Smooth Muscle Cell Deletion of Low-Density Lipoprotein Receptor–Related Protein 1 Augments Angiotensin II–Induced Superior Mesenteric Arterial and Ascending Aortic Aneurysms

Frank M. Davis, Debra L. Rateri, Anju Balakrishnan, Deborah A. Howatt, Dudley K. Strickland, Selen C. Muratoglu, Christopher M. Haggerty, Brandon K. Fornwalt, Lisa A. Cassis, Alan Daugherty

Objective—Low-density lipoprotein receptor–related protein 1 (LRP1), a multifunctional protein involved in endocytosis and cell signaling pathways, leads to several vascular pathologies when deleted in vascular smooth muscle cells (SMCs). The purpose of this study was to determine whether LRP1 deletion in SMCs influenced angiotensin II–induced arterial pathologies.

Approach and Results—LRP1 protein abundance was equivalent in selected arterial regions, but SMC-specific LRP1 deletion had no effect on abdominal and ascending aortic diameters in young mice. To determine the effects of LRP1 deficiency on angiotensin II vascular responses, SMC-specific LRP1 (smLRP1+/+) and smLRP1-deficient (smLRP1−/−) mice were infused with saline, angiotensin II, or norepinephrine. Several smLRP1−/− mice died of superior mesenteric arterial (SMA) rupture during angiotensin II infusion. In surviving mice, angiotensin II profoundly augmented SMA dilation in smLRP1−/− mice. SMA dilation was blood pressure dependent as demonstrated by a similar response during norepinephrine infusion. SMA dilation was also associated with profound macropage accumulation, but minimal elastin fragmentation. Angiotensin II infusion led to no significant differences in abdominal aorta diameters between smLRP1+/+ and smLRP1−/− mice. In contrast, ascending aortic dilation was exacerbated markedly in angiotensin II–infused smLRP1−/− mice, but norepinephrine had no significant effect on either aortic region. Ascending aortas of smLRP1−/− mice infused with angiotensin II had minimal macropage accumulation but significantly increased elastin fragmentation and mRNA abundance of several LRP1 ligands including MMP-2 (matrix metalloproteinase-2) and uPA (urokinase plasminogen activator).

Conclusions—smLRP1 deficiency had no effect on angiotensin II–induced abdominal aortic aneurysm formation. Conversely, angiotensin II infusion in smLRP1−/− mice exacerbated SMA and ascending aorta dilation. Dilation in these 2 regions had differential association with blood pressure and divergent pathological characteristics. (Arterioscler Thromb Vasc Biol. 2015;35:155-162. DOI: 10.1161/ATVBAHA.114.304683.)

Key Words: angiotensin II ■ aortic aneurysm ■ LRP1 protein, mouse

Low-density lipoprotein receptor–related protein 1 (LRP1) is a multifunctional member of the low-density lipoprotein receptor gene family that interacts with numerous ligands. Ligand engagement can result in either removal of the ligand from the extracellular environment or stimulation of specific intracellular signaling pathways. Determination of the contribution of LRP1 to vascular diseases was hindered initially by the embryonic lethality of whole body deletion. Subsequent vascular studies have demonstrated a role for LRP1 using cell-specific deletion, with particular emphasis on macrophages and smooth muscle cells (SMC). LRP1 deficiency in either of these cell types augments atherosclerosis in hypercholesterolemic mice and neointimal formation. SMC-specific deletion of LRP1 (smLRP1−/−) in aged mice also leads to several vascular phenotypes, including extended aortic length, aberrant superior mesenteric artery structure, and ascending aorta dilation.

Recent studies have suggested a role for LRP1 in human vascular pathologies, particularly aortic aneurysms. Genome-wide association studies have implicated the rs1466535 polymorphism of the LRP1 gene with abdominal aortic aneurysms (AAAs), although the 2 studies differ.

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in which allele confers risk.\textsuperscript{11,12} A role for LRP1 in human AAAs has also been inferred by reduced abundance of LRP1 protein in aneurysmal tissue.\textsuperscript{13} In addition to an implied role in AAAs, exome sequencing of LRP1 identified a missense mutation in patients with thoracic aortic aneurysms who are afflicted with Marfan syndrome.\textsuperscript{14} These recent studies provide the basis for a potential role of LRP1 within aortic aneurysm formation.

Numerous studies have demonstrated a role for angiotensin II in several vascular pathologies, particularly atherosclerosis and aortic aneurysms.\textsuperscript{15–17} Angiotensin II is one of the few mediators, that regulates abundance of LRP1 protein in SMCs.\textsuperscript{18} In addition, angiotensin II regulates expression of many LRP1 ligands, some of which have been implicated in aneurysm formation and compromised vascular integrity. These include plasminogen activator inhibitor 1,\textsuperscript{19} transforming growth factor-\(\beta\),\textsuperscript{20} selected MMPs (matrix metalloproteinases),\textsuperscript{21} and connective tissue growth factor.\textsuperscript{22} Given the potential for angiotensin II to augment many LRP1 ligands that affect vascular integrity, we speculated that LRP1 deficiency would influence vascular pathologies formed during chronic infusion of angiotensin II.

