Cardiac-specific overexpression of farnesyl pyrophosphate synthase induces cardiac hypertrophy and dysfunction in mice

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Aims
Farnesyl pyrophosphate synthase (FPPS) is a key enzyme in the mevalonate pathway. In our previous study, we found that inhibition of FPPS attenuates cardiac hypertrophy in spontaneously hypertensive rats (SHRs) and prevents angiotensin (Ang) II-induced hypertrophy in cardiomyocytes. Here, we further investigate the role of FPPS in cardiac hypertrophy and heart failure (HF) using a transgenic (Tg) model, and its mechanisms.

Methods and results
Tg mice with cardiac-specific expression of FPPS were studied as an experimental model. The results showed that Tg mice with overexpression of FPPS exhibited cardiac hypertrophy, fibrosis, and HF, as well as increased synthesis of farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate in heart tissue. These pathological changes were associated with the activation of RhoA and other known kinases in the hypertrophic signalling pathway, such as extracellular signal-related kinases 1/2 and p38. Adenoviral infection of FPPS in cultured neonatal cardiomyocytes induced a hypertrophic response characterized by an increased cell size and an increased extent of sarcomeric organization, as well as an increased activation profile of small GTPases and downstream protein kinases concordant with those seen in vivo. Further investigation showed a marked increase of FPPS protein levels in hypertrophic ventricles of patients with valvular heart disease.

Conclusion
Taken together, these results suggest that FPPS may function as a potent regulator in myocardial remodelling. The FPPS-regulated signalling pathway is relevant to the pathological changes in cardiac hypertrophy and HF.

Keywords
Cardiac hypertrophy • Molecular biology • Signal transduction • Heart failure

1. Introduction
Cardiac hypertrophy and heart failure (HF) are leading causes of morbidity and mortality worldwide. Cardiac hypertrophy is thought to be an adaptive response to pressure and/or volume overload, which enables the heart to normalize ventricular-wall tension and improve pump function.¹ However, a sustained or excessive hypertrophic response is considered to be maladaptive on the basis of the progression towards HF.²,³ It is well known that pathological hypertrophy is characterized by significant changes in the size, shape, wall thickness, and contractile function of cardiac chambers.⁴,⁵ These are (at least in part) associated with changes in the expression of certain genes, including embryonic genes such as B-type natriuretic peptide (BNP) and β-myosin heavy chain (β-MHC),⁶ as well as others involved in contractile function.

Farnesyl pyrophosphate synthase (FPPS) is an essential enzyme in the mevalonate pathway. FPPS catalyses isopentenyl pyrophosphate and dimethylallyl pyrophosphate to form geranyl pyrophosphate and farnesyl pyrophosphate (FPP) (see Supplementary material online, Figure S1). FPP is a substrate for squalene synthase (SQS), which catalyses the first key step leading to cholesterol biosynthesis.⁷ FPP is also a substrate for the synthesis of geranylgeranyl
pyrophosphate (GGPP), which is necessary for protein geranylgeranylation and activation of small GTPases such as RhoA. RhoA controls the formation of actin structures. Recent studies have suggested that RhoA participates in the hypertrophic response of cardiomyocytes. Overexpression of RhoA in the heart leads to the development of congestive heart failure with bradycardia, impaired contractile function, and the induction of interstitial fibrosis.

For a long time, the mevalonate pathway was considered to play a part in lipid metabolism. Our previous studies suggested that the expression level of FPPS is significantly increased in angiotensin (Ang) II-treated cardiomyocytes as well as in hypertrophic heart tissues and vascular remodelling. In addition, we demonstrated that inhibition of FPPS attenuates cardiac hypertrophy and vascular remodelling. Also, knockdown of FPPS expression with small interfering RNAs or inhibition of FPPS prevents Ang II-induced hypertrophy in cultured cardiomyocytes. Therefore, FPPS might serve as a regulator of cardiac hypertrophy. However, the studies described above had limitations. SHRs are not the perfect model to elucidate the function of a specific gene due to their complex genetic background. Also, we could not exclude the other pharmacological effects of FPPS inhibitors that could influence cardiac and vascular remodelling. In the present study, transgenic (Tg) mice with cardiac-specific expression of FPPS were used to explore the potential role of FPPS on cardiac hypertrophy and the development of HF.

