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ORIGINAL RESEARCH

Vascular Contractility Relies on Integrity of Progranulin Pathway: Insights Into Mitochondrial Function

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BACKGROUND: The complex interplay between vascular contractility and mitochondrial function is central to cardiovascular disease. The progranulin gene (*GRN*) encodes glycoprotein PGRN (progranulin), a ubiquitous molecule with known anti-inflammatory property. However, the role of PGRN in cardiovascular disease remains undefined. In this study, we sought to dissect the significance of PGRN in the regulation vascular contractility and investigate the interface between PGRN and mitochondrial quality.

METHODS AND RESULTS: We used aortae from male and female C57BL6/J wild-type (PGRN+/+) and B6(Cg)-Grntm1.1Aidi/J (PGRN–/–) mice. Our results showed suppression of contractile activity in PGRN–/–, followed by reduced α-smooth muscle actin expression. Mechanistically, PGRN deficiency suppressed mitochondrial respiration, induced mitochondrial fission, and disturbed autophagy process and redox signaling, while restoration of PGRN levels in aortae from PGRN–/– mice via lentivirus delivery ameliorated contractility and boosted mitochondria activity. In addition, in vivo treatment with mitochondrial fission inhibitor restored mitochondrial quality and vascular contractility, while vascular smooth muscle cells overexpressing PGRN displayed higher lysosome biogenesis, accelerated mitophagy flux, and mitochondrial respiration accompanied by vascular hypercontractility. Finally, angiotensin II failed to induce vascular contractility in PGRN–/–, suggesting a key role of PGRN to maintain the vascular tone.

CONCLUSIONS: Our findings suggest that PGRN preserves the vascular contractility via regulating mitophagy flux, mitochondrial activity and dynamics, and redox signaling. Therefore, loss of PGRN function appears as a pivotal risk factor in cardiovascular disease.

Key Words: progranulin ■ vascular contractility ■ vasculature

Cardiovascular diseases (CVD) encompass a broad array of conditions that have an impact on the heart and blood vessels. In the year 2020, CVD were responsible for an estimated 19.1 million deaths across the world.[1] They can manifest as congenital anomalies or be acquired, and some may also have a hereditary component.[2] Vascular smooth muscle cells (VSMC) are the predominant cell type in the arterial wall and normally adopt a quiescent, contractile phenotype to regulate vascular tone, which is a key process in cardiovascular homeostasis.[3]

Although the pathogenesis of CVD is multifaceted, recent findings suggest that mitochondrial dysfunction plays a central role in both the development and

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RESEARCH PERSPECTIVE

What Is New?

- Our study reveals that deficiency of PGRN (progranulin) disturbs mitochondrial dynamics and recycling, subsequently suppressing vascular smooth muscle cell contraction and facilitating the remodeling of conductance arteries.
- In this study, we demonstrate that PGRN can have a negative impact on vascular remodeling and aortic contractility, highlighting the importance of PGRN integrity in aortic diseases related to reduced contractility.

What Question Should be Addressed Next?

- Additional research is necessary to clarify how PGRN influences autophagy and mitophagy fluxes, the mechanisms by which PGRN affects capacitance versus resistance arteries, and its role in other hypertension models should also be assessed.

Nonstandard Abbreviations and Acronyms

Ang-II	angiotensin II
GRN	granulin
mtROS	mitochondria-derived reactive oxygen species
OCR	oxygen consumption rate
PGC1	peroxisome proliferator-activated receptor gamma coactivator 1-α
PGRN	progranulin
TFEB	transcription factor EB
VSMC	vascular smooth muscle cell

progression of CVD and its associated complications.[4] Mitochondrial dysfunction has been identified as a significant factor in the development of arterial dysfunction.[5–7] In CVD, mitochondrial dysfunction leads to the generation of mitochondria-derived reactive oxygen species (mtROS) and decreased ATP production, thereby resulting in loss of contraction of VSMC. However, the precise mechanisms underlying mitochondrial functions in VSMC remain largely unknown. Hence, there is a persistent need to explore innovative therapeutic and preventive strategies focused on revitalizing vascular mitochondrial function. This pursuit involves leveraging key components responsible for maintaining mitochondrial homeostasis, which encompasses a complex network of cellular processes including mitochondrial fission, fusion, biogenesis, and mitophagy—key components in a regulation of mitochondrial homeostasis. Notably, in the context of CVD, abnormalities in mitochondrial structure and function are linked to decreased levels of mitophagy, highlighting the potential importance of mitophagy in sustaining mitochondrial homeostasis and preserving VSMC function.[8,9]

PGRN (progranulin), an anti-inflammatory glycoprotein, demonstrates widespread expression across diverse tissues and cell types, with its complex roles encompassing embryogenesis, wound healing, neurodegenerative processes, and lysosomal function.[10] Within the neurodegenerative disorders setting, specifically frontotemporal lobar degeneration, mutations in the granulin gene result in PGRN protein haploinsufficiency, precipitating neurodegeneration.[11] In the cardiorenal domain, PGRN has been identified as a protector of cardiac and renal integrity during ischemia–reperfusion injury.[12,13] Furthermore, PGRN exhibits anti-inflammatory effects within the vascular endothelium, bestowing atheroprotective benefits that include elevating nitric oxide levels.[14] Our group recently demonstrated that the lack of PGRN induces endothelial dysfunction, affecting the vascular contractility of small arteries and resulting in elevated blood pressure.[15] In VSMC, PGRN acts as a mitigator of calcification[16] and regulator of cell migration via modulating IL-8 (interleukin-8) secretion.[17] Although the vascular protective effects of PGRN have been described in other scenarios and in resistance arteries, it remains unclear whether it regulates the vascular function of large vessels such as the aorta.

Herein we show a novel face of PGRN's function, wherein PGRN preserves mitochondrial balance through mtROS signaling, mitochondrial function and dynamic, and mitophagy flux in VSMCs. We further demonstrate that deficit in PGRN induces loss of VSMC contractility in conductance arteries. This discovery implies that PGRN may present a highly promising therapeutic avenue for tackling cardiovascular diseases by modulating mitochondrial function and cellular bioenergetics and further suggest that individuals with PGRN deficiency display cardiovascular risk along with neurodegenerative diseases.

METHODS

Data Availability Statement

The data underlying this article will be shared upon reasonable request to the corresponding author.

Mice

Twelve- to 16-week-old male and female C57BL6/J wild type (PGRN+/+), global PGRN mutant B6(Cg)-Grn$^{tm1.1Aidi}$/J (PGRN–/–), and male mt-Keima mice were used. All mice were fed with standard mouse

chow and tap water was provided ad libitum. Mice were housed in an American Association of Laboratory Animal Care–approved animal care facility in the Rangos Research Building at the Children's Hospital of Pittsburgh of the University of Pittsburgh. CO_2 overdose was used as a model of euthanasia. The Institutional Animal Care and Use Committee from University of Pittsburgh approved all protocols (protocol #22061179). All procedures conform to the guidelines from the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*.

Aorta were harvested for functional and structural evaluations and cell culture preparation. For further experimental details and list of primers and antibodies, please see Tables S1 and S2. Additional methods can be found in Data S1.

Statistical Analysis

Concentration–response curves were analyzed using a nonlinear interactive fitting program (GraphPad Prism 9.0; GraphPad Software Inc., San Diego, CA). This statistical approach was employed to create a curve that depicts the relationship between the concentrations of agonists (phenylephrine and thromboxane A2) and the resulting contraction. This allows for the evaluation of the maximal effect (Emax) and pD2 values for phenylephrine and thromboxane A2. Here, "Emax" refers to the maximum effect achievable by potassium chloride (KCl), phenylephrine, and thromboxane A2, and "pD2" represents the negative logarithm of the molar concentration of phenylephrine and thromboxane required to achieve 50% of the maximum effect (EC50).