The purpose of this study was to determine whether the absence of SMC LRP1 influenced angiotensin II–induced arterial pathologies. The primary expectation was that smLRP1 deficiency would promote angiotensin II–induced AAAs. However, this was not observed. In contrast, angiotensin II infusion profoundly augmented aneurysms in both the superior mesenteric artery (SMA) and ascending aorta. Despite the commonality of arterial dilation, the 2 regions differed markedly in response to elevated blood pressure and tissue pathology.

**Materials and Methods**

Materials and Methods are available in the online-only Data Supplement.

**Results**

**LRP1 Abundance in Arterial Vasculature**

We first determined the abundance of LRP1 protein in selected arterial regions. LRP1 protein abundance was found to be uniform across all regions of the aorta and SMA (Figure 1A). Regional abundance of LRP1 protein was also determined in LRPI\textsuperscript{+/+} and LRPI\textsuperscript{−/−} mice to confirm that SM22-driven Cre effectively mediated recombination of the homozygous LRPI\textsuperscript{flox/flox} gene to prominently deplete the LRPI gene in vascular SMCs (Figure 1B), as described previously.\textsuperscript{9}

Baseline aortic measurements were acquired before initiating infusions, while smLRP1\textsuperscript{+/+} and smLRP1\textsuperscript{−/−} mice were \(\approx 8\) weeks of age, and these were predicted to have no overt vascular pathology. In agreement with this prediction, smLRP1 genotype had no significant difference in ascending or abdominal aortic diameters (Figure 1C and 1D).
SMC Depletion of LRP1 Exacerbat ed SMA Dilation in a Blood Pressure-Dependent Manner

After baseline measurements, smLRP1+/+ and smLRP1−/− mice were infused with either saline or angiotensin II (1000 ng/kg per day) for 28 days. Chronic angiotensin II infusion increased systolic blood pressure in all mice, with no significant difference between genotypes (Table I in the online-only Data Supplement). Several smLRP1−/− mice died during angiotensin II infusion. On necropsy, mesenteric hematomas were noted (Figure 2A and 2B). In surviving mice, in vivo MRI demonstrated that angiotensin II–infused smLRP1−/− mice had pronounced SMA dilation (Figure 2C; Figure IA and IB in the online-only Data Supplement). Ex vivo measurements after 28 days of infusion demonstrated that SMA diameters of saline-infused smLRP1+/+ and smLRP1−/− mice were not significantly different (Figure 3A and 3B). In contrast, SMA diameters were dilated markedly during infusion of angiotensin II (P<0.05; Figure 3A and 3B).

Because angiotensin II infusion increased systolic blood pressure, we determined its contribution to the pronounced SMA dilation. smLRP1+/+ and smLRP1−/− mice were infused with either saline or norepinephrine (5.6 mg/kg per day) for 28 days. As described previously,23,24 this infusion rate of norepinephrine produced systolic blood pressure increases that were equivalent to angiotensin II infusion (Table I in the online-only Data Supplement). As with angiotensin II infusion, so also norepinephrine infusion promoted pronounced SMA dilation (Figure 3A and 3B).

Dilated SMA tissue was characterized by increased accumulation of CD68+ cells in the adventitia and media of smLRP1−/− mice infused with either angiotensin II or norepinephrine (Figure 3C, 3E, 3G, 3I, 3K, and 3M). In contrast, no CD68+ cells were detected in the media SMAs of

Figure 2. Angiotensin (Ang II) infusion in smooth muscle cell–specific LRP1–deficient (smLRP1−/−) mice promoted superior mesenteric artery (SMA) dilation and rupture. A and B, Rupture of SMA caused a mesenteric hematoma. *Hematoma within the mesenteric fat pad; #small intestine. C, Representative 3D magnetic resonance images performed using a 7.0-T Clinscan system.
smLRP1+/+ mice that had been infused with saline, angiotensin II, or norepinephrine, although there was a sparse presence of CD68+ cells in the adventitia (Figure 3C, 3G, and 3K). Movat staining demonstrated a relatively modest, but significant, increase in elastin fragmentation in SMAs of smLRP1−/− mice after infusion of both angiotensin II and norepinephrine (NE). Bar represents 400 μm. B. Bar graphs of SMA dilation after saline, Ang II, or NE infusion. Histobars represent means, and errors are SEMs (n=5 per group). *P<0.05 by 2-way ANOVA with multiple comparisons post hoc Holm–Sidak test by all genotypes and infusion groups. ns indicates no statistical significance. C to N. Representative SMA micrographs of smLRP1−/− (C, D, G, H, K, L) and smLRP1+/+ mice (E, F, I, J, M, N) infused with saline (C–F), Ang II (G–J), or NE (K–N). Cross-sections of SMA were immunostained for CD68+ cells (C, E, G, I, K, M) and stained with Movat (D, F, H, J, L, N). CD68+ cells are red and elastin stains black. Sections are oriented with the lumen at the top of the image.

