

# Peptidyl-prolyl isomerases: a full cast of critical actors in cardiovascular diseases

Gianluca Lorenzo Perrucci<sup>1</sup>, Aoife Gowran<sup>2</sup>, Marco Zanobini<sup>1,3</sup>,  
Maurizio Colognesi Capogrossi<sup>4</sup>, Giulio Pompilio<sup>1,2,3</sup>, and Patrizia Nigro<sup>2\*</sup>

<sup>1</sup>Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy; <sup>2</sup>Laboratory of Vascular Biology and Regenerative Medicine, Centro Cardiologico Monzino-IRCCS, Via Parea 4, Milan 20138, Italy; <sup>3</sup>Department of Cardiovascular Surgery, Centro Cardiologico Monzino-IRCCS, Milan 20138, Italy; and <sup>4</sup>Laboratory of Vascular Pathology, Istituto Dermopatico dell'Immacolata-IRCCS, Rome 00167, Italy

Received 30 October 2014; revised 13 January 2015; accepted 30 January 2015; online publish-ahead-of-print 6 March 2015

Time for primary review: 35 days

## Abstract

Peptidyl-prolyl *cis-trans*-isomerases are a highly conserved family of immunophilins. The three peptidyl-prolyl *cis-trans*-isomerase subfamilies are cyclophilins, FK-506-binding proteins, and parvulins. Peptidyl-prolyl *cis-trans*-isomerases are expressed in multiple human tissues and regulate different cellular functions, e.g. calcium handling, protein folding, and gene expression. Moreover, these subfamilies have been shown to be consistently involved in several cardiac and vascular diseases including heart failure, arrhythmias, vascular stenosis, endothelial dysfunction, atherosclerosis, and hypertension. This review provides a concise description of the peptidyl-prolyl *cis-trans*-isomerases and presents an incisive selection of studies focused on their relationship with cardiovascular diseases.

## Keywords

Cyclophilins • FKBP • Parvulins • Cardiovascular disease

## 1. Introduction

Cardiovascular diseases (CVDs) are a broad spectrum of pathologies. Presently, CVDs cause over 4 million deaths per year and remain the leading cause of approximately half of all deaths in Europe.<sup>1</sup> In the last 10 years, scientific research has highlighted the relevance of a specific protein family, the peptidyl-prolyl *cis-trans*-isomerases (PPlases), in a variety of CVDs.

PPlases are immunophilins, which catalyze the isomerization of peptide bonds from *trans* to *cis* conformation to accelerate protein folding.<sup>2,3</sup> They have specific catalytic isomerase activity at the level of X-Pro peptide bond (X represents any amino acid, a.a.). PPlases comprise several protein subfamilies, which are well conserved in all organisms.<sup>4</sup> Several of these were discovered because of their high specific affinity for immunosuppressant drugs, such as cyclosporin A (CSA), tacrolimus (FK-506), and sirolimus (rapamycin), which are inhibitors of PPlase enzymatic function.<sup>5–7</sup> Depending on drug-binding abilities, PPlases showing affinity with CSA have been classified as cyclophilins (Cyps),<sup>2,8</sup> while molecules sensitive to tacrolimus and sirolimus have been named FK-506-binding proteins (FKBPs).<sup>9–11</sup> Ultimately, the number of proteins encompassed by the PPlase family is growing and many have no affinity to immunosuppressive drugs, such as the peptidyl-prolyl *cis-trans*-isomerase NIMA-interacting 1 (Pin1), a member of the parvulin (Pars) family of PPlases. Besides not having affinity with immunosuppressive drugs, Pars do not show a high

degree of sequence homology with other subfamilies of PPlases, apart from their catalytic domain.<sup>12</sup> All three PPlase subfamilies play a central role in the regulation of several physiological functions and a wide spectrum of diseases with different pathological mechanisms. This review highlights the main features of PPlases and clarifies their role in CVDs.

## 2. Cyclophilins

Cyps are a highly conserved protein subfamily that includes 18 isoenzymes encoded by 17 genes. Although the function of most cyclophilin isoforms is unknown, six are implicated in CVDs (summarized in Table 1).

### 2.1 Cyclophilin A

CypA was the first PPlase to be discovered.<sup>13</sup> It is the most abundant Cyp expressed in all tissues and localizes to the cytosol, nucleus, and extracellular space.<sup>14–16</sup> Many biological activities have been reported for CypA which converge on CypA acting as a key protein involved in protein folding, trafficking, and assembly, immune modulation, and cell signalling.<sup>17</sup> CypA was identified as the primary cytosolic-binding protein of the immunosuppressive drug CSA.<sup>18,19</sup> In mammals, the CSA-CypA complex binds to and inhibits calcineurin, a calcium-calmodulin-activated serine/threonine-specific phosphatase.<sup>20</sup> Calcineurin inhibition blocks nuclear

\* Corresponding author. Tel: +39 02 58002028; fax: +39 02 58002342, Email: patrizia.nigro@ccfm.it

**Table 1** PPIases involved in CVDs

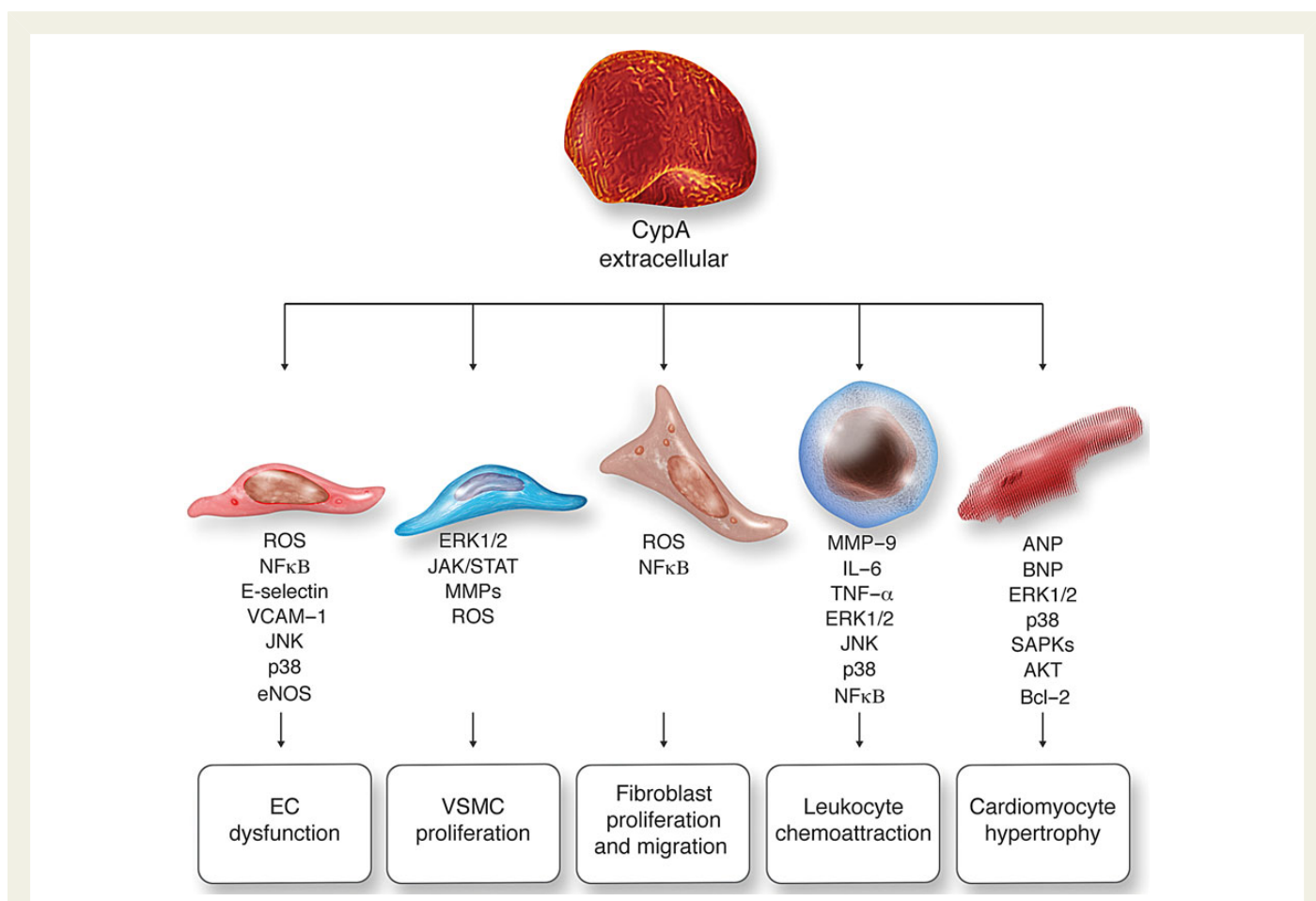
PPIase subfamilies	Protein/alternative name	Gene	MW (kDa)	Cell localization	Tissue distribution	CVD involvement	References
Cyps	CypA/Cyp18	PPIA	18.012	Cytoplasm, nucleus, extracellular space	Ubiquitous	Vascular remodelling, AAA, cardiac hypertrophy, atherosclerosis, I/R injury, hypertension, arterial thrombosis, CAD, essential hypertension, diabetes	16,26,27,37–39,40–45
	CypB/Cyp23/SCYLP	PPIB	23.742	ER, nucleus, extracellular space	Ubiquitous	Hypertension, HF	66,67
	CypC/Cyp22a	PPIC	22.763	ER, Golgi apparatus, extracellular space	Kidney	Middle cerebral artery ischaemia	77
	CypD/Cyp22/CypF/Cyp3	PPIF	22.040	Mitochondria	Ubiquitous	I/R injury, HF, arterial thrombosis, cardiac hypertrophy, atherosclerosis, diabetes	80,82,84,96,97,102,104,105,107,108
FKBPs	CypJ/Cyp18.1	PPIL3	18.155	Nucleus, cytoplasm	Ubiquitous	Congenital heart defects	123
	Cyp40	PPID	40.764	Nucleus, cytoplasm	Ubiquitous		
	FKBP12/FKBP1A	FKBP1A	11.951	Cytoplasm, SR	Skeletal muscle, heart, brain	Cardiac development, hypertension, arrhythmia	133–136
	FKBP12.6/FKBP1B	FKBP1B	11.782	Cytoplasm, SR	Skeletal muscle, heart, brain	HF, AF, CPVT, ARVC, arrhythmias in DMD, hypertrophy, diabetic cardiomyopathy	142,144,147,149–152,154,157,159
Pars	FKBP6/FKBP36	FKBP6	37.214	Nucleus	Skeletal muscle, heart, brain	SVAS in William's syndrome	164
	Pin1	PIN1	18.200	Nucleus, cytoplasm	Ubiquitous	Cardiac hypertrophy, restenosis, hypertension, diabetes	173–176

factor of activated T cells (NFAT) translocation from the cytosol to the nucleus, thus preventing the transcription of genes encoding cytokines, e.g. IL-2. We and others have provided evidence that CypA is secreted in response to inflammatory stimuli such as reactive oxygen species (ROS), hypoxia, and infection.<sup>21–24</sup> Secreted CypA, partially through CD147, acts as a paracrine and autocrine factor that mediates cell-to-cell communication.<sup>25</sup> In fact, extracellular CypA induces endothelial cell (EC) dysfunction,<sup>21,26</sup> vascular smooth muscle cell (VSMC), and fibroblast proliferation, and promotes cardiomyocyte hypertrophy<sup>16,27</sup> (Figure 1). Furthermore, extracellular CypA is a potent chemoattractant for inflammatory cells.<sup>23,28</sup> CypA has been implicated in the following pathologies: viral infections,<sup>29</sup> neurodegeneration,<sup>30</sup> cancer,<sup>31</sup> rheumatoid arthritis,<sup>32</sup> sepsis,<sup>33</sup> asthma,<sup>34</sup> periodontitis,<sup>35</sup> and ageing.<sup>36</sup>

Recently, we have demonstrated the involvement of both intracellular and extracellular CypA in several CVDs. Using a complete carotid ligation model in wild-type (WT), CypA knockout (CypA<sup>-/-</sup>), and CypA overexpressing mice (specifically in VSMCs), we understood that CypA is critically involved in 'vascular remodelling' (neointima formation as well as medial and adventitial thickening).<sup>16</sup> Additionally, we demonstrated that deletion of CypA in ApoE<sup>-/-</sup> mice prevents the formation of abdominal aortic aneurysm (AAA)<sup>37</sup> and cardiac hypertrophy<sup>27</sup> in response to angiotensin II infusion and the development of atherosclerosis in mice fed a high-fat diet.<sup>26</sup> Mechanistic studies revealed that deletion of CypA in all models reduced inflammation, oxidative stress, and extracellular matrix degradation.<sup>38</sup> Seizer et al.<sup>39</sup> reported that CypA is involved in myocardial 'ischaemia and reperfusion (I/R) injury' by the regulation of macrophage and neutrophil recruitment into the damaged tissue. CypA was also implicated in 'arterial thrombosis' by a mechanism involving the regulation of Ca<sup>2+</sup> in platelets.<sup>40</sup> The involvement of CypA in the pathogenesis of hypertension has been suggested by the finding that CypA regulates the activity of the atrial natriuretic factor and its receptor, the membrane-bound guanylate cyclase-A, which regulates blood pressure.<sup>41</sup> Consistently, our studies demonstrated that CypA modulates endothelial nitric oxide synthase (eNOS) expression, a critical protein for nitric oxide (NO) generation and blood pressure regulation.<sup>26</sup> A clear involvement of CypA in pulmonary hypertension (PH) was found by a mechanism of ERK1/2 activation and secretion of cytokines/chemokines and growth factors, e.g. PDGF-BB.<sup>42</sup> Interestingly, high plasma levels of CypA, which predicted poor prognosis, were found in PH patients. Additionally, CypA has been proposed as a valuable biomarker for coronary artery disease (CAD),<sup>43,44</sup> essential hypertension,<sup>45</sup> and type-2 diabetes.<sup>46</sup> Hence, the development of drugs blocking its deleterious effects may offer a successful novel approach for the treatment of cardiovascular pathologies.