3. Results

3.1 Generation of Tg mice

To test directly the effect of FPPS on hypertrophic responses in vivo, we derived independent lines of Tg mice harbouring different levels of overexpression under control of the cardiac-specific-MHC promoter.

2. Methods

The study protocol conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, 8th Edition, 2011), and was approved by the Institutional Animal Care and Use Committee of Zhejiang University (Zhejiang, China). Additional methodological details are also provided in the Supplementary material online.

2.1 Generation of α-MHC-FPPS transgenic mice

A ~1.1 kb mouse FPPS cDNA was subcloned into a pBluescript-based Tg vector between the 5.5 kb murine α-MHC promoter (a gift from Prof. J. Robbins, University of Cincinnati, Cincinnati, OH, USA) and HGH polyadenylation sequences (see Supplementary material online, Figure S2A and B).

2.2 Echocardiographic and haemodynamic evaluation

Echocardiography was performed on anaesthetized (1.5% isoflurane) mice, using a Sonos 5500 Echocardiographic 95 System (Philips Medical Systems, Andover, MA, USA). The invasive haemodynamic measurements were performed in anaesthetized (1.5% isoflurane) mice using cardiac catheterization. The adequacy of anaesthesia was monitored by the lack of the pedal withdrawal reflex, slow constant breathing, and no response to surgical manipulation.

2.3 Blood pressure

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a Tail-cuff Pressure Analysis System (BP-98A; Softron, Tokyo, Japan) at 4 and 9 months of age while the mice were conscious. Data were averaged for six to seven consecutive measurements.

2.4 Histopathology

Mice were anaesthetized with isoflurane and euthanized by rapid cervical dislocation, then the whole hearts were weighed, fixed in 10% (v/v) formalin, embedded in paraffin, and sectioned at 5 μm intervals.

2.5 Replication-deficient adenovirus production and culture of primary cardiomyocytes, immunofluorescence microscopy

Adenoviral constructs encoding rat FPPS cDNA were created using the Admax™ Adenoviral Vector System (Microbix Biosystems, Ontario, Canada) according to manufacturer instructions. Neonatal cardiomyocytes were prepared from 1 to 2-day-old Wistar rats obtained from the Experimental Animal Center, Chinese Academy of Sciences (Shanghai, China) as described previously. To isolate ventricles, neonates were sacrificed by swift decapitation.

2.6 Real-time polymerase chain reaction

mRNA expression levels of genes were analysed by real-time polymerase chain reaction (RT–PCR).

2.7 Western blot analyses

Protein samples from cells and tissues were prepared according the protocols of the manufacturer (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

2.8 Assays for cholesterol, FPP, and GGPP

Levels of total and free cholesterol were determined by enzymatic means using Total and Free Cholesterol Assay kits (Applygen Technologies, Beijing, China). Levels of FPP and GGPP were quantified by high-performance liquid chromatography-fluorescence as described previously.

2.9 Activation of RhoA, Rac1, Cdc42, and Ras

RhoA, Rac1, and Cdc42 activity was determined by an absorbance-based G-LISA Activation Assay Biochemistry kit (Kit #BK124; #BK127; #BK128; Cytoskeleton, Denver, CO, USA) according to manufacturer instructions. Ras activity was determined by an enzyme-linked immunoassay (ELISA) kit according to manufacturer instructions (Ras GTPase Chemi ELISA Kit, Active Motif, Carlsbad, CA, USA).

2.10 Human heart samples

Informed consent was obtained from all participants in accordance with the guidelines of the Human Subjects Committee of the Medical Ethical Commission of the First Affiliated Hospital of Zhejiang University (Zhejiang, China). The investigation conforms with the principles outlined in the Declaration of Helsinki.

2.11 Statistical analyses

Results are the mean ± SEM. For direct comparison of differences between two groups, Student’s t-test was used. Analysis of variance (ANOVA) in the case of serial measurements, followed by the multiple-comparison Bonferroni t-test, was used to assess differences among groups. P < 0.05 was considered significant.