For comparisons of multiple groups, 2-way ANOVA to compare strain (PGRN+/+ or PGRN–/–) and treatment (vehicle and scramble lentivirus or pharmacological or lentivirus treatments), followed by the Tukey posttest was used. Differences between the 2 groups were determined using Student's *t* test. The vascular function data are expressed as a maximal response. The concentration–response curves were fitted by nonlinear regression analysis. Maximal response was determined and used to determine if there was difference between the groups. Analyses were performed using Prism 10.0 (GraphPad Software, La Jolla, CA). A difference was considered statistically significant when $P \leq 0.05$.

RESULTS

Deficiency in PGRN Affects Vascular Contractility

To analyze whether PGRN regulates vascular contraction, we examined the effects of KCl (120 mM), phenylephrine, or thromboxane A2 analog (U46619) (Figure 1A and 1B) in endothelium-denuded aortic rings from PGRN+/+ and PGRN–/– mice via wire myograph. Deficiency in PGRN decreased the vascular contractility to all 3 agents in male (Figure 1A) and female mice (Figure 1B) and diminished the protein expression for alpha-smooth muscle actin but not mRNA level (Figure S1A and S1B). In addition, lack of PGRN did not affect the vascular thickness and fibrosis at least in mice at 12 to 14 weeks of age (Figure S1C).

We further examined the role of PGRN regulating vascular contraction in primary VSMC isolated from PGRN+/+ and PGRN–/–. By performing the collagen gel disc assay, we observed that PGRN–/– VSMC contracted less after 48 hours compared with PGRN+/+ VSMC (Figure 1C). In addition, we found attenuated F-actin (Figure 1D) and alpha-smooth muscle actin levels, with no difference in alpha-smooth muscle actin mRNA expression (Figure S1D and S1E). Finally, deficiency in PGRN triggered vascular inflammation in aortae from male and female mice characterized by elevated ICAM (intercellular adhesion molecule), VCAM (vascular cell adhesion molecule), IL-1β (interleukin-1 beta), TNFα (tumor necrosis factor alpha), and IL-6 mRNA (Figure S2A and S2B).

PGRN Is a Major Regulator of Vascular Contraction Via Regulating Mitochondria Quality

In conditions like diabetic nephropathy[18] or in neuroblastoma cells,[19] there is evidence that PGRN plays a role in the regulation of mitochondrial quality. Consequently, we sought to investigate whether PGRN also has an impact on mitochondrial profile within the vasculature. In VSMC, lack of PGRN attenuated oxygen consumption rate (OCR; analyzed by Seahorse) (Figure 2A) followed by suppression of ATP levels, mitochondrial complex I activity, and nicotinamide adenine dinucleotide+ (NAD^+)/nicotinamide adenine dinucleotide hydrogen (NADH) ratio (Figure 2B), but it did not affect the mitochondria number (mtDNA/nDNA) (Figure 2C). Because we observed suppressed complex I activity, we analyzed the complexes expression by using the oxidative phosphorylation antibody, which revealed that deficiency in PGRN reduces complexes I and II expression (Figure 2D). Furthermore, mitochondria from PGRN–/– VSMC demonstrated impaired membrane potential, which was measured by JC-1 stain. Carbonyl cyanide chlorophenylhydrazone was used as a positive control in PGRN+/+ VSMC (Figure S3A). Finally, complex I related genes and peroxisome proliferator-activated receptor γ (a complex I gene regulator[20]) levels were decreased in aortae from male and female PGRN–/– mice (Figure S3B and S3C).

Mitochondrial dysfunction has been associated with exacerbated mtROS formation. By using MitoSox

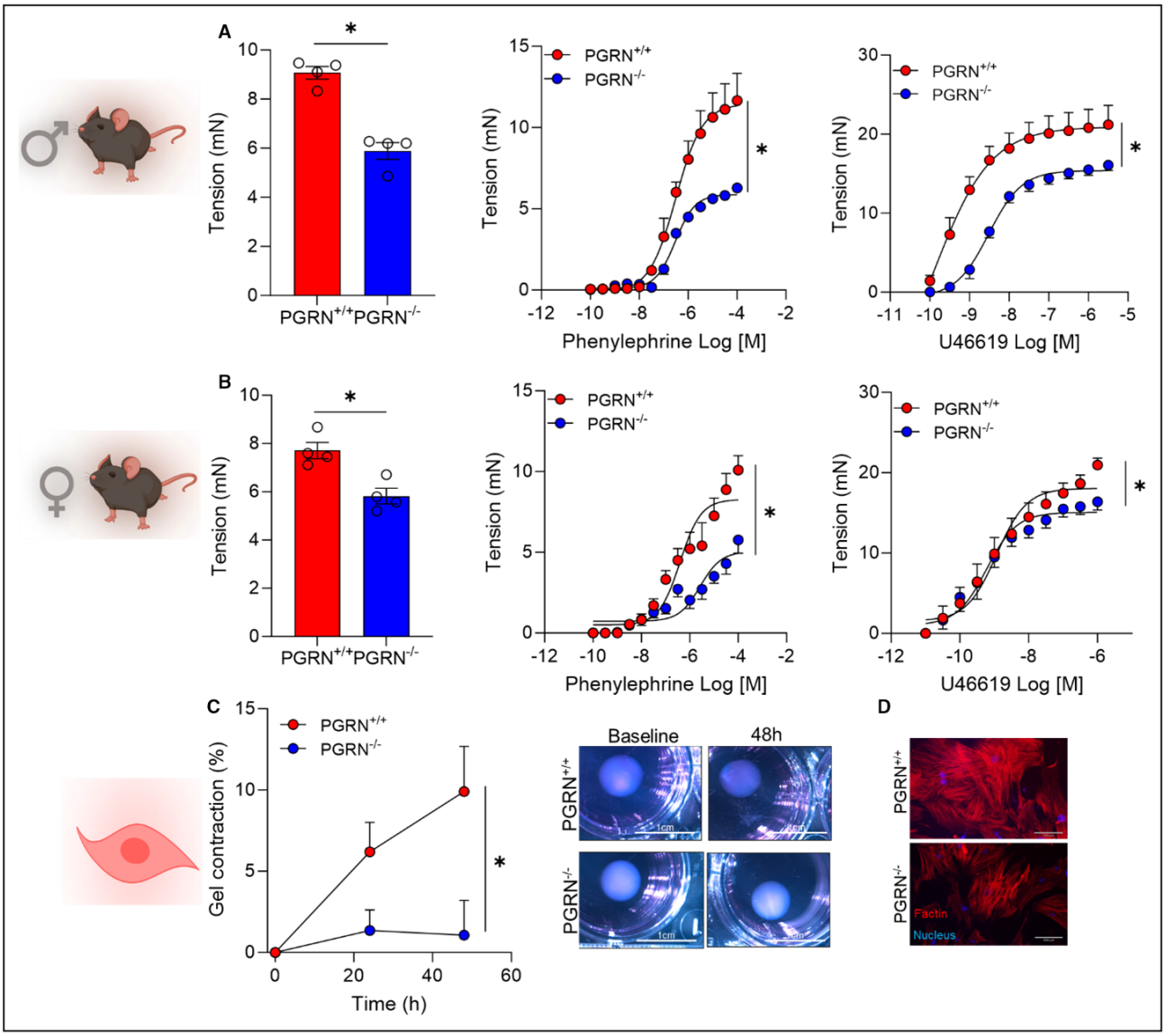

Figure 1. Deficiency in PGRN impairs vascular contractility ex vivo.
Ex vivo wire myography showing KCl (120 mM) response and concentration-effect curves to phenylephrine and thromboxane A2 analog (U46619) (**A** and **B**) in endothelium-denuded aortic rings from male (**A**) and female (**B**) PGRN–/– and PGRN+/+ mice. Collagen contraction assay in PGRN+/+ and PGRN–/– VSMC. Gel area variation on day 2 post-gelation normalized to baseline area (**C**). Scale bar, 1 cm. Representative image of Actin filaments in PGRN+/+ and PGRN–/– VSMC. In red, Actin filaments stained with rhodamine-phalloidin; in blue, nuclei stained with DAPI (**D**). Scale bar, 100 μm. Data are presented as mean±SEM and analyzed by Student's *t* test (n=3–6). **P*<0.05 vs PGRN+/+ samples. VSMC indicates aortic vascular smooth muscle cells; KCl, potassium chloride; and PGRN, progranulin.

we found that lack of PGRN induced mtROS formation (Figure 2E), which is exacerbated by antimycin A (a potent mitochondrial ROS inducer) (Figure 2F). ERK1/2 (extracellular signal-regulated kinase 1/2), a MAPK (mitogen-activated protein kinase) member and ROS-sensitive protein, was overactivated in PGRN–/– VSMC (Figure S3D), which was not further affected by PDGF-BB (platelet-derived growth factor-BB) compared with PGRN+/+ (Figure S3E).

