in the development of cardiomyopathy caused by *LMNA* mutations. They further provide proof-of-principle for JNK inhibition as a novel therapeutic option to prevent or delay the cardiomyopathy in humans with mutations in *LMNA*.

1. Introduction

Mutations in *LMNA* encoding A-type nuclear lamins are responsible for at least three severe diseases involving striated muscles: autosomal Emery–Dreifuss muscular dystrophy [1], limb girdle muscular dystrophy type 1B [2], and dilated cardiomyopathy type 1A [3]. A common feature of these disorders is dilated cardiomyopathy, which is characterized by an age of onset generally in the third decade of life and frequently associated with a progressive conduction system disease leading to implantation of defibrillators [4]. Affected individuals eventually develop heart failure, for which there is currently no curative treatment, ultimately necessitating cardiac transplantation.

Identification of *LMNA* mutations in patients with dilated cardiomyopathy raised intriguing questions about the relationship between A-type nuclear lamins and dilated cardiomyopathy, since lamins are not known to contribute in force transmission or generation in cardiomyocytes. The link between cardiomyopathy and abnormalities in A-type lamins is poorly understood and only a few hypotheses have been raised concerning pathophysiology. We have recently reported 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 (MAP kinase) signalling cascade in hearts of *Lmna* H222P knock-in mice, a model of autosomal Emery–Dreifuss muscular dystrophy [5]. Male $Lmna^{H222P/H222P}$ mice developed left ventricular (LV) dilatation and depressed contractile function starting at approximately 8–10 weeks of age and invariably developed LV dilatation and decreased cardiac contractility at 16 weeks of age with death typically occurring between 16 and 36 weeks [6]. On the basis of our observations that ERK and JNK are activated in these mice before the onset of clinically detectable cardiomyopathy, as well as our demonstration that lamin A variants that cause striated muscle disease activate both of these protein kinases when expressed in cultured cells, we hypothesized that abnormal activation of ERK and

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JNK plays a primary pathogenic role in the development of cardiomyopathy [5]. Recently, we showed that blocking ERK signalling in male *Lmna*$^{H222P/H222P}$ mice, using a small molecule inhibitor of extracellular signal-regulated kinase kinase (MEK) that activates ERK, induced normal LV diameters and cardiac ejection fraction (EF) at the age of 16 weeks, when placebo-treated mice had significant abnormalities in these parameters [7]. In the present study, we sought to determine if pharmacological inhibition of JNK signaling would similarly prevent or delay the development of dilated cardiomyopathy in *Lmna*$^{H222P/H222P}$ mice. To test this hypothesis, we treated *Lmna*$^{H222P/H222P}$ mice with SP600125, an inhibitor of JNK.

2. Methods

2.1. Mice

Lmna$^{H222P/H222P}$ mice were generated and genotyped as previously described [6]. Genotyping was performed by polymerase chain reaction using genomic DNA isolated from tail clippings and oligonucleotides with sequences 5′-cagccatcacctctcctttg-3′ and 5′-agcaccagggagaggacagg-3′. Mice were fed a chow diet and housed in a disease-free barrier facility with 12-h/12-h light/dark cycles. The Institutional Animal Care and Use Committee at Columbia University Medical Center approved the use of animals and the study protocol. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. JNK inhibitor

The anthrapyrazolone inhibitor of JNK, SP600125 (Calbiochem), was dissolved in dimethyl sulfoxide (DMSO) (Sigma) at a concentration of 0.5 mg/ml and administered at a dose of 3 mg/kg/day for 5 days a week. The inhibitory activity of SP600125 is selective for all JNK isoforms (IC_{50} = 40 nM for JNK-1 and JNK-2 and 90 nM for JNK-3) [8]. The placebo consisted of DMSO alone and was delivered in the same volume. Placebo and SP600125 were administered by intraperitoneal injection using a 27 5/8-gauge syringe. Treatment was initiated when mice were 8 weeks of age and continued until 16 weeks of age.

2.3. Thansthoracic echocardiography

At 16 weeks of age, mice were anesthetized with 1.5% isoflurane in O_2 and placed on a heating pad (37 °C). Echocardiography was performed using a Visualsonics Vevo 770 ultrasound with a 30-MHz transducer applied to the chest wall. Cardiac ventricular dimensions and EF were measured in 2D mode and M-mode three times for the number of animals indicated. A “blinded” echocardiographer (J.S.), unaware of the genotype or treatment, performed the examinations and interpreted the results.

2.4. Histopathological analysis

Mice were sacrificed at 16 weeks of age after being examined by echocardiography and freshly removed hearts were fixed in 4% formaldehyde for 48 hours, embedded in paraffin, sectioned at 5 µm, and stained with Gomori's trichrome or hematoxylin and eosin. Representative stained sections were photographed using a Microphot SA (Nikon) light microscope attached to a Spot RT Slide camera (Diagnostic Instruments). Images were processed using Adobe Photoshop CS (Adobe Systems).