FPPS induces cardiac hypertrophy and dysfunction in Tg mice
Six independent FPPS Tg lines were generated (line 35; line 36; line 40; line 42; line 43; and line 80) (see Supplementary material online, Figure S2C). FPPS expression was increased significantly in heart tissue as determined by RT–PCR, western blot analyses, and immunohistochemical analyses (Figure 1A–C). We also analysed levels of FPP, GGPP, and cholesterol (downstream products of the mevalonate pathway) in the heart. Cholesterol levels in the liver (where cholesterol is synthesized) were detected. Because FPP and GGPP are necessary for the protein prenylation and activation of small GTPases such as Ras and RhoA, we chose line 40 with high overexpression of FPPS which was predicted to have more synthesis of FPP and GGPP. As expected, levels of cholesterol, FPP, and GGPP were significantly increased in the hearts of Tg mice (see Supplementary material online, Tables 1 and 2), whereas cholesterol levels in the liver were not significantly different between Tg and non-transgenic (NTg) mice (data not shown). Other lines were not analysed further.

### 3.2 Tg mice developed cardiac hypertrophy, fibrosis, and left ventricular dysfunction but not hypertension

Heart-to-body weight (HW/BW) was significantly increased in Tg mice compared with NTg mice at 6 weeks and 4 months (see Supplementary material online, Table 3). Haematoxylin and eosin (H&E) and Masson’s trichrome-stained sections of 4-month-old mice exhibited a phenotype of hypertrophy and cardiac fibrosis (Figure 2). Masson’s trichrome-stained heart sections revealed diffuse interstitial fibrosis in Tg mice (Figure 2B and D). Histological examination showed increased cell size and large nuclei in the cardiomyocytes of Tg mice, thereby confirming myocyte hypertrophy (Figure 2C and E). Multiple foci of myocyte disarray were observed in Tg mouse tissue, which were not observed in the hearts of NTg mice (Figure 2C).

To assess cardiac morphology in the functioning heart, echocardiography, and invasive haemodynamic measurements were conducted.
on FPPS Tg and NTg mice. Four-month-old and 9-month-old FPPS Tg mice had increased septum wall thickness, left ventricular (LV) posterior wall thickness, and LV chamber dimension, which were associated with reduced ejection fraction (EF) and fraction shortening (FS) (see Supplementary material online, Table 4 and Figure 1D). Importantly, in 9-month-old Tg mice, EF, FS, and the minimum rate of isovolumic LV pressure change (LV \( \frac{dP}{dt} \)) were reduced by 72, 117, and 44\% compared with those in NTg mice, respectively \((P < 0.001 \text{ or } P < 0.05)\), whereas left ventricular diastolic pressure (LVDP) was increased by 188\% \((P < 0.01)\) (see Supplementary material online, Tables 4 and 5). Although there was no significant difference between the two groups at the age of 4 months for \( \frac{dP}{dt} \) or LVDP, the LVDP was increased by 121\% and the maximum LV \( \frac{dP}{dt} \) was decreased by 26\% in Tg mice compared with those in NTg mice, respectively. These results demonstrated that FPPS Tg mice had cardiac hypertrophy, fibrosis, and LV dysfunction.

Cardiac remodelling and LV dysfunction are important causes of mortality, so the survival of Tg-FPPS mice was observed on a monthly basis for 12 months. The lifespan curve showed that a large increase in the mortality of Tg-FPPS mice compared with that seen in NTg mice occurred between the 8th and 12th month after birth \((P < 0.01)\) (see Supplementary material online, Figure S3).

FPPS inhibitors can lower blood pressure slightly in SHRs.\(^{15,16}\) Hence, SBP and DBP in this model were measured with the tail-cuff apparatus. However, there were no significant differences in arterial pressure between Tg mice and NTg mice in the 4- and 9-month age groups (data not shown).