Additionally, we assessed whether alterations in mitochondrial function were evident in whole aortae as well. Using Oroboros O2k respirometry, we examined mitochondrial respiration in fresh aortae and found that mitochondrial complex I activity is impaired in PGRN–/– with no changes in complex II (coupled or uncoupled) (Figure 3A). Furthermore, aortae from PGRN–/– displayed reduced complex I, III, and IV protein expression (Figure 3B). Circulating PGRN plays a major role in regulating cardiovascular biology.[15] Therefore, we treated PGRN+/+ and PGRN–/– with recombinant PGRN (20 μg/day/mouse) for 7 days via osmotic mini-pump[15]; we found that PGRN treatment did not affect mitochondrial respiration (Figure S4A). Similarly, recombinant

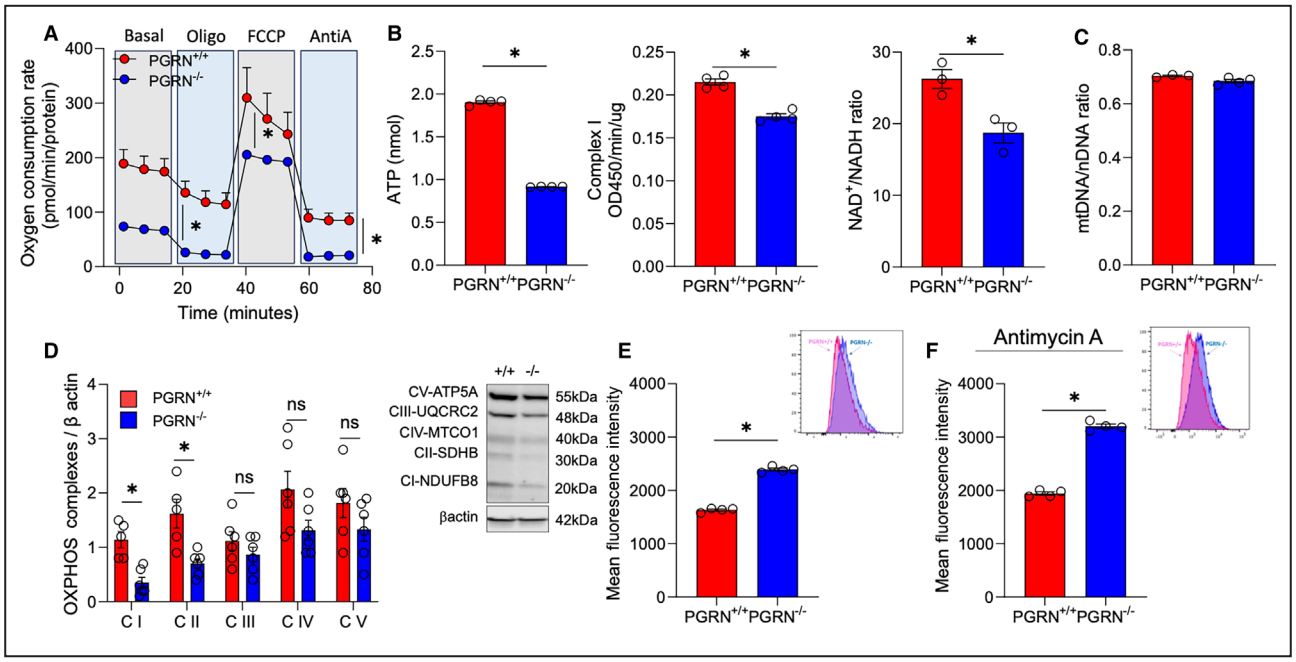

Figure 2. Loss of PGRN affects mitochondrial quality in aVSMC.
Mitochondrial oxygen consumption rates in PGRN+/+ and PGRN−/− VSMC measured using the Seahorse XF Extracellular Flux 96 analyzer (**A**), analysis was performed in presence of oligomycin, 7 carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone, and antimycin A. ATP content, mitochondrial complex I activity, and NAD+/NADH ratio (**B**) in PGRN+/+ and PGRN−/− aVSMC. **C**, Quantitative polymerase chain reaction analysis of mtDNA copy number relative to nDNA in PGRN+/+ and PGRN−/− VSMC. **D**, Western blot for protein expression of OXPHOS in PGRN+/+ and PGRN−/− VSMC. β-Actin was used as load control. Mitochondrial reactive oxygen species levels measured by MitoSox stain via flow cytometry in PGRN−/− and PGRN+/+ VSMC at basal condition (**E**) or under antimycin A treatment (**F**). Data are presented as mean±SEM and analyzed by Student's *t* test (n=3–6). **P*<0.05 vs PGRN+/+ samples. Analysis of oxygen consumption rate was performed comparing area vs area of basal, oligomycin, FCCP, and antimycin A from PGRN+/+ and PGRN−/− VSMC. VSMC indicates aortic vascular smooth muscle cell; KCl, potassium chloride; NAD^+/NADH, nicotinamide adenine dinucleotide+/nicotinamide adenine dinucleotide hydrogen; OXPHOS, oxidative phosphorylation; and PGRN, progranulin.

PGRN did not affect OCR in PGRN−/− VSMC in vitro (Figure S4B). Thus, we focused on understanding the intracellular role of PGRN by reexpressing PGRN via lentivirus delivery. After a time-course to find the best time of lentivirus incubation, we treated the aortae from PGRN+/+ and PGRN−/− with lentivirus encoding PGRN for 24 hours and analyzed the mitochondrial respiration and vascular contractility. Lentivirus increased PGRN expression in PGRN+/+ and repopulated PGRN in PGRN−/− (Figure 3C). In addition, lentivirus increased complex I activity (Figure 3D) and vascular contractility to KCl, phenylephrine, and U46619 in PGRN−/− arteries (Figure 3E) but did not affect mitochondrial respiration (Figure S5A) and vascular contractility to KCl, phenylephrine, and U46619 (Figure S5B through S5D) PGRN+/+ arteries.

Overexpression of PGRN Enhances Mitochondria Capacity and Generates Contractile VSMCs

Because we found these exciting effects of PGRN on regulating vascular contractility and mitochondrial respiration, we created VSMC overexpressing PGRN via lentivirus delivery (SMC^{PGRN}), which was confirmed by Western blot in cell lysate (Figure S6A) or ELISA in supernatant (Figure S6B). Compared with SMC^{Ctrl} (rat aortic SMC treated with scramble lentivirus), SMC^{PGRN} demonstrated an increase in ATP levels (Figure S6C), complex I activity (Figure S6D), and NAD^+/NADPH ratio (Figure S6E). Furthermore, overexpressing PGRN reduced mtROS formation (Figure S6F) and partially protected against antimycin A-induced mtROS production (Figure S6G). In addition, SMC^{PGRN} demonstrated higher cell contraction, which could be observed in collagen gel disc assay (Figure S6H and S6I), elevated F-actin stain (more F-actin and smaller cell size) (Figure S6J), and higher alpha-smooth muscle actin content (Figure S6K).