## 2.2 Cyclophilin B

CypB is an abundant protein expressed in all tissues, at levels lower than CypA,<sup>47</sup> and shares 65% sequence homology with CypA. It localizes within the endoplasmic reticulum (ER), nucleus, and extracellular space. The major functions of CypB were found to be related to the control of ER redox homeostasis,<sup>48</sup> collagen folding,<sup>49</sup> ribosome biogenesis,<sup>50</sup> Ca<sup>2+</sup> homeostasis,<sup>51</sup> and prolactin signalling.<sup>52</sup> Both anti- and pro-inflammatory effects were reported for CypB. For instance, CypB was demonstrated as an essential protectant against ROS<sup>53</sup> and pro-inflammatory stimuli.<sup>54,55</sup> Conversely, extracellular CypB, like CypA, was found to induce the chemotaxis of inflammatory cells into damaged tissues.<sup>23,28,56,57</sup> In particular, CypB induced integrin-mediated cell adhesion by its interaction with CD147, CD98, and beta-1



**Figure 1** Cellular effects of CypA. CypA modulates different cardiovascular cell functions. Particularly, CypA, by activating the signalling proteins depicted in the figure, provokes endothelial dysfunction, increases proliferation of vascular smooth muscle cells and fibroblasts, acts as chemoattractant mediator for monocytes and other inflammatory cells, and stimulates cardiomyocyte hypertrophy.

integrins,<sup>58</sup> which was dependent on protein kinase (PK) C-delta activation and was critical for ERK1/2-mediated signalling. CypB has been associated with osteogenesis imperfecta,<sup>49,59</sup> cancer,<sup>55,60,61</sup> CMV infection,<sup>62,63</sup> HIV,<sup>64</sup> neurodegeneration,<sup>65</sup> asthma,<sup>34</sup> and ageing.<sup>66</sup>

A study by Kainer and Doris<sup>67</sup> suggested the importance of CypB in hypertension as they showed increased CypB levels in the renal proximal tubules of spontaneous hypertensive rats (SHR). These data indicate that CypB may participate in the abnormal functioning of renal transport-epithelium in SHR. Moreover, the beneficial effect of shock wave therapy (SWT) in 'ischaemic heart failure' (HF) was proposed to be mediated by CypB in parallel with Toll-like receptor 3 activation in ECs.<sup>68</sup> These molecular events are the basis for the pro-inflammatory response characteristic of the early response to SWT. The involvement of CypB in cardiovascular pathologies was also suggested by Berk and collaborators whom identified CypB in conditioned medium from VSMCs treated with a ROS generator (LY83583).<sup>56</sup> This secreted CypB mediated ROS-induced activation of ERK1/2 and regulated the effects of ROS on vascular function.

### 2.3 Cyclophilin C

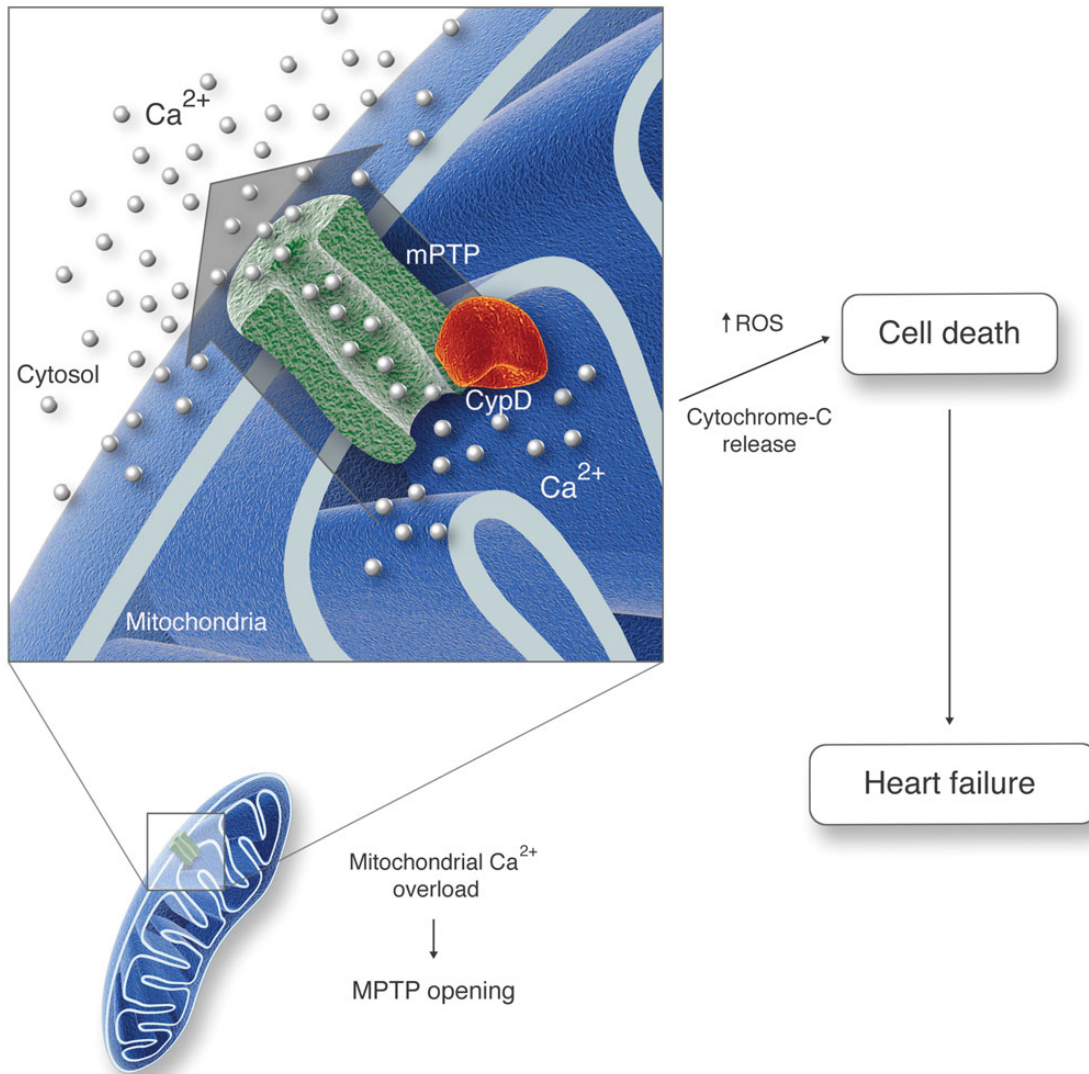
CypC differs from CypA and CypB as it displays a restricted tissue distribution, with the most abundant expression observed in the kidney.<sup>69</sup> CypC localizes in the ER, Golgi, and extracellular space,<sup>70,71</sup> is inhibited

by CSA,<sup>72</sup> regulates ER redox homeostasis,<sup>48</sup> and degrades DNA *in vitro*.<sup>73</sup> Interestingly, CypC associates with a secreted glycoprotein, CypC-associated protein (CypCAP)<sup>74</sup> which modulates macrophage activation (via NFAT),<sup>75</sup> endotoxin signalling,<sup>76</sup> and metalloproteinase-13 expression.<sup>77</sup>

Interestingly, Shimizu *et al.*,<sup>78</sup> by modelling middle cerebral artery occlusion (MCAO) ischaemia in rat, reported increased expression of CypC and CyCAP predominantly in microglia of the ischaemic core 7 days after MCAO. Although the cellular role of the proteins remains somewhat unclear, the authors suggested that CypC and CyCAP might participate in neuroprotection by modulating neuroinflammation.

### 2.4 Cyclophilin D

CypD is expressed in all human cell types, however at lower levels compared with CypA.<sup>79</sup> In light of its mitochondrial targeting,<sup>80</sup> CypD is known to play a pivotal role in regulating mitochondrial permeability transition pore (mPTP) opening and mitochondrial Ca<sup>2+</sup> homeostasis control, ensuring optimal metabolic function<sup>81,82</sup> and appropriate cell death activation<sup>83–86</sup> (Figure 2). The hypothesis that CypD contributes to mPTP opening has been corroborated by genetic studies demonstrating that CypD deficiency reduces the propensity of the mPTP to open<sup>83–85,87</sup> while overexpression increases opening.<sup>88,89</sup> Recent investigations revealed that CypD is involved in muscular dystrophy,<sup>90,91</sup>



**Figure 2** Intracellular role of CypD in heart failure. Mitochondrial calcium ( $\text{Ca}^{2+}$ , blue dots) concentration overload is the stimulus required for CypD-mPTP coupling. This provokes mPTP opening,  $\text{Ca}^{2+}$  release into the cytoplasm, and subsequent mitochondrial-mediated process leading to cardiac cell death.

Alzheimer's disease,<sup>92,93</sup> Parkinson's disease,<sup>94</sup> multiple sclerosis,<sup>95</sup> and ageing.<sup>96</sup>

Numerous studies utilizing CypD<sup>-/-</sup> mice determined that CypD knockout provides protection in several I/R models, including injury to the heart,<sup>83,85,97,98</sup> brain,<sup>84</sup> and kidney.<sup>99,100</sup> However, some factors, such as duration of ischaemia<sup>101</sup> or age,<sup>102</sup> were shown to shift CypD from a pro-survival protein to a cell death mediator. A cardioprotective effect of CypD knockdown was also shown using a CypD-siRNA-based approach followed by two-photon imaging in perfused rat hearts subjected to I/R injury.<sup>103</sup> Furthermore, mitochondrial-targeted CSA consistently improved cytoprotection in isolated rat cardiomyocytes subjected to transient glucose and oxygen deprivation, a pseudo-I/R model.<sup>104</sup> Interesting results were also found in a HF model where mice lacking CypD showed decreased infarct size and adverse left ventricular (LV) remodelling in addition to improved heart function after myocardial infarction (MI).<sup>105</sup> Moreover, loss of CypD blocked Ca<sup>2+</sup>-influx-induced necrosis of cardiomyocytes, isoproterenol-induced

premature cell death, and HF.<sup>106</sup> Surprisingly, decreased cytoprotection was observed in CypD<sup>-/-</sup> mice subjected to ischaemic preconditioning.<sup>107</sup> Similar negative effects were observed in a model of pressure overload-induced HF.<sup>81</sup> In fact, these mice exhibited substantially greater cardiac hypertrophy, fibrosis and reduced myocardial function compared with WT mice. Even more remarkably, physiological exercise (swimming) in CypD<sup>-/-</sup> mice worsened cardiac hypertrophy in comparison to control mice. Mechanistically, the maladaptive cardiac phenotype of CypD<sup>-/-</sup> mice was associated with an alteration in mPTP-mediated Ca<sup>2+</sup>-efflux, resulting in elevated levels of mitochondrial matrix Ca<sup>2+</sup> and enhanced activation of Ca<sup>2+</sup>-dependent dehydrogenases. This alteration, in turn, led to increased glucose oxidation relative to fatty acid, thereby limiting the metabolic flexibility of the heart that is critically involved in compensation during stress. The involvement of CypD in metabolic pathways was confirmed recently by Menazza et al.<sup>82</sup> who used proteomic and metabolomic analysis to show that CypD<sup>-/-</sup> hearts have altered levels of proteins involved in

the Krebs cycle, branch chain a.a. degradation, and pyruvate metabolism. CypD was also implicated in platelet activation and arterial thrombosis.<sup>108</sup> In fact, in an embolic-stroke model, thrombosis was found to be markedly accelerated in CypD-deficient mice. Other studies associated CypD with atherosclerosis and diabetes. Genetic ablation of CypD in adult mice maintained on a high-fat diet, normalized glucose and insulin responses to acute glucose challenge, and prevented diabetes in Pdx1-deficient mice.<sup>109</sup> Thus, CypD is engaged in many cardiovascular pathologies, likely due to its critical role in the regulation of the mPTP which underpins metabolism and cell death.

