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INVITED REVIEW

The NO signalling pathway in aortic aneurysm and dissection

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Recent studies have shown that NO is a central mediator in diseases associated with thoracic aortic aneurysm, such as Marfan syndrome. The progressive dilation of the aorta in thoracic aortic aneurysm ultimately leads to aortic dissection. Unfortunately, current medical treatments have neither halt aortic enlargement nor prevented rupture, leaving surgical repair as the only effective treatment. There is therefore a pressing need for effective therapies to delay or even avoid the need for surgical repair in thoracic aortic aneurysm patients. Here, we summarize the mechanisms through which NO signalling dysregulation causes thoracic aortic aneurysm, particularly in Marfan syndrome. We discuss recent advances based on the identification of new Marfan syndrome mediators related to pathway overactivation that represent potential disease biomarkers. Likewise, we propose iNOS, sGC and PRKG1, whose pharmacological inhibition reverses aortopathy in Marfan syndrome mice, as targets for therapeutic intervention in thoracic aortic aneurysm and are candidates for clinical trials.

KEYWORDS
aortic aneurysm, cGMP, Marfan syndrome, NO, NOS, PRKG1, sGC

1 | INTRODUCTION

Aortic diseases comprise of a variety of inherited and acquired disorders, including aneurysmal dilation and dissection, which account for 1%–2% of all deaths in developed countries. Aneurysms progress through the continuous expansion of the artery, resulting in destabilization and eventually dissection or rupture of the aortic wall, causing sudden death due to extensive haemorrhaging. Aortic aneurysms (AA) are classified according to their anatomical location in either the thoracic ascending aorta or the abdominal aorta. Although both

Abbreviations: ACEI, ACE inhibitor; ACTA2, smooth muscle α-actin 2; Alk5, activin receptor-like kinase 5; BH4, tetrahydro-l-biopterin/sapropterin; BKCa, Ca^{2+}-activated K^{+}; CaM, calmodulin; DetaNO, DetaNONOate; FBN1, fibrillin-1/sapropterin; MLCP, myosin light chain phosphatase; MYH1, smooth muscle myosin heavy chain 11; MYLK, myosin light chain kinase; MYPT1, myosin phosphatase target subunit 1; MYPT1K, MYPT1-associated kinase; PRKG1, protein kinase cGMP-dependent type 1; ROCK, Rho-associated protein kinase; sGC, soluble guanylate cyclase; TGFBR2, TGF-β receptor type II/transforming growth factor beta receptor 2; VASP, vasodilator-stimulated phosphoprotein; WT, wild-type.

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aneurysm types share pathophysiological features, they differ in their aetiology, population prevalence and modes of inheritance (Lindsay & Dietz, 2011). Abdominal aortic aneurysm is the most common form and is mainly considered of atherosclerotic origin, with associated risk factors such as smoking, male sex, hypercholesterolaemia, hypertension and diabetes (Lindsay & Dietz, 2011; Pinard et al., 2019). Abdominal aortic aneurysm is typically characterized by the presence of atheroma plaque, infiltration of inflammatory cells, destructive extracellular matrix remodelling, and dysfunction and depletion of vascular smooth muscle cells (Lindsay & Dietz, 2011; Pinard et al., 2019). In contrast, thoracic aortic aneurysm occurs in the young and the old alike, is usually associated with genetic influences and does not always show an association with cardiovascular risk factors (Lindsay & Dietz, 2011; Pinard et al., 2019). Inherited forms of thoracic aortic aneurysm commonly feature extracellular matrix remodelling (with fragmentation and disarray of elastic fibres and accumulation of proteoglycans), vascular smooth muscle cell dysplasia and a less prominent inflammatory component without atheroma (Ladich et al., 2016; Pinard et al., 2019).

Heritable thoracic aortic aneurysm has been linked to more than 15 highly penetrant genes (Ostberg et al., 2020; Renard et al., 2018) that can be categorized clinically as either syndromic (associated with additional systemic features, such as Marfan syndrome [MFS] or Loeys-Dietz syndrome) or non-syndromic, showing an essentially vascular phenotype. Syndromic thoracic aortic aneurysm and dissection is linked to genes that encode extracellular matrix-related proteins or proteins involved in transforming growth factor (TGF)-β signalling, such as **asprosin (fibrillin 1; FBN1)**, **TGF-β receptor type II (TGFBR2)** or SMAD3 (Ostberg et al., 2020; Pinard et al., 2019). Familial non-syndromic thoracic aortic aneurysm and dissection is mainly associated with genes that encode structural proteins and kinases that control the contractile apparatus of smooth muscle cells, such as smooth muscle α-actin 2 (*ACTA2*), **myosin light chain kinase (MYLK)**, smooth muscle myosin heavy chain 11 (*MYH11*) and **protein kinase cGMP-dependent type 1 (PRKG1)** (Ostberg et al., 2020; Pinard et al., 2019).

Marfan syndrome (MFS), the classical syndromic form of thoracic aortic aneurysm and dissection, affects 1 in every 3000–5000 people (Pearson et al., 2008). Marfan syndrome patients show progressive dilation of the aortic root culminating in aortic dissection and rupture, which is the major cause of morbidity and mortality in these patients (Cañadas et al., 2010a). Marfan syndrome is an autosomal dominant connective tissue disorder caused by mutations in the *FBN1* gene on chromosome 15. The multiple manifestations of these *FBN1* mutations include oculo-musculo-skeletal abnormalities, ectopia lentis, pectus deformities, high stature or pulmonary emphysema with spontaneous pneumothorax.

Fibrillin-1 is a large (350-kDa) glycoprotein that is a major component of extracellular matrix microfibrils, providing a scaffold for elastic fibre formation and maturation (Sakai et al., 2016). *FBN1* mutations disorganize microfibril formation, ultimately destabilizing the aortic wall and rendering it vulnerable to haemodynamic damage (Romaniello et al., 2014). Marfan syndrome patients and mouse models show medial degeneration, which is characterized by poor elastic fibre alignment, disorganization of entire lamellar units, vascular smooth muscle cell apoptosis, and excessive accumulation of collagen (vascular fibrosis) and proteoglycans (Cañadas et al., 2010a; Cikach et al., 2018; Humphrey & Tellides, 2019; Ladich et al., 2016). The aortic walls of Marfan syndrome patients and mouse models show a consistent signature of increased TGF-β signalling (Dietz, 2010; Gomez et al., 2009), prompting the hypothesis that *FBN1* mutations caused aortic disease by perturbing the sequestration of the inactive TGF-β complex in the extracellular matrix (Neptune et al., 2003). However, mice lacking the TGF-β-binding site in *Fbn1* do not have Marfan syndrome symptoms (Charbonneau et al., 2010). Moreover, recent studies indicate that TGF-β is vasculoprotective in Marfan syndrome and that its blockade increases disease severity (Daugherty et al., 2017; Mallat et al., 2017). Although its precise contribution remains unclear, it thus seems that TGF-β has dual effects and its activation should be considered as a consequence rather than a cause of disease (Muiño-Mosquera & De Backer, 2019; Takeda et al., 2018).