Mitochondrial Fission Drives Lack of PGRN-Induced Mitochondrial Dysfunction and Loss of Vascular Contraction

The alteration of mitochondrial biogenesis and dynamics is essential for maintaining mitochondrial respiration. Therefore, we investigated whether PGRN plays a role in orchestrating the processes of mitochondrial biogenesis and fusion and fission. Lack of PGRN

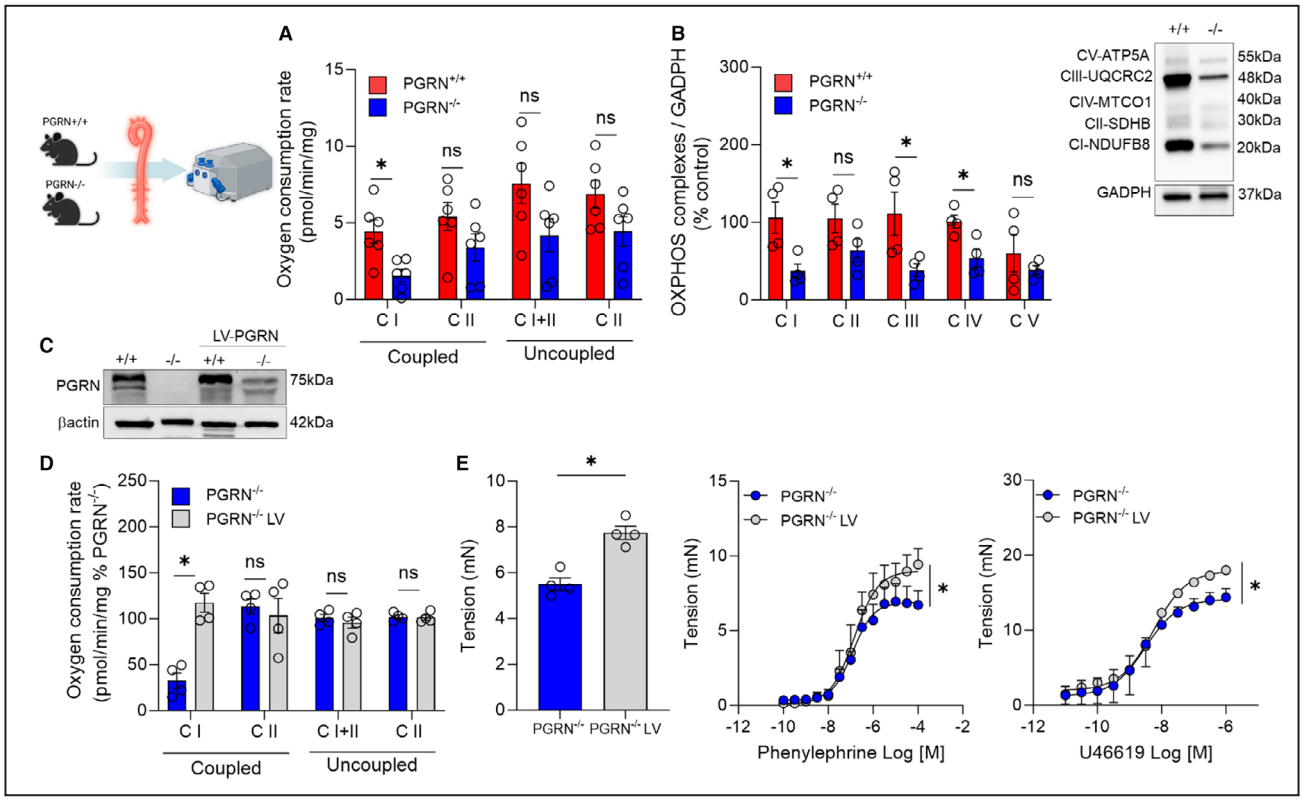

Figure 3. Reexpression of PGRN restores mitochondrial respiration and vascular contractility in PGRN-deficient arteries. Mitochondrial oxygen consumption rates in fresh aortae from PGRN+/+ and PGRN–/– measured using the Oroboros O2k respirometry (**A**). Western blot for protein expression of OXPHOS. GAPDH was used as load control (**B**). Western blot for protein expression of PGRN after lentivirus delivery for 24 h. β-Actin was used as load control (**C**). Mitochondrial oxygen consumption rates in fresh aortae from PGRN–/– mice after lentivirus delivery (**D**). Ex vivo wire myography showing KCl (120 mM) response and concentration-effect curves to phenylephrine and thromboxane A2 analog (U46619) (**E**) in endothelium-denuded aortic rings from male PGRN–/– mice after lentivirus delivery. Data are presented as mean±SEM and analyzed by Student's *t* test (n=3–4). **P*<0.05 vs PGRN+/+ samples. KCl indicates potassium chloride; LV, lentivirus; OXPHOS, oxidative phosphorylation; and PGRN, progranulin.

suppressed PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1-α, a marker of mitochondria biogenesis) in aortae and VSMC (Figure 4A and 4B) and induced mitochondria fission, characterized by upregulation of DRP-1 (mitochondrial dynamin-related protein 1) expression in aortae (Figure 4A) and VSMC (Figure 4B) and downregulated MFN1 (mitofusin 1) expression (marker of fusion) in aortae (Figure 4A) and VSMC (Figure 4B). To understand whether fragmented mitochondria was driving loss of vascular contractility and OCR suppression, we treated PGRN+/+ and PGRN–/– mice with a DRP-1 inhibitor (mitochondrial division inhibitor-1) and found that inhibition of DRP-1 restored vascular contractility in aortae to KCl, phenylephrine, and U46619 (Figure 4C) and enhanced the OCR in aortae (Figure 4D). Ultimately, SMC^{PGRN} demonstrated no difference for mitochondrial number (Figure S7A) and biogenesis (Figure S7B) but increased MFN1 (Figure S7C) and diminished DRP-1 expression (Figure S7D). In summary, lack of PGRN triggers loss of vascular contractility via inducing mitochondrial fragmentation.

Deficiency in PGRN Disrupts Vascular Autophagy and Mitophagy

Studies conducted on kidneys have demonstrated that PGRN regulates autophagy.[18] However, it remains unclear whether PGRN has an impact on vascular mitophagy and subsequently mitochondrial quality. Considering this, our investigation aimed to determine whether the disruption of PGRN signaling would influence mitochondrial recycling. We observed that VSMC from PGRN–/– present elevated PTEN induced putative kinase 1 (PINK1) (66 and 33 kDa), E3 ubiquitin protein ligase (PARKIN), LC3A/B ratio, and p62 accumulation suggesting a dysfunctional mitophagy (Figure 5A). Furthermore, we found an accumulation of lysosomal-associated membrane protein 1 (LAMP1) (marker of lysosome) (Figure 5B) and higher transcription factor EB (TFEB) activity, characterized by higher TFEB content in the nuclei (Figure 5B). This suggests that TFEB activation is higher in PGRN–/– VSMCs, which leads to higher lysosome formation. However, despite the higher lysosome formation, they may be dysfunctional.