## 2.5 Cyclophilin J and cyclophilin 40

CypJ is a novel member of the Cyp family which shares 50% of its sequence identity with CypA. The biological functions of CypJ are still unclear. Studies demonstrated that CypJ is involved in cancer biology, e.g. CypJ overexpression up-regulates drug resistance-related genes and may play a role in the clinical resistance to chemotherapy.<sup>110</sup> It was reported that CypJ gene expression may be correlated with the development of human glioma and might control the conformation of apoptin, a pro-apoptotic protein in tumour cells.<sup>111</sup>

Cyp40 is a large ubiquitously expressed protein with an immunophilin-like domain together with a conserved tetratricopeptide repeat (TPR) domain which is involved in protein interaction.<sup>112</sup> Cyp40 localizes predominantly to the nuclei. However, evidence of diffuse staining within the cytoplasm has been reported.<sup>113</sup> Cyp40 contributes to protein folding, ligand binding, and glucocorticoid-, estrogen-, progesterone-, and aryl-receptor signalling.<sup>114–117</sup> Interestingly, Cyp40 regulates the ATPase activity of heat shock protein 90 (Hsp90) favouring assembly into chaperone protein-folding machinery.<sup>118</sup> Additionally, Cyp40 is required for the activity of microRNAs in *Arabidopsis thaliana* and may chaperone Argonaute1 (AGO1) or a protein that is critical for AGO1 function (R.S. Poethig, personal communication).<sup>119</sup> Intriguingly, either the up-regulation of Cyp40 gene expression or loss of function might have pro-tumorigenic effects.<sup>120–122</sup> Moreover, Cyp40 was altered in prenatal alcohol-exposed mice suggesting its involvement in learning deficits.<sup>123</sup>

CypJ and Cyp40 were found to be implicated in the congenital heart defects observed in helicase-like transcription factor (Hltf) null mice<sup>124</sup> which die a few hours after birth because of reduced cardiac output. A genome-wide transcriptome profiling of Hltf null post-partum hearts revealed that CypJ and Cyp40 were down-regulated 2.57- and 2.71-fold, respectively. Although more studies are necessary, these results link CypJ and Cyp40 activity to heart development and cardiac functions.

## 3. FK-506-binding proteins

The FKBP subfamily includes >20 members which are named on the basis of their molecular weight. A number of FKBP genes have been cloned, but few cases suggest a specific cellular function.<sup>6</sup> FKBP associated with CVDs are summarized in Table 1.

### 3.1 FKBP12

FKBP12 is one of the smallest and most extensively studied FKBP identified to date.<sup>125</sup> FKBP12 displays an overall cytoplasmic and sarcoplasmic reticulum (SR) expression profile and is strongly involved in protein–protein interactions.<sup>126</sup> FKBP12 binds both isoforms of ryanodine receptors (RyR1 and RyR2), with higher selectivity for RyR1 which is mainly expressed in skeletal muscle. FKBP12-RyR1 binding induces

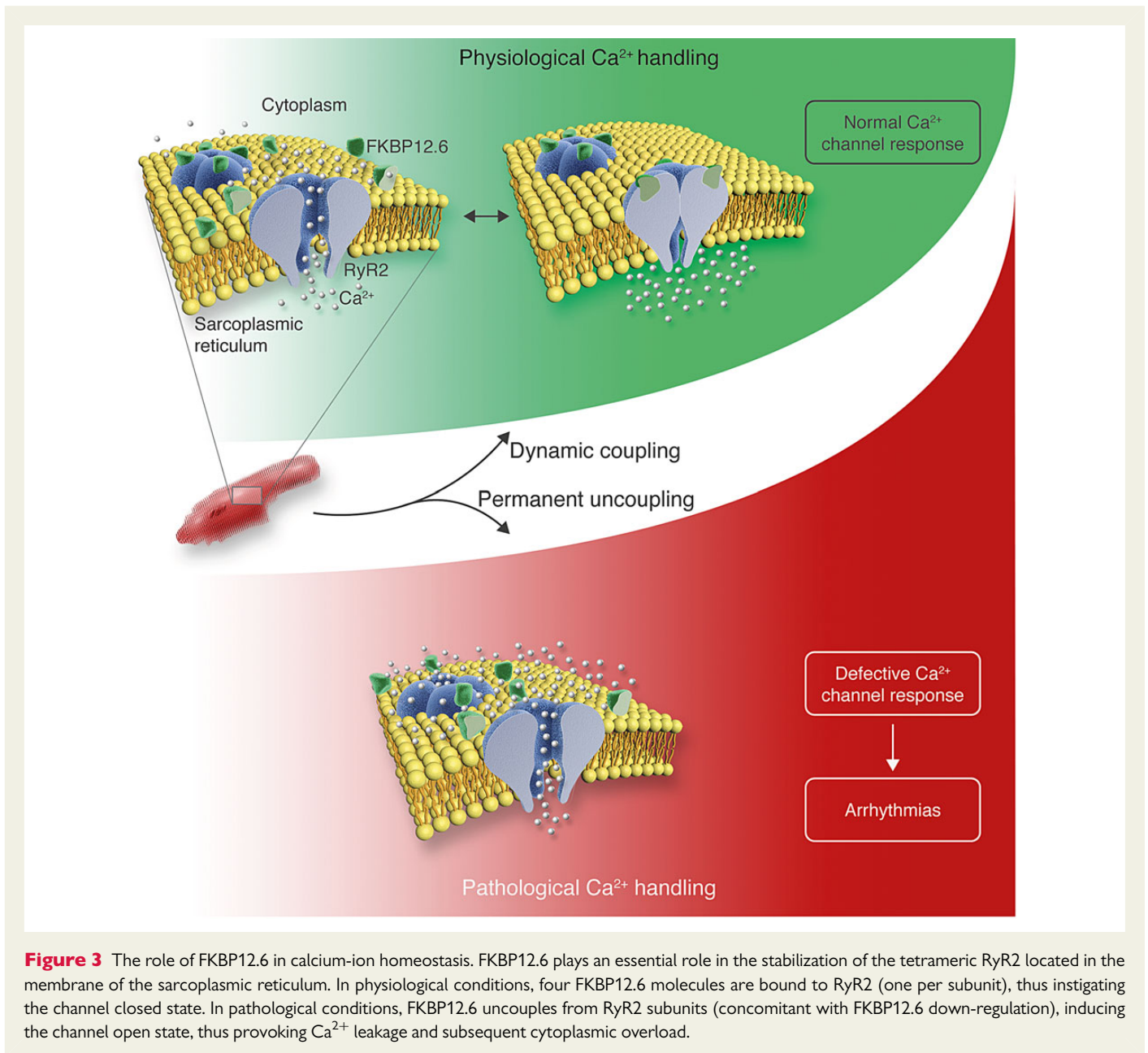
allosteric mechanisms to stabilize the closed-channel state.<sup>127–129</sup> Rapamycin and FK-506 inhibit FKBP12-RyRs binding. Further, FKBP12 also interacts with inositol trisphosphate receptors via PKA phosphorylation. Furthermore, FKBP12 interacts and inhibits calcineurin and mTOR<sup>126,130–133</sup> which limit T-cell translocation toward inflammatory loci by inhibiting cytokine production, e.g. IL-2.<sup>130</sup>

FKBP12<sup>-/-</sup> mice die *in utero*, due to cardiac abnormalities including: severe dilated cardiomyopathy, hypertrabeculation, ventricular non-compaction, and ventricular septal defects, suggesting an essential physiological function of FKBP12 in cardiac development.<sup>134,135</sup> However, further experiments highlighted the important role for FKBP12 in the regulation of ionic currents such as Na<sup>+</sup>, voltage-dependent K<sup>+</sup>, transient outward K<sup>+</sup>, sustained K<sup>+</sup>, L-type and transient Ca<sup>2+</sup> currents.<sup>136</sup> In light of this regulation, FKBP12 was found to be a critical regulator of heart rhythm. In fact, Maruyama *et al.* has illustrated the role of FKBP12 in cardiac arrhythmia using two different approaches: mice overexpressing FKBP12 and a conditional FKBP12 knockout model (cardiomyocyte restriction under the control of alpha-myosin heavy chain). In both models a significantly enlarged heart, related to the dysregulation of the voltage-gated sodium current I(Na), was observed.<sup>136</sup> In 2011, Chiasson *et al.* showed that FKBP12 deficiency leads to the development of hypertension.<sup>137</sup> Consistently, immunosuppressive drugs inhibiting FKBP12 (and also FKBP12.6) were able to cause arterial hypertension, reducing vasodilation and also acting on vasoconstriction.<sup>138</sup>

### 3.2 FKBP12.6

FKBP12.6 shares 85% sequence homology with FKBP12<sup>126,139</sup> and also contains a single FK-506-binding domain. FKBP12.6 plays an important role in RyR2 stabilization<sup>140,141</sup> and colocalizes with RyR2 in the heart<sup>142</sup> and vascular tissues, where it is the predominant isoform.<sup>143</sup> FKBP12.6-immunosuppressant drug complexes inhibit calcineurin<sup>130,131</sup> and bind mTOR,<sup>132,133</sup> inducing the previously mentioned inhibitory effects on cytokine production and cytotoxic T-cell proliferation.<sup>130,132,133</sup>

The main mechanism explaining the involvement of FKBP12.6 in several CVDs is its role in the regulation of intracellular Ca<sup>2+</sup> handling. During HF aetiology, PKA hyperphosphorylates RyR2, this in turn leads to detachment of FKBP12.6 from RyR2, negative feedback for FKBP12.6 expression, and defective Ca<sup>2+</sup> channel function (Figure 3).<sup>141,144–148</sup> Indeed, Hu *et al.* demonstrated reduced expression of FKBP12.6, RyR2, and SERCA2a in a rat model of HF, showing the contribution of Ca<sup>2+</sup> leakage and reduced Ca<sup>2+</sup> uptake to the development of HF.<sup>144</sup> Furthermore, FKBP12.6 plays an important role in several arrhythmogenic diseases, such as atrial fibrillation (AF),<sup>149–151</sup> catecholaminergic polymorphic ventricular tachycardia (CPVT),<sup>152</sup> and arrhythmogenic right ventricular cardiomyopathy (ARVC).<sup>153,154</sup> Hyperphosphorylated RyR2 were isolated from the atria of canines affected by AF.<sup>149,150</sup> Atrial cardiomyocytes isolated from FKBP12.6-deficient mice showed enhanced SR Ca<sup>2+</sup> leakage, in addition to an increased propensity for developing AF.<sup>151</sup> PKA-induced RyR2 hyperphosphorylation was also highlighted in cardiac RyR2 of diabetic rats where FKBP12.6 levels were depleted.<sup>155</sup> Moreover, Ca<sup>2+</sup> sparks showed a time-dependent decay together with progression of diabetic cardiomyopathy potentially due to the alteration of FKBP12.6 levels.<sup>156</sup> Lehnart *et al.*<sup>157</sup> have shown SR Ca<sup>2+</sup> leakage during diastole in FKBP12.6<sup>-/-</sup> mice, implicating FKBP12.6 deficiency in triggering cardiac arrhythmias. In 2008, a conditional cardiac-specific overexpression of FKBP12.6 demonstrated that increased FKBP12.6-RyR2 binding prevents stress-evoked ventricular tachycardia in normal hearts



**Figure 3** The role of FKBP12.6 in calcium-ion homeostasis. FKBP12.6 plays an essential role in the stabilization of the tetrameric RyR2 located in the membrane of the sarcoplasmic reticulum. In physiological conditions, four FKBP12.6 molecules are bound to RyR2 (one per subunit), thus instigating the channel closed state. In pathological conditions, FKBP12.6 uncouples from RyR2 subunits (concomitant with FKBP12.6 down-regulation), inducing the channel open state, thus provoking  $\text{Ca}^{2+}$  leakage and subsequent cytoplasmic overload.