Figure 3. Increased systolic blood pressure promoted superior mesenteric artery (SMA) dilation characterized by macrophage accumulation in smooth muscle cell–specific LRP1–deficient (smLRP1−/−) mice. A. Hematoxylin and eosin stained SMA cross-sections were obtained 0.2 mm from bifurcation with aorta. smLRP1+/+ and smLRP1−/− mice were infused with saline, angiotensin (Ang) II, or norepinephrine (NE). B. Bar graphs of SMA dilation after saline, Ang II, or NE infusion. Histobars represent means, and errors are SEMs (n=5 per group). *P<0.05 by 2-way ANOVA with multiple comparisons post hoc Holm–Sidak test by all genotypes and infusion groups. ns indicates no statistical significance.

smLRP1−/− mice was confirmed by ex vivo measurements, even though mice had the same body length (Figure IIIB and IIIC in the online-only Data Supplement). The aortic elongation was caused by increased length of both the thoracic and abdominal aorta (Figure IIID and IIIE in the online-only Data Supplement).

Aortic aneurysmal pathology was examined after 28 days of infusion. Against expectations, smLRP1−/− had no effect on angiotensin II–induced AAA formation as measured in vivo by ultrasound and ex vivo by measurement of diameters of suprarenal aortas (Figure IV in the online-only Data Supplement).

Dimensions of the ascending aorta were also measured in both smLRP1+/+ and smLRP1−/− mice. As described previously,17,25 angiotensin II infusion significantly increased ascending aortic diameter in smLRP1−/− mice, independent of blood pressure elevation. This dilation was significantly exacerbated in smLRP1−/− mice infused with angiotensin II (P<0.05; Figure 4A and 4B). Unlike SMA dilation,
development of dilation in the ascending aorta was independent of systolic blood pressure because norepinephrine infusion did not significantly enhance expansion of ascending aortas from smLRP1−/− mice (Figure 4B).

Concomitant with luminal expansion were pathological changes in the media of ascending aortas after 28 days of angiotensin II infusion. No significant increase in CD68+ cell accumulation was discernible in aortic media of any group (Figure 5A, 5D, 5G, and 5J). However, CD68+ cell accumulation was visible in adventitia of the ascending aorta in angiotensin II–infused smLRP1−/− mice (Figure 5J). Elastin fragmentation was increased greatly in angiotensin II–infused smLRP1+/+ and smLRP1−/− mice compared with saline-infused mice. Angiotensin II–infused smLRP1−/− mice had the most significant increase in elastin disruption compared with all other groups (Figure 5B, 5C, 5E, 5F, 5H, 5I, and 5K–5M).

Angiotensin II Upregulated Expression of LRP1 Ligands in Ascending Aortas of smLRP1−/− Mice

To identify a potential mechanism by which angiotensin II exacerbates smLRP1−/− ascending aortic dilation, we quantified abundance of known LRP1 ligands. In these experiments, mRNA was extracted from ascending aortas of saline- or angiotensin II–infused smLRP1+/+ and smLRP1−/− mice and analyzed for mRNA abundance of LRP1 ligands. Given that norepinephrine infusion in smLRP1−/− mice did not produce ascending aortic dilation, we did not analyze expression of LRP1 ligands in these mice (Table II in the online-only Data Supplement). mRNA of several extracellular proteases including MMP-2 and uPA (urokinase plasminogen activator) was increased significantly in angiotensin II–infused smLRP1−/− ascending aortas (Figure 6A and 6B). Furthermore, mRNA abundance of plasminogen activator inhibitor 1 and thrombospondin 2 was increased (Figure 6C and 6D).

Discussion

Angiotensin II infusion into mice promotes formation of aneurysms in the abdominal and ascending aortic regions, and augments atherosclerosis when associated with hypercholesterolemia.15–17 Although these vascular pathologies are caused by the interaction of angiotensin II with AT1a receptors,26–28 it has to be defined which cell type is stimulated by angiotensin II to form these diseases, except in the case of deleting endothelial AT1a receptors on the development of ascending aortic aneurysms.26,29,30 LRP1 is expressed in many cell types where it exerts protective effects from vascular pathology. Angiotensin II regulates many LRP1 ligands, but the interactions of angiotensin II, LRP1, and vascular disease have not been studied in vivo. The present study demonstrated that LRP1 protein abundance was equivalent throughout all arterial regions examined. Surprisingly, deletion of LRP1 in SMCs had no effect on AAA, whereas it promoted angiotensin II–induced SMA and ascending aortic aneurysms by disparate mechanisms.