2.5. Protein extraction and immunoblotting

Heart tissue was homogenized in extraction buffer as previously described [7]. Protein samples were subjected to SDS–PAGE, transferred to nitrocellulose membranes, and blotted with primary antibodies against JNK (Santa Cruz), phosphorylated JNK (no. 9251; Cell Signaling), phosphorylated c-Jun (no. Sc-822; Santa Cruz), ERK1/2 (no. Sc-94; Santa Cruz), and phosphorylated ERK1/2 (no. 9101; Cell Signaling). Secondary antibodies were horseradish peroxidase–conjugated (Amersham). Recognized proteins were visualized by enhanced chemiluminescence (ECL; Amersham). The signal generated using antibody against GAPDH was used as an internal control to normalize the amounts of protein between immunoblots.

2.6. Quantitative real-time RT–PCR analysis

Total RNA was extracted using the RNeasy Isolation Kit (Qiagen) as previously described [7]. cDNA was synthesized using Superscript first strand synthesis system according to the manufacturer's instructions (Invitrogen) on total RNA. For each replicate in each experiment, RNA from tissue samples of different animals was used. Primers were designed correspond to mouse RNA sequences using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) for *Nppa* (forward 5′-gcttccaggccatattggag-3′, reverse 5′-ccctgcttcctcagtctgct-3′), *Nppb* (forward 5′-ggaccaaggcctcacaaaag-3′, reverse 5′-tacagcccaaacgactgacg-3′), *Myl4* (forward 5′-cccaagcctgaagagatgag-3′, reverse 5′-agacaacagctgctccacct-3′), *Myl7* (forward 5′-tcaaggaagccttcagctgc-3′, reverse 5′-cggaacacttaccctcccg-3′), *Myh7* (forward 5′-tgcagcagttcttaaccac-3′, reverse 5′-tcgaggcttctggaagttgt-3′), and *JunD* (forward 5′-atgtgcacgaaaatggaaca-3′, reverse 5′-cctgacccgaaaagtagctg-3′). The real-time RT–PCR reaction contained iQ SYBR Green Super Mix (Bio-Rad), 200 nM of each primer, and 0.2 µl of template in a 25-µl reaction volume. Amplification was carried out using the MyiQ Single-Color Real-Time PCR Detection System (Bio-Rad) with an initial denaturation at 95 °C for 2 min followed by 50 cycles at 95 °C for 30 s and 62 °C for 30 s. Relative levels of mRNA expression were calculated using the $\Delta\Delta C_T$ method [7]. Individual expression values were normalized by comparison with *Gapdh* mRNA (forward 5′-tgcaccaccaactgcttag-3′, reverse 5′-ggatgcagggatgatgttc-3′).

2.7. Statistical analysis

Results of immunoblots, real-time RT–PCR, and fibrosis quantification were compared using the Student unpaired *t*-test. Comparisons of the echocardiographic parameters between DMSO-treated *Lmna*$^{H222P/H222P}$ and *Lmna*$^{+/+}$ mice and between SP600125-treated and DMSO-treated *Lmna*$^{H222P/H222P}$ were performed using the Student unpaired *t*-test; to validate these results, a nonparametric test (Mann–Whitney) was performed and concordance was checked. Statistical analyses were performed using GraphPad Prism software.

3. Results

3.1. Effect of SP600125 on JNK activity

Systemic administration of SP600125 to *Lmna*$^{H222P/H222P}$ mice partially blocked phosphorylation of JNK in hearts as shown by immunoblot (Fig. 1A). At 3 mg/kg/day (5 times a week), we did not detect inhibition of phosphorylation of ERK in the hearts (Fig. 1A). Quantification of the immunoblot signals for JNK showed that DMSO-treated *Lmna*$^{H222P/H222P}$ mice had a 2.5-fold increase of phosphorylated JNK expression compared to *Lmna*$^{+/+}$ mice but *Lmna*$^{H222P/H222P}$ treated with SP600125 had a significantly reduced level of phosphorylated JNK similar to *Lmna*$^{+/+}$ mice (Fig. 1A). Phosphorylation of the downstream target, c-Jun, was also significantly reduced by SP600125