### 3.3 Altered expression of cardiac hypertrophy and fibrosis-associated genes in FPPS Tg mice

The 'molecular programme' of cardiac hypertrophy is often associated with an altered expression of contractile genes or foetal encoded genes in the heart. Accordingly, in the present study, RT–PCR was done to quantify expression levels of \( \alpha\)-MHC, \( \beta\)-MHC, atrial

![Figure 2](https://academic.oup.com/cardiovascres/article-abstract/97/3/490/275338/493)

**Figure 2** Characterization of cardiac phenotypes in FPPS Tg mice. (A) Gross morphology of hearts from FPPS Tg mice and NTg mice. (B) Histological assessment of cardiac fibrosis by Masson’s trichrome staining in FPPS Tg mice and NTg mice. Scale bar, 50 \( \mu \)m for \( \times 40 \); 200 \( \mu \)m for \( \times 10 \). (C) Haematoxylin and eosin staining of ventricles. All images are shown at identical magnification. Original magnification \( \times 40 \); scale bar, 20 \( \mu \)m. (D) Quantification of the fibrosis area (blue) from trichrome-stained sections in NTg mice vs. Tg mice \((n = 3)\). ***\( P < 0.001 \). (E) Quantification of cardiomyocyte areas from haematoxylin and eosin-stained sections in NTg mice vs. Tg mice \((n = 3)\). ***\( P < 0.001 \).
natriuretic peptide (ANP), and BNP. Expression of these genes was significantly up-regulated in FPPS Tg hearts compared with those in age- and sex-matched NTg hearts \( (P < 0.01) \), whereas the expression of \( \alpha \)-MHC was not significantly changed (Figure 3A). Additionally, we analysed certain marker genes for cardiac fibrosis. Expression of transforming growth factor 2 (TGF-\( \beta \)2), TGF-\( \beta \)3, and connective tissue growth factor (CTGF), but not TGF-\( \beta \)1, was significantly increased in FPPS Tg hearts compared with those in age- and sex-matched NTg hearts (Figure 3B). These changes in gene expression suggested an activation of the molecular programme for cardiac hypertrophy and fibrosis in FPPS Tg mice.

To examine whether FPPS could influence cardiac development, we compared ANP and BNP mRNA expression in neonatal hearts of NTg and Tg mice by RT–PCR. ANP and BNP mRNA expression in the neonatal hearts of Tg mice was not altered compared with those in the neonatal hearts of NTg mice (data not shown). We

**Figure 3** Quantification of hypertrophy- and fibrosis-associated gene expression, as well as total and phosphorylated protein levels in FPPS transgenic hearts. (A and B) RT–PCR analysis of genes. \( \beta \)-Actin was the loading control. Error bars represent the SEM \( (n = 8) \). \*\( P < 0.05 \); \**\( P < 0.01 \). (C) Levels of total and phosphorylated proteins. \( \beta \)-Actin was the loading control. Controls were NTg mice. (D) Relative expression levels of proteins are shown as the ratio between phosphorylated proteins and total proteins. Error bars represent the SEM \( (n = 3) \). \*\( P < 0.05 \).
used the α-MHC promoter to generate Tg-FPPS mice, so most of the exogenous FPPS gene (driven by the α-MHC promoter in the hearts of Tg mice) would be expressed after birth. The results suggested that sustained overexpression of FPPS in the postnatal heart regulated the expression of ANP and BNP in vivo.

3.4 FPPS increased phosphorylation of ERK1/2 and P38 as well as RhoA expression

FPPS-catalysed isoprenoid intermediates are vital for the functions of the Rho family, whereas Rho family members regulate extracellular signal-related kinases (ERK1/2) and p38. There was a 1.7-fold increase in ERK1/2 phosphorylation and ~1.6-fold increase in p38 phosphorylation in FPPS Tg hearts, but significant changes in phosphorylated protein kinase B (AKT) or glycogen synthase kinase-3β (GSK3β) were not observed. Levels of ERK1/2, p38, AKT, and GSK3β protein did not vary between FPPS Tg and NTg hearts (Figure 3C and D).