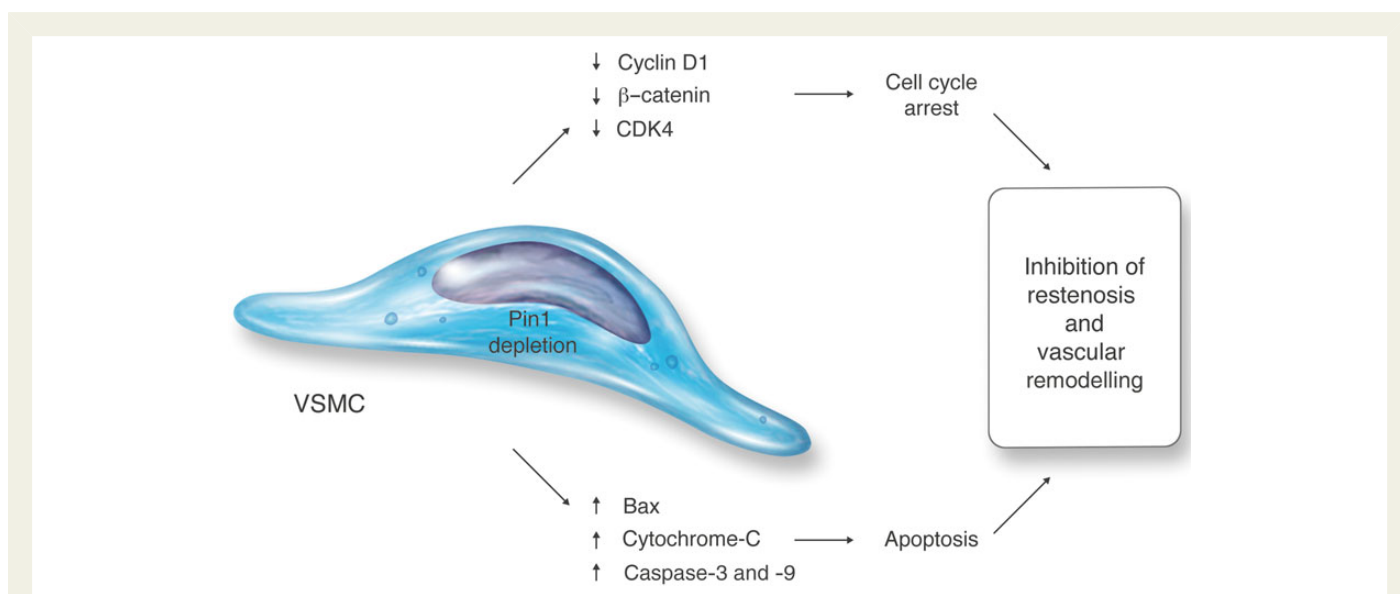
potentially by reducing diastolic SR  $\text{Ca}^{2+}$  leakage.<sup>146,152,158</sup> Thus, the FKBP12.6-RyR2 complex is an evident target for future pharmacological treatments of ventricular tachycardia.<sup>146</sup> Indeed, decreased FKBP12.6 expression has been linked to the increased probability of RyR2 in the open-channel state in naturally occurring canine models of ARVC. However, sequence analysis of the canine FKBP12.6 promoter regions did not identify any mutations,<sup>153,154</sup> indicating that FKBP12.6 is not an ARVC-associated gene, despite PPIase involvement in the pathological process. Fauconnier *et al.* demonstrated that FKBP12.6 expression was down-regulated, resulting in  $\text{Ca}^{2+}$  leakage in the 'mdx' mouse model. These results suggest that FKBP12.6 is implicated in the arrhythmogenic events related to muscular dystrophy.<sup>159</sup> Liu *et al.*<sup>160</sup> generated a FKBP12.6<sup>-/-</sup> mouse model with a conditional expression of FKBP12.6 in heart tissue which exhibited the rescue of the cardiac hypertrophic phenotype through reduced abnormal calcium release. More controversial results stem from the cardiac effects of FKBP12.6 overexpression. Some authors have provided results showing that

FKBP12.6 overexpression leads to hypertrophy and hyperplasticity, with increased activation of p38 MAPK and ERK1/2 and levels of apoptotic factors.<sup>161</sup> Conversely, other studies have shown a protective effect of FKBP12.6 overexpression on LV hypertrophy progression in hypertensive mice.<sup>158</sup>

### 3.3 FKBP6

FKBP6, the most recently discovered member of the FKBP subfamily of immunophilins, has a three-unit TPR motif at its C-terminal which is essential for mediating protein–protein interactions and the assembly of protein complexes.<sup>162,163</sup> FKBP6 has a nuclear intracellular localization with an expression level similar to that of other FKBP6s; higher levels in heart and skeletal muscle, and lower levels in the brain.<sup>112</sup>

In 2004, a segment of murine chromosome 12, which includes the FKBP6 gene, was shown to correspond to a region which is deleted in Williams–Beuren Syndrome (WBS), a disease characterized by congenital cardiovascular anomalies.<sup>164</sup> Hemizyosity of the deleted genes



**Figure 4** Cellular effects of Pin1 on vascular remodelling. Pin1 deficiency in vascular smooth muscle cells determines both cell cycle arrest and enhancement of apoptosis. These effects are mediated by down-regulation of cyclin D1, beta-catenin, CDK4, increased Bax, released cytochrome-C, and activation of caspase-3 and -9.

in WBS, and possibly also the FKBP6 gene, is responsible for supravalvular aortic stenosis (SVAS), which involves ascending aortic branch narrowing, as well as connective tissue manifestations.<sup>165</sup> In fact, a SVAS phenotype with concomitant deficits in the ELN, GTF2IRD1, and FKBP6 genes has been reported in three WBS patients.<sup>166</sup>

## 4. Parvulins

Pars are a PPLase subfamily, which show no significant sequence homology with other PPLases<sup>12</sup> and show no affinity for immunosuppressant drugs. There are two human Pars, Pin1 and Pin14. To date, only Pin1 is linked to CVDs (Table 1).

### 4.1 Pin1

The PPLase domain of Pin1 is a rare example of high specificity in substrate recognition as binding requires a 'phospho-X-Pro' a.a. sequence, where phospho-X may be phospho-serine (p-Ser) or phospho-threonine (p-Thr).<sup>167</sup> Pin1 is a nuclear protein, and it is involved in cell cycle progression<sup>167,168</sup> and in the control of oncogenic pathways.<sup>169</sup>

In particular, Lv *et al.*<sup>170,171</sup> provided *in vitro* evidence on the involvement of Pin1 signalling in VSMC cell cycle progression and apoptosis (Figure 4). Since post-injury VSMC apoptosis may limit neointima formation, these results underline a potentially critical role of Pin1 in restenosis after endovascular damage. Recent data have shown that reduced Pin1 expression in VSMCs treated with nectandrin B (a potent eNOS activator) blocks cell proliferation through stimulation of the adenosine monophosphate-activated protein kinase pathway.<sup>172</sup> In 2008, the interaction between Pin1 and inducible nitric oxide synthase (iNOS), an important endothelial inflammation mediator, was discovered. Given the high sequence homology between iNOS and eNOS, Ruan *et al.*<sup>173</sup> demonstrated the phosphorylation-dependent interaction of Pin1 with eNOS, resulting in Pin1-induced eNOS inactivation due to conformational changes. This inactivation was proposed to be mediated by either direct impairment of the eNOS catalytic site or indirectly

by making eNOS more or less susceptible to phosphorylation/dephosphorylation and enzyme degradation.<sup>173</sup> Thus, Pin1 activity can be easily considered as an 'on-off' switch where the activity of downstream proteins, such as phosphatases, depend on its function.<sup>174</sup> Ruan *et al.* showed impaired NO production due to increased Pin1; however, Chiasson *et al.*<sup>175</sup> uncovered a concerted down-regulation of NO and Pin1. Furthermore, treatment with juglone (a specific Pin1 inhibitor) or Pin1 gene deletion caused both hypertension and endothelium dysfunction (phosphorylated eNOS and decreased NO production) in mice.<sup>175</sup> Moreover, Paneni *et al.* have recently shown that Pin1<sup>-/-</sup> diabetic mice were protected against endothelial impairment in a hyperglycaemic setting. Pin1 expression and activity increased specifically in EC during hyperglycaemia, which plays a key role in triggering diabetic vascular disease. Indeed, Pin1 facilitates p66<sup>Shc</sup> mitochondrial translocation, inducing ROS production, while impairing NO availability.<sup>176</sup> Recently, it has been noted that Pin1<sup>-/-</sup> mice were protected from pressure overload-induced cardiac hypertrophy. However, surprisingly, cardiomyocytes overexpressing Pin1 also displayed resistance to hypertrophy.<sup>177</sup> To reconcile these paradoxical findings, the study found that Pin1 overexpression reduces MEK activation via inhibitory Raf<sup>Ser259</sup> autophosphorylation, thus leading to an overall decrease in hypertrophic signalling. Recently, statins have been shown to exert their pleiotropic antihypertrophic effect partly through Pin1 inactivation.<sup>178</sup> Given these experimental findings, it can be suggested that Pin1 may play a significant role in CVD by acting as a regulator of NOS and hypertrophic cell signals. However, more studies are necessary to elucidate a putative strategy for Pin1-targeted drug therapy.

## 5. Conclusion and clinical perspectives

PPLases are a class of proteins that play a central role in multiple biological processes, such as protein folding, trafficking, and assembly, as well as

intracellular calcium handling, chemotaxis, and cell cycle progression. In particular, there is a vast amount of information on individual members of this family of proteins in the development of several CVDs, such as vascular stenosis, hypertension, atherosclerosis, cardiac hypertrophy, arrhythmias, ischaemic and non-ischaemic cardiomyopathy, and HF. The mechanisms underlying this involvement are related to their strong association with key functional regulators of the cardiovascular system, including eNOS, scavenger receptors, calcineurin, RyR, inositol trisphosphate receptor, and mTOR. In spite of the recent advances on the role of PPlases in the cardiovascular field, several aspects related to PPlases pathophysiological function *in vitro* and *in vivo* remain poorly understood. Therefore, a detailed analysis of their interaction with critical molecular partners and/or receptors in addition to characterization of the signalling pathways may shed light on this protein family.

It is important to point out that some controversies exist between the different classes of PPlases. For instance, while Cyps and Pin1 loss prevents several CVD, FKBP12.6 deficiency leads to AF and cardiac arrhythmias. Even within the same subfamily, some contrasting results have come to light depending on cardiac disease type. An example is offered by CypD knockdown which provides cardioprotection following I/R injury while a maladaptive cardiac phenotype is evident in HF. Another point that should be considered regarding the extracellular PPlases is the dose-dependent effect. Specifically, studies in cultured ECs have shown that exogenously administered CypA at low concentrations enhances cell proliferation, capillary-like structure development, migration, invasive properties as well as MMP-2 secretion. In contrast, at high concentrations, CypA inhibits HUVEC migration and viability.<sup>179</sup>

In light of these considerations, future investigations should be focused on finding inhibitors targeting specific PPlases and in well-characterized disease models. The achievement of the drug specificity for homogenous protein families is a very difficult task and requires a concerted effort between medicinal chemistry as well as of the specific biochemical and pharmacological experts. Indeed, currently many efforts are focused on the development of novel PPlase inhibitors by intelligent structure-based drug design methodologies.