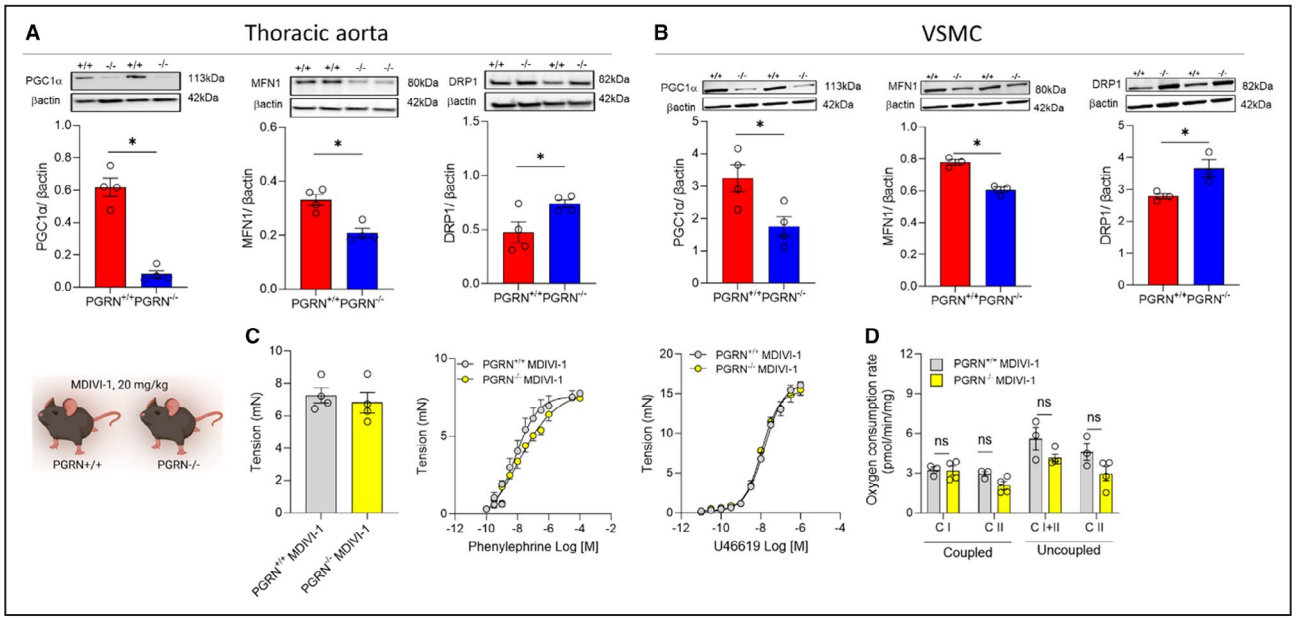

Figure 4. PGRN deficiency suppresses mitochondria respiration and vascular contraction via mitochondria fragmentation. Western blot for protein expression of PGC1α, mitofusin 1, and DRP1 in PGRN+/+ and PGRN−/− in aortae from PGRN+/+ and PGRN−/− mice (**A**). β-Actin was used as load control. Western blot for protein expression of PGC1α, mitofusin 1, and DRP1 in PGRN+/+ and PGRN−/− in VSMC (**B**). β-Actin was used as load control. KCl response (120 mM) and concentration-effect curves to phenylephrine and thromboxane A2 analog (U46619) (**C**) in endothelium-denuded aortic rings of aortae from PGRN+/+ and PGRN−/− mice treated with MDIVI-1 (DRP1 inhibitor). Mitochondrial oxygen consumption rates measured using the Oroboros O2k respirometry in fresh aortae from PGRN+/+ and PGRN−/− mice treated with MDIVI-1 (**D**). Data are presented as mean±SEM and analyzed by Student's *t* test (n=3–4). **P*<0.05 vs PGRN+/+ aVSMC. aVSMC indicates aortic vascular smooth muscle cell; DRP1, mitochondrial dynamin-related protein 1; KCl, potassium chloride; MDIVI-1, mitochondrial division inhibitor-1; MFN1, mitofusin 1; OXPHOS, oxidative phosphorylation; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-α; and PGRN, progranulin.

In VSMC overexpressing PGRN (SMCs^{PGRN}) we observed reduced PINK1 and no difference for PARK (Figure 5C). We also found that expression of PGRN increases LC3II and suppress p62 (Figure 5D). Bafilomycin A1 (a vacuolar H+-ATPase inhibitor and disruptor of autophagic flux) led to a further accumulation of LC3II (Figure 5D) and increased p62 expression (Figure 5D). Furthermore, we used aortae from mt-Keima mice to track mitophagy in freshly isolated aortae. Thus, we overexpressed PGRN via lentivirus delivery (24 hours of incubation) in aortae from these mice and tracked mitochondrial flux. PGRN caused a remarkable increase of mitochondria in lysosome (red) compared with control arteries (Figure 5E). Finally, we found that overexpression of PGRN did not affect LAMP expression (Figure 5F) but triggered TFEB activation (accumulation in the nuclei of SMCs^{PGRN}) (Figure 5F). These findings suggest that PGRN induces lysosome biogenesis and autophagy/mitophagy.

Spermidine, an Autophagy Inducer, Rescues Mitochondrial Respiration and Vascular Contractility in PGRN-Deficient Mice

We used spermidine as an autophagy inducer to analyze whether restoring autophagy flux would improve mitochondrial respiration and vascular contractility (schematic in Figure S8A). To confirm that spermidine caused autophagy we measured LC3II/I ratio in hearts from PGRN+/+ and PGRN−/− and observed that spermidine restored LC3II/I ratio in PGRN−/− (Figure S8B). Furthermore, spermidine increased the mitochondrial respiration (Figure S8C) and vascular contractility for KCl (Figure S8D), phenylephrine (Figure S8E), and U46619 (Figure S8F) in the aortae of PGRN−/− mice. We did not see any effect of spermidine in arteries from PGRN+/+ mice in terms of OCR (Figure S9A) and vascular contractility for KCl (Figure S9B), phenylephrine (Figure S9C), and U46619 (Figure S9D).

PGRN-Deficient Mice Do Not Respond to Angiotensin-II-Induced Vascular Contractility

To analyze the impact of PGRN on a pathophysiological scenario, we challenged the PGRN+/+ and PGRN−/− mice with angiotensin-II (Ang-II, an inducer of vascular contraction and remodeling and mitochondria dysfunction). We first analyzed whether Ang-II changes PGRN expression in aortae from PGRN+/+, which revealed that Ang-II upregulated PGRN protein expression (Figure 6A). To investigate whether vascular