Thoracic aortic aneurysm and dissection, in general, and Marfan syndrome, in particular, remain incurable. Although patient lifespan can be increased by prophylactic surgery, there are currently no effective pharmacological treatments for thoracic aortic aneurysm and dissection. There is therefore a need to identify therapeutic targets that would allow the development of effective pharmacological strategies to manage thoracic aortic aneurysm and dissection. In this context, our recent work showed that syndromic thoracic aortic aneurysm and dissection in mouse models of Marfan syndrome is caused by overactivation of the **NO** signalling pathway and identified potential targets for therapeutical intervention (de la Fuente-Alonso et al., 2021; Oller et al., 2017). Here, we review general mechanisms involved in NO signalling and focus on the mechanisms by which NO signalling dysregulation leads to thoracic aortic aneurysm and dissection. We discuss these findings in the context of discovering potential targets for intervention in human Marfan syndrome and identifying circulating biomarkers that could be useful for Marfan syndrome disease monitoring and clinical follow-up. In addition, we debate how drugs that chronically activate the NO pathway may be detrimental to aortic homeostasis.

2 | THE NO SIGNALLING PATHWAY

2.1 | NOS isoforms

NO is a small signalling molecule that, although initially described as an endothelium-derived relaxing factor, is essential in nearly all body systems. The diverse functions of NO are conditioned by the distribution, expression and regulation of the three nitric oxide synthases (NOSs): brain or **neuronal NOS (nNOS, encoded by NOS1)**, **inducible NOS (iNOS, encoded by NOS2)** and **endothelial NOS (eNOS, encoded by NOS3)**. NOS enzymes are homodimeric oxidoreductases that use **L-arginine** as the substrate in NO production (Stuehr & Haque, 2019). Changes in the concentrations of L-arginine and **BH4**

(sapropterin), an essential cofactor, or high levels of ROS can trigger 'NOS uncoupling', resulting in the production of superoxide anions instead of NO and thus altering the NO/ROS balance (Alkaitis & Crabtree, 2012; Crabtree & Channon, 2011; Otani, 2009).

eNOS is principally expressed in endothelial cells and eNOS-derived NO mediates vascular smooth muscle cell relaxation and vasodilation in small arteries (Palmer et al., 1988). eNOS is also present in cardiomyocytes (Petroff et al., 2001), platelets and some neurons (Farah et al., 2018; Wallerath et al., 1997). Although expressed constitutively, eNOS expression is regulated by epigenetic modifications, shear stress and ROS (Farah et al., 2018). Of note, eNOS *S*-glutathionylation leads to inactivation by eNOS uncoupling (Chen et al., 2010). *nNOS* is mainly expressed in neurons (Bredt et al., 1990), but it is also present in other cell types, including vascular smooth muscle cells (Boulanger et al., 1998), endothelial cells (Lührs et al., 2002) and cardiomyocytes (Xu et al., 1999). Indeed, the importance of nNOS in vascular physiology and disease has become increasingly recognized (Costa et al., 2016). The binding of eNOS and nNOS to **calmodulin (CaM)**, and therefore their activity, is regulated by Ca^{2+}. *iNOS* is mainly found in macrophages (Cho et al., 1992), but its expression can be stimulated in virtually any cell type, including leukocytes, fibroblasts, endothelial cells, vascular smooth muscle cells and cardiomyocytes (Farah et al., 2018; Oller et al., 2015). The very high affinity of iNOS for CaM ensures that these proteins interact at very low Ca^{2+} concentrations and that iNOS is therefore not regulated by Ca^{2+}. Consequently, once induced under oxidative or pro-inflammatory conditions, iNOS is constantly active.

2.2 | Signalling components downstream of NO

NO modulates BP and vascular tone through its canonical pathway, stimulating the **soluble guanylate cyclase (sGC)** and **PRKG1** (Figure 1a). However, NO can also regulate additional physiological processes in a sGC-independent manner (Figure 1b), including post-translational modification of proteins such as *S*-nitrosylation and, in the context of excessive ROS production, protein nitration (Radi, 2013).

2.2.1 | Canonical pathway

Upon activation, sGC catalyses the production of **cGMP**, which subsequently activates PRKG1 (Figure 1a). This kinase modulates

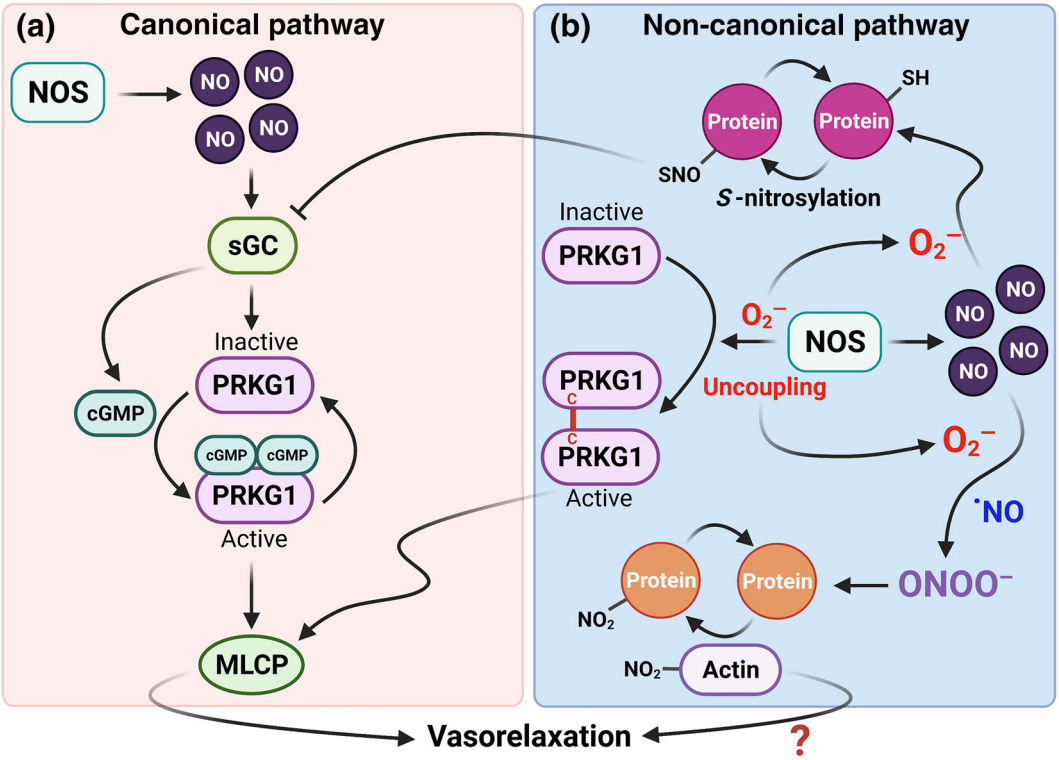

FIGURE 1 Canonical and non-canonical NO signalling pathways. In the canonical pathway (a), NOS-produced NO activates sGC, inducing cGMP production, which binds to and activates protein kinase cGMP-dependent type 1 (PRKG1). PRKG1 activation leads to vasorelaxation through various mechanisms, including the activation of myosin light chain phosphatase (MLCP). In the non-canonical pathway (b), superoxide anion (O_2^-), derived from NOS uncoupling among other sources, activates PRKG1 to induce vasorelaxation in a cGMP-independent manner by promoting PRKG1 dimerization through C42. Additionally, O_2^- and NO converge to generate peroxinitrite anion ($ONOO^-$), which is the intermediary molecule necessary to induce Tyr and Trp nitration of various proteins, including smooth muscle actin, a process that dysregulates actin polymerization dynamics and might induce vasorelaxation. *S*-nitrosylation of Cys residues in sGC by NO inhibits cGMP production in a negative feedback loop. Cartoon created with BioRender.com

several processes, such as actin filament and myosin dynamics and intracellular Ca^{2+} handling, leading to vasorelaxation (Lincoln et al., 2006).

sGC is a heterodimeric cytosolic enzyme formed by two homologous subunits, α and β, linked by a ferrous *b*-type **haem**. The haem domain, present in the β unit, is responsible for NO sensing, which activates the catalytic conversion of GTP to cGMP (Winger & Marletta, 2005). cGMP can induce vasodilation through the modulation of several downstream signalling components, including **cGMP-hydrolysing PDEs** (PDE5, PDE6 and PDE9), cGMP-dependent kinases such as PRKG1 and cGMP-gated ion channels (Murad, 2006; Warner et al., 1994). Prolonged exposure of sGC to NO results in its desensitization, which appears to be caused by the *S*-nitrosylation of certain Cys residues (Beuve et al., 2016; Mayer et al., 2009; Sayed et al., 2007). sGC is also inactivated by pro-oxidative conditions, because oxidation of the haem domain interrupts NO signalling, an event that is overridden by BH4 supplementation (Schmidt et al., 2012).