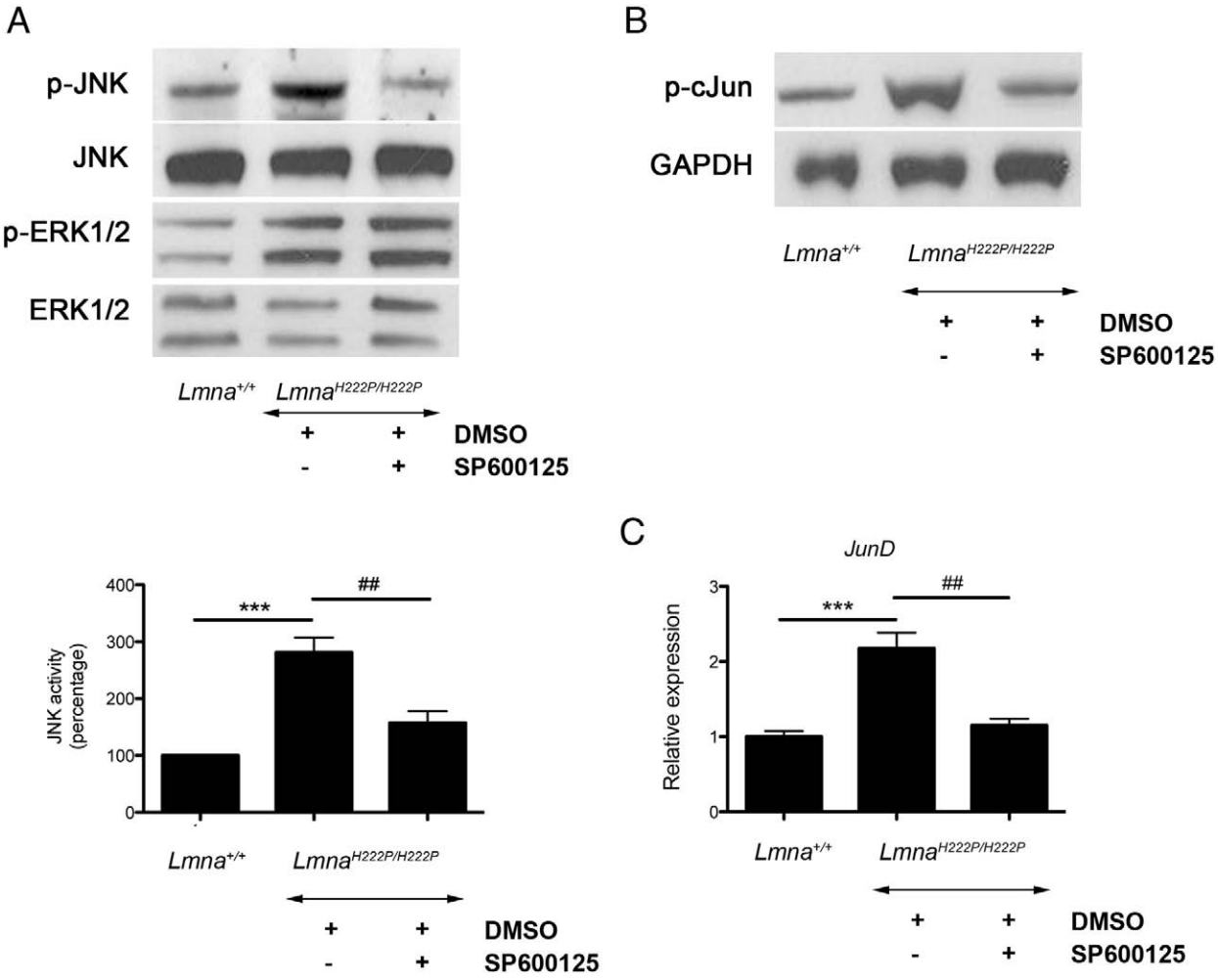

Fig. 1. Treatment of male $Lmna^{H222P/H222P}$ mice with SP600125 inhibits phosphorylation of JNK signaling. (A) Representative immunoblots using antibodies against phosphorylated JNK (p-JNK), total JNK, phosphorylated ERK1/2 (p-ERK1/2), and total ERK1/2 to probe proteins extracted from hearts from $Lmna^{H222P/H222P}$ mice treated with SP600125 or DMSO. Blots of proteins extracted from hearts of $Lmna^{+/+}$ mice are shown for comparison. Data in bar graphs show quantification of phosphorylated JNK compared with total JNK measured by scanning immunoblots and using ImageJ64 software. Values are means ± standard errors for $n = 5$ samples from different animals per group. Comparison between DMSO-treated $Lmna^{H222P/H222P}$ and $Lmna^{+/+}$ mice was performed using the Student unpaired *t*-test, $^{***}P<0.0005$. Comparison between SP600125-treated and DMSO-treated $Lmna^{H222P/H222P}$ was performed using the Student unpaired *t*-test, $^{\#\#}P<0.005$. (B) Representative immunoblots using antibody against phosphorylated cJun (p-cJun) to probe proteins extracted from hearts from $Lmna^{H222P/H222P}$ mice treated with SP600125 or DMSO. Blot of proteins extracted from hearts of $Lmna^{+/+}$ mice is shown for comparison. Antibody against GAPDH is used as a loading control. (C) Quantitative real-time RT–PCR showing the expression of mRNA from *JunD*. Results from hearts from $Lmna^{+/+}$ mice are shown for comparison. Bars indicate the fold overexpression of the indicated mRNA calculated by the $\Delta\Delta C_T$ method. Values are means ± standard errors for $n = 5$ samples from different animals per group. Reactions were performed in triplicate for each different RNA sample. Comparison between DMSO-treated $Lmna^{H222P/H222P}$ and $Lmna^{+/+}$ mice was performed using the Student unpaired *t*-test, $^{***}P<0.0005$. Comparison between SP600125-treated and DMSO-treated $Lmna^{H222P/H222P}$ was performed using the Student unpaired *t*-test, $^{\#\#}P<0.005$.

(Fig. 1B), as well as the expression of *JunD* mRNA (Fig. 1C), confirming the efficacy of the small molecule inhibitor in the heart at the given dose.

