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Alan Daugherty and Lisa A. Cassis

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ATVB In Focus
Abdominal Aortic Aneurysms: Pathophysiological Mechanisms and Clinical Implications

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• Powell JT, Brady AR. Detection, management, and prospects for the medical treatment of small abdomial aortic aneurysms. 2004;24:241–245.

Mouse Models of Abdominal Aortic Aneurysms

Alan Daugherty, Lisa A. Cassis

Abstract—Many mouse models of abdominal aortic aneurysms have been developed that use a diverse array of methods for producing the disease, including genetic manipulation and chemical induction. These models could provide insight into potential mechanisms in the development of this disease. Although experimental studies on abdominal aortic aneurysms (AAAs) have used a variety of mammalian and avian approaches, there is an increasing reliance on the use of mice. The models recapitulate some facets of the human disease including medial degeneration, inflammation, thrombus formation, and rupture. Most of the mouse models of AAA are evoked either by genetically defined approaches or by chemical means. The genetic approaches are spontaneous and engineered mutations. These include defects in extracellular matrix maturation, increased degradation of elastin and collagen, aberrant cholesterol homeostasis, and enhanced production of angiotensin peptides. The chemical approaches include the intraluminal infusion of elastase, periaortic incubations of calcium chloride, and subcutaneous infusion of AngII. A common feature of these models is the reduction of AAA incidence and severity by the prophylactic administration of matrix metalloproteinase (MMP) inhibitors or genetically engineered deficiencies of specific members of this proteolytic protein family. The validation of mouse models of AAAs will provide insight into the mechanisms of progression of the human disease. **(*Arterioscler Thromb Vasc Biol*. 2004;24:429-434.)**

Key Words: aneurysms ■ mice

By definition, an aneurysm is a permanent dilation of the arterial wall. However, even this seemingly simple criterion has not led to a uniform consensus on the definition of a pathologically relevant aneurysm. Some favor an absolute measurement of the abdominal segment of the aorta, but this criterion is mired by changes in aortic diameter with age and differences between genders.[1] Another criterion is an arterial width measurement that is compared with a normal segment, but this is compromised by the ability to define a truly "normal" segment.[2]

There is also a paucity of information on the sequence of events that culminate in the initiation, maturation, and eventual rupture of human AAAs. By the nature of the disease, acquisition of tissue in the formative phase of the disease is not a practical option, even if such pathology was routinely identified as an early stage of development. Therefore, we are restricted to descriptive pathology of tissues segments that are generally acquired during surgical repair of AAAs that are in excess of 5 cm. In addition to the arterial wall being grossly distorted at this stage, such aneurysmal aortas provide limited insight into the events preceding the development of this aberrant tissue.

A basic premise of animal models of disease is that they mimic the cellular and biochemical characteristics in the progression of the human disease. In the case of AAAs, there is a relative paucity of information to gauge the fidelity that animal models reproduce the human disease. Several models of AAAs that have been created in large animals have been

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From the Department of Medicine (A.D.), Graduate Center for Nutritional Sciences (A.D., L.A.C.), Department of Physiology (A.D.), and Division of Pharmaceutical Sciences (L.A.C.), University of Kentucky, Lexington.
Correspondence to Alan Daugherty, Gill Heart Institute, Wethington Building, Room 521, University of Kentucky, Lexington, KY 40536-0230. E-mail Alan.Daugherty@uky.edu
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Characteristics of Mouse Models of Abdominal Aortic Aneurysms