Protein farnesylation and geranylgeranylation are necessary for the activation of small GTPases such as Ras and RhoA. The present study showed that RhoA activity was significantly increased in the hearts of Tg mice (Figure 4A) (by about three-fold; \( P < 0.01 \)) compared with that in NTg control mice. However, our findings did not show significant changes in Ras activity (Figure 4B). Also, the Rac1 and Cdc42 activity were not significantly changed (Figure 4C and D). Thus, the results suggested a direct relationship between the FPPS gene and RhoA. This implied that FPPS was involved in cardiac hypertrophy through RhoA activation.

3.5 FPPS adenovirus induced cardiomyocyte hypertrophy in vitro

To further investigate the ability of FPPS to induce a hypertrophic response in vitro, an adenovirus expressing FPPS was generated to allow infection of neonatal rat ventricular cardiomyocytes. Approximately 90–95% of cultured primary neonatal cardiomyocytes were infected with the FPPS-encoding adenovirus (Ad-FPPS) and green fluorescent protein adenovirus (Ad-GFP) compared with uninfected cells (Figure 5A). FPPS adenoviral infection induced a hypertrophic response in cultured neonatal cardiomyocytes, as characterized by an increased cell size and the extent of sarcomeric organization (Figure 5B). Ad-FPPS infection resulted in an average cell-surface area of 2452 ± 111 μm² compared with 1424 ± 73 μm² in Ad-GFP-infected myocytes (\( P < 0.001 \)) (Figure 5C). FPPS inhibitor alendronate attenuated the increase in the cell-surface area induced by Ad-FPPS infection (1662 ± 67 μm², \( P < 0.001 \)) (Figure 5C). BNP expression was increased significantly in Ad-FPPS-infected cardiomyocytes compared with Ad-GFP-infected or control cardiomyocytes (\( P < 0.01 \)) (Figure 5D). Alendronate attenuated the increased expression of BNP in Ad-FPPS-infected cardiomyocytes (\( P < 0.01 \)) (Figure 5D). Additionally, the increased BNP mRNA expression induced by Ad-FPPS infection was attenuated by a RhoA inhibitor C3 exoenzyme, but not by the Ras inhibitor FTS (\( P < 0.01 \)) (Figure 5E).

Ad-FPPS infection induced a seven-fold-to-eight-fold increase in FPPS protein expression (Figure 6A and B). Consistent with the in vivo data, Ad-FPPS infection resulted in a 1.5-fold increase in ERK1/2 and p38 phosphorylation, respectively, compared with Ad-GFP-infected or control cardiomyocytes, which were blocked by alendronate (Figure 6C). However, Ad-FPPS infection did not

Figure 4 RhoA and Ras expression in Tg mice and NTg mice. (A) RhoA activity in the hearts of NTg mice and Tg mice (n = 6) \( **P < 0.01 \). (B) Ras activity in the hearts of NTg mice and Tg mice (n = 6). (C) Rac1 activity in the hearts of NTg mice and Tg mice (n = 7). (D) Cdc42 activity in the hearts of NTg mice and Tg mice (n = 7).
influence GSK3β or AKT phosphorylation, whereas total protein levels of these genes remained invariable after 48 h of infection (Figure 6A and B). As indicated, cultures were infected in the presence of the FPPS inhibitor alendronate (30 μM) (ALN). Scale bar, 50 μm. (C) The cell-surface area was measured in α-actinin-stained cardiomyocytes using confocal microscopy and digitized imaging. **p < 0.01, vs. Ad-GFP, ###p < 0.001 vs. Ad-FPPS. (D) RT–PCR analyses of BNP mRNA expression in cardiomyocytes. As indicated, cultures were infected in the presence of the FPPS inhibitor alendronate (ALN). β-Actin was the loading control. Identical results were obtained in three independent experiments. #p < 0.05 vs. Ad-GFP, ###p < 0.001 vs. Ad-FPPS. (E) RT–PCR analyses of BNP mRNA expression in cardiomyocytes. As indicated, cultures were infected in the presence of C3 exoenzyme (30 ng/mL) or FTS (50 μM). β-Actin was the loading control. Identical results were obtained in three independent experiments. **p < 0.01 vs. Ad-GFP, ###p < 0.001 vs. Ad-FPPS.