PRKG1/PKG1 is one of the two cGMP-dependent serine/threonine kinases present in mammals; PRKG1 is a ubiquitous protein mainly expressed in smooth muscle cells, cerebellum and platelets, whereas **PRKG2** is present in intestinal mucosa, lung, brain and kidney (Kuo, 1974; Lohmann et al., 1997; Pfeifer et al., 1998). Cellular processes regulated by PRKG1 include platelet activation and aggregation (Massberg et al., 1999) and smooth muscle relaxation (Sausbier et al., 2000). The PRKG1 NH_2-terminal domain participates in interactions with other proteins or dimerization, and the enzyme also contains a regulatory domain with two cGMP-binding sites and the catalytic domain (Hofmann et al., 2006). PRKG1 is regulated by several transcriptional, post-transcriptional and post-translational mechanisms (Francis et al., 2010; Sellak et al., 2013) and can be activated via direct oxidation by ROS (Burgoyne et al., 2007).

2.2.2 | Non-canonical pathway

NO can also regulate many cardiovascular processes in a cGMP-independent manner, triggering *N*-nitrosylation and nitrosative stress or overproducing superoxide anion upon NOS uncoupling, which in combination with high NO levels generates peroxynitrite (Figure 1b) (Alkaitis & Crabtree, 2012; Crabtree & Channon, 2011; Otani, 2009; Rochette et al., 2013). Peroxynitrite induces nitrosative stress accompanied by protein nitration, oxidative damage and the activation of several signalling pathways (Farah et al., 2018; Förstermann & Sessa, 2012; Kraehling & Sessa, 2017; Palacios-Callender et al., 2004). Interestingly, we recently found that numerous tyrosine residues in smooth muscle actin are nitrated in the aortas of Marfan syndrome mice (de la Fuente-Alonso et al., 2021), a feature that might contribute to aortic dysregulation in Marfan syndrome, because actin nitration impairs actin polymerization dynamics (Aslan, 2012).

Some studies have reported interactions between the canonical and non-canonical signalling effects of NO (Wu et al., 2018). As mentioned, PRKG1α is directly activated by disulfide dimerization, a process that takes place under pro-oxidant conditions (Burgoyne et al., 2007; Prysyazhna & Eaton, 2015) (Figure 1b). As discussed below, this regulatory mechanism might play a critical role in cardiovascular homeostasis, contributing to processes such as BP regulation and cell adhesion and migration by pulmonary vascular smooth muscle cells (Negash et al., 2009; Prysyazhna & Eaton, 2015).

2.2.3 | PRKG1 downstream signalling

PRKG1, the key component of both canonical and non-canonical pathways, reduces smooth muscle cell contractility through various mechanisms (Figure 2), including by directly phosphorylating telokin, RhoA and the myosin light chain phosphatase (MLCP) subunit MYPT1 (myosin phosphatase target subunit 1) (Ellerbroek et al., 2003; Sawada et al., 2001; Walker et al., 2001; Wooldridge et al., 2004). PRKG1-mediated phosphorylation of RhoA-Ser-188 hampers the activation **of Rho-associated protein kinase (ROCK)** and, consequently, the ROCK-mediated inactivating phosphorylation of MYPT1-Thr-696 (Wooldridge et al., 2004). MYPT1-Ser-695 phosphorylation by PRKG1 has no direct effect on MLCP phosphatase activity but protects MYPT1 from inactivating phosphorylation by ROCK or MYPT1-associated kinase (MYPT1K) (Nakamura et al., 2007; Wooldridge et al., 2004). Together, these mechanisms contribute to sustaining MLCP in an active state and therefore maintaining myosin light chain unphosphorylated and in its relaxed state (Somlyo, 2007).

NO also mediates vasorelaxation by modulating Ca^{2+} handling in vascular smooth muscle cells (Figure 2). This is achieved by PRKG1α-mediated decreases in total Ca^{2+} influx through the activation of **Ca^{2+}-activated K^+ (BKCa) channels** (Barman et al., 2003; Fukao et al., 1999), reductions in Ca^{2+} release from **inositol trisphosphate (IP3)** through inositol 1,4,5-triphosphate receptor associated 1 (IRAG1) phosphorylation (Vanderheyden et al., 2009) and increases in Ca^{2+} import to the sarcoplasmic reticulum through phospholamban phosphorylation (Koller et al., 2003; Schlossmann et al., 2000). These processes lower vascular smooth muscle cell intracellular Ca^{2+} concentration, leading to vasorelaxation (Sausbier et al., 2000). Moreover, NO regulates vascular smooth muscle cell proliferation and cytoskeleton remodelling through the PRKG1 downstream target vasodilator-stimulated phosphoprotein (VASP) (Chen et al., 2004; Defawe et al., 2010).

3 | NO IN AORTIC ANEURYSM

eNOS is the NOS in charge of equilibrating cardiovascular homeostasis and disease. Accordingly, eNOS dysfunction is widely related to cardiovascular disease and risk factors (Garcia & Sessa, 2019). nNOS and iNOS also play crucial roles in some cardiovascular disorders, such as hypertension, atherosclerosis, myocardial infarction and heart failure (Lee et al., 2016). In this section, we focus on the implication of NO in abdominal aortic aneurysm and thoracic aortic aneurysm, leaving its implication in Marfan syndrome for a later section.