Mode of AAA Induction	Characteristics	Comments	Ref.
Genetically Determined			
Blotchy	MD		5
Lox deficiency	MD	Elastin and collagen cross-linking defect with death from aneurysmal rupture in full-term fetus	6
MMP-3 or TIMP-1 deficiencies	MD	Medial degeneration that occurred in both the thoracic and abdominal segment	7–9
LDL receptor−/−	MD	Aneurysms localized to the suprarenal segment in mice fed diets enriched with saturated fat, cholesterol, and cholate	13,14
ApoE−/−	MD, A		13
ApoE−/− × eNOS−/−	MD, T, A		16
SMC-specific LRP−/− × LDL receptor−/−	MD, A	Large AAAs in abdominal aorta with aortic arch thickening and vessel elongation.	18
Transgenic mice overexpressing renin and angiotensinogen	MD	Rupture of abdominal and thoracic aorta aneurysms within 10 days of increased salt intake	21
Chemically Induced			
Elastase	MD, I	Infusion into infrarenal aorta leads to delayed dilation and inflammation.	23
Calcium chloride	MD, I	Progressive dilation and inflammation at site of application.	27,28
AngII infusion into LDL receptor −/−	MD, I, T, A	AAAs form in the supra-renal aorta.	29
ApoE−/− mice	MD, I, T, A		30
C57BL/6 mice	MD, I, T		34

MD indicates medial degeneration; I, inflammatory components; T, thrombus; A, atherosclerosis.

reviewed recently.[3,4] In the current review, we focus on recent advances in the development of models of AAA in mice (Table). Mice have become dominant in biomedical research for reasons that include their small size, their relative cheapness, the ability to compare well-documented genetic backgrounds, and the ability to delete or overexpress specific genes. One area of need in AAA research is a model to test surgical interventions. In this respect, the physical constraints of mouse models of AAAs will probably have limited usefulness. However, it is likely that mice will be the major species applied to provide mechanistic insight into the aneurysmal process.

Spontaneously Mutated and Genetically Engineered Mice

Defects of Extracellular Matrix

The blotchy mouse has a mutation on the X chromosome that leads to abnormal intestinal copper absorption. Because copper is a required co-factor for lysyl oxidase (Lox), these mice have an inability to crosslink both elastin and collagen because of reduced Lox levels. These mice have a propensity for AAA, with a gender bias in rupture that is frequent in males and rare in females.[5,6] However, there are more generalized problems such as aneurysms in other regions of the vasculature and emphysema that limit the usefulness of the mice.[4]

Similarly, mice with a genetically engineered deficiency in Lox developed to full term but were not viable because of aortic rupture from large aneurysms in the thoracic aorta.[7] Electron microscopy of the aortic walls showed highly fragmented elastic fibers and discontinuity in the smooth muscle cell layers in Lox −/− fetuses. Thus, while deficiencies of Lox in mice can result in aneurysm formation and increase the propensity for aortic rupture, these vascular pathologies occur predominately in the thoracic rather than the abdominal aorta and are associated with generalized health problems limiting the usefulness of these mice in AAA research.

Matrix Metalloproteinases

Aneurysmal development has been noted in a number of mice with genetically engineered deficiencies of components of the MMP system. These include mice deficient in MMP-3[8] and TIMP-1.[9,10] However, in both strains of mice, the medial dissections and small aneurysmal structures were present on the thoracic and abdominal segments of the aorta. Therefore, the lack of specificity for the abdominal region may reflect a more generalized destruction of arterial extracellular matrix than occurs in human AAAs.

Hyperlipidemic Mice

The use of mice in atherosclerosis research was escalated by the development of mice that are deficient in either apoE[11,12] or LDL receptors.[13] Although the majority of the reports on these mice have focused on the development of atherosclerotic lesions, there have been some limited reports on AAA development. In the initial description of en face analysis of aortic lesions in mice, it was noted that abdominal aneurysms were a frequent occurrence in both LDL receptor−/− and apoE−/− mice that were fed a high-fat diet for protracted intervals.[14] These AAAs developed in the suprarenal area of the aorta, just above the left renal artery.