3.2. Effect of SP600125 on cardiac expression of natriuretic peptides and myosin

A feature of dilated cardiomyopathy is the up-regulation of natriuretic peptides [9]. The up-regulation of genes involved in sarcomere organization also occurs in dilated cardiomyopathy [5]. In hearts from male $Lmna^{H222P/H222P}$ mice, the expressions of *NppA* and *NppB* mRNAs encoding natriuretic peptides precursors were significantly increased compared to $Lmna^{+/+}$ mice (Fig. 2A). Similarly, the expression of *Myl7* and *Myl4* mRNAs encoding myosin light chain and the expression of *Myh7* mRNA encoding myosin heavy chain were significantly increased compared to $Lmna^{+/+}$ mice (Fig. 2B). SP600125-treated $Lmna^{H222P/H222P}$ mice had a significantly decreased cardiac expression of *Myl7*, *Myl4*, *Myh7*, *NppA*, and *NppB* compared to DMSO-treated $Lmna^{H222P/H222P}$ mice (Figs. 2A and B).

3.3. Effect of JNK inhibition on cardiac function

Histopathological analysis of hearts at 16 weeks of age showed that DMSO-treated $Lmna^{H222P/H222P}$ mice had an increase in fibrotic tissue compared to $Lmna^{+/+}$ mice ($P<0.0005$; Fig. 3A). As assessed by quantification of collagen staining, SP600125-treated $Lmna^{H222P/H222P}$ mice had a statistically significant decrease of fibrosis compared to DMSO-treated $Lmna^{H222P/H222P}$ ($P<0.05$; Fig. 3A). Hence, treatment with SP600125 prevents cardiac fibrosis in $Lmna^{H222P/H222P}$ mice. To confirm the degree of fibrosis, we further determined the expression of *Col1a1* and *Col1a2* encoding type I collagen by quantitative real-time RT–PCR. At 16 weeks of age, DMSO-treated $Lmna^{H222P/H222P}$ mice