Many studies have reported aortic aneurysm formation during the initial few days of angiotensin II infusion. Death is commonly attributable to rupture of the suprarenal or ascending aorta.11,12 Although arterial rupture also occurred in a limited number of smLRP1−/− mice during angiotensin II infusion, this was attributed to SMA rupture rather than compromise of aortic integrity. Although SMA enlargement has been described previously in aged smLRP1−/− mice,43 it has not been documented during angiotensin II infusion. To further define the differences between SMA and aortic aneurysms in smLRP1−/− mice, we investigated the effects of hypertension on aneurysmal disease within these arterial regions. It has been previously demonstrated that increases of blood pressure are not directly involved in angiotensin II–augmented aneurysmal formation of the suprarenal and ascending aortic
regions. This conclusion was primarily based on comparisons with pathology in mice with hemodynamic equivalent infusions of norepinephrine. Using the same strategy in this study, hemodynamic equivalent infusions of either angiotensin II or norepinephrine promoted a similar extent of dilation.33 Therefore, the presence of elastin fragmentation in angiotensin II– and norepinephrine-infused mice could be responsible for the pronounced macrophage accumulation. However, this relationship differed in the ascending aorta of angiotensin II–infused mice where there was extensive elastin fragmentation but minimal vascular macrophage accumulation. The cause and consequence of macrophage accumulation in the SMA need further study.

Previous studies in mice have inferred that LRP1 deficiency led to formation of AAAs, but did not provide direct evidence. In humans, LRP1 has been linked to the development of AAAs on the basis of genetic associations and changes of LRP1 protein abundance in aneurysm tissue.11–13 Therefore, it was predicted that SMC deletion of LRP1 would promote development of AAAs in normocholesterolemic mice during angiotensin II infusion. Because normocholesterolemic mice usually have a low incidence of angiotensin II–induced AAAs, they provided a model to determine augmentation of AAAs. Contrary to this prediction, LRP1 absence did not lead to overt AAA formation during angiotensin II infusion, or any measurable change in aortic diameters compared with LRP1-proficient mice. LRP1 was effectively deleted in this aortic region, so the absence of alteration in AAA pathology was not attributable to any technical shortcomings. This lack of effect may not necessarily contradict the inference of LRP1 in human AAA, which is derived from weak associations that have generated discrepant allelic associations.

There has been considerable interest on the role of angiotensin II in the development of ascending aortic aneurysms, both experimentally and clinically. Interest was stimulated primarily by demonstrating that an AT1 receptor antagonist, losartan, prevented thoracic aortic aneurysms in mice harboring a C1039G fibrillin-1 mutation.38 Conversely, angiotensin II infusion promotes expansion of the ascending aorta in both normo- and hypercholesterolemic mice. SMC-specific LRP1 deficiency also promotes expansion of the aortic root and descending aorta in aged mice. The present study demonstrated that lack of SMC LRP1 greatly augmented angiotensin II–induced ascending aortic expansion. As described for angiotensin II–induced ascending aortic dilation in LRP1-proficient mice, norepinephrine infusion at rates that induced equivalent systolic blood pressure increases did not mimic effects of angiotensin II. Also, although LRP1 deficiency greatly augmented rates of ascending aortic expansion, there was no profound difference in pathological characteristics of tissue sections. These were characterized by profound elastin degradation, but a relative paucity of medial macrophage accumulation. Other characteristics include the lack of any

Figure 5. Ascending aortic sections from angiotensin (Ang) II–infused smooth muscle cell–specific LRP1–deficient (smLRP1−/−) mice have increased elastin fragmentation. A to L, Representative ascending aortic sections of smLRP1−/− (A–C, G–I) or smLRP1+/- mice (D–F, J–L) infused with saline or Ang II infusion. Macrograph accumulation was identified by CD68+ cells (A, D, G, J), and elastin fragmentation was examined by Movat (B, E, H, K). Additional magnification of Movat stained sections is seen in the green boxes (C, F, I, L). Sections are oriented with the lumen at the top of the image. Yellow arrows indicate regions of marked elastin fragmentation. M, Elastin fragmentation counted in cross-sections of ascending aortas from saline or Ang II–infused smLRP1−/− and smLRP1+/- mice. Histobars represent group means (n=4 per group), and errors are SEM. Lines represent P<0.05 by 2-way ANOVA followed by a post hoc Holm–Sidak test.
neointimal formation and a predominance of medial changes on the adventitial aspect of the aorta. Overall, although concomitant angiotensin II infusion and SMC-specific LRP1 deficiency promote dilation of the ascending aorta and SMA, the disparate roles of blood pressure and pathological appearances differ different mechanisms for aneurysm formation.