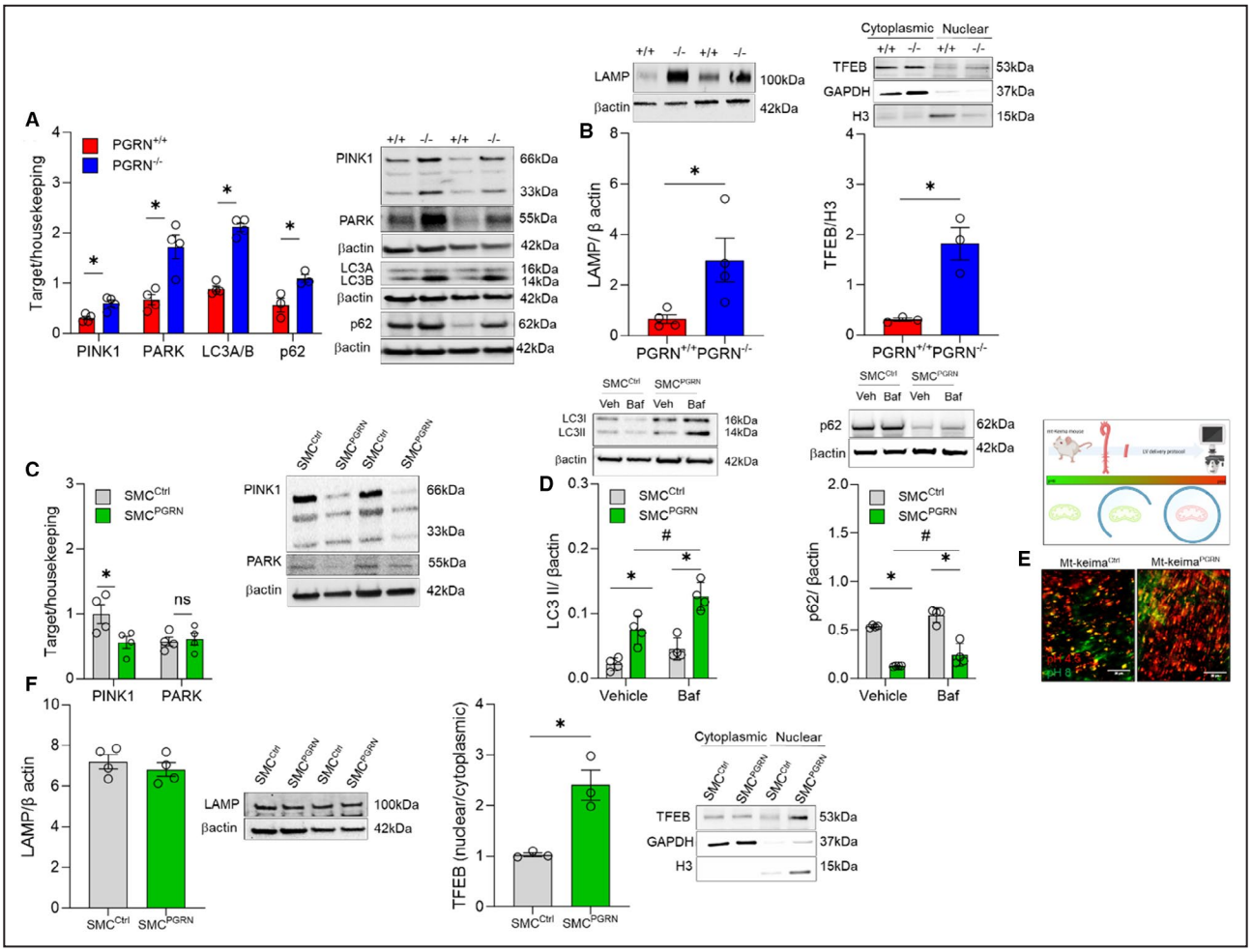

Figure 5. PGRN deficiency triggers dysregulated lysosome formation and disturbs mitophagy flux.
Western blot for mitophagy and autophagy markers—PINK1 (66 And 33 kDa), PARK, LC3A/B ratio, p62 (**A**), lysosome levels (LAMP) in cell lysate from PGRN+/+ and PGRN–/– VSMC and TFEB activation (**B**). Western blot for protein expression for mitophagy markers—PINK1 (66 and 33 kDa) and PARK in lysate from rat aortic smooth muscle cells treated with scramble lentivirus (SMC^{Ctrl}) and SMC treated with PGRN-lentivirus (SMC^{PGRN}) (**C**). LC3II and p62 expression in SMC^{Ctrl} and SMC^{PGRN} in presence or absence of Baf (50 μM for 2 h) (**D**). Track of mitophagy in fresh aortae from Mt-Keima mice treated with scramble lentivirus or PGRN-lentivirus encoding PGRN for 24 h (**E**). Green represents mitochondria in pH8 (likely cytosolic), and red represents mitochondria in pH4.5 (likely lysosome). LAMP expression in cell lysate from SMC^{Ctrl} and SMC^{PGRN} and TFEB activation in SMC^{Ctrl} and SMC^{PGRN} (**F**). TFEB activation was measured by quantifying TFEB content in cytosolic and nuclear fractions. GADPH was used as a marker of cytoplasm, and histone3 as a marker of nuclei. β-Actin was used as load control for all W estern blots. Data are presented as mean±SEM and analyzed by Student's *t* test or 2-way ANOVA followed by the Tukey posttest (n=3–4). **P*<0.05 vs PGRN+/+ or vs SMC^{Ctrl}. #*P*<0.05 vs Baf+SMC^{PGRN} vs Baf+SMC^{Ctrl}. VSMC indicates aortic vascular smooth muscle cell; Baf, bafilomycin A1; DRP1, mitochondrial dynamin-related protein 1; H3, histone3; KCl, potassium chloride; MDIVI-1, mitochondrial division inhibitor-1; MFN1, mitofusin 1; OXPHOS, oxidative phosphorylation; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-α; PGRN, progranulin; SMC, smooth muscle cell; and TFEB, transcription factor EB.

levels of PGRN are exclusively responsive to Ang-II, we treated mice with aldosterone (600 ng/kg for 14 days), but this did not lead to any changes in PGRN expression (Figure S10). This indicates that vascular PGRN levels are not influenced by any model of hypertension and appear to be specifically sensitive to Ang-II. We next analyzed mitochondria respiration and vascular function in PGRN+/+ and PGRN–/–. We found that Ang-II did not change the mitochondria respiration in PGRN–/– compared with PGRN+/+ (the difference between PGRN+/+ and PGRN–/– persisted) (Figure 6B) and did not affect vascular contractility to KCl, phenylephrine, and U46619 (Figure 6C) in endothelium-denude aortic rings from PGRN–/– mice. Furthermore, Ang-II similarly affected the wall thickness in PGRN+/+ and PGRN–/–, but PGRN–/– were more sensitive to Ang-II-induced vascular fibrosis (Figure 6D). Next, we assessed whether lack of PGRN affects Ang-II-induced contractility in vivo and blood pressure. At baseline condition, deficiency of PGRN caused high mean arterial pressure ([mean±SEM]=PGRN+/+: 95.1±4 versus PGRN–/–: 108.5±5*, **P*<0.05 versus PGRN+/+ [N=3,