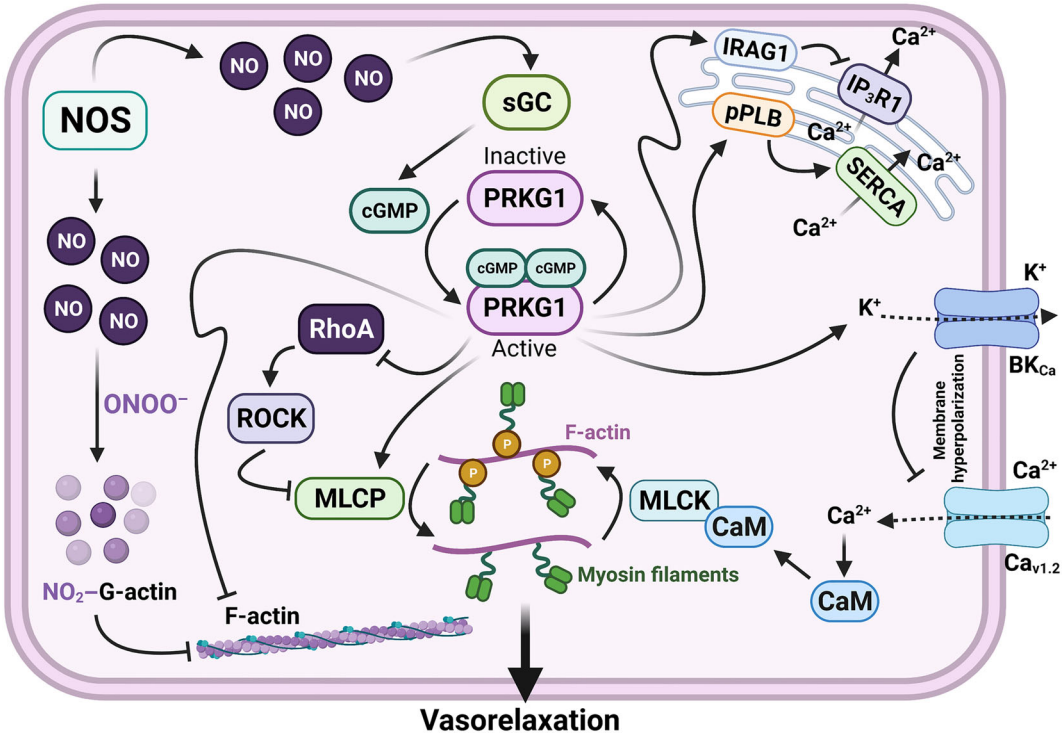

FIGURE 2 NO induces vasorelaxation by inhibiting the contractile machinery of vascular smooth muscle cells. sGC activated by NOS-derived NO produces cGMP. protein kinase cGMP-dependent type 1 (PRKG1) activation by cGMP leads to vasorelaxation through the phosphorylation of multiple targets. PRKG1 reduces smooth muscle cell (SMC) contractility by directly phosphorylating MYPT1 on Ser-695, impairing the MYPT1-mediated inhibitory phosphorylation of ROCK on Thr-696 and therefore maintaining MLCP active. PKKG1-mediated phosphorylation of RhoA on Ser-188 impairs RhoA activation of ROCK. As a result, MLCP remains active and dephosphorylates the myosin regulatory light chain of myosin filaments, reducing SMC contractility. PRKG1 modulates intracellular Ca^{2+} concentration through complementary mechanisms: (i) inositol 1,4,5-triphosphate receptor associated 1 (IRAG1) phosphorylation suppresses Ca^{2+} release from the sarcoplasmic reticulum (SR) to the cytosol via **IP_3R1**; (ii) phospholamban (PLB) phosphorylation stimulates Ca^{2+} intake in the SR through **sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA)**; (iii) phosphorylation of BKCa channels activates them, causing smooth muscle cell (SMC) hyperpolarization and consequently a decrease in Ca^{2+} import from the extracellular milieu through voltage-dependent **$Ca_V1.2$** channels. Low cytosolic Ca^{2+} impairs myosin light chain kinase (**MLCK**) activation through Ca^{2+}-activated calmodulin (CaM) binding, thus contributing to the maintenance of underphosphorylated myosin filaments. Additionally, NO, together with ROS, produces peroxynitrite anion ($ONOO^-$), which elicits G-actin nitration, potentially altering actin polymerization dynamics and therefore contributing to NO-mediated vasorelaxation. Cartoon created with BioRender.com

3.1 | NO regulates vascular smooth muscle cell phenotype

Under normal conditions, activation of NO–sGC–PRKG1 signalling leads to vasodilation. The NO produced in endothelial cells by eNOS diffuses to vascular smooth muscle cells, activating sGC–PRKG1 signalling (Ataei Ataabadi et al., 2020). NO can also be provided by neurons or inflammatory cells through the action of nNOS and iNOS, respectively (Chachlaki & Prevot, 2020; Cho et al., 1992; Farah et al., 2018), and, remarkably, by vascular smooth muscle cells themselves (Hecker et al., 1999; Nettersheim et al., 2021; Oller et al., 2017). To vasodilate, vascular smooth muscle cells need to be quiescent and contractile, a state known as the contractile phenotype. After mechanical damage or biochemical stimulation, vascular smooth muscle cells undergo a phenotypic switch to a synthetic phenotype, characterized by a highly proliferative and low contractile profile and the overproduction of extracellular matrix (Alexander & Owens, 2012). The ability of vascular smooth muscle cells to respond to stimuli and switch from one phenotype to other is known as phenotypic plasticity.

It is widely accepted that the NO–sGC–PRKG1 pathway modulates the vascular smooth muscle cell phenotype switch. NO pathway activation has been reported to impact the vascular smooth muscle cell phenotype by promoting cell adhesion via the inhibition of RhoA GTPase and the cell-surface up-regulation of β1 and β3 integrins (Weinmeister et al., 2008). However, there is controversy about whether these events inhibit or promote vascular smooth muscle cell proliferation (Dey et al., 2005; Feil et al., 2005; Lincoln et al., 2006). The first study on this topic reported that NO donors and the cGMP analogue 8-Br-cGMP inhibited proliferation of rat vascular smooth muscle cells (Garg & Hassid, 1989). Subsequent studies showed that adenovirus-mediated gene transfer of *PRKG1* in combination with the administration of NO donors or 8-Br-cGMP activated pro-apoptotic signalling in cultured vascular smooth muscle cells (Chiche et al., 1998). Seemingly in contrast, cGMP was shown to amplify **FGF-2**-induced proliferation of primary rat vascular smooth muscle cells

(Hassid et al., 1994), whereas another study claimed that sGC–PRKG1 signalling activates MAPKs, leading to increased proliferation of freshly isolated rat vascular smooth muscle cells. Similarly, vascular smooth muscle cell proliferation was increased by forced expression of VASP (Chen et al., 2004). These conflicting findings on the role of NO signalling in vascular smooth muscle cell proliferation may reflect the use of primary versus subcultured cells (Lehners et al., 2018), because it is known that cultured cells have a predominantly synthetic profile, less similar to homeostatic vascular smooth muscle cells in vivo (Chamley-Campbell et al., 1979).

A number of studies have provided evidence for an influence of NO–sGC–PRKG1 pathway components on abdominal aortic aneurysm and thoracic aortic aneurysm and dissection, with *iNOS* in particular being linked to abdominal aortic aneurysm and inflammation. NO has been implicated in vessel dilation in mouse models of cerebral aneurysm and abdominal aortic aneurysm, but reports provide contradictory data depending on whether pharmacological or targeted gene deletion approaches were used (Fukuda et al., 2000; Johanning et al., 2001, 2002; Kuhlencordt et al., 2001; Lee et al., 2001; Sadamasa et al., 2003). Moreover, contradictory data have even been obtained with similar experimental approaches. In the case of iNOS, diverse reports have shown either inhibitory or stimulatory roles in abdominal aortic aneurysm models (Johanning et al., 2001, 2002; Kuhlencordt et al., 2001; Lee et al., 2001). Contrasting results on the role of iNOS have also been reported in cerebral aneurysm. Pharmacological inhibitors of iNOS indicate a critical role in its development (Fukuda et al., 2000), whereas, in genetic studies, *Nos2*$^{-/-}$ mice had a similar incidence of cerebral aneurysm to wild-type (WT) mice (Sadamasa et al., 2003). In abdominal aortic aneurysm patients, increased iNOS expression has been found in the aortic media and adventitia, particularly in macrophages, lymphocytes and vascular smooth muscle cells, and has been proposed to promote oxidative vascular injury (Liao et al., 2006; Zhang et al., 2003).

Recent studies have revealed a major role of iNOS in thoracic aortic aneurysm, with iNOS and NO levels both strongly increased in the aortas of mice deficient in the Adamts1 metalloproteinase (*Adamts1*$^{+/-}$ mice), which develop a syndromic form of AA similar to Marfan syndrome (Oller et al., 2017). In this case, genetic and pharmacological studies both demonstrate involvement of iNOS in aortic dilation and medial degeneration. NOS inhibition with L-NAME prevented the aortopathy induced by Adamts1 knockdown and fully reversed aortic dilation in mice with established aortic disease. Similarly, genetic approaches indicated that *Nos2*$^{-/-}$ mice are resistant to the development of aortopathy upon Adamts1 silencing (Oller et al., 2017). Thus, the pathological role of NO in this model is mediated by iNOS, which is not normally expressed in resting cells but is strongly induced transcriptionally upon ***Adamts1*** silencing *in vivo* (Oller et al., 2017).