The mechanism by which LRP1 deficiency enhances selected angiotensin II–induced vascular pathologies is unclear. Angiotensin II generates vascular pathologies through stimulation of AT1a receptors, but deletion of this receptor in SMCs had no effect on angiotensin II–induced atherosclerosis and aneurysm.29 The pronounced enhancement of angiotensin II–induced vascular pathologies in the absence of SMC LRP1 is likely because of augmentation of ligands that interact with LRP1. The present study has demonstrated increased mRNA abundance for several proteases that have been indicated in aneurysmal tissue.9 Furthermore, although we have defined mRNA abundance for these proteases and arterial tissue, LRP1 ligands could have originated from extracellular sources. Defining the role of these proteases will require future studies that systematically delete these genes in mice with SMC-specific deletion of LRP1.

Overall, these studies demonstrate that the activity of LRP1 in SMCs is a major factor regulating development of angiotensin II–induced vascular pathologies. These studies also demonstrate regional disparate effects of elevating systolic blood pressure. It will be of interest in future studies to determine the specific LRP1 ligands that are enhanced by angiotensin II to compromise vascular integrity.

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**Disclosures**

None.

**References**


Low-density lipoprotein receptor–related protein 1 (LRP1) is a multifunctional protein that engages many ligands to perform endocytosis and signaling. It has been implicated in vascular pathologies in both humans and mice. Angiotensin II is well known to promote experimental vascular pathologies. In this study, the effects of LRPI deletion in smooth muscle cells were determined during angiotensin II–induced vascular pathologies. Unexpectedly, angiotensin II infusion led to deaths because of superior mesenteric arterial rupture that was associated with pronounced dilation of this vessel in smooth muscle cell–specific deficient LRP−/− mice. Also unexpectedly, there was no effect of smooth muscle cell deficiency of LRP1 on formation of abdominal aortic aneurysms during angiotensin II infusion. However, the absence of LRPI led to a major promotion of ascending aortic dilation during angiotensin II infusion. Therefore, angiotensin II infusion in mice deficient of LRPI in smooth muscle cells had a highly regional effect on development of vascular pathologies.
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Smooth Muscle Cell Deletion of LDL Receptor Related Protein-1 Augments AngII-Induced Superior Mesenteric Arterial and Ascending Aortic Aneurysms

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MATERIALS AND METHODS

Mice and diet

The LRP1flox mouse was developed using cre-mediated recombination resulting in a deletion of a portion of the LRP1 promoter and transcription site, as well as exons 1 (signal peptide coding region) and 2. A search in the Entrez data base for miRNA expressed in the mouse LRP1 gene revealed no known miRNA present. Smooth muscle-specific LRP1 deletion was accomplished by breeding SM22 Cre transgenic mice to LRP1 floxed mice. Male smLRP1-/- and littermate +/+ mice in a mixed background were separated into saline and AngII infusion groups. Male mice were housed 5 per cage in individually ventilated cages with negative air pressure (Allentown, Inc; Allentown, NJ). Aspen hardwood chips (Sani-Chip; Cat # 7090A; Harlan; Madison, WI) were used as bedding. Drinking water was filtered by reverse osmosis (pH 6.0-6.2). Light cycle of the room was 14 hours of light and 10 hours of dark. Ambient temperature ranged from 68 to 74°F and humidity was 50-60%. All mice were 6-8 weeks old at the start of study and were fed normal laboratory mouse diet (Cat # 2918; Harlan Teklad Global 18% Protein Rodent Diet; Madison, WI). Necropsy was performed to determine cause of death during infusion. Genotypes were confirmed at termination using DNA isolated from tails and PCR using Cre primers. All procedures were approved by the University of Kentucky IACUC.

Infusions

Saline, AngII and NE were infused via subcutaneous pumps as described previously. Osmotic mini-pumps (Alzet Model #2004; Durect; Cupertino, CA) were filled with saline, AngII (1,000 ng/kg/min dissolved in saline) or NE (5.6 mg/kg/day; dissolved in saline containing 0.2% ascorbic acid). Mice were sedated with Isoflurane (Isothesia; Butler Animal Health Supply; Dublin, OH) and pumps were implanted subcutaneously on the right side of the mice. Surgical staples were used to close incision sites and a topical anesthetic cream (LMX4; Ferndale Laboratories; Ferndale, MI) was applied.

Ultrasonic imaging

Ascending and suprarenal regions of aortas were imaged in vivo using a Vevo 2100 with a MicroScan MS400 transducer (18-38 MHz, resolution 30 µm; VisualSonics; Toronto, Ontario, Canada). Mice were anesthetized using Isoflurane (Isothesia; Butler Animal Health Supply; Dublin, OH) and hair was removed from the chest and belly using a hair clipper and depilatory cream (Nair; Church and Dwight Co; Princeton, NJ). Ultrasonic gel (Medline; Mundelein, IL) was placed on the shaved areas and a transducer was used to capture images. B-Mode ultrasonic images were measured to obtain the maximum systolic diameter of ascending and suprarenal regions of the aorta. A mean of three cardiac cycles was used for diameter measurements in each mouse.