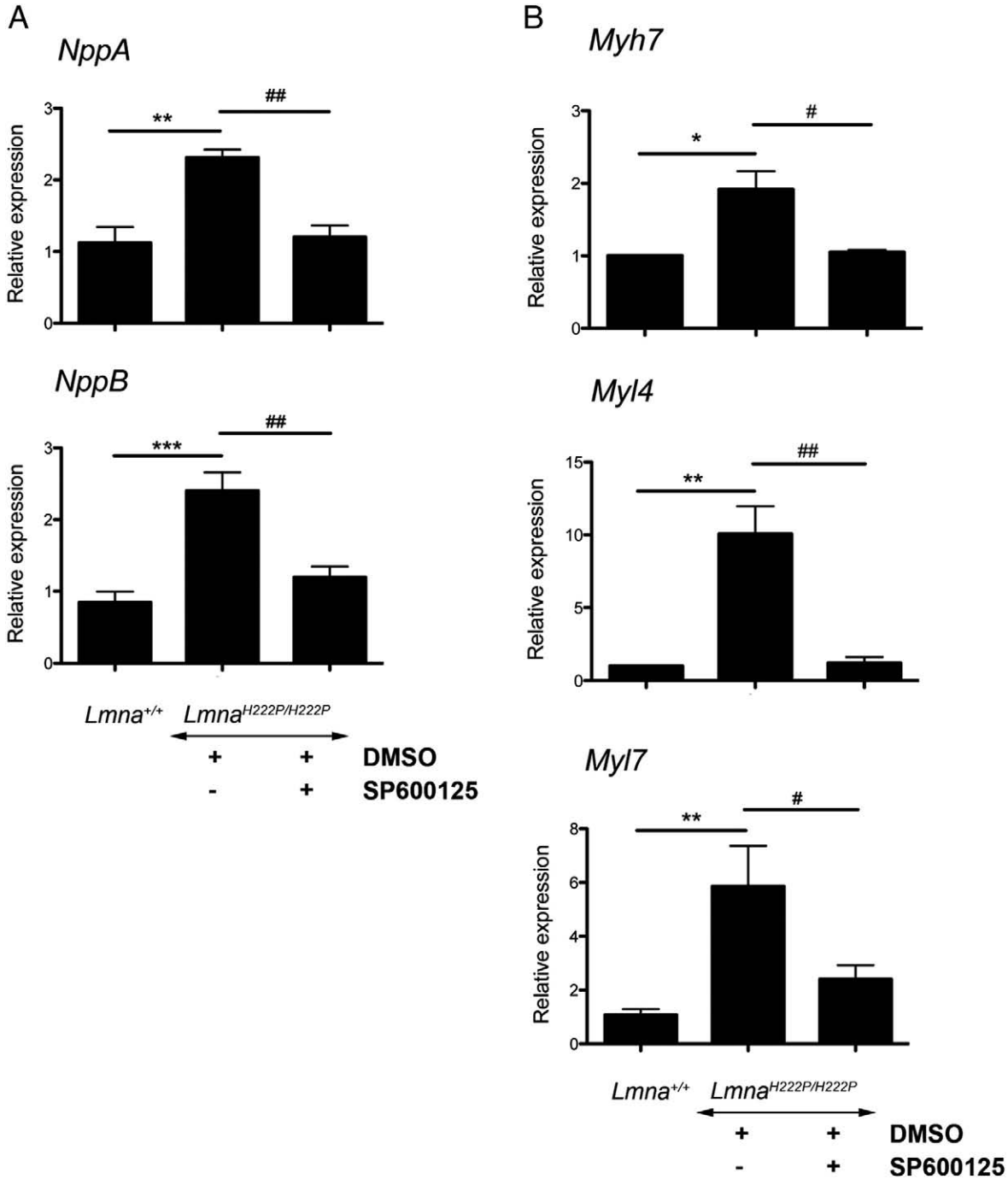

Fig. 2. Treatment of male *Lmna*$^{H222P/H222P}$ mice with SP600125 inhibits expression of natriuretic peptides and myosin chains. Quantitative real-time RT–PCR showing the expression of (A) mRNAs from *NppA* and *NppB* genes encoding natriuretic peptide precursors A and B, respectively, and (B) mRNAs from *Myh7* encoding myosin heavy chain and *Myl4* and *Myl7* encoding myosin light chain. Results from hearts from *Lmna*$^{+/+}$ mice are shown for comparison. Bars indicate the fold overexpression of the indicated mRNA calculated by the $\Delta\Delta C_T$ method. Values are means ± standard errors for $n = 5$ samples from different animals per group. Reactions were performed in triplicate for each different RNA sample. Comparison between DMSO-treated *Lmna*$^{H222P/H222P}$ and *Lmna*$^{+/+}$ mice was performed using the Student unpaired *t*-test, $^{*}P<0.05$, $^{**}P<0.005$, $^{***}P<0.0005$. Comparison between SP600125-treated and DMSO-treated *Lmna*$^{H222P/H222P}$ was performed using the Student unpaired *t*-test, $^{‡}P<0.05$, $^{‡‡}P<0.005$.

had a significantly increased expression of both genes in the heart compared to *Lmna*$^{+/+}$ mice (Fig. 3B). In DMSO-treated *Lmna*$^{H222P/H222P}$ mice, *Col1a1* and *Col1a2* mRNAs were increased by 3-fold compared to *Lmna*$^{+/+}$ mice (Fig. 3B). Treatment with SP600125 significantly lowered the expression of both *Col1a1* and *Col1a2* mRNAs in the heart of *Lmna*$^{H222P/H222P}$ mice compared to the DMSO-treated *Lmna*$^{H222P/H222P}$ mice (Fig. 3B).

We recently reported alterations in nuclear morphology, including abnormal elongation of nuclei, in cardiomyocytes of *Lmna*$^{H222P/H222P}$ mice [7]. At 16 weeks of age, DMSO-treated *Lmna*$^{H222P/H222P}$ mice had a significant increase in nuclear length, compared to *Lmna*$^{+/+}$ mice (Fig. 4A). Cardiomyocyte nuclei in SP600125-treated *Lmna*$^{H222P/H222P}$ mice had an overall length that was similar to those in *Lmna*$^{+/+}$ mice (Fig. 4A). Mean lengths of cardiomyocyte nuclei in placebo-treated *Lmna*$^{H222P/H222P}$ mice were significantly longer than in *Lmna*$^{+/+}$ mice and SP600125-treated *Lmna*$^{H222P/H222P}$ mice (Fig. 4B).

Compared with *Lmna*$^{+/+}$ mice, *Lmna*$^{H222P/H222P}$ mice treated with DMSO had significant increases in LV end-diastolic and end-systolic