Some studies pointed to TGF-β as a critical mediator of thoracic aortic aneurysm pathology (Dietz, 2010; Gomez et al., 2009), but further research suggested an early protective role of TGF-β in a mouse model of Marfan syndrome (Cook et al., 2015). Moreover, neutralizing antibodies against TGF-β prevented neither the aortic dilation nor the medial degeneration in a mouse model of thoracic aortic aneurysm induced by *Adamts1* silencing (Oller et al., 2017). Furthermore, whereas iNOS is induced early during disease onset, TGF-β signalling only occurs in late stages of the disease in this mouse model (Oller et al., 2017), strongly suggesting a secondary role for TGF-β activation in aortopathy onset.

eNOS has also been linked to thoracic aortic aneurysm and abdominal aortic aneurysm. Research on eNOS in thoracic aortic aneurysm showed that its expression is reduced in aneurysmal versus control human aortas (Pimiento et al., 2008), suggesting a protective role. However, the thoracic aortic aneurysm samples analysed in this study came from older patients than those from control donors. A mouse study using animals deficient in the eNOS cofactor BH4 showed that eNOS uncoupling promoted abdominal aortic aneurysm formation upon treatment with **angiotensin II (Ang II)** (Gao et al., 2012). Interestingly, oral administration of **folic acid**, which recouples eNOS by increasing **dihydrofolate reductase (DHFR)** function, completely prevented Ang II-induced abdominal aortic aneurysm formation and progressive eNOS uncoupling. Abdominal aortic aneurysm was also prevented by eNOS recoupling upon DHFR overexpression, suggesting that abdominal aortic aneurysm involves abnormal eNOS function (Gao et al., 2012). A later study reproduced the protective role of folic acid in the Ang II-infused *ApoE*$^{-/-}$ null mouse abdominal aortic aneurysm model, sharply reducing abdominal aortic aneurysm incidence and restoring NO bioavailability (Siu et al., 2014). Taken together, these results suggest that normal eNOS function protects against abdominal aortic aneurysm formation.

Little is known about the contribution of *sGC* to aortic aneurysm, but increased expression of non-functional sGC splice variants has been found in aortic aneurysm patients (Martin et al., 2014). In contrast, more is known about the role of *PRKG1* in aortic aneurysm. Together with *MYH11*, *MYLK* and *ACTA2*, *PRKG1* is one of the few key genes in which mutations are linked to familial non-syndromic thoracic aortic aneurysm and dissection (Ostberg et al., 2020). Indeed, the gain-of-function Arg177Gln mutation in *PRKG1* has been detected in familial non-syndromic thoracic aortic aneurysm and dissection (Guo et al., 2013). This mutation induces a structural change that impairs the ability of the N-terminal regulatory domain to inhibit PRKG1 activity in the absence of cGMP, rendering the kinase constitutively active (Guo et al., 2013). Increased PRKG1 activity leads to decreased phosphorylation of the myosin regulatory light chain, causing vascular smooth muscle cell relaxation. Among patients harbouring this variant, 63% have acute aortic dissections at mean age of 31 years, and 37% have aortic root enlargement (Guo et al., 2013). The same PRKG1 variant was also linked to thoracic aortic aneurysm and dissection in an independent study (Gago-Díaz et al., 2016), and an additional variant in the PRKG1 ATP-binding motif, Gly370Ser, was linked to aortic dissection in another report (Zhang et al., 2018). Remarkably, a knock-in mouse model bearing the Arg177Gln variant developed aortic dilation and showed increased elastic fibre fragmentation, apoptosis and oxidative stress in aortic tissue, and these features were prevented by administrating the antioxidant vitamin B12 analogue cobinamide

(Schwaerzer et al., 2019). However, these mice did not develop aortic dissections unless they were subjected to transverse aortic constriction.

4 | NO–sGC–PRKGI SIGNALLING IN MARFAN SYNDROME

In the past few years, several studies have linked NO to Marfan syndrome aortopathy, establishing implications in endothelial dysfunction, arterial wall degeneration and aneurysm formation (Chung et al., 2007; Krishna et al., 2012; Lomelí et al., 2018; Yang et al., 2010). However, the mechanisms by which NO mediates pathological aortic remodelling remain unclear.

4.1 | NO signalling in Marfan syndrome

Myography-based studies suggest that NO might be involved in aortopathy through the non-canonical pathway, with abnormal NO levels and eNOS uncoupling stimulating the generation of ROS that impair aortic contractility and endothelium-dependent relaxation (Yang et al., 2010). We later showed that iNOS is overexpressed in the aortic medial layer of Marfan syndrome mice (*Fbn1*$^{C1039G/+}$), particularly in smooth muscle cells (Oller et al., 2017), and similar results have been published recently by another group (Nettersheim et al., 2021). As in the aortopathy in *Adamts1*-deficient mice, we found that NO is also a signature of Marfan syndrome. Our analysis detected high NO levels in the aortas of Marfan syndrome mice and showed that pharmacological iNOS inhibition with **1400W** reversed Marfan syndrome-associated aortopathy. These results suggest that iNOS-mediated NO production plays an essential role in Marfan syndrome and in the aorthopathy triggered by *Adamts1* deficiency. These two aortic diseases are likely to be mechanistically linked, because Adamts1 deficiency is a feature of Marfan syndrome mice. Indeed, we propose that *Adamts1* deficiency might account for some or all of the aortic phenotype of Marfan syndrome mice. More importantly, elevated iNOS expression is detected in the aortas of Marfan syndrome patients, whereas *ADAMTS1* is markedly down-regulated (Oller et al., 2017), suggesting that this down-regulation may underlie the aortic phenotype of Marfan syndrome patients (Figure 3). Although the precise mechanisms leading to NO production in Marfan syndrome remain to be elucidated, iNOS activation may turn out to be a common process in other diseases that occur with aortic dilation and