Systolic blood pressure measurements

A non-invasive tail cuff system (Kent Scientific; Torrington, CT) was used to measure systolic blood pressure at baseline and during week 3 of infusion. Conscious mice were restrained on a heated platform. Blood pressure was measured the same
time each day for at least three consecutive days. Accepted data criteria were 10 of 20 measurements (50%) and a SD <30.

**Magnetic Resonance Imaging**

Magnetic resonance imaging (MRI) was performed using a 7.0-T Clinscan system (Bruker, Ettlingen, Germany) with a 30 cm inner diameter quadrature radiofrequency coil. Mice were anesthetized with Desflurane and positioned in the supine position in the imaging unit. Body temperature was maintained at 36.5 ± 0.5 °C using a heated water circuit. Electrocardiographic data, body temperature and respiration rate were continuously monitored throughout the scan using an MRI-compatible system (SA Instruments, Stony Brook, NY).

To span the length of the aorta from top of the arch to iliac bifurcation, two transverse stacks of cardiorespiratory-gated gradient echo bright blood images were acquired with in-plane resolution of 0.1198 x 0.1198 mm², slice thickness of 0.6 mm, no slice gaps, and 14-15 phases per cardiac cycle. In each case, the thoracic stack (43 slices) was acquired first, then the mouse bed was manually re-positioned with respect to the center of the cylinder coil, followed by acquisition of an abdominal stack (35-40 slices). Four fiducial markers (100 IU vitamin E tablets) were aligned and positioned under the mouse serving as reference points between the two imaging positions to ensure overlap between the slice stacks. Separate respiratory-gated, T1-weighted sagittal localizer stacks were acquired to visualize and define positions of these markers in both bed positions. Additional gradient echo images were acquired in oblique views of the aortic arch for qualitative assessment.

**MRI Post-processing**

Transverse image stacks were interpolated to obtain an approximately isotropic 3-dimensional image volume. The aorta (inclusive of the takeoffs of the head and neck vessels, the superior mesenteric artery takeoff and the iliac bifurcation) was semi-automatically segmented in a single cardiac phase using custom software written in MATLAB (The Mathworks, Natick, MA). To merge segmented aortic masks, one set of fiducial marker images was separately segmented with a 3D level set segmentation algorithm. Translation of a single marker between positions was determined based on visual registration of the 3D segmentation mask with the corresponding marker in the second localizer stack. The resulting translation was imposed on the 3D image position coordinates for the appropriate transverse image stacks. Additional small adjustments to the registration were made in some cases to maintain consistency with anatomic landmarks. Once merged, the segmented isosurface was defined and complete reconstruction was exported for geometric feature extraction.

Geometric analysis was performed using the Vascular Modeling Toolkit (VMTK; vmtk.org), with results visualized in ParaView (paraview.org). The 3D surface was first smoothed for 30 iterations with a passband of 0.1, and the vessel centerline calculated and smoothed (100 iterations; smoothing factor 0.1). From this centerline, maximum and average radii of the aorta and superior mesenteric arteries (based on the maximum inscribed sphere radius at each point along the centerline), length of the aorta from left
subclavian artery takeoff to iliac bifurcation, and aortic tortuosity from left subclavian artery takeoff to iliac bifurcation were calculated. Tortuosity (T) was defined as the ratio of the centerline distance traveled versus the straight line distance connecting the two end points.

**Isolation and culture of vascular SMCs**

Mice were euthanized with a ketamine/xylazine solution (90/10 mg/kg, respectively; Henry Schein, Oshkosh, WI). Arterial vasculature was perfused through the left ventricle of the heart with sterile PBS. Aortas were excised, and dissected free of adventitia and fat. Aortas were separated into thoracic (from the aortic valve to the last intercostal) and abdominal aortic regions (from the last intercostal to the bifurcation). Vascular SMCs were isolated from aortas as described previously. SMCs from 4 pooled mice were plated 1 well of a 6 well plate and grown in DMEM, FBS(20%) and penicillin and streptomycin (1%) and placed in a 5% CO$_2$, 37°C incubator. Vascular SMC phenotype was determined by immunostaining cells for α-actin (Abcam, ab5694, Cambridge, MA). Cells were grown to 80-90% confluency and serum-starved for 24 hours before use.

**Western blot analyses**

Aortic tissue homogenate or total cell lysates of primary vascular SMCs harvested from either thoracic or abdominal aortic regions were prepared in RIPA lysis buffer. Protein concentration was measured using the Bradford assay (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein samples (2.5-10 g) were resolved by 4-20% SDS-PAGE and transferred electrophoretically to PVDF membranes. After blocking, antibodies against the following proteins were used to probe the membranes: LRP1 (Abcam, ab92544, Cambridge, MA) and β-actin (Catalog# A5441, Sigma-Aldrich, St. Louis, MO). Then membranes were incubated with either biotinylated goat anti-rabbit or biotinylated rabbit anti-rat secondary antibodies (Cat # T1101 and T10813, Vector Laboratories; Burlingame, CA), and immune complexes were visualized by chemiluminescence (Catalog number 34080; Thermo Scientific, Waltham, MA) and quantified using a Thermo Scientific myECL Imager.