To date, various drugs targeting these proteins have been discovered comprising FK506, sirolimus/rapamycin, cyclosporine, and tacrolimus.<sup>180</sup> Several FKBP-binding macrocyclic drugs, everolimus, zotarolimus, and temsirolimus are in Phase III trials as targets for cell proliferation, immunosuppression, and anti-cancer effects.<sup>181</sup>

One of the problems associated with these inhibitors, however, is their off-target effects, particularly and not surprisingly, non-deliberate immunosuppression. Thus, concerted efforts to generate compounds lacking immunosuppressive activity have resulted in varied outcomes. In particular, Debio 025 (Alisporivir) and NIM81182–84 have shown great promise in multiple therapeutic areas.<sup>182–184</sup> Similarly, the development of cell impermeable, non-immunosuppressive CSA analogues has permitted the inhibition of extracellular CypA in mouse models of inflammation.<sup>185</sup>

Finally, because many of the PPlase family members are secreted, they might hold great promise to be a valuable biomarkers for diagnostic/prognostic tests for cardiovascular-related diseases.

It is muted that the development of agents that selectively inactivate PPlases or block the binding to their molecular targets or modulating the secretory pathways may be an appealing approach to fully elucidate the pathological mechanisms and provide treatments for CVDs, pathologies with a huge global impact in the world.

**Conflict of interest:** none declared.

## Funding

The authors are supported by funding from the FP7-PEOPLE-2011-CIG-294016 and National Institutes of Health RF-2010-2321151.

## References

- Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. *Eur Heart J* 2014;**35**:2950–2959.
- Galat A. Peptidylprolyl cis/trans isomerases (immunophilins): biological diversity-targets-functions. *Curr Top Med Chem* 2003;**3**:1315–1347.
- Suzuki R, Nagata K, Yumoto F, Kawakami M, Nemoto N, Furutani M, Adachi K, Maruyama T, Tanokura M. Three-dimensional solution structure of an archaeal FKBP with a dual function of peptidyl prolyl cis-trans isomerase and chaperone-like activities. *J Mol Biol* 2003;**328**:1149–1160.
- Galat A. Sequence diversification of the FK506-binding proteins in several different genomes. *Eur J Biochem* 2000;**267**:4945–4959.
- Harding MW, Galat A, Uehling DE, Schreiber SL. A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* 1989;**341**:758–760.
- Marks AR. Cellular functions of immunophilins. *Physiol Rev* 1996;**76**:631–649.
- Gothel SF, Marahiel MA. Peptidyl-prolyl cis-trans isomerases, a superfamily of ubiquitous folding catalysts. *Cell Mol Life Sci* 1999;**55**:423–436.
- Adams B, Musiyenko A, Kumar R, Barik S. A novel class of dual-family immunophilins. *J Biol Chem* 2005;**280**:24308–24314.
- Chang JY, Sehgal SN, Bansbach CC. FK506 and rapamycin: novel pharmacological probes of the immune response. *Trends Pharmacol Sci* 1991;**12**:218–223.
- Morris RE. Rapamycin: FK506's fraternal twin or distant cousin? *Immunol Today* 1991;**12**:137–140.
- Kunz J, Hall MN. Cyclosporin A, FK506 and rapamycin: more than just immunosuppression. *Trends Biochem Sci* 1993;**18**:334–338.
- Rahfeld JU, Rucknagel KP, Schelbert B, Ludwig B, Hacker J, Mann K, Fischer G. Confirmation of the existence of a third family among peptidyl-prolyl cis/trans isomerases. Amino acid sequence and recombinant production of parvulin. *FEBS Lett* 1994;**352**:180–184.
- Fischer G, Bang H, Mech C. Determination of enzymatic catalysis for the cis-trans-isomerization of peptide binding in proline-containing peptides. *Biomed Biochim Acta* 1984;**43**:1101–1111.
- Zhu C, Wang X, Deinum J, Huang Z, Gao J, Modjtahedi N, Neagu MR, Nilsson M, Eriksson PS, Hagberg H, Luban J, Kroemer G, Blomgren K. Cyclophilin A participates in the nuclear translocation of apoptosis-inducing factor in neurons after cerebral hypoxia-ischemia. *J Exp Med* 2007;**204**:1741–1748.
- Bannon JH, O'Donovan DS, Kennelly SM, Mc Gee MM. The peptidyl prolyl isomerase cyclophilin A localizes at the centrosome and the midbody and is required for cytokinesis. *Cell Cycle* 2012;**11**:1340–1353.
- Satoh K, Matoba T, Suzuki J, O'Dell MR, Nigro P, Cui Z, Mohan A, Pan S, Li L, Jin ZG, Yan C, Abe J, Berk BC. Cyclophilin A mediates vascular remodeling by promoting inflammation and vascular smooth muscle cell proliferation. *Circulation* 2008;**117**:3088–3098.
- Galat A. Peptidylproline cis-trans-isomerases: immunophilins. *Eur J Biochem* 1993;**216**:689–707.
- Handschoemacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW. Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science* 1984;**226**:544–547.
- Colgan J, Asmal M, Yu B, Luban J. Cyclophilin A-deficient mice are resistant to immunosuppression by cyclosporine. *J Immunol* 2005;**174**:6030–6038.
- Liu J, Farmer JD Jr, Lane WS, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* 1991;**66**:807–815.
- Jin ZG, Melaragno MG, Liao DF, Yan C, Haendeler J, Suh YA, Lambeth JD, Berk BC. Cyclophilin A is a secreted growth factor induced by oxidative stress. *Circ Res* 2000;**87**:789–796.
- Seko Y, Fujimura T, Taka H, Mineki R, Murayama K, Nagai R. Hypoxia followed by reoxygenation induces secretion of cyclophilin A from cultured rat cardiac myocytes. *Biochem Biophys Res Commun* 2004;**317**:162–168.
- Sherry B, Yarlett N, Strupp A, Cerami A. Identification of cyclophilin as a proinflammatory secretory product of lipopolysaccharide-activated macrophages. *Proc Natl Acad Sci USA* 1992;**89**:3511–3515.
- Suzuki J, Jin ZG, Meoli DF, Matoba T, Berk BC. Cyclophilin A is secreted by a vesicular pathway in vascular smooth muscle cells. *Circ Res* 2006;**98**:811–817.
- Satoh K, Nigro P, Berk BC. Oxidative stress and vascular smooth muscle cell growth: a mechanistic linkage by cyclophilin A. *Antioxid Redox Signal* 2010;**12**:675–682.
- Nigro P, Satoh K, O'Dell MR, Soe NN, Cui Z, Mohan A, Abe J, Alexis JD, Sparks JD, Berk BC. Cyclophilin A is an inflammatory mediator that promotes atherosclerosis in apolipoprotein E-deficient mice. *J Exp Med* 2011;**208**:53–66.
- Satoh K, Nigro P, Zeidan A, Soe NN, Jaffre F, Oikawa M, O'Dell MR, Cui Z, Menon P, Lu Y, Mohan A, Yan C, Blaxall BC, Berk BC. Cyclophilin A promotes cardiac hypertrophy in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* 2011;**31**:1116–1123.