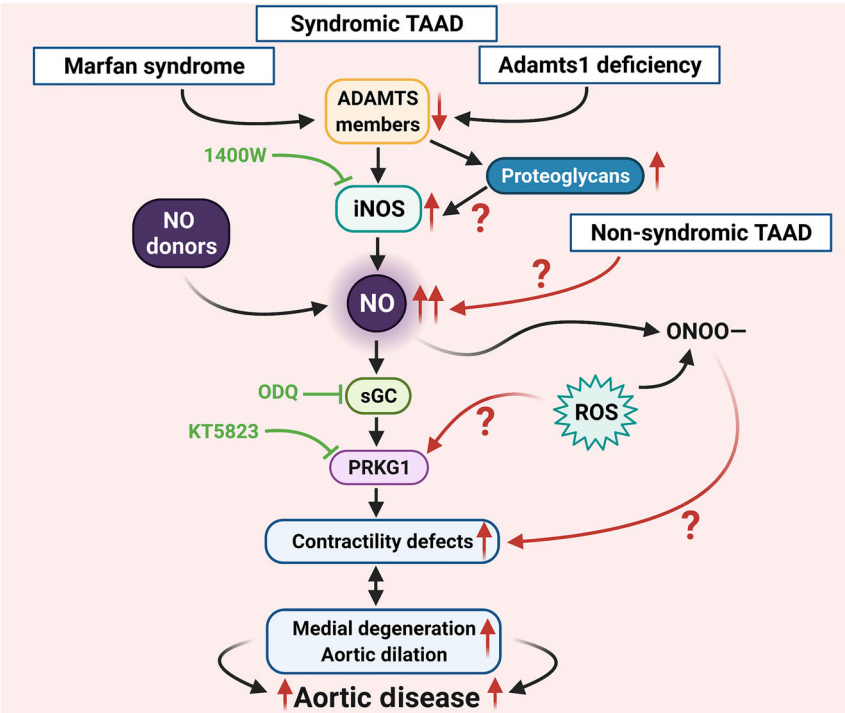

FIGURE 3 Proposed NO signalling pathways in thoracic aortic aneurysm and dissection (TAAD). In *Adamts1*-deficient mice and Marfan syndrome mouse models and patients, low levels of ADAMTS members in aortic tissue lead to iNOS overexpression through mechanisms that, although not fully characterized, might involve proteoglycan accumulation. Activation of the canonical pathway by a sharp increase in NOS-derived NO or by administration of high doses of NO donors causes contractility defects, which ultimately lead to aortic dilation and medial degeneration. The extracellular matrix remodelling characteristic of medial degeneration might further hamper vascular smooth muscle cell (VSMC) contractility in a positive feedback loop. ROS might also contribute to aortic disease in thoracic aortic aneurysm and dissection (TAAD) by directly activating protein kinase cGMP-dependent type 1 (PRKG1) or by dysregulating actin cytoskeleton dynamics through actin nitration. Whether NO mediates aortopathy in non-syndromic TAAD remains unknown. Pharmacological inhibition of iNOS (with 1400W), sGC (**ODQ**) and PRKG1 (KT5823) regresses aortic disease in mouse models of TAAD. Cartoon created with BioRender.com

medial degeneration. In line with an essential role of NO in Marfan syndrome, another study found that serum levels of the NO-derived metabolites NO_3^- and NO_2^- were significantly higher in Marfan syndrome patients (Lomelí et al., 2018). Increased ROS levels and tyrosine nitration (a post-translational modification induced by NO in pro-oxidant conditions) were reported in aneurysmal aortic tissue of Marfan syndrome patients and in cultured vascular smooth muscle cells (Jiménez-Altayó et al., 2018). Moreover, **NADPH oxidase 4 (NOX4)** gene deletion in the *Fbn1*$^{C1039G/+}$ Marfan syndrome mouse model prevented aortopathy progression, as revealed by the presence of less fragmented elastic fibres and milder aortic root dilation (Jiménez-Altayó et al., 2018). Elevated ROS production was also observed in the mg$\Delta^{loxPneo}$ Marfan syndrome mouse model. However, these mice did not show reduced aortic dilation and elastic fibre fragmentation upon inhibition of ROS production with the anti-oxidant **lipoic acid** (Guido et al., 2018).

The presence of high ROS levels in two independent Marfan syndrome mouse models suggested the possibility that medial iNOS produces superoxide anion rather than NO in Marfan syndrome. However, the elevated circulating cGMP in Marfan syndrome patients and mice provides evidence of sGC overactivation and therefore of NO overproduction (de la Fuente-Alonso et al., 2021). The same study reported elevated VASP-S239 phosphorylation in aortic tissue from Marfan syndrome patients and mice that was reversed upon pharmacological PRKG inhibition or lentiviral-mediated *Prgk1* silencing in Marfan syndrome mice, indicating that not only sGC but also PRKG1 is overactivated in Marfan syndrome. Further supporting supraphysiological production of NO in Marfan syndrome, nitrated protein levels in Marfan syndrome patients and mouse plasma and in Marfan syndrome mouse aorta were higher than in control samples (de la Fuente-Alonso et al., 2021). Moreover, the same study reported that NO is not only necessary but also sufficient for Marfan syndrome-associated aortopathy, because treatment of healthy WT mice with high doses of NO donors (**isosorbide mononitrate** or DetaNONOate [DetaNO]) induced ascending and abdominal aortic dilation and medial degeneration similar to the phenotype of age-matched Marfan syndrome mice (Figure 3).

The influence of sex on thoracic aortic aneurysm has become particularly evident in Marfan syndrome patients as several studies found that men are at higher risk than women for aortic dilatation and dissection or for needing prophylactic surgery (Detaint et al., 2010; Franken et al., 2016; Renard et al., 2017). Accordingly, increased risk of aortic events in males has been observed also in a mouse model of Marfan syndrome and in mice deficient in activin receptor-like kinase 5 (Alk5) now known as *Tgfbr1* (Renard et al., 2017; Schmit et al., 2015). Importantly, women with Marfan syndrome should have cardiovascular assessment prior to pregnancy, particularly those with a dilating aortic root, because the risk of aortic dissection increases notably during pregnancy and up to several months postpartum (Milewicz et al., 2021). Similarly, pregnancy adversely affects aortic disease in a mouse model of Marfan syndrome with multiparous female mice showing aortic diameters and elastic fibres fragmentations comparable with those in male mice (Renard et al., 2017). Although we have not specifically investigated whether the NO–sGC–PRKG1 signalling pathway is more active in males or females, we found that inhibition of this pathway regresses aortic disease in both (de la Fuente-Alonso et al., 2021). These results therefore urge to use mouse models for comparing the activation degree of the NO pathway in males and females and in pregnancy.

Because NO–sGC–PRKG1 signalling causes vasorelaxation, its overactivation might account for the mild hypotension observed in *Fbn1*$^{C1039G/+}$ Marfan syndrome mice (de la Fuente-Alonso et al., 2021; Oller et al., 2017). Intriguingly, normal BP in these mice was achieved upon PRKG1 inhibition and *Prkg1* silencing, but not following sGC inhibition (de la Fuente-Alonso et al., 2021). This potential discrepancy might be attributable to the sGC-independent oxidation of PRKG1 on cysteine 42, which maintains the enzyme active despite sGC inhibition (Burgoyne et al., 2007). PRKG1 is directly activated by disulfide dimerization, a process that takes place under pro-oxidant conditions (Prysyazhna & Eaton, 2015). *Prkg1*^{Cys42Ser} knock-in mice cannot form interprotein disulfide bonds and are hypertensive (Prysyazhna et al., 2012). Therefore, we propose that PRKG1-mediated hypotension results not only from canonical sGC–PRKG1 signalling but also from the pro-oxidant conditions reported in Marfan syndrome.

4.2 | Biomarkers in Marfan syndrome patients

Despite advances in the understanding of the processes underlying inherited thoracic aortic aneurysm and dissections, translating these findings into new therapies or diagnostic approaches is still at an early stage. Aortic dissection and rupture are the major causes of morbidity and mortality in Marfan syndrome, and most thoracic aortic aneurysms are silent until dissection occurs. Furthermore, treatment is associated with a risk of post-surgery complications. Unfortunately, dissection very often occurs when the aortic diameter is below the recommended threshold for elective prophylactic surgery (Pape et al., 2007). Aorta size is a poor predictor of dissection risk and efforts are therefore needed to identify biomarkers of the degree of vessel-wall deterioration, thus providing a sound prediction of the risk of dissection as the basis of the decision to operate.