### 3.6 Gene expression in the mevalonate pathway

FPPS is only one of the key enzymes in the mevalonate pathway. Therefore, it was necessary to investigate the expression of other enzymes in the mevalonate pathway in our model. There was no significant difference in mRNA expression in the other enzymes between Tg mice and NTg mice, including farnesyltransferase α, farnesyltransferase β, geranylgeranyltransferase type I (GGTase-I), geranylgeranyl pyrophosphate synthase (GGPS), 3-hydroxy-3-methylglutaryl-coenzyme A reductase, isopentenyl diphosphate isomerase and SQS (data not shown). These results suggested that up-regulated FPPS in this experimental model selectively promoted FPP and GGPP levels without the disturbance of other enzymes in the mevalonate pathway.

### 3.7 FPPS expression is increased in hypertrophic human hearts

To explore the potential role of FPPS in cardiac hypertrophy, we first examined FPPS expression in LV myocardium samples from individuals who had mitral stenosis (MS) combined with aortic stenosis as well as hypertrophy and subjects with MS without hypertrophy. FPPS was significantly up-regulated in patients with LV hypertrophy (see Supplementary material online, Figure S4A and B). RT–PCR analyses showed an increase in the mRNA levels of foetal genes in LV hypertrophic hearts (see Supplementary material online, Figure S4C). These results strengthened the notion of the involvement of FPPS in cardiac hypertrophy.
4. Discussion

To investigate the role of FPPS as a potential regulator of cardiac hypertrophy in vivo, we generated Tg mice with tissue-specific expression of FPPS in the heart. This was characterized as overexpression of FPPS and significantly increased synthesis of FPP and GGPP in the myocardium. We showed here, for the first time, that moderate up-regulation of FPPS could result in cardiac hypertrophy, fibrosis, and LV dysfunction.

Our previous studies have shown that FPPS expression is significantly up-regulated in the hypertrophic hearts of SHRs as well as in Ang II-treated cardiomyocytes.12–14 and that chronic inhibition of FPPS attenuates cardiac hypertrophy and fibrosis in SHRs.15 Chen et al.16 reported that chronic inhibition of FPPS improves endothelial function in SHRs. In addition, knockdown of FPPS by RNA interference or inhibition of FPPS prevents Ang-II-mediated cardiac hypertrophy.12,25 These studies suggest that FPPS plays an important part in cardiac and vascular remodelling in SHRs. Therefore, it is reasonable to speculate that increased FPPS expression might cause cardiac hypertrophy. In the tissue-specific Tg mice used in the present study, a phenotype of cardiac hypertrophy, fibrosis, and LV dysfunction was detected, as evidenced by reduced EF and FS, increased interstitial fibrosis, and increased ANP, BNP, β-MHC mRNA expression. Consistent with in vivo findings, FPPS adenovirus infection also induced increases in BNP mRNA expression, cell size, and the extent of sarcomeric organization in cultured cardiomyocytes.

The Rho family of small GTP-binding proteins, including the Rho, Rac, and Cdc42 subfamilies, regulates cytoskeletal function in many aspects.26 In addition, Ras small G-protein isoforms activate a kinase cascade involving Raf, mitogen-activated protein kinase (MAPK) and ERK phosphorylates, which in turn induce cell proliferation and growth regulation that can lead to cardiovascular remodelling. Transfection or microinjection of constitutively activated mutants of Rho and Ras proteins leads to changes in hypertrophic gene expression in cardiac and vascular remodelling.
cultered myocytes and, in some cases, induce myofibrillogenesis. These in vitro data suggest that RhoA and Ras display similar functional effects in primary neonatal rat cardiomyocytes, and are important signalling proteins for the study of cardiac hypertrophy. Moreover, Tg mice expressing a constitutively activated form of these small proteins in the myocardium have extended in vitro characterization. Protein geranylgeranylation with GGPP is necessary for the activation of RhoA, whereas protein farnesylation with FPP is necessary for Ras activation. We demonstrated in the present study that FPPS induced RhoA activation in vitro and in vivo. This may be explained that FPPS overexpression increased the synthesis GGPP, affected the geranylgeranylation of RhoA, and subsequently activated RhoA. However, despite a significant increase in the FPP level, Ras activation was not observed in the present study. This result is consistent with that of a previous study. This finding might be explained by the fact that endogenous FPP is already saturated for FTase function, or that FTase activity at the site of Ras farnesylation is limited because FPP is compartmentalized within the cell, which ultimately limits Ras activation. In addition, Rac1 and Cdc42 activation were also not identified in the present study. The reason is unknown. However, it showed that reciprocal effects both in vitro and in vivo on the activation of Rac1/Cdc42 and RhoA in response to cardiac hypertrophy, which indicated that Rac1/Cdc42 and RhoA may have different roles when modulating cardiac hypertrophy.