**Pathology**

At experimental termination, mice were anesthetized with an i.p. injection of ketamine/xylazine (90/10 mg/kg, respectively; Henry Schein, Oshkosh, WI). Blood was collected by cardiac puncture, and aortas were flushed with saline. For the measurement of length and ex vivo photographs, aortas with hearts and kidneys were dissected free and tissues were immersion fixed overnight in 10% formalin. Extraneous tissue was removed and ascending aortas were photographed. The regional length of the aorta in either the thoracic + suprarenal region (from left subclavian artery to left renal artery) or the infrarenal region (from the left renal artery to bifurcation) was measured ex vivo using Image Pro Version 7.0 software. For immunostaining and pathology, aortas and SMAs were dissected free and extraneous tissue was removed. Ascending aortas and SMAs were placed in OCT without fixation.
Ascending aortas and SMAs were serially sectioned (10 μm thickness; multiple series of ten slides with 9 sections/slide). H&E and Movat’s pentachrome stains were used to examine cellularity and elastin fibers. Immunostaining was performed to detect specific cell types. SMAs were quantified using artery cross sections that were stained with H&E at 0.9mm-1.8mm from the bifurcation of the SMA and aorta. Anterior-posterior (AP) and transverse (trans) diameters were measured on 9 serial 10 μm sections per mouse. A mean was determined for measurements from these 9 serial sections to give a SMA diameter for each mouse. For immunostaining, the following antibodies were used: α-actin for smooth muscle cells (Cat # ab5694; Abcam; Cambridge, MA) and CD68 for macrophages (Cat# MCA1957GA, Serotec; Raleigh, NC). Reactivity was determined using biotin labeled secondary antibodies (Cat # BA-4001 and BA-6000; Vector Laboratories; Burlingame, CA), horseradish peroxidase ABC kits (Peroxidase Elite Standard; Cat # PK-6100; Vector Laboratories; Burlingame, CA) and 3-amino-9-ethylcarbazole for chromogen (ImmPACT™; Cat # SK-4205; Vector Laboratories; Burlingame, CA). Non-immune primary antibodies, secondary only, and no primary or secondary antibody slides were run as negative controls to confirm specificity of primary and secondary antibodies, and ablation of endogenous tissue peroxidase, respectively. Elastin breaks were counted at the maximum ascending aortic or superior mesenteric artery expansion. For each mouse, fragmentation was counted in 5 serial 10 μm sections and then meaned. Four mice were used per group, and group mean elastin fragmentation was reported.

**Real Time PCR**

Ascending aortas were dissected free and adventitia left intact. Total RNA was extracted from ascending aortas using the Qiagen Rneasy Fibrous Kit (Qiagen, Hilden, Germany) according to manufacturer’s instructions. Reverse transcription and real time PCR were performed using an iCycler (Bio-Rad). RNA (100 ng) was reverse transcribed using the i-script cDNA synthesis kit (Bio-Rad). PCR product accumulation was monitored using a custom designed 96-well Taqman gene expression assay plate according to manufacturer’s instructions (Life Sciences, Grand Island, NY). The gene expression assays included on the custom 96-well plates are in Supplemental Table III. mRNA abundance was calculated using the ΔΔCt method with normalization to either β-actin or GADPH.

**Statistical analyses**

Statistical tests included standard parametric procedures for a single response and repeated measures. Analyses were performed using SigmaPlot version 12 (Systat Software Inc, San Jose, CA). The specific statistical test used is identified in each figure legend. Holm-Sidak for post hoc testing was used to ascertain group differences in two-way ANOVA. Data are represented as individual and mean ± SEM. P<0.05 was considered statistically significant. All positive phenotypes attained a power of greater than 0.8.
REFERENCES
SUPPLEMENTAL MATERIAL

Supplemental Table I: Systolic blood pressure measurements.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Systolic Blood Pressure (mmHg)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>smLRP1+/+</td>
<td>smLRP1-/-</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>145 ± 3.5</td>
<td>137 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>Saline Infusion</td>
<td>143 ± 7.0</td>
<td>139 ± 7.0</td>
<td></td>
</tr>
<tr>
<td>AngII Infusion</td>
<td>187 ± 6.0*</td>
<td>180 ± 7.0*</td>
<td></td>
</tr>
<tr>
<td>NE Infusion</td>
<td>180 ± 8.8*</td>
<td>175 ± 8.8*</td>
<td></td>
</tr>
</tbody>
</table>

Data represented mean ± SEM;

* denoted P<0.05 by Student’s t test for comparisons between saline and AngII or NE infusion within each genotype.
**Supplemental Table II**: Relative abundance of mRNA in the ascending aorta using a gene expression assay plate assay.