A more detailed analysis of AAAs in apoE −/− mice revealed that prolonged feeding of a high-fat diet led to “pseudo-microaneurysms” in which destruction of the media was counteracted by thickening of the adventitia to prevent extravasation of blood.[15] In this study, female mice were placed on a diet enriched in saturated fat, cholesterol, and cholate for approximately 6 months. This pathology was ablated in apoE−/− mice that were also deficient in urokinase. The decrease in aneurysm formation in urokinase

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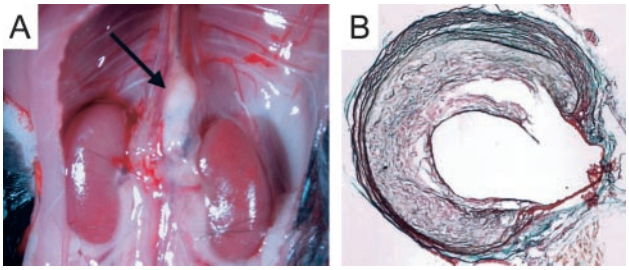

AAA present in an aged female apoE−/− mouse. A, The presence of an AAA is readily apparent in the suprarenal region of the aorta. B, A cross-section stained with Gomori demonstrates the extensive dilation and extracellular matrix deposition.

deficient mice was attributed to an inability to activate MMP-12 via a plasmin-dependent pathway.

AAAs formed under mature atherosclerotic lesions after prolonged feeding of LDL receptor −/− mice with diets enriched in saturated fat, cholesterol, and cholate.[16] Medial elastolysis and vessel dilation in the abdominal aorta occurred under atherosclerotic lesions that had necrotic cores with a predominant lipid component. Elastin degradation and ectasia was ablated by the administration of a broad-spectrum MMP inhibitor, CGS 27023A, without exerting any discernable effect on the extent and characteristics of atherosclerotic lesions.

Many of the current publications that describe AAAs in hyperlipidemic mice have used dietary interventions to promote pronounced hypercholesterolemia. However, we have recently noted the appearance of a large AAA in the suprarenal aorta of a 13-month-old apoE−/− female mouse that had been maintained on a normal diet. This large AAA was grossly visible (Figure 1A) and contained a marked dilation of the lumen and considerable arterial remodeling (Figure 1B). These data demonstrate that even endogenous hyperlipidemia can promote the development of AAA in sufficiently aged mice without the need for a dietary stimulus.

Compound-Deficient Hyperlipidemic Mice

Mice that have a combined deficiency of both apoE and endothelial nitric oxide synthase have been developed.[17] These mice were fed a high-fat diet from weaning for 4 to 6 months. Twenty five percent of the male mice had AAAs in the suprarenal region. These AAAs were characterized by thrombotic or fibrous material accumulating in the perimedial region. No AAAs were reported in female mice. Lowering the elevated blood pressure that occurs in the absence of endothelial NOS with hydralazine administration did not alter the incidence of AAA.[18] This has lead to the suggestion of other mechanisms including changes in vascular smooth muscle proliferation, leukocyte-endothelial interactions, and modulation of platelet aggregation. These effects may be related to the enhanced synthesis of angiotensins since the ACE inhibitor enalapril reduces some forms of vascular pathology in apoE and eNOS compound-deficient mice.[19]

A recent study focused on the role of low-density lipoprotein receptor-related protein (LRP) in smooth muscle cells on vascular disease.[20] LRP is a multifunctional protein that binds a variety of biologically diverse ligands.[21] Mice with LRP specifically depleted in smooth muscle cells (SMC) were bred to LDL receptor −/− mice and were fed either normal or high-fat diets. Pathology developed in the aortic arch and abdominal aorta of these mice with substantial lengthening, dilatation, and thickening. In addition, large aneurysms developed in the abdominal aortic region. Aortas from these mice exhibited an increased number of SMCs and grossly distorted elastin laminae. LRP1 exerts some of its effects on SMC through interactions with intracellular signaling processes for platelet-derived growth factor (PDGF) receptor-bb. Thus it was proposed that the vascular pathology in the absence of LRP1 is caused by increased activity of the PDGF receptor-bb. This option was explored by treating these mice with the tyrosine kinase inhibitor, Gleevec, which increased the stability of the elastin. Although PDGF has well characterized effects on SMC growth, the mechanism of its role in elastin integrity and AAA formation have not been defined.