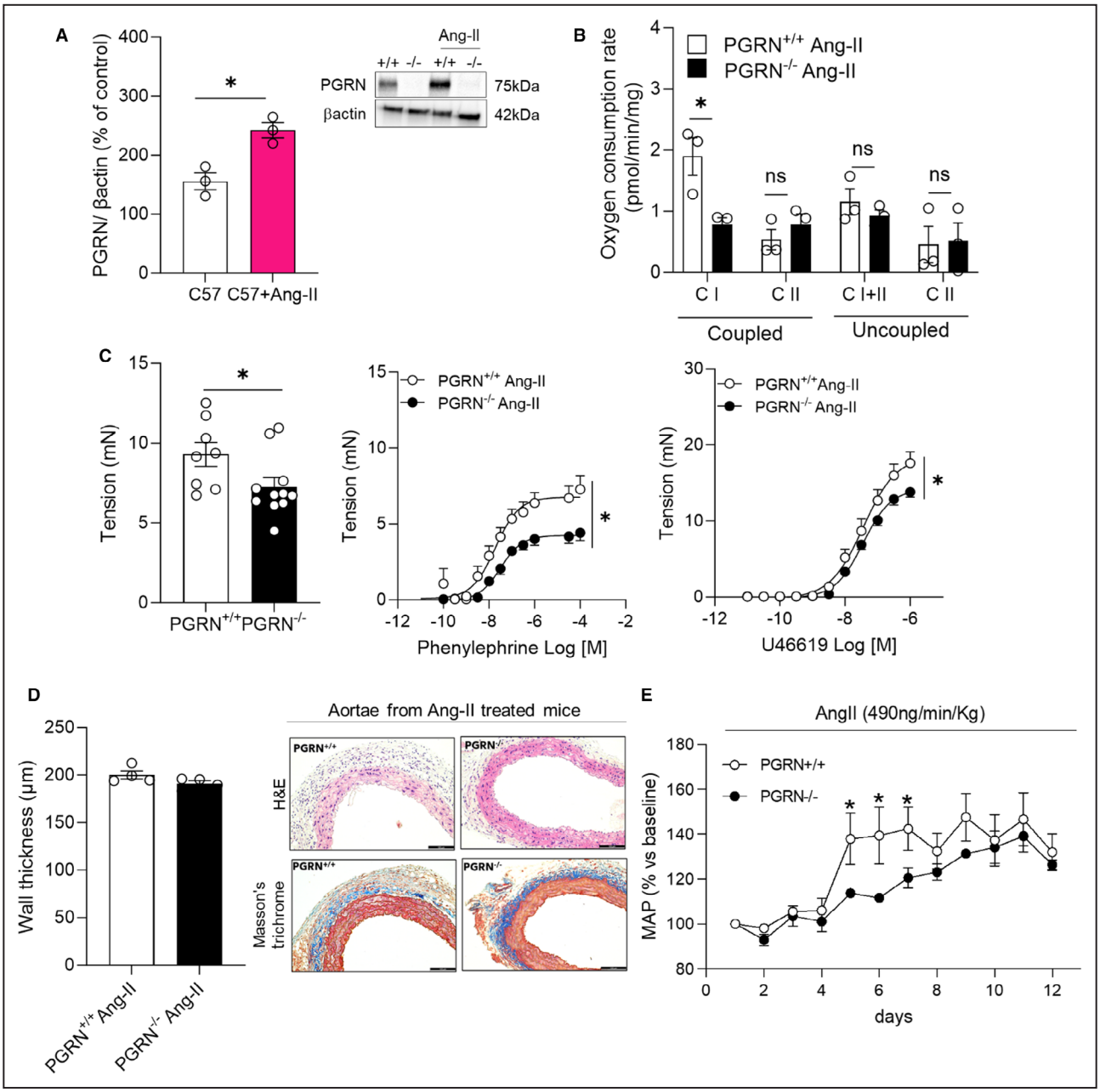

Figure 6. PGRN-deficient mice fail to respond to Ang-II-induced vascular hypercontractility.
Western blot for PGRN expression in aortae from PGRN+/+ and PGRN−/− mice treated with Ang-II. β-Actin was used as load control (**A**). Mitochondrial oxygen consumption rates measured using the Oroboros O2k respirometry in fresh aortae of PGRN+/+ and PGRN−/− mice treated with Ang-II (**B**). Ex vivo wire myography showing KCl (120 mM) response and concentration-effect curves to phenylephrine and thromboxane A2 analog (U46619) (**C**) in endothelium-denuded aortic rings of aortae from PGRN+/+ and PGRN−/− mice treated with Ang-II. Aortic wall thickness derived from hematoxylin and eosin stain and Masson's trichrome stains in aortae from PGRN+/+ and PGRN−/− mice treated with Ang-II (**D**). Scale bar, 100 µm. MAP of PGRN+/+ and PGRN−/− mice treated with Ang-II (**E**). Ang-II treatment consisted of 490 ng/kg/day for 14 d, via osmotic mini-pump. Data are presented as mean±SEM and analyzed by Student's *t* test (n=3–4). **P*<0.05 vs PGRN−/−. Ang-II indicates angiotensin-II; H&E, hematoxylin and eosin; KCl, potassium chloride; MAP, mean arterial pressure; and PGRN, progranulin.

average of 5 days, mm Hg]), as we have reported before.[15] After 14 days, Ang-II treatment induced high mean arterial pressure similarly in both PGRN+/+ and PGRN−/− male mice. However, PGRN-deficient mice exhibited a delayed pressure response compared with their counterparts (Figure 6E). These findings indicate that Ang-II-induced vascular contractility in large vessels depends on the integrity of the PGRN signaling pathway, which differs from what we have previously demonstrated in resistance arteries of PGRN−/− mice.[15]

Additionally, it appears that PGRN plays a pivotal role as a gatekeeper in the formation of fibrosis.

DISCUSSION

Loss of vascular contraction is a critical factor in CVD pathogenesis, limiting the normal function of the artery and increasing the risk of organ damage because of impaired blood flow.[21] However, the mechanisms involved in this phenotype are not well understood. In this study, we uncover a novel and major role for PGRN, which consists of maintaining the VSMC contractility via adjusting mitochondrial quality and dependent on lysosome biogenesis, mitophagy flux, and complex I biogenesis pathways.

PGRN loss-of-function mutations cause neuronal ceroid lipofuscinosis and frontotemporal dementia-granulin in a dosage-dependent way.[22] PGRN regulates development, survival, function, and maintenance of mammalian cells including vascular cells (VSMC[23] and endothelial cells[15]). Recently, Gerrits et al[24] identified that the neurovascular unit is severely affected in GRN-associated frontotemporal dementia. In VSMC, PGRN exerts antimigratory effects via modulating IL-8 formation in atherosclerotic setting and its full-length (~75 kDa) acts inhibiting the calcification in calcific aortic valve disease[16] indicating that PGRN plays a major role in regulating VSMC quality. Herein, for the first time, we are describing that deficiency in PGRN strikingly affects the contraction of VSMC likely suppressing mitochondrial quality.

In neuronal[19] and renal cells[18] and in *Caenorhabditis elegans*[25] PGRN acts as a regulator of mitochondrial function via preserving mitochondrial dynamics, biogenesis, and mitochondrial recycling. Mitochondrial dysfunction is part of premature aging and contributes to appearance of inflammation, mitochondria-associated oxidative stress, cell senescence, and apoptosis, which are leading causes of CVD[26–28] including atherosclerosis and aneurysm.[29–31] From a mechanistic perspective, our study revealed that the deficiency of PGRN had a multifaceted impact on mitochondrial quality within VSMC. Remarkably, VSMC deficient in PGRN exhibited mitochondrial fragmentation and a decrease in the expression of complex I genes and mass. These changes subsequently exerted a profound influence on mitochondrial respiration, as evidenced by a reduction in complex I activity, a disrupted NAD+/NADH ratio, and diminished ATP levels. The compromised mitochondrial function culminated in an excessive generation of mtROS. It is known that reduction in mitochondrial complex I mass or activity and mitochondrial fragmentation are recognized as significant factors contributing to redox imbalance and the development of cardiovascular disease.[32] In our study, we observed that the disruption of PGRN signaling led to an excessive production of mtROS and worsened sensitivity to antimycin A-induced mtROS generation. Significantly, the overexpression of PGRN in VMSC resulted in the development of high-capacity mitochondria characterized by enhanced mitochondria fusion, increased complex I activity, a higher NAD+/NADH ratio, elevated ATP levels, and improved antioxidant properties (inhibition of mtROS formation). This was followed by VSMC hypercontractility. From a therapeutic standpoint, our findings suggest that the overexpression of PGRN in VSMC could potentially regulate mitochondrial capacity and provide protection against CVD. Although the specific mechanisms by which PGRN regulates mitochondrial complex I activity and biogenesis were not investigated in this study, previous research in neuroblastoma cell lines has shown that peroxisome proliferator-activated receptor γ activation can rescue mitochondrial function when complex I is inhibited. Therefore, the observed suppression of peroxisome proliferator-activated receptor γ in aortae from both male and female PGRN-deficient mice may indicate impaired formation of genes related to complex I.[20]

Mitochondria are exceptionally dynamic organelles that undergo synchronized cycles of fission and fusion, a phenomenon referred to as mitochondrial dynamics. These processes are essential for maintaining mitochondrial shape, distribution, and size.[28,33] In a positive feedback loop, an excess of ROS triggers mitochondrial fission,[34] and mitochondria can rapidly fragment, accompanied by an increase in ROS generation.[35] To confirm that lack of PGRN affects mitochondrial quality and vascular contractility by inducing mitochondrial fragmentation, we inhibited DRP-1, a GTPase that regulates mitochondrial fission. Inhibiting DRP-1 in vivo with mitochondrial division inhibitor-1 restored mitochondrial respiration and vascular contractility in aorta. Therefore, within the vasculature, PGRN may exert its antioxidant properties by regulating complex I activity and mitochondrial dynamics, as previously observed in nonvascular cell types.[19]

Deficiency in PGRN regulates the expression of PGC1α in nonvascular tissues.[18,36] In line with this observation, we noticed a significant decrease in PGC1α in VSMC and aortae deficient for PGRN. However, this suppression did not result in changes in mitochondrial quantity. It is important to note that the method employed for detecting mitochondria number may not accurately correlate biogenesis and mitochondrial quantity. When mitochondria become fragmented, smaller ones contain their own DNA.[37] Consequently, detecting higher numbers of small mitochondria via mtDNA quantification may lead to an overestimation of total mitochondrial count, therefore a clear correlation between PGC1α and mitochondrial number in our study might be miscalculated. Thus, further research is necessary to explore the interface between PGRN and PGC1α in cardiovascular biology, as well as to elucidate if a deficit of PGC1α results in less mitochondria biogenesis.