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Relative mRNA Expression in Ascending Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>smLRP1+/+ &amp; Saline</td>
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<tr>
<td>MMP-2</td>
<td>1.0 ± 0.15</td>
</tr>
<tr>
<td>MMP-13</td>
<td>1.0 ± 0.15</td>
</tr>
<tr>
<td>MMP-14</td>
<td>1.0 ± 0.15</td>
</tr>
<tr>
<td>Htra1</td>
<td>1.0 ± 0.08</td>
</tr>
<tr>
<td>Tpsb2</td>
<td>1.0 ± 0.26</td>
</tr>
<tr>
<td>Tgfb1</td>
<td>1.0 ± 0.20</td>
</tr>
<tr>
<td>Tgfr2</td>
<td>1.0 ± 0.42</td>
</tr>
<tr>
<td>Igfbp3</td>
<td>1.0 ± 0.21</td>
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<tr>
<td>LRP5</td>
<td>1.0 ± 0.20</td>
</tr>
<tr>
<td>Ctsd</td>
<td>1.0 ± 0.20</td>
</tr>
<tr>
<td>Ctgf</td>
<td>1.0 ± 0.08</td>
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<tr>
<td>Agtr1a</td>
<td>1.0 ± 0.27δ</td>
</tr>
<tr>
<td>Agtr1b</td>
<td>1.0 ± 0.33</td>
</tr>
<tr>
<td>Agtr2</td>
<td>1.0 ± 0.24</td>
</tr>
<tr>
<td>Thbs2</td>
<td>1.0 ± 0.16</td>
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<tr>
<td>Plau</td>
<td>1.0 ± 0.77</td>
</tr>
<tr>
<td>Adamts5</td>
<td>1.0 ± 0.23</td>
</tr>
<tr>
<td>Cpa3</td>
<td>1.0 ± 0.45</td>
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<tr>
<td>Pdgfb</td>
<td>1.0 ± 0.15</td>
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<tr>
<td>Serpine1</td>
<td>1.0 ± 0.47</td>
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<tr>
<td>Mdk</td>
<td>1.0 ± 0.06</td>
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</table>

* P<0.05 for comparison to smLRP1+/+ & saline
δ P<0.05 for comparison to all other groups within a given target gene
# P<0.05 for comparison to smLRP1+/+ & saline and smLRP1-/- & saline
All statistical tests were by two way ANOVA. Post-hoc multiple comparisons were
performed using the Holm-Sidak test.
### Supplemental Table III: Taqman Gene Expression Assays of LRP1 Ligands

<table>
<thead>
<tr>
<th>Gene of Interest</th>
<th>Taqman Gene Expression Assay</th>
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<tr>
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<td>Thbs2</td>
<td>Mm01279240_m1</td>
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<td>Mmp9</td>
<td>Mm00600164_g1</td>
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<td>Mmp14</td>
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<td>MMP-13</td>
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<td>Lrp1</td>
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<td>Lrp5</td>
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<td>m18S</td>
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<td>Actb</td>
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<tr>
<td>Gapdh</td>
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</table>
Figure I: Representative MR images from smLRP1/- mice demonstrated profound dilation of the SMA and tortuosity of the aortic arch. Representative MRI of aortas from AngII-infused smLRP1+/+ and -/- mice. 
A,B. MRI transverse scan of the abdominal aorta (\(^\wedge\)). * denotes SMA. 
C,D. Oblique scan of the aortic arch.
Figure II: **SMA dilation was associated with modest elastin fragmentation.** Bar graph of elastin fragmentation counted in cross sections of SMAs from Saline, AngII or NE-infused smLRP1+/+ and +/- mice. Histobars represent group means (n=4 per group) and errors are SEM. Lines denote P<0.05 by two way ANOVA followed by a post hoc Holm-Sidak test.
Figure III: smLRP1/- resulted in an increased aortic length.  

A. Representative MRI of aortas from smLRP1+/+ and -/- mice.  Black lines indicate boundaries of regional aortic length measurements.

B. Overall aortic length was measured ex vivo from left subclavian artery to bifurcation.

C. Mouse body length was measured from snout to anus. Circles represent individual mice (n=8-16 per group).  Diamonds are group means and error bars are SEM.

D-E. The aortic length was further divided into regions with the thoracic + suprarenal aortic region (measured from left subclavian artery to left renal artery) and infrarenal aortic region (measured from the left renal artery to bifurcation).  * denotes P<0.01.  Statistical comparisons were performed using Student’s t test.
Figure IV: smLRP1-/- did not affect AngII-induced AAA formation.  
A. Maximum suprarenal abdominal aortic diameters in vivo were measured by ultrasound.  
B. Maximal suprarenal abdominal aortic diameters were measured ex vivo. Circles represent individual mice (n=4-14 per group). Diamonds are group means and error bars are SEM. No statistical differences were detected among groups by two way ANOVA with Holm-Sidak test multiple comparisons.