Several studies have used proteomics approaches to analyse differentially expressed proteins in human thoracic aortic aneurysm (Pilop et al., 2009; Serhatli et al., 2014). However, the thoracic aortic aneurysm molecular profiles generated in these studies are limited. To our knowledge, no previous studies have used high-throughput quantitative approaches to achieve a dynamic analysis of the deep proteome in specific studies of patients with syndromic or non-syndromic aortic disease. Given its potential role in the progression of Marfan syndrome aortopathy, circulating TGF-β is an attractive biomarker of disease progression and for guiding medical treatment. Nevertheless, further studies are needed to ensure reliability (adjusting for platelet activation) and international consistency (Fletcher et al., 2020). Similarly, several studies have established the use of **MMP-2** and **MMP-9** as prognostic biomarkers of aortic disease severity (Ikonomidis

et al., 2005; Shen et al., 2015). Elevated plasma MMP-2 has been linked to enhanced aortic diameter and aortic stiffness, although no studies to date have shown links to outcomes (Tzemos et al., 2010; Wang et al., 2010).

Our findings showing that aortopathy in Marfan syndrome is mediated by NO pathway overactivation may allow the identification of prognostic and diagnostic biomarkers. For example, we found that plasma levels of cGMP are up-regulated in Marfan syndrome mice and in various Marfan syndrome patient cohorts (de la Fuente-Alonso et al., 2021). Circulating cGMP appears to be a faithful readout of sGC activity and can be easily measured in plasma or serum. High-throughput proteomics revealed that the high NO levels in Marfan syndrome result in nitration of tyrosine and tryptophan (stable post-translational modifications induced by NO) in plasma proteins from Marfan syndrome patients and mice (de la Fuente-Alonso et al., 2021). The same study also showed that pVASP-S239, a marker of PRKG activation, is increased in the aortas of Marfan syndrome mice and patients (de la Fuente-Alonso et al., 2021). Future experiments will be required to determine if pVASP-S239, analysed in samples from patients undergoing prophylactic aortic surgery, is a prognostic indicator of their risk of subsequent type B dissection.

It is important to note that elevated cGMP has been reported in other diseases (Chawla et al., 1980; Ogawa et al., 1984; Reginauld et al., 2019), and it is likely that protein nitration is also a feature of other diseases that occur with NO pathway activation, such as infection, inflammation, allergy and sepsis (Bae et al., 2011; Sanyal et al., 2017). Therefore, although the identity of the nitrated plasma proteins may differ between conditions, plasma cGMP and tyrosine/typtophan-nitrated proteins might find use less as specific diagnostic Marfan syndrome biomarkers and more as tools for monitoring and clinical follow-up. Future clinical studies in large cohorts will be needed to assess the suitability of these markers for monitoring or predicting the course of disease and for determining the efficacy of future treatments.

4.3 | Novel treatments for aortic disorders in Marfan syndrome patients

Because aortic dissection is the main cause of morbidity and mortality in Marfan syndrome, efforts must focus on decreasing the risk of aortic rupture. Unfortunately, current medical treatment neither halts abnormal aortic growth nor prevents aortic dissection or death, and the only available measures are life-style adaptation, routine monitoring of the rate of aortic growth and surgical repair of the aorta once the aneurysm grows beyond a critical threshold (Gersony et al., 2007; Keane & Pyeritz, 2008).

Conventional therapies have focused on reducing aortic wall stress by lowering systemic vascular resistance and BP (Fletcher et al., 2020; Keane & Pyeritz, 2008). The first-line in the prevention of aortic complications in Marfan syndrome is treatment with β-adrenoceptor antagonists (β-blockers). These drugs have been used in clinical practice since 1971 and their potential benefits in Marfan syndrome are attributed to a reduction in haemodynamic stress in the proximal aorta (Cañadas et al., 2010b; Keane & Pyeritz, 2008; Pearson et al., 2008). Several clinical trials and retrospective studies have been conducted to determine whether β-adrenoceptor antagonists slow the progression of aortic dilation. In 1994, a randomized trial of β-adrenoceptor antagonists treatment in Marfan syndrome patients suggested that **propranolol** decreased the rate of aortic root growth and reduced aortic complications (Shores et al., 1994). However, a later meta-analysis showed no clinical benefit of β-adrenoceptor antagonists therapy in Marfan syndrome patients (Gersony et al., 2007) and additional studies suggested that β-adrenoceptor antagonists do not even slow the rate of aortic growth in Marfan syndrome (Phomakay et al., 2014; Selamet Tierney et al., 2007; Yetman et al., 2005).

An alternative pharmacological treatment suggested for Marfan syndrome is calcium channel blockers, owing to the effective drop in BP with these drugs. However, there have been calls for this suggestion to be reconsidered because calcium channel blockers cause deleterious effects in Marfan syndrome mice and increase the risk of aortic dissection and the need for aortic surgery in patients (Doyle et al., 2015).

The preponderance of markers of excessive TGF-β signalling in the aortas of Marfan syndrome patients and mouse models, and the possibility that these markers might be induced by excessive angiotensin II signalling, prompted the investigation of the therapeutic potential of angiotensin receptor antagonists and angiotensin-converting enzyme inhibitors (ACEIs) in Marfan syndrome.

ACEIs inhibit the renin-angiotensin system (RAS) by blocking the conversion of **Ang I** to Ang II, leading to decreased signalling through both **AT_1** and **AT_2** receptor pathways. In this context, blockade of AT_2, but not AT_1 receptor, reduced apoptosis in the aortic wall of Marfan syndrome patients, suggesting that AT_2 antagonists could provide extra protection of the aortic wall in patients treated with ACEIs (Cañadas et al., 2010b). However, further studies suggested that AT_2 receptor signalling attenuates aortic aneurysm (Habashi et al., 2011). Additional studies are therefore needed to determine the clinical implications of simultaneous blockade of both receptors in Marfan patients.

Several studies have demonstrated that the AT_1 antagonist **losartan** reduces the rate of aortic root dilatation in rodent models of Marfan syndrome (Habashi et al., 2006) and in children with severe Marfan syndrome (Brooke et al., 2008). Indeed, losartan is recommended by current treatment guidelines because of its unique anti-remodelling properties (Habashi et al., 2006), which are not observed with β-adrenoceptor antagonists or ACEIs (Phomakay et al., 2014). However, the promising results obtained in mice have not been reproduced in human studies, which demonstrated no significant effects on clinical endpoints. For instance, in a large randomized trial of 5- to 60-year-old Marfan syndrome patients, losartan failed to slow aortic enlargement more effectively than conventional treatment with atenolol after 3 years of follow-up (Forteza et al., 2016). However, a similar study with a median follow-up period of 8 years showed a decreased number of composite clinical endpoints in

Marfan syndrome patients treated with a combination of losartan and a β-adrenoceptor antagonist (van Andel et al., 2020). The apparent discrepancy between these studies may be attributable to the longer follow-up period and the highly aggressive disease in the patients selected for the later study. Recently, another AT_1 antagonist, **irbesartan**, was found to modestly reduce aortic root growth in children and young adults (Mullen et al., 2019). Although irbesartan and losartan both block AT_1 receptor, irbesartan has a longer half-life and higher bioavailability, suggesting that the difference in their effects may be in part due to an insufficient duration of the action of losartan. Although these studies suggest that AT receptor antagonists might offer some clinical benefit to Marfan syndrome patients, the results do not fulfil the expectations based on the impressive results in Marfan syndrome mouse models. Thus, strong incentives remain to find alternative therapeutic strategies to prevent acute aortic dissection and the associated mortality and morbidity in Marfan syndrome patients.