It appears that PGRN exerts its cardiovascular effects primarily in its secreted form, as demonstrated previously in endothelial cells.[15] To investigate this further, we sought to restore circulating PGRN levels in PGRN-deficient mice using osmotic mini-pump delivery and by treating isolated VSMC. Surprisingly, our observations indicated that circulating PGRN did not have a significant impact on mitochondrial function in freshly isolated aortae or in cells treated with recombinant PGRN. These findings strongly suggest that PGRN does not maintain vascular contractility and mitochondrial quality through autocrine or paracrine mechanisms. Instead, it appears that PGRN may function as an intracellular molecule. To further investigate this concept, we reconstituted PGRN levels in freshly isolated aortae from PGRN-deficient mice via lentivirus delivery, allowing us to assess mitochondria respiration and vascular contractility. Remarkably, the reintroduction of PGRN in fresh aortae led to the restoration of mitochondrial capacity, particularly through the enhancement of complex I activity, and a significant improvement in vascular contractility. This crucial piece of data strongly indicates that PGRN exerts its regulatory effects on vascular contractility and complex I activity from within the cell, rather than in its circulating form.

Mammalian cells have developed finely tuned mechanisms to ensure the preservation of functional mitochondria, aligning with energy demand of cells.[38] Mitophagy is a selective autophagy process responsible for eliminating accumulated damaged or dysfunctional mitochondria from cells, contributing to the maintenance of mitochondrial homeostasis.[38] In diabetic nephropathy, PGRN is crucial in maintaining renal integrity via mitophagy.[18] In VSMC we observed that PGRN deficiency leads to an impaired mitophagy flux (accumulation of p62, LC3A/B, PINK1, and PARKIN), whereas overexpressing PGRN in fresh aortae from mt-Keima mice or in VSMC leads to an accelerated mitophagy process. To explore the potential therapeutic avenue of reinvigorating autophagy in individuals with PGRN deficiency, we treated mice with spermidine, a natural polyamine with autophagic characteristics[39] and life span-extending effect in mammalian organisms.[29,39,40] Spermidine effectively induced autophagy and improved the mitochondria function rescuing the vascular function in PGRN-deficient mice. These exciting findings suggest that the reinstatement of mitophagy flux holds promise as a pharmacological strategy to mitigate cardiovascular risk in patients with loss-of-function mutations in PGRN. This therapeutic approach offers a possibility for addressing cardiovascular concerns in individuals who are PGRN deficient.

PGRN is a guardian of lysosome biology[41] by regulating lysosome biogenesis in a TFEB-dependent manner[42] and lysosome function. Lack of PGRN triggers accumulation of p62 (marker of damaged lysosome[43]) and LAMP1/2.[44,45] In line with previous findings, PGRN-deficient VSMC demonstrated accumulation of p62 and LC3I/II, increased LAMP content, and greater nuclear TFEB levels, although there is more TFEB in the nuclear fraction—an indicative of higher activity—the flux autophagic is interrupted (increased p62 and LC3I/II accumulation), indicating that TFEB is more active, but the formed lysosomes are imperfect. This exacerbated activation of TFEB may be indicative of an effort to stimulate the formation of suitable lysosomes and subsequently establish a proper autophagy flux, through an inefficient pathway though. Interestingly, a high amount of PGRN promoted more TFEB translocation into the nuclei and led to a prominent p62 reduction and increased LC3I/II. These observations strongly suggest that PGRN plays a role in triggering TFEB activation within lysosomes, subsequently influencing autophagy flux. Therefore, it becomes evident that mitochondrial complex I activity and biogenesis, mitochondrial dynamic, and the disturbance of mitophagy are key downstream pathways influenced by PGRN.

Because lack of vascular PGRN pathway is a cardiovascular risk, we challenged mice with Ang-II for 2 distinct reasons: (1) to analyze whether ablation of PGRN would aggravate Ang-II-induced mitochondria dysfunction and (2) to examine whether Ang-II could induce the vascular contractility in PGRN-deficient mice. Normally, Ang-II treatment provokes vascular hypercontractility,[46] upregulates contractile proteins,[47] and triggers mitochondria dysfunction.[48] At least with Ang-II treatment, lack of PGRN did not affect mitochondrial function, but it blunted the vascular hypercontractility, thus vascular PGRN pathway is essential to maintaining the vascular tone and induce vascular contraction. It suggests that PGRN plays a major role adjusting the vascular contractility pathways. Our recent study revealed that endothelium-intact mesenteric arteries from global PGRN-deficient mice exhibit increased contractility in response to noradrenaline, accompanied by elevated blood pressure.[15] Herein, our findings demonstrate that endothelium-denuded aortic rings from PGRN–/– mice show reduced contraction to different agonists. Furthermore, we observed that Ang-II induces a similar aortic contraction and increase in blood pressure in both PGRN+/+ and PGRN–/– male mice, however, with a delay of approximately 3 days in the blood pressure elevation in PGRN–/–.

In this study, we propose that the PGRN pathway plays a pivotal role in the regulation of vascular tone of large artery through the modulation of mitochondrial quality and using 2 distinct and sequential mechanisms. PGRN regulates mitochondrial complex I biogenesis and activity and orchestrates mitochondria recycling via maintaining lysosome quality. Subsequently, it preserves vascular bioenergetic pathway and redox balance inhibiting an exacerbated mtROS formation, which conserves

the vascular tone. Future research should explore the long-term consequences of PGRN deficiency in the context of CVD and evaluate how PGRN levels correlate with disease severity and progression in human patients. Finally, our study opens new opportunities not only to cardiovascular biology but also to examine this pathway in neurodegenerative diseases associated with PGRN mutations, which have recently been associated with perturbation of neurovascular compartment[24] as well.

Interestingly, loss of vascular contraction appears to be limited to the aorta and not resistance arteries, because our previous study performed in mesenteric arteries and focused on endothelial cells demonstrated that lack of PGRN increases vascular contractility in intact mesenteric arteries and leads to elevated blood pressure. We can speculate that this discrepancy in PGRN's effects on large and small vessels may be related to their differing roles in accommodating blood flow. For instance, although large arteries play a minimal role in blood pressure regulation, they become significant in diseases associated with hypertension. In contrast, small arteries are thought to play a crucial role in the pathogenesis of high blood pressure by actively modulating blood flow and altering their diameter in response to physiological changes, thereby influencing overall blood pressure regulation.[49] Furthermore, resistance arteries are densely innervated by sympathetic fibers and show a high expression of contractile smooth muscle proteins (*ACTA2*, *TAGLN*, *CNN1*, *CALD1*, and *MYH11*). In contrast, the aorta expresses fewer sympathetic components and contractile proteins.[50–52] Yet, the absence of endothelium in our current study could an additional explanation for the difference in vascular contractility of capacitance versus resistance arteries. The endothelium plays a critical role in regulating vascular tone by controlling the release of vasodilators and vasoconstrictors in response to environmental conditions, thus making a significant contribution to vascular function.[53] Lastly, our prior studies have shown that in the context of obesity or leptin treatment—both of which can lead to high blood pressure—the aorta exhibits reduced contractility,[54,55] a phenomenon distinct from the observed responses in small arteries.[56] Hence, we can speculate that the heterogeneity between the aorta and mesenteric arteries in our studies may contribute to this inconsistency. Factors like the regulation of vascular resistance, impact on blood flow, level of sympathetic innervation and contractile proteins, presence of endothelium, and adaptive mechanisms, including mitochondrial profile, may differ between these vessel types. Further research is needed to thoroughly evaluate these differences.

Conclusions

Overall, our findings offer new mechanistic insights into the regulation of vascular contractility in conductance arteries and place PGRN as an important protein in aortic diseases associated with loss of contractile properties such as aneurysm and aortic dissection. Mechanistically, PGRN affects aortic mitochondrial recycling, dynamics, and oxidative stress, thus influencing VSMC quality, we demonstrate that PGRN modulates VSMC contraction and remodeling by managing mitochondrial quality. Unlike our previous findings in resistance arteries. Therefore, reduced PGRN levels may directly contribute to dysfunctional VSMC in capacitance vessels.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Data S1

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