28. Xu Q, Leiva MC, Fischkoff SA, Handschumacher RE, Lyttle CR. Leukocyte chemotactic activity of cyclophilin. *J Biol Chem* 1992;**267**:11968–11971.
29. Zhou D, Mei Q, Li J, He H. Cyclophilin A and viral infections. *Biochem Biophys Res Commun* 2012;**424**:647–650.
30. Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, Holtzman DM, Betsholtz C, Armulik A, Sallstrom J, Berk BC, Zlokovic BV. Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature* 2012;**485**:512–516.
31. Obchoei S, Wongkhan S, Wongkham C, Li M, Yao Q, Chen C. Cyclophilin A: potential functions and therapeutic target for human cancer. *Med Sci Monit* 2009;**15**:RA221–RA232.
32. Wang L, Wang CH, Jia JF, Ma XK, Li Y, Zhu HB, Tang H, Chen ZN, Zhu P. Contribution of cyclophilin A to the regulation of inflammatory processes in rheumatoid arthritis. *J Clin Immunol* 2010;**30**:24–33.
33. Zhang PH, Yang LR, Li LL, Zeng JZ, Ren LC, Liang PF, Huang XY. Proteomic change of peripheral lymphocytes from scald injury and *Pseudomonas aeruginosa* sepsis in rabbits. *Burns* 2010;**36**:82–88.
34. Stemmy EJ, Benton AS, Lerner J, Alcalá S, Constant SL, Freishtat RJ. Extracellular cyclophilin levels associate with parameters of asthma in phenotypic clusters. *J Asthma* 2011;**48**:986–993.
35. Liu L, Li C, Cai C, Xiang J, Cao Z. Cyclophilin A (CypA) is associated with the inflammatory infiltration and alveolar bone destruction in an experimental periodontitis. *Biochem Biophys Res Commun* 2010;**391**:1000–1006.
36. Li J, Xie H, Yi M, Peng L, Lei D, Chen X, Jian D. Expression of cyclophilin A and CD147 during skin aging. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2011;**36**:203–211.
37. Satoh K, Nigro P, Matoba T, O'Dell MR, Cui Z, Shi X, Mohan A, Yan C, Abe J, Illig KA, Berk BC. Cyclophilin A enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. *Nat Med* 2009;**15**:649–656.
38. Nigro P, Pompilio G, Capogrossi MC. Cyclophilin A: a key player for human disease. *Cell Death Dis* 2013;**4**:e888.
39. Seizer P, Ochmann C, Schonberger T, Zach S, Rose M, Borst O, Klingel K, Kandolf R, MacDonald HR, Nowak RA, Engelhardt S, Lang F, Gawaz M, May AE. Disrupting the EMMPRIN (CD147)-cyclophilin A interaction reduces infarct size and preserves systolic function after myocardial ischemia and reperfusion. *Arterioscler Thromb Vasc Biol* 2012;**31**:1377–1386.
40. Elvers M, Herrmann A, Seizer P, Munzer P, Beck S, Schonberger T, Borst O, Martin-Romero FJ, Lang F, May AE, Gawaz M. Intracellular cyclophilin A is an important Ca(2+) regulator in platelets and critically involved in arterial thrombus formation. *Blood* 2012;**120**:1317–1326.
41. Chen ZJ, Vetter M, Chang GD, Liu S, Che D, Ding Y, Kim SS, Chang CH. Cyclophilin A functions as an endogenous inhibitor for membrane-bound guanylate cyclase-A. *Hypertension* 2004;**44**:963–968.
42. Satoh K, Sato T, Kikuchi N, Omura J, Kurosawa R, Suzuki K, Sugimura K, Aoki T, Nochioka K, Tatebe S, Miyamichi-Yamamoto S, Miura M, Shimizu T, Ikeda S, Yaoita N, Fukumoto Y, Minami T, Miyata S, Nakamura K, Ito H, Kadomatsu K, Shimokawa H. Basigin mediates pulmonary hypertension by promoting inflammation and vascular smooth muscle cell proliferation. *Circ Res* 2014;**115**:738–750.
43. Yan J, Zang X, Chen R, Yuan W, Gong J, Wang C, Li Y. The clinical implications of increased cyclophilin A levels in patients with acute coronary syndromes. *Clin Chim Acta* 2012;**413**:691–695.
44. Satoh K, Fukumoto Y, Sugimura K, Miura Y, Aoki T, Nochioka K, Tatebe S, Miyamichi-Yamamoto S, Shimizu T, Osaki S, Takagi Y, Tsuburaya R, Ito Y, Matsumoto Y, Nakayama M, Takeda M, Takahashi J, Ito K, Yasuda S, Shimokawa H. Plasma cyclophilin A is a novel biomarker for coronary artery disease. *Circ J* 2013;**77**:447–455.
45. Chang CS, Su SL, Chang CC, Lee KW, Kuo CL, Huang CS, Tseng WM, Liu CS. Cyclophilin-A: a novel biomarker for untreated male essential hypertension. *Biomarkers* 2013;**18**:716–720.
46. Ramachandran S, Kartha CC. Cyclophilin-A: a potential screening marker for vascular disease in type-2 diabetes. *Can J Physiol Pharmacol* 2012;**90**:1005–1015.
47. Hasek KW, Glass JR, Godbout M, Sutcliffe JG. An endoplasmic reticulum-specific cyclophilin. *Mol Cell Biol* 1991;**11**:3484–3491.
48. Stocck P, Chapman DC, Beach LA, Williams DB. Depletion of cyclophilins B and C leads to dysregulation of endoplasmic reticulum redox homeostasis. *J Biol Chem* 2014;**289**:23086–23096.
49. Barnes AM, Carter EM, Cabral WA, Weis M, Chang W, Makareeva E, Leikin S, Rotimi CN, Eyre DR, Raggio CL, Marini JC. Lack of cyclophilin B in osteogenesis imperfecta with normal collagen folding. *N Engl J Med* 2010;**362**:521–528.
50. Dieriks B, Van Oostveldt P. Spatiotemporal behavior of nuclear cyclophilin B indicates a role in RNA transcription. *Int J Mol Med* 2012;**29**:1031–1038.
51. Bram RJ, Crabtree GR. Calcium signalling in T cells stimulated by a cyclophilin B-binding protein. *Nature* 1994;**371**:355–358.
52. Ryczyn MA, Reilly SC, O'Malley K, Clevenger CV. Role of cyclophilin B in prolactin signal transduction and nuclear retrotranslocation. *Mol Endocrinol* 2000;**14**:1175–1186.
53. Kim K, Kim H, Jeong K, Jung MH, Hahn BS, Yoon KS, Jin BK, Jahng GH, Kang I, Ha J, Choe W. Release of overexpressed CypB activates ERK signaling through CD147 binding for hepatoma cell resistance to oxidative stress. *Apoptosis* 2012;**17**:784–796.
54. Marcant A, Denys A, Melchior A, Martinez P, Deligny A, Carpentier M, Allain F. Cyclophilin B attenuates the expression of TNF-alpha in lipopolysaccharide-stimulated macrophages through the induction of B cell lymphoma-3. *J Immunol* 2012;**189**:2023–2032.
55. Jeong K, Kim H, Kim K, Kim SJ, Hahn BS, Jahng GH, Yoon KS, Kim SS, Ha J, Kang I, Choe W. Cyclophilin B is involved in p300-mediated degradation of CHOP in tumor cell adaptation to hypoxia. *Cell Death Differ* 2014;**21**:438–450.
56. Liao DF, Jin ZG, Baas AS, Daum G, Gygi SP, Aebersold R, Berk BC. Purification and identification of secreted oxidative stress-induced factors from vascular smooth muscle cells. *J Biol Chem* 2000;**275**:189–196.
57. Pakula R, Melchior A, Denys A, Vanpouille C, Mazurier J, Allain F. Syndecan-1/CD147 association is essential for cyclophilin B-induced activation of p44/42 mitogen-activated protein kinases and promotion of cell adhesion and chemotaxis. *Glycobiology* 2007;**17**:492–503.
58. Melchior A, Denys A, Deligny A, Mazurier J, Allain F. Cyclophilin B induces integrin-mediated cell adhesion by a mechanism involving CD98-dependent activation of protein kinase C-delta and p44/42 mitogen-activated protein kinases. *Exp Cell Res* 2008;**314**:616–628.
59. Cabral WA, Perdivara I, Weis M, Terajima M, Blissett AR, Chang W, Perosky JE, Makareeva EN, Mertz EL, Leikin S, Tomer KB, Kozloff KM, Eyre DR, Yamauchi M, Marini JC. Abnormal type I collagen post-translational modification and crosslinking in a cyclophilin B KO mouse model of recessive osteogenesis imperfecta. *PLoS Genet* 2014;**10**:e1004465.
60. Williams PD, Owens CR, Dziegielewska J, Moskaluk CA, Read PW, Larner JM, Story MD, Brock WA, Amundson SA, Lee JK, Theodorescu D. Cyclophilin B expression is associated with in vitro radioresistance and clinical outcome after radiotherapy. *Neoplasia* 2011;**13**:1122–1131.
61. Fang F, Flegler AJ, Du P, Lin S, Clevenger CV. Expression of cyclophilin B is associated with malignant progression and regulation of genes implicated in the pathogenesis of breast cancer. *Am J Pathol* 2009;**174**:297–308.
62. Weng L, Tian X, Gao Y, Watashi K, Shimotohno K, Wakita T, Kohara M, Toyoda T. Different mechanisms of hepatitis C virus RNA polymerase activation by cyclophilin A and B in vitro. *Biochim Biophys Acta* 2012;**1820**:1886–1892.
63. Hanouille X, Badillo A, Wieruszkeski JM, Verdegem D, Landrieu I, Bartenschlager R, Penin F, Lippens G. Hepatitis C virus NS5A protein is a substrate for the peptidyl-prolyl cis/trans isomerase activity of cyclophilins A and B. *J Biol Chem* 2009;**284**:13589–13601.
64. Luban J, Bossolt KL, Franke EK, Kalpana GV, Goff SP. Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell* 1993;**73**:1067–1078.
65. Oh Y, Kim EY, Kim Y, Jin J, Jin BK, Jahng GH, Jung MH, Park C, Kang I, Ha J, Choe W. Neuroprotective effects of overexpressed cyclophilin B against Abeta-induced neurotoxicity in PC12 cells. *Free Radic Biol Med* 2011;**51**:905–920.
66. Lam YW, Tam NN, Evans JE, Green KM, Zhang X, Ho SM. Differential proteomics in the aging Noble rat ventral prostate. *Proteomics* 2008;**8**:2750–2763.
67. Kainer DB, Doris PA. Cyclophilin B expression in renal proximal tubules of hypertensive rats. *Hypertension* 2000;**35**:958–964.
68. Holfeld J, Tepekoylu C, Kozaryn R, Urbschat A, Zacharowski K, Grimm M, Paulus P. Shockwave therapy differentially stimulates endothelial cells: implications on the control of inflammation via toll-Like receptor 3. *Inflammation* 2014;**37**:65–70.
69. Friedman J, Weissman I, Alpert S. An analysis of the expression of cyclophilin C reveals tissue restriction and an intriguing pattern in the mouse kidney. *Am J Pathol* 1994;**144**:1247–1256.
70. Ohe Y, Ishikawa K, Itoh Z, Tatemoto K. Cultured leptomeningeal cells secrete cerebrospinal fluid proteins. *J Neurochem* 1996;**67**:964–971.
71. Wang P, Mariman E, Keijer J, Bouwman F, Noben JP, Robben J, Renes J. Profiling of the secreted proteins during 3T3-L1 adipocyte differentiation leads to the identification of novel adipokines. *Cell Mol Life Sci* 2004;**61**:2405–2417.
72. Friedman J, Weissman I. Two cytoplasmic candidates for immunophilin action are revealed by affinity for a new cyclophilin: one in the presence and one in the absence of CsA. *Cell* 1991;**66**:799–806.
73. Montague JW, Hughes FM Jr, Cidlowski JA. Native recombinant cyclophilins A, B, and C degrade DNA independently of peptidylprolyl cis-trans-isomerase activity. Potential roles of cyclophilins in apoptosis. *J Biol Chem* 1997;**272**:6677–6684.
74. Friedman J, Trahey M, Weissman I. Cloning and characterization of cyclophilin C-associated protein: a candidate natural cellular ligand for cyclophilin C. *Proc Natl Acad Sci USA* 1993;**90**:6815–6819.
75. Yamaguchi R, Hosaka M, Torii S, Hou N, Saito N, Yoshimoto Y, Imai H, Takeuchi T. Cyclophilin C-associated protein regulation of phagocytic functions via NFAT activation in macrophages. *Brain Res* 2011;**1397**:55–65.
76. Trahey M, Weissman I. Cyclophilin C-associated protein: a normal secreted glycoprotein that down-modulates endotoxin and proinflammatory responses in vivo. *Proc Natl Acad Sci USA* 1999;**96**:3006–3011.
77. Kong W, Longaker MT, Lorenz HP. Cyclophilin C-associated protein is a mediator for fibronectin fragment-induced matrix metalloproteinase-13 expression. *J Biol Chem* 2004;**279**:55334–55340.
78. Shimizu T, Imai H, Seki K, Tomizawa S, Nakamura M, Honda F, Kawahara N, Saito N. Cyclophilin C-associated protein and cyclophilin C mRNA are upregulated in penumbral neurons and microglia after focal cerebral ischemia. *J Cereb Blood Flow Metab* 2005;**25**:325–337.