It has been reported that small GTPases (including RhoA) participate in the activation of the ERK pathway and control p38-MAPK signaling. MAPK is a widely distributed group of enzymes ending in three terminal MAPK branches: p38-MAPK, ERK1/2, and c-Jun NH2-terminal kinases (JNK). There is considerable evidence that MAPK cascades are involved in cardiac hypertrophy. For example, ERK1/2 Tg mice display concentric and compensated cardiac hypertrophy. Tg mice overexpressing the MAPKKK signalling factor TAK1 show significant p38 activation and diluted hypertrophic cardiomyopathy. As reported in our previous study, inhibition of FPPS reduces RhoA activation as well as ERK1/2 and p38 phosphorylation. The present study showed that FPPS overexpression increased phosphorylated ERK1/2 and p38 levels in vitro and in vivo. These results suggest that FPPS overexpression increases RhoA activation, and subsequently increases levels of ERK1/2 and p38 phosphorylation, which might be responsible for cardiac hypertrophy. The lack of FPPS-induced Akt and GSK3β phosphorylation in FPPS Tg mice may be explained a lack of Ras activation because phosphoinositide-3-kinase and Akt are downstream of the Ras pathway.

It is well known that small GTPases also activate the TGF-β cascade. Previous studies demonstrated increased myocardial TGF-β expression during cardiac hypertrophy and fibrosis. Tg mice overexpressing TGF-β result in cardiac hypertrophy and interstitial fibrosis. Conversely, TGF-β suppression protects against maladaptive remodelling in cardiomyocytes, and reduces the fibrosis induced by pressure overload in the heart. CTGF is responsible for some of the effects of TGF-β, and plays an important part in fibrogenic processes in the heart. The present study showed that overexpression of FPPS induced RhoA activation, and the increase of cardiac fibrosis in Tg mice was accompanied by marked increases in TGF-β and CTGF expression in the LV. Thus, we assumed that RhoA signalling participated in cardiac hypertrophy and fibrosis through its influence on TGF-β expression in FPPS Tg mice.

The mevalonate pathway is an important metabolic pathway that plays a key part in multiple cellular processes. Previous studies have shown changes in the expression of different enzymes in the mevalonate pathway during cardiovascular remodelling in SHRs. Therefore, we assessed the expression of other enzymes in Tg mice. The changes in the expression of other enzymes were not found in Tg mice. This finding suggested that up-regulated FPPS independently increased FPP and GGPP levels in this animal model.

Results from animal and in vitro studies suggest that FPPS has an important role in cardiovascular remodelling. However, it is not clear if FPPS participates in hypertrophic heart disease in humans. Hence, we examined FPPS expression in heart tissue samples from patients with clinically evident hypertrophy. The results showed that the level of FPPS protein was increased significantly in the hypertrophic myocardium, which were consistent with SHRs data. The present study is the first to demonstrate an increase in FPPS expression in human cardiac hypertrophy. This suggests that FPPS may participate in hypertrophic heart disease in human.

In conclusion, we provide here important experimental evidence that elevated FPPS expression can lead to cardiac hypertrophy, fibrosis, and LV dysfunction. These are accompanied by increased FPP and GGPP levels, increased RhoA activation, up-regulated ERK1/2 and p38 phosphorylation, and increased TGF-β expression.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

Conflict of interest: none declared.

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References


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