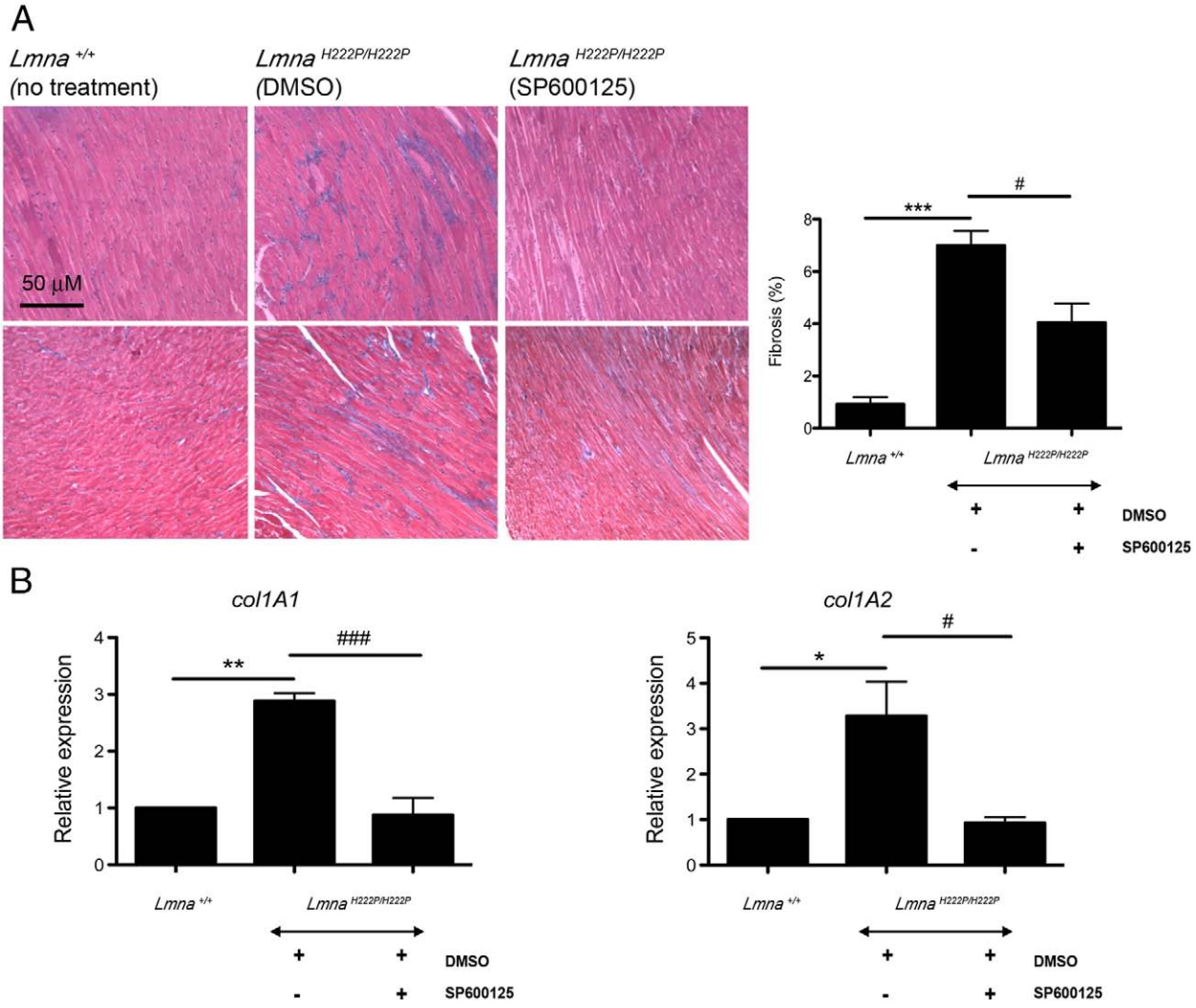

Fig. 3. Treatment with SP600125 prevents cardiac fibrosis in *Lmna*$^{H222P/H222P}$ mice. (A) Representative heart tissue sections from *Lmna*$^{H222P/H222P}$ mice treated with SP600125 or DMSO stained with Gomori's trichrome are shown. Heart tissue section from *Lmna*$^{+/+}$ mice is shown for comparison. Scale bar: 50 μm. Bar graph represents the quantification of fibrotic area in hearts from mice. Micrographs ($n=3$) for each heart were processed (JMicroVision software), and blue staining fibrotic tissue was quantified (ImageJ64 software). Bars indicate the percentage of fibrosis in heart from *Lmna*$^{+/+}$ and *Lmna*$^{H222P/H222P}$ mice treated with SP600125 or DMSO. Values are means ± standard errors for $n=3$ mice per group. Comparison between DMSO-treated *Lmna*$^{H222P/H222P}$ and *Lmna*$^{+/+}$ mice was performed using the Student unpaired *t*-test, $^{***}P<0.0005$. Comparison between SP600125-treated and DMSO-treated *Lmna*$^{H222P/H222P}$ was performed using the Student unpaired *t*-test, $^{\ddagger}P<0.05$. (B) Effect of SP600125 inhibitor on cardiac expression of collagens genes in *Lmna*$^{H222P/H222P}$ mice. Bars indicate the expression of *Col1a1* and *Col1a2* in heart from *Lmna*$^{+/+}$ and *Lmna*$^{H222P/H222P}$ mice treated with SP600125 or placebo (DMSO). Values are means ± standard errors for $n=3$ hearts. Comparison between DMSO-treated *Lmna*$^{H222P/H222P}$ and *Lmna*$^{+/+}$ mice was performed using the Student unpaired *t*-test, $^{*}P<0.05$, $^{**}P<0.005$. Comparison between SP600125-treated and DMSO-treated *Lmna*$^{H222P/H222P}$ mice was performed using the Student unpaired *t*-test, $^{\ddagger}P<0.05$, $^{\ddagger\ddagger}P<0.0005$.

diameters and decreases in interventricular septal diameter, EF, and fractional shortening (Table 1), consistent with what has been described in previous studies [6,7]. We then analyzed the effects of the JNK inhibitor compared to the placebo on echocardiographic parameters. The analysis was performed with heart rates similar between the two groups (358.47 ± 81.75 beats/min for DMSO-treated *Lmna*$^{H222P/H222P}$ mice and 355 ± 80.77 beats/min for SP600125-treated *Lmna*$^{H222P/H222P}$ mice). When treated with SP600125, *Lmna*$^{H222P/H222P}$ mice had approximately 5% smaller mean LV end-diastolic diameter, although the difference compared to placebo-treatment mice did not reach statistical significance. When treated with SP600125, *Lmna*$^{H222P/H222P}$ mice had a 15% smaller mean LV end-systolic diameter compared to DMSO-treated *Lmna*$^{H222P/H222P}$ mice ($P<0.05$). Systemic treatment with SP600125 had a positive effect on cardiac function leading to an EF approximately 20% higher than in DMSO-treated *Lmna*$^{H222P/H222P}$ mice ($P<0.005$). Hence, treatment with SP600125 for 8 weeks prevented or delayed the development of significant cardiac contractile dysfunction in *Lmna*$^{H222P/H222P}$ mice.