79. Bergsma DJ, Eder C, Gross M, Kersten H, Sylvester D, Appelbaum E, Cusimano D, Livi GP, McLaughlin MM, Kasyan K. The cyclophilin multigene family of peptidyl-prolyl isomerases. Characterization of three separate human isoforms. *J Biol Chem* 1991;**266**: 23204–23214.
80. Hazelton JL, Petrasheuskaya M, Fiskum G, Kristian T. Cyclophilin D is expressed predominantly in mitochondria of gamma-aminobutyric acidergic interneurons. *J Neurosci Res* 2009;**87**:1250–1259.
81. Elrod JW, Wong R, Mishra S, Vagnozzi RJ, Sakthivel B, Goonasekera SA, Karch J, Gabel S, Farber J, Force T, Brown JH, Murphy E, Molkenin JD. Cyclophilin D controls mitochondrial pore-dependent Ca(2+) exchange, metabolic flexibility, and propensity for heart failure in mice. *J Clin Invest* 2010;**120**:3680–3687.
82. Menazza S, Wong R, Nguyen T, Wang G, Gucek M, Murphy E. CypD(-/-) hearts have altered levels of proteins involved in Krebs cycle, branch chain amino acid degradation and pyruvate metabolism. *J Mol Cell Cardiol* 2013;**56**:81–90.
83. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, Brunskill EV, Sayen MR, Gottlieb RA, Dorn GW, Robbins J, Molkenin JD. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* 2005;**434**:658–662.
84. Schinzel AC, Takeuchi O, Huang Z, Fisher JK, Zhou Z, Rubens J, Hetz C, Danial NN, Moskowitz MA, Korsmeyer SJ. Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. *Proc Natl Acad Sci USA* 2005;**102**:12005–12010.
85. Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H, Inohara H, Kubo T, Tsujimoto Y. Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* 2005;**434**:652–658.
86. Di Lisa F, Bernardi P. A CaPful of mechanisms regulating the mitochondrial permeability transition. *J Mol Cell Cardiol* 2009;**46**:775–780.
87. Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P. Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. *J Biol Chem* 2005;**280**: 18558–18561.
88. Li Y, Johnson N, Capano M, Edwards M, Crompton M. Cyclophilin-D promotes the mitochondrial permeability transition but has opposite effects on apoptosis and necrosis. *Biochem J* 2004;**383**:101–109.
89. Matas J, Young NT, Bourcier-Lucas C, Aschah A, Marcil M, Deschepper CF, Burelle Y. Increased expression and intramitochondrial translocation of cyclophilin-D associates with increased vulnerability of the permeability transition pore to stress-induced opening during compensated ventricular hypertrophy. *J Mol Cell Cardiol* 2009;**46**: 420–430.
90. Millay DP, Sargent MA, Osinska H, Baines CP, Barton ER, Vuagniaux G, Sweeney HL, Robbins J, Molkenin JD. Genetic and pharmacologic inhibition of mitochondrial-dependent necrosis attenuates muscular dystrophy. *Nat Med* 2008;**14**:442–447.
91. Palma E, Tiepolo T, Angelin A, Sabatelli P, Maraldi NM, Basso E, Forte MA, Bernardi P, Bonaldo P. Genetic ablation of cyclophilin D rescues mitochondrial defects and prevents muscle apoptosis in collagen VI myopathic mice. *Hum Mol Genet* 2009;**18**: 2024–2031.
92. Du H, Guo L, Fang F, Chen D, Sosunov AA, McKhann GM, Yan Y, Wang C, Zhang H, Molkenin JD, Gunn-Moore FJ, Vonsattel JP, Arancio O, Chen JX, Yan SD. Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. *Nat Med* 2008;**14**:1097–1105.
93. Du H, Guo L, Zhang W, Ryzewska M, Yan S. Cyclophilin D deficiency improves mitochondrial function and learning/memory in aging Alzheimer disease mouse model. *Neurobiol Aging* 2011;**32**:398–406.
94. Thomas B, Banerjee R, Starkova NN, Zhang SF, Calingasan NY, Yang L, Wille E, Lorenzo BJ, Ho DJ, Beal MF, Starkov A. Mitochondrial permeability transition pore component cyclophilin D distinguishes nigrostriatal dopaminergic death paradigms in the MPTP mouse model of Parkinson's disease. *Antioxid Redox Signal* 2012;**16**:855–868.
95. Forte M, Gold BG, Marracci G, Chaudhary P, Basso E, Johnsen D, Yu X, Fowlkes J, Rahder M, Stem K, Bernardi P, Bourdette D. Cyclophilin D inactivation protects axons in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. *Proc Natl Acad Sci USA* 2007;**104**:7558–7563.
96. Luvisetto S, Basso E, Petronilli V, Bernardi P, Forte M. Enhancement of anxiety, facilitation of avoidance behavior, and occurrence of adult-onset obesity in mice lacking mitochondrial cyclophilin D. *Neuroscience* 2008;**155**:585–596.
97. Belaidi E, Decorsis J, Augeul L, Durand A, Ovize M. Endoplasmic reticulum stress contributes to heart protection induced by cyclophilin D inhibition. *Basic Res Cardiol* 2013;**108**:363.
98. Lim SY, Davidson SM, Mocanu MM, Yellon DM, Smith CC. The cardioprotective effect of necrostatin requires the cyclophilin-D component of the mitochondrial permeability transition pore. *Cardiovasc Drugs Ther* 2007;**21**:467–469.
99. Devalaraja-Narashimha K, Diener AM, Padanilam BJ. Cyclophilin D gene ablation protects mice from ischemic renal injury. *Am J Physiol Renal Physiol* 2009;**297**:F749–F759.
100. Park JS, Pasupulati R, Feldkamp T, Roesser NF, Weinberg JM. Cyclophilin D and the mitochondrial permeability transition in kidney proximal tubules after hypoxic and ischemic injury. *Am J Physiol Renal Physiol* 2011;**301**:F134–F150.
101. Ruiz-Meana M, Inserte J, Fernandez-Sanz C, Hernando V, Miro-Casas E, Barba I, Garcia-Dorado D. The role of mitochondrial permeability transition in reperfusion-induced cardiomyocyte death depends on the duration of ischemia. *Basic Res Cardiol* 2011;**106**:1259–1268.
102. Wang X, Carlsson Y, Basso E, Zhu C, Rousset CI, Rasola A, Johansson BR, Blomgren K, Mallard C, Bernardi P, Forte MA, Hagberg H. Developmental shift of cyclophilin D contribution to hypoxic-ischemic brain injury. *J Neurosci* 2009;**29**:2588–2596.
103. Kato M, Akao M, Matsumoto-Ida M, Makiyama T, Iguchi M, Takeda T, Shimizu S, Kita T. The targeting of cyclophilin D by RNAi as a novel cardioprotective therapy: evidence from two-photon imaging. *Cardiovasc Res* 2009;**83**:335–344.
104. Dube H, Selwood D, Malouitre S, Capano M, Simone MI, Crompton M. A mitochondrial-targeted cyclosporin A with high binding affinity for cyclophilin D yields improved cytoprotection of cardiomyocytes. *Biochem J* 2012;**441**:901–907.
105. Lim SY, Hausenloy DJ, Arjun S, Price AN, Davidson SM, Lythgoe MF, Yellon DM. Mitochondrial cyclophilin-D as a potential therapeutic target for post-myocardial infarction heart failure. *J Cell Mol Med* 2010;**15**:2443–2451.
106. Nakayama H, Chen X, Baines CP, Kleivitsky R, Zhang X, Zhang H, Jaleel N, Chua BH, Hewett TE, Robbins J, Houser SR, Molkenin JD. Ca2+- and mitochondrial-dependent cardiomyocyte necrosis as a primary mediator of heart failure. *J Clin Invest* 2007;**117**: 2431–2444.
107. Hausenloy DJ, Lim SY, Ong SG, Davidson SM, Yellon DM. Mitochondrial cyclophilin-D as a critical mediator of ischaemic preconditioning. *Cardiovasc Res* 2010;**88**:67–74.
108. Jobe SM, Wilson KM, Leo L, Raimondi A, Molkenin JD, Lentz SR, DiPaola J. Critical role for the mitochondrial permeability transition pore and cyclophilin D in platelet activation and thrombosis. *Blood* 2008;**111**:1257–1265.
109. Fujimoto K, Chen Y, Polonsky KS, Dorn GW 2nd. Targeting cyclophilin D and the mitochondrial permeability transition enhances beta-cell survival and prevents diabetes in Pdx1 deficiency. *Proc Natl Acad Sci USA* 2010;**107**:10214–10219.
110. Chen S, Zhang M, Ma H, Saiyin H, Shen S, Xi J, Wan B, Yu L. Oligo-microarray analysis reveals the role of cyclophilin A in drug resistance. *Cancer Chemother Pharmacol* 2008;**61**:459–469.
111. Huo DH, Yi LN, Yang J. Interaction with Ppi3 leads to the cytoplasmic localization of Apoptin in tumor cells. *Biochem Biophys Res Commun* 2008;**372**:14–18.
112. Nair SC, Rimerman RA, Toran EJ, Chen S, Prapapanich V, Butts RN, Smith DF. Molecular cloning of human FKBP51 and comparisons of immunophilin interactions with Hsp90 and progesterone receptor. *Mol Cell Biol* 1997;**17**:594–603.
113. Mark PJ, Ward BK, Kumar P, Lahooti H, Minchin RF, Ratajczak T. Human cyclophilin 40 is a heat shock protein that exhibits altered intracellular localization following heat shock. *Cell Stress Chaperones* 2001;**6**:59–70.
114. Pratt WB, Galigniana MD, Harrell JM, DeFranco DB. Role of hsp90 and the hsp90-binding immunophilins in signalling protein movement. *Cell Signal* 2004;**16**: 857–872.
115. Ratajczak T, Ward BK, Minchin RF. Immunophilin chaperones in steroid receptor signaling. *Curr Top Med Chem* 2003;**3**:1348–1357.
116. Reynolds PD, Ruan Y, Smith DF, Scammell JG. Glucocorticoid resistance in the squirrel monkey is associated with overexpression of the immunophilin FKBP51. *J Clin Endocrinol Metab* 1999;**84**:663–669.
117. Lu TC, Bhattacharya P, Chan WK. Cyclophilin-40 has a cellular role in the aryl hydrocarbon receptor signaling. *FEBS Lett* 2008;**582**:3167–3173.
118. Carrello A, Allan RK, Morgan SL, Owen BA, Mok D, Ward BK, Minchin RF, Toft DO, Ratajczak T. Interaction of the Hsp90 cochaperone cyclophilin 40 with Hsc70. *Cell Stress Chaperones* 2004;**9**:167–181.
119. Smith MR, Willmann MR, Wu G, Berardini TZ, Moller B, Weijers D, Poethig RS. Cyclophilin 40 is required for microRNA activity in Arabidopsis. *Proc Natl Acad Sci USA* 2009;**106**:5424–5429.
120. Pearson JD, Mohammed Z, Bacani JT, Lai R, Ingham RJ. The heat shock protein-90 co-chaperone, Cyclophilin 40, promotes ALK-positive, anaplastic large cell lymphoma viability and its expression is regulated by the NPM-ALK oncoprotein. *BMC Cancer* 2012;**12**:229.
121. Kumar P, Mark PJ, Ward BK, Minchin RF, Ratajczak T. Estradiol-regulated expression of the immunophilins cyclophilin 40 and FKBP52 in MCF-7 breast cancer cells. *Biochem Biophys Res Commun* 2001;**284**:219–225.
122. Ward BK, Kumar P, Turbett GR, Edmondston JE, Papadimitriou JM, Laing NG, Ingram DM, Minchin RF, Ratajczak T. Allelic loss of cyclophilin 40, an estrogen receptor-associated immunophilin, in breast carcinomas. *J Cancer Res Clin Oncol* 2001;**127**: 109–115.
123. Allan AM, Goggin SL, Caldwell KK. Prenatal alcohol exposure modifies glucocorticoid receptor subcellular distribution in the medial prefrontal cortex and impairs frontal cortex-dependent learning. *PLoS ONE* 2014;**9**:e96200.
124. Helmer RA, Martinez-Zaguilan R, Dertien JS, Fulford C, Foreman O, Peiris V, Chilton BS. Helicase-like transcription factor (Hltf) regulates g<sup>2</sup>m transition, wt1/gata4/hif-1a cardiac transcription networks, and collagen biogenesis. *PLoS ONE* 2013;**8**:e80461.
125. Liu F, Wang YQ, Meng L, Gu M, Tan RY. FK506-binding protein 12 ligands: a patent review. *Expert Opin Ther Pat* 2013;**23**:1435–1449.
126. Kang CB, Hong Y, Dhe-Paganon S, Yoon HS. FKBP family proteins: immunophilins with versatile biological functions. *Neuro-Signals* 2008;**16**:318–325.
127. Huang F, Shan J, Reiken S, Wehrens XH, Marks AR. Analysis of calstabin2 (FKBP12.6)-ryanodine receptor interactions: rescue of heart failure by calstabin2 in mice. *Proc Natl Acad Sci USA* 2006;**103**:3456–3461.
128. Tiermerman AP, Wiederrecht G, Marcy A, Fleischer S. Characterization of an exchange reaction between soluble FKBP-12 and the FKBP-ryanodine receptor complex.