Our results indicate that aortic homeostasis depends on the precise maintenance of NO levels and signalling in the aorta. We have shown that acute NO-pathway stimulation of WT mice with high doses of NO donors (isosorbide mononitrate or DetaNO) suffices to induce an Marfan syndrome-like aortopathy, triggering aortic dilation and elastic fibre fragmentation (de la Fuente-Alonso et al., 2021). Current therapies for angina, heart failure, pulmonary hypertension and erectile dysfunction are based on activating NO signalling, either by increasing NO production and bioavailability or by targeting signalling elements downstream of NO production (Farah et al., 2018). Accumulated clinical experience strongly suggests that patients treated with nitrates (NO donors), the PDE5 inhibitor **sildenafil** or sGC activators (such as **ataciguat**, **vericiguat**, **riociguat** and TY-55002) do not develop harmful aortic dilation. In this regard, sildenafil failed to affect aortic root dilation in Marfan syndrome mice (White et al., 2019). However, our observations with high NO-donor doses in mouse models (de la Fuente-Alonso et al., 2021) suggest that these patients might be at risk of mild aortic dilation and warrant consideration of the potential detrimental effects on aortic homeostasis of drugs that chronically activate the NO–sGC–PRKG signalling pathway.

We reported that iNOS is induced in vascular smooth muscle cells from Marfan syndrome mice and patients and, more importantly, that pharmacological iNOS inhibition rapidly reverses aortic dilation and medial degeneration in Marfan syndrome mice and *Adatms1*-deficient mice (Oller et al., 2017). These findings constituted the first proof of concept that NO signalling blockade could be an effective treatment for thoracic aortic aneurysm and dissection in humans. Indeed, the powerful and extremely fast action of iNOS inhibition in reversing aortopathy in mouse models urges the evaluation of iNOS inhibitors in preclinical and clinical trials for the treatment of Marfan syndrome. Because iNOS inhibitors have been found to be safe in clinical trials for other diseases, trials for Marfan syndrome treatment could be implemented with minimal delay. Recent findings from our laboratory have shed light on how the NO–sGC–PRKG signalling pathway mediates aortopathy in a mouse model of Marfan syndrome and identified sGC and PRKG1 as potential therapeutic targets for intervention in human Marfan syndrome (de la Fuente-Alonso et al., 2021). These findings are consistent with recent studies demonstrating that a gain-of-function mutation in *PRKG1* predisposes to thoracic aortic aneurysm and dissection by disrupting the contractile unit (Guo et al., 2013; Schwaerzer et al., 2019), suggesting that NO–sGC–PRKG signalling might be an essential factor not only in Marfan syndrome but also in non-syndromic familial thoracic aortic aneurysm and dissection. In an apparent paradox, Marfan syndrome mice expressing constitutively active eNOS ($Nos3^{S1176D}$) or overexpressing eNOS showed a slower rate of aortic growth than control Marfan syndrome mice, suggesting that endothelial NO release might be beneficial for the aorta in Marfan syndrome mice (Sellers et al., 2018). Additional research to integrate these apparently conflicting results is undoubtedly required; however, the $Nos3^{S1176D}$ mice have not been made available to us (Kashiwagi et al., 2013).

A causal role for sGC and PRKG1 activation in Marfan syndrome aortopathy is supported by the regression of aortic dilation and medial degeneration in Marfan syndrome mice treated with a lentivirus that knocks down aortic *Prkg1* expression or with pharmacological inhibitors of sGC or PRKG1 (de la Fuente-Alonso et al., 2021). This result strongly suggests that these inhibitors would be useful for the treatment of patients with Marfan syndrome or even other forms of thoracic aortic aneurysm and dissection. Another interesting strategy for these patients would be the use of a long-acting siRNA able to block aortic iNOS or PRKG1 expression.

5 | CONCLUSIONS AND FUTURE DIRECTIONS

Thoracic aortic aneurysm and dissections like those that occur in Marfan syndrome are one of the most devastating manifestations of cardiovascular disease; however, the therapeutic options to delay, prevent or treat their progression are still very limited. There are also no clear criteria for assessing the degree of deterioration of a diseased vascular wall and its propensity to dissect. It is therefore crucial to develop effective pharmacological strategies and identify biomarkers that can help to make timely surgical decisions.

The last decade has seen substantial advances in the understanding of the molecular mechanisms underlying thoracic aortic aneurysm and dissection, including the identification of activated signalling pathways, such as those involving TGF-β and Ang II. However, it remains unclear whether activation of these pathways causes this disease or is only a consequence. We have shown that NO mediates syndromic aortic disease in mice and that pathologically increased iNOS expression and iNOS-derived NO are found in a mouse model of Marfan syndrome and in *Adamts1*-deficient mice, in which iNOS inhibition rapidly regresses aortopathy (Oller et al., 2017). These results indicate not only that NO is an essential mediator of aortic disease but also that specific inhibition of iNOS is a potential treatment for the human disease. Further analysis of the downstream NO-signalling components mediating these effects identified sGC and PRKG1 as critical

mediators of Marfan syndrome aortic disease (de la Fuente-Alonso et al., 2021). The observation that gain-of-function mutations in PRKG1 predispose to thoracic aortic aneurysm and dissection (Gago-Díaz et al., 2016; Guo et al., 2013; Schwaerzer et al., 2019; Zhang et al., 2018) suggests that NO–sGC–PRKG signalling is also involved in non-syndromic aortic diseases. However, additional research is required to address this important issue.

It will also be important to identify the upstream components that regulate the NO pathway in Marfan syndrome. These studies could, for example, shed light on how *FBN1* mutations lead to NO overproduction. These mutations may lead, by as yet unknown mechanisms, to the reported decrease in Marfan syndrome in the levels of the metalloprotease ADAMTS1 (Oller et al., 2017) or of other ADAMTS family members. This decrease might cause an accumulation of ADAMTS proteoglycan substrates, which might eventually trigger signals leading to iNOS induction (Figure 3). An experimental programme addressing this important question is ongoing in our laboratory.

The studies summarized in this review also point to the potential for identifying factors linked to NO–sGC–PRKG1 pathway overactivation that could serve as disease biomarkers. Potential candidates include circulating nitrated proteins, cGMP and nitrites/nitrates, all of which are elevated in Marfan syndrome patient plasma, as well as aortic tissue markers such as ADAMTS1 down-regulation and the up-regulated expression of iNOS and pVASP-S239 (de la Fuente-Alonso et al., 2021; Lomelí et al., 2018; Oller et al., 2017). As discussed above, future validation of these markers in larger and independent patient cohorts will be needed to determine the true usefulness of these mediators as prognostic or disease-status biomarkers in Marfan syndrome and potentially in other syndromic or non-syndromic thoracic aortic aneurysm and dissection presentations.

Aside from iNOS inhibition, aortopathy in Marfan mice can also be reversed by pharmacological blockade of sGC or PRKG1. This not only supports a causal role for these mediators in the disease but also suggests a promising alternative for the treatment or prevention of aortic disease. Although the example of losartan in Marfan syndrome illustrates the need for caution in extrapolating conclusions from mouse models to human disease, the accumulated evidence highlights iNOS, sGC and PRKG1 as strong candidates for therapeutic targeting in Marfan syndrome. It is especially important to note that iNOS induction and the activation of sGC and PRKG1 are consistently detected both in Marfan syndrome mice and in Marfan patients (de la Fuente-Alonso et al., 2021; Oller et al., 2017). Clinical trials are urgently required to determine the therapeutic potential of drugs that inhibit these targets in Marfan syndrome and potentially other thoracic aortic aneurysm and dissection presentations.

5.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander et al., 2021).

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CONFLICT OF INTERESTS

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

M.T., and A.d.l.F.-A. prepared figures; M.T., A.d.l.F.-A., M.R.C. and J.M.R. wrote the manuscript; M.R.C., and J.M.R. edited and revised the manuscript. All authors read and approved the manuscript.

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