4. Discussion

Our results show that abnormal activation of the stress-induced JNK signalling pathway contributes to the pathogenesis of cardiomyopathy caused by mutations in *LMNA* encoding A-type lamins. It remains unclear how A-type lamins with amino acid substitutions activate JNK signalling. Some investigators have hypothesized that alterations in response to stress may underlie the development of cardiac disease caused by *LMNA* mutation [10]. Abnormal responses to stress in cardiomyocytes with abnormalities in A-type lamins could therefore potentially have an impact on activation of JNK. This hypothesis remains to be tested.

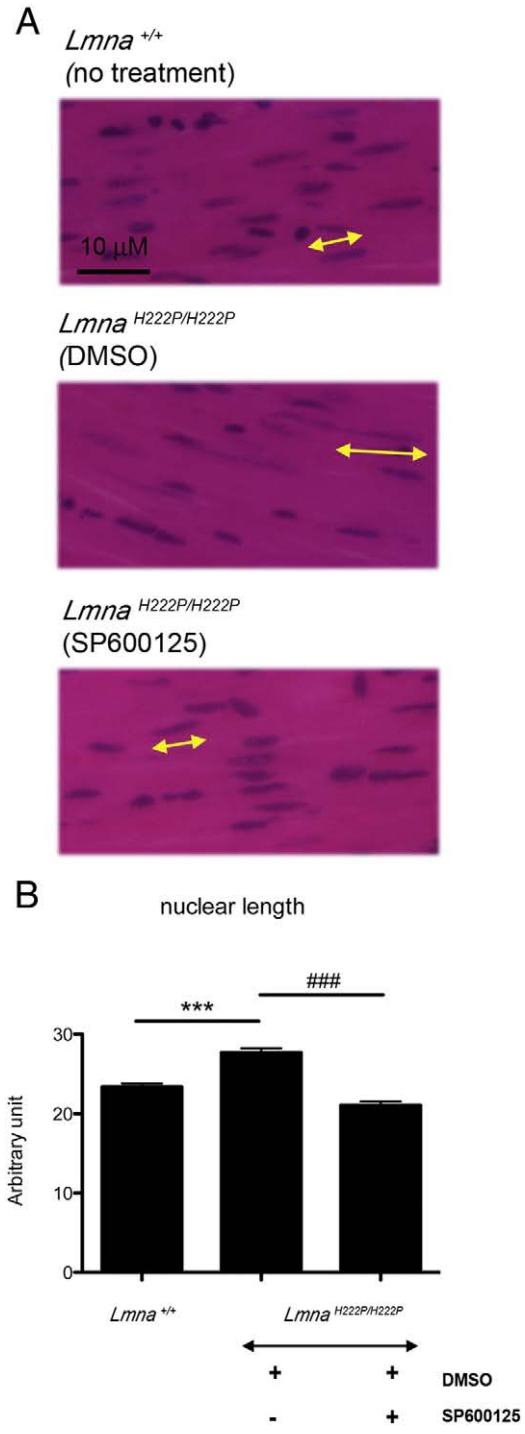

Fig. 4. Treatment with SP600125 prevents abnormal elongation of cardiomyocyte nuclei in *Lmna*$^{H222P/H222P}$ mice. (A) Histological analysis of cross sections of hearts from *Lmna*$^{H222P/H222P}$ mice treated with SP600125 or placebo (DMSO). Heart from *Lmna*$^{+/+}$ mice was used for comparison. Sections are stained with hematoxylin and eosin. Yellow lines with arrowheads demonstrate the measurement of nuclear length. Scale bar: 10 μm. (B) Quantification of nuclear elongation in cardiomyocytes from mice. Cardiomyocyte nuclei are measured along the yellow lines with arrowheads. Bars indicate the length of cardiomyocyte nuclei in the indicated hearts. Values are means ± standard errors for $n = 230$ cardiomyocytes. Comparison between DMSO-treated *Lmna*$^{H222P/H222P}$ and *Lmna*$^{+/+}$ mice was performed using the Student unpaired *t*-test, $^{***}P < 0.0005$. Comparison between SP600125-treated and DMSO-treated *Lmna*$^{H222P/H222P}$ mice was performed using the Student unpaired *t*-test, $^{\#\#\#}P < 0.0005$.

We have demonstrated that partial pharmacological inhibition of JNK *in vivo*, using SP600125, prevents significant cardiomyopathy in male *Lmna*$^{H222P/H222P}$ mice at an age when placebo-treated controls have detectable cardiac dysfunction. We recently reported that the partial pharmacological blockade of ERK signalling for the same duration in *Lmna*$^{H222P/H222P}$ mice of the same age similarly prevents cardiomyopathy [7]. Data in our previous study [7] and our current results show that inhibiting either the ERK or the JNK branches of the MAP kinase signalling cascade prevents the re-expression of fetal genes such as those encoding myosins, the up-regulation in expression of natriuretic peptides, LV dilatation, and decreased cardiac contractility. We have also shown that the JNK inhibitor prevents onset of cardiac fibrosis in 16-week-old *Lmna*$^{H222P/H222P}$ mice.