- Modulation by FKBP mutants deficient in peptidyl-prolyl isomerase activity. *J Biol Chem* 1995;**270**:2451–2459.
129. Edlich F, Fischer G. Pharmacological targeting of catalyzed protein folding: the example of peptide bond cis/trans isomerases. *Handb Exp Pharmacol* 2006;**172**:359–404.
  130. Fruman DA, Klee CB, Bierer BE, Burakoff SJ. Calcineurin phosphatase activity in T lymphocytes is inhibited by FK 506 and cyclosporin A. *Proc Natl Acad Sci USA* 1992;**89**:3686–3690.
  131. Sewell TJ, Lam E, Martin MM, Leszyk J, Weidner J, Calaycay J, Griffen P, Williams H, Hung S, Cryan J. Inhibition of calcineurin by a novel FK-506-binding protein. *J Biol Chem* 1994;**269**:21094–21102.
  132. Brown EJ, Beal PA, Keith CT, Chen J, Shin TB, Schreiber SL. Control of p70 s6 kinase by kinase activity of FRAP in vivo. *Nature* 1995;**377**:441–446.
  133. Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* 1991;**253**:905–909.
  134. Shou W, Aghdasi B, Armstrong DL, Guo Q, Bao S, Charng MJ, Mathews LM, Schneider MD, Hamilton SL, Matzuk MM. Cardiac defects and altered ryanodine receptor function in mice lacking FKBP12. *Nature* 1998;**391**:489–492.
  135. Chen H, Zhang W, Sun X, Yoshimoto M, Chen Z, Zhu W, Liu J, Shen Y, Yong W, Li D, Zhang J, Lin Y, Li B, VanDusen NJ, Snider P, Schwartz RJ, Conway SJ, Field LJ, Yoder MC, Firulli AB, Carlesso N, Towbin JA, Shou W. Fkbp1a controls ventricular myocardium trabeculation and compaction by regulating endocardial Notch1 activity. *Development* 2013;**140**:1946–1957.
  136. Maruyama M, Li BY, Chen H, Xu X, Song LS, Guatimosim S, Zhu W, Yong W, Zhang W, Bu G, Lin SF, Fishbein MC, Lederer WJ, Schild JH, Field LJ, Rubart M, Chen PS, Shou W. FKBP12 is a critical regulator of the heart rhythm and the cardiac voltage-gated sodium current in mice. *Circ Res* 2011;**108**:1042–1052.
  137. Chiasson VL, Talreja D, Young KJ, Chatterjee P, Banes-Berceli AK, Mitchell BM. FK506 binding protein 12 deficiency in endothelial and hematopoietic cells decreases regulatory T cells and causes hypertension. *Hypertension* 2011;**57**:1167–1175.
  138. Long C, Cook LG, Hamilton SL, Wu GY, Mitchell BM. FK506 binding protein 12/12.6 depletion increases endothelial nitric oxide synthase threonine 495 phosphorylation and blood pressure. *Hypertension* 2007;**49**:569–576.
  139. Jayaraman T, Brillantes AM, Timmerman AP, Fleischer S, Erdjument-Bromage H, Tempst P, Marks AR. FK506 binding protein associated with the calcium release channel (ryanodine receptor). *J Biol Chem* 1992;**267**:9474–9477.
  140. Wehrens XH, Marks AR. Altered function and regulation of cardiac ryanodine receptors in cardiac disease. *Trends Biochem Sci* 2003;**28**:671–678.
  141. Kushnir A, Marks AR. The ryanodine receptor in cardiac physiology and disease. *Adv Pharmacol* 2010;**59**:1–30.
  142. Timmerman AP, Jayaraman T, Wiederrecht G, Onoue H, Marks AR, Fleischer S. The ryanodine receptor from canine heart sarcoplasmic reticulum is associated with a novel FK-506 binding protein. *Biochem Biophys Res Commun* 1994;**198**:701–706.
  143. Timmerman AP, Onoue H, Xin HB, Barg S, Copello J, Wiederrecht G, Fleischer S. Selective binding of FKBP12.6 by the cardiac ryanodine receptor. *J Biol Chem* 1996;**271**:20385–20391.
  144. Boulos S, Meloni BP, Arthur PG, Majda B, Bojarski C, Knuckey NW. Evidence that intracellular cyclophilin A and cyclophilin A/CD147 receptor-mediated ERK1/2 signalling can protect neurons against in vitro oxidative and ischemic injury. *Neurobiol Dis* 2007;**25**:54–64.
  145. Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosembly N, Marks AR. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 2000;**101**:365–376.
  146. Gellen B, Fernandez-Velasco M, Brier F, Vinet L, LeQuang K, Rouet-Benzineb P, Benitah JP, Pezet M, Palais G, Pellegrin N, Zhang A, Perrier R, Escoubet B, Marniquet X, Richard S, Jaisser F, Gomez AM, Charpentier F, Mercadier JJ. Conditional FKBP12.6 overexpression in mouse cardiac myocytes prevents triggered ventricular tachycardia through specific alterations in excitation-contraction coupling. *Circulation* 2008;**117**:1778–1786.
  147. Zhang X, Tallini YN, Chen Z, Gan L, Wei B, Doran R, Miao L, Xin HB, Kotlikoff MI, Ji G. Dissociation of FKBP12.6 from ryanodine receptor type 2 is regulated by cyclic ADP-ribose but not beta-adrenergic stimulation in mouse cardiomyocytes. *Cardiovasc Res* 2009;**84**:253–262.
  148. Zhang Y, Huang ZJ, Dai DZ, Feng Y, Na T, Tang XY, Dai Y. Downregulated FKBP12.6 expression and upregulated endothelin signaling contribute to elevated diastolic calcium and arrhythmogenesis in rat cardiomyopathy produced by L-thyroxine. *Int J Cardiol* 2008;**130**:463–471.
  149. Chelu MG, Sarma S, Sood S, Wang S, van Oort RJ, Skapura DG, Li N, Santonastasi M, Muller FU, Schmitz W, Schotten U, Anderson ME, Valderrabano M, Dobrev D, Wehrens XH. Calmodulin kinase II-mediated sarcoplasmic reticulum Ca<sup>2+</sup> leak promotes atrial fibrillation in mice. *J Clin Invest* 2009;**119**:1940–1951.
  150. Vest JA, Wehrens XH, Reiken SR, Lehnart SE, Dobrev D, Chandra P, Danilo P, Ravens U, Rosen MR, Marks AR. Defective cardiac ryanodine receptor regulation during atrial fibrillation. *Circulation* 2005;**111**:2025–2032.
  151. Sood S, Chelu MG, van Oort RJ, Skapura D, Santonastasi M, Dobrev D, Wehrens XH. Intracellular calcium leak due to FKBP12.6 deficiency in mice facilitates the inducibility of atrial fibrillation. *Heart Rhythm* 2008;**5**:1047–1054.
  152. Lehnart SE, Wehrens XH, Laitinen PJ, Reiken SR, Deng SX, Cheng Z, Landry DW, Kontula K, Swan H, Marks AR. Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak. *Circulation* 2004;**109**:3208–3214.
  153. Ahmad F. The molecular genetics of arrhythmogenic right ventricular dysplasia-cardiomyopathy. *Clin Invest Med* 2003;**26**:167–178.
  154. Oyama MA, Reiken S, Lehnart SE, Chittur SV, Meurs KM, Stern J, Marks AR. Arrhythmogenic right ventricular cardiomyopathy in Boxer dogs is associated with calstabin2 deficiency. *J Vet Cardiol* 2008;**10**:1–10.
  155. Yaras N, Ugur M, Ozdemir S, Gurdal H, Purali N, Lacampagne A, Vassort G, Turan B. Effects of diabetes on ryanodine receptor Ca release channel (RyR2) and Ca<sup>2+</sup> homeostasis in rat heart. *Diabetes* 2005;**54**:3082–3088.
  156. Zhao SM, Wang YL, Guo CY, Chen JL, Wu YQ. Progressive decay of Ca<sup>2+</sup> homeostasis in the development of diabetic cardiomyopathy. *Cardiovasc Diabetol* 2014;**13**:75.
  157. Lehnart SE, Terrenoire C, Reiken S, Wehrens XH, Song LS, Tillman EJ, Mancarella S, Coromilas J, Lederer WJ, Kass RS, Marks AR. Stabilization of cardiac ryanodine receptor prevents intracellular calcium leak and arrhythmias. *Proc Natl Acad Sci USA* 2006;**103**:7906–7910.
  158. Vinet L, Pezet M, Bito V, Brier F, Biesmans L, Rouet-Benzineb P, Gellen B, Previlon M, Chimenti S, Vilaine JP, Charpentier F, Sipido KR, Mercadier JJ. Cardiac FKBP12.6 overexpression protects against triggered ventricular tachycardia in pressure overloaded mouse hearts. *Basic Res Cardiol* 2012;**107**:246.
  159. 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.
  160. Liu Y, Chen H, Ji G, Li B, Mohler PJ, Zhu Z, Yong W, Chen Z, Xu X, Xin H, Shou W. Transgenic analysis of the role of FKBP12.6 in cardiac function and intracellular calcium release. *Assay Drug Dev Technol* 2011;**9**:620–627.
  161. Zhong J, Chen J, Cao T, Wang L, Zhang W, Liu D, Zhu Z. Adenovirus-mediated FKBP12.6 overexpression induces hypertrophy and apoptosis in cultured neonatal cardiomyocytes. *Clin Exp Pharmacol Physiol* 2009;**36**:135–140.
  162. Smith RL, Redd MJ, Johnson AD. The tetratricopeptide repeats of Ssn6 interact with the homeo domain of alpha 2. *Genes Dev* 1995;**9**:2903–2910.
  163. Tzamaris D, Struhl K. Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. *Genes Dev* 1995;**9**:821–831.
  164. Noguchi J, Kobayashi E, Akiyama K, Kawai Y, Ozawa M, Ohnuma K, Kikuchi K, Kaneko H, Kunieda T. Fine mapping of a region of rat chromosome 12 close to the aspermia (as) locus and comparison with the human orthologous regions. *Exp Anim* 2004;**53**:429–435.
  165. Meng X, Lu X, Morris CA, Keating MT. A novel human gene FKBP6 is deleted in Williams syndrome. *Genomics* 1998;**52**:130–137.
  166. Tomita-Mitchell A, Mahnke DK, Struble CA, Tuffnell ME, Stamm KD, Hidestrand M, Harris SE, Goetsch MA, Simpson PM, Bick DP, Broeckel U, Pelech AN, Tweddell JS, Mitchell ME. Human gene copy number spectra analysis in congenital heart malformations. *Physiol Genomics* 2012;**44**:518–541.
  167. Ranganathan R, Lu KP, Hunter T, Noel JP. Structural and functional analysis of the mitotic rotamase Pin1 suggests substrate recognition is phosphorylation dependent. *Cell* 1997;**89**:875–886.
  168. Toko H, Hariharan N, Konstantin MH, Ormachea L, McGregor M, Gude NA, Sundararaman B, Jyo E, Jyo AY, Collins B, Din S, Mohsin S, Uchida T, Sussman MA. Differential regulation of cellular senescence and differentiation by prolyl isomerase Pin1 in cardiac progenitor cells. *J Biol Chem* 2014;**289**:5348–5356.
  169. Bao L, Kimzey A, Sauter G, Sowadski JM, Lu KP, Wang DG. Prevalent overexpression of prolyl isomerase Pin1 in human cancers. *Am J Pathol* 2004;**164**:1727–1737.
  170. Lv L, Zhang J, Zhang L, Xue G, Wang P, Meng Q, Liang W. Essential role of Pin1 via STAT3 signalling and mitochondria-dependent pathways in restenosis in type 2 diabetes. *J Cell Mol Med* 2009;**17**:989–1005.
  171. Lv L, Zhou Z, Huang X, Zhao Y, Zhang L, Shi Y, Sun M, Zhang J. Inhibition of peptidyl-prolyl cis/trans isomerase Pin1 induces cell cycle arrest and apoptosis in vascular smooth muscle cells. *Apoptosis* 2013;**15**:41–54.
  172. Ki SH, Lee JW, Lim SC, Hien TT, Im JH, Oh WK, Lee MY, Ji YH, Kim YG, Kang KW. Protective effect of nectandrin B, a potent AMPK activator on neointima formation: inhibition of Pin1 expression through AMPK activation. *Br J Pharmacol* 2012;**168**:932–945.
  173. Ruan L, Torres CM, Qian J, Chen F, Mintz JD, Stepp DW, Fulton D, Venema RC. Pin1 prolyl isomerase regulates endothelial nitric oxide synthase. *Arterioscler Thromb Vasc Biol* 2010;**31**:392–398.
  174. Erol A. Pin1 as a protector of vascular endothelial homeostasis. *Hypertension* 2012;**59**:e14.
  175. Chiasson VL, Munshi N, Chatterjee P, Young KJ, Mitchell BM. Pin1 deficiency causes endothelial dysfunction and hypertension. *Hypertension* 2011;**58**:431–438.
  176. Paneni F, Costantino S, Castello L, Battista R, Capretti G, Chianotto S, D'Amario D, Scavone G, Villano A, Rustighi A, Crea F, Pitocco D, Lanza G, Volpe M, Del Sal G, Luscher TF, Cosentino F. Targeting prolyl-isomerase Pin1 prevents mitochondrial oxidative stress and vascular dysfunction: insights in patients with diabetes. *Eur Heart J* 2014.
  177. Toko H, Konstantin MH, Doroudgar S, Ormachea L, Jyo E, Jyo AY, Din S, Gude NA, Collins B, Volkens M, Thuerauf DJ, Glembocki CC, Chen CH, Lu KP, Muller OJ, Uchida T, Sussman MA. Regulation of cardiac hypertrophic signaling by prolyl isomerase Pin1. *Circ Res* 2013;**112**:1244–1252.

178. Sakai S, Shimojo N, Kimura T, Tajiri K, Maruyama H, Homma S, Kuga K, Mizutani T, Aonuma K, Miyauchi T. Involvement of peptidyl-prolyl isomerase Pin1 in the inhibitory effect of fluvastatin on endothelin-1-induced cardiomyocyte hypertrophy. *Life Sci* 2014;**102**:98–104.
179. Kim SH, Lessner SM, Sakurai Y, Galis ZS. Cyclophilin A as a novel biphasic mediator of endothelial activation and dysfunction. *Am J Pathol* 2004;**164**:1567–1574.
180. Erlejan AG, Lagadari M, Galigniana MD. Hsp90-binding immunophilins as a potential new platform for drug treatment. *Future Med Chem* 2013;**5**:591–607.
181. Galat A. Functional diversity and pharmacological profiles of the FKBP and their complexes with small natural ligands. *Cell Mol Life Sci* 2013;**70**:3243–3275.
182. Flisiak R, Feinman SV, Jablkowski M, Horban A, Kryczka W, Pawlowska M, Heathcote JE, Mazzella G, Vandelli C, Nicolas-Metral V, Groscurin P, Liz JS, Scalfaro P, Porchet H, Crabbe R. The cyclophilin inhibitor Debio 025 combined with PEG IFNalpha2a significantly reduces viral load in treatment-naive hepatitis C patients. *Hepatology* 2009;**49**:1460–1468.
183. Arora K, Gwinn WM, Bower MA, Watson A, Okwumabua I, MacDonald HR, Bukrinsky MI, Constant SL. Extracellular cyclophilins contribute to the regulation of inflammatory responses. *J Immunol* 2005;**175**:517–522.
184. Damsker JM, Okwumabua I, Pushkarsky T, Arora K, Bukrinsky MI, Constant SL. Targeting the chemotactic function of CD147 reduces collagen-induced arthritis. *Immunology* 2009;**126**:55–62.
185. Balsley MA, Malesevic M, Stemmy EJ, Gigley J, Jurjus RA, Herzog D, Bukrinsky MI, Fischer G, Constant SL. A cell-impermeable cyclosporine A derivative reduces pathology in a mouse model of allergic lung inflammation. *J Immunol* 2010;**185**:7663–7670.