This preclinical study in mice assessed primary (LV dilatation, EF) and secondary (expression of natriuretic factors) end points that are used in many human clinical heart failure trials. The measurements of LV function we used are strictly correlated to prognosis, and in many human clinical trials, their behaviour parallels changes in mortality with treatment [11]. For example, LV end-systolic volume is the major determinant of survival in human subjects after recovery from myocardial infarction and after coronary artery bypass grafting for impaired LV function [12,13]. While mortality is a reasonable end point in phase III clinical trials for advanced heart failure, it is rarely, if ever, used in the initial drug assessment phase or in treatment of subjects with early heart disease [11], as were both the case in our study. The effect of JNK inhibition at later treatment onset, after the development of decreased cardiac ejection fraction, is currently being evaluated in our laboratory, as many patients will be diagnosed at an advance stage. Nonetheless, our previous [7] and current results clearly provide proof-of-principle that specific inhibitors that target both JNK and ERK signalling could prevent or delay the onset of cardiomyopathy caused by *LMNA* mutations and indicate that additional studies are warranted. Future studies of the effects of ERK and JNK signalling pathway inhibitors on cardiac conduction defects could also be interesting, given that early conduction abnormalities usually occur in human subjects with *LMNA* mutations.

JNK has been shown to play a central role in tissue remodelling through its ability to interact to AP-1-mediated transcription [14,15]. AP-1 function is regulated both through changes in the abundance of its Jun and Fos components and posttranslational modification by phosphorylation [16]. Of interest, AP-1 modulates the regulation of type I collagen [17,18]. This is consistent with our observation that SP600125 decreased the expression of *JunD* mRNA as well as *Col1a1* and *Col1a2* mRNAs in hearts of *Lmna*$^{H222P/H222P}$ mice. We also showed that there was a markedly decreased amount of myocardial fibrosis in hearts of 16-week-old *Lmna*$^{H222P/H222P}$ mice treated with the JNK inhibitor. This antifibrotic action of SP600125 could be secondary to the beneficial effect on the cardiac structure and function.

Additional preclinical research should be performed before initiating clinical trials of ERK and JNK inhibition in human subjects with cardiomyopathy caused by *LMNA* mutations. To resolve the possible but unlikely issue that off-target effects are providing benefits on cardiomyopathy in *Lmna*$^{H222P/H222P}$ mice, these animals should be treated with other drugs in these classes, such as JNK inhibitors of different structure that recognize a different interaction site [19]. For ERK signalling, several compounds of different structures that act at various sites in the pathway are currently in clinical development and several have already been used in human subjects [20]. The effects of longer-term ERK and JNK inhibition on various tissues also need to be examined in experimental animals, especially on skeletal muscle that is often simultaneously affected in individuals with *LMNA* mutations that cause cardiomyopathy [1,2].

Conflict of interest

H.J.W. and A.M. are inventors on a pending PCT patent application on methods for treating and/or preventing cardiomyopathies by ERK and JNK inhibition filed by the Trustees of Columbia University in the City of New York.

Table 1
Echocardiographic data at 16 weeks of age for *Lmna*$^{+/+}$ mice and *Lmna*$^{H222P/H222P}$ mice treated with DMSO or SP600125.

Genotype (treatment)	*n*	LVEDD (mm)	LVESD (mm)	IVSD (mm)	EF (%)	FS (%)
Lmna$^{+/+}$ (no treatment)	13	3.35 ± 0.09	2.00 ± 0.09	0.72 ± 0.02	76.82 ± 2.01	44.21 ± 1.77
Lmna$^{H222P/H222P}$ (DMSO)	15	$3.59 \pm 0.09^{*}$	$2.68 \pm 0.14^{***}$	$0.65 \pm 0.03^{*}$	$56.89 \pm 2.87^{***}$	$29.70 \pm 1.87^{***}$
Lmna$^{H222P/H222P}$ (SP600125)	13	3.46 ± 0.07	$2.30 \pm 0.12^{‡}$	$0.72 \pm 0.02^{‡}$	$69.24 \pm 2.66^{‡‡}$	$38.70 \pm 2.05^{‡‡}$

LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; IVSD, interventricular septum diameter; EF, ejection fraction; FS, fractional shortening. Values are means ± standard errors.
Comparison between DMSO-treated *Lmna*$^{H222P/H222P}$ and *Lmna*$^{+/+}$ mice was performed using the Student unpaired *t*-test, $^{*}P<0.05$, $^{**}P<0.005$, $^{***}P<0.0005$.
Comparison between SP600125-treated and DMSO-treated *Lmna*$^{H222P/H222P}$ was performed using the Student unpaired *t*-test and results validated by a nonparametric test (Mann–Whitney), $^{‡}P<0.05$, $^{‡‡}P<0.005$.

Acknowledgments

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