Overproduction of Angiotensin Peptides

The Tsukuba hypertensive mouse was produced by cross-breeding of strains that expressed transgenes of human renin and angiotensinogen.[22] Although neither of the single transgenic mice had elevations of arterial blood pressure, the double transgenic animal had modest elevations of ≈20 mm Hg. There was no overt vascular pathology in these mice when provided with a standard nutritional base. However, when these mice were provided with drinking water containing 1% sodium chloride, there was a marked increase in mortality for the first 10 days of salt administration. These deaths were caused by ruptured aortic aneurysm in both the thoracic and abdominal region. The rupture site in those dying of abdominal aortic rupture was between the celiac artery and the left renal artery.[23] The formation of AAA was not associated with changes in blood pressure. Therefore, interpretation of the mechanism of the AAA formation in these mice awaits further description of the pathology and the effects of the salt diet on angiotensin peptide production.

Chemically-Induced AAA

Elastase Infusion

The infusion of elastase into the infrarenal segment of rat aortas has been a frequently used model of AAAs.[24] The rationale for its development was based on the disrupted nature of elastin in AAAs and its requirement for the maintenance of the structural integrity of the artery. This rat model has been modified to produce AAAs in the aortic infrarenal region of mice.[25] The procedure involves the introduction of a catheter at the iliac bifurcation and isolation of a segment of the abdominal aorta by a distal suture. Porcine pancreatic elastase was introduced into the lumen and incubated for 5 minutes before restoration of flow. The infusion induced an immediate and sustained dilation that was presumably caused by the mechanical effects of the procedure. A dilated state was maintained at constant dimensions for a further 7 days whether vehicle or elastase was infused. During this interval, there was minimal change to the medial layers of the vessel and elastin fibers remained intact. By day 14, dilation was present in mice infused with elastase, whereas the mice infused with heat-inactivated enzyme re-

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tained the same external dimensions. At this interval, there was extensive destruction of the elastic lamellae and the adventitial region was occupied by a pronounced inflammatory infiltrate, predominantly containing macrophages. Neutrophils were present on the peripheral aspects of the adventitia surrounding the aorta. The presence of AAA was defined as an increase in aortic diameter that was 100% greater than the size of vessel before perfusion. It should be noted that the source of the elastase has important effects on the outcome of these studies, because AAA development may have been attributable to a contaminant in the commercially available preparations.[26]

Administration of doxycycline significantly attenuated elastase-induced AAA formation, presumably because of its property as a broad-spectrum inhibitor of MMPs.[23] Furthermore, deficiency of MMP-9 totally ablated the formation of elastin-induced AAA. Conversely, MMP-12 deficiency, either alone or in combination with MMP-9, exerted no effect on the dimensions of elastase-induced AAAs.[25] The pathology of elastase-induced AAAs was accompanied by decreased expression of both endothelial and neural nitric oxide but a marked increase in the inducible form of this enzyme. The enhanced production of nitric oxide was indirectly demonstrated by the presence of immunostainable nitrotyrosine. Although inducible forms of NOS have been ascribed to a causal role in inflammatory disease and the development of vascular diseases, the size of elastase-induced AAAs was not influenced in male mice that were deficient in inducible NOS. On the contrary, the absence of inducible NOS enhanced the size of AAAs in female mice.[27]

Calcium Chloride

Periaortic application of calcium chloride promoted arterial wall thickening in rabbits.[28] When calcium chloride was combined with thioglycollate and applied to arteries of hyperlipidemic rabbits, it promoted the formation of AAA.[29] This approach has been applied to mice by the peri-aortic incubation of calcium chloride. This has been achieved either through placement of a gauze soaked in a calcium chloride solution or through direct placement of a concentrated solution on the aorta between the renal branches and the iliac bifurcation.[30,31] This procedure did not provoke an immediate increase in aortic diameter in one study, although by 2 weeks there was a significant increase in diameter that progressed in the next week.[30] In another study, there was progressive increase in aortic diameter for the 4 weeks of observation.[31] These modes of incubating the infrarenal aorta with calcium chloride led to increases of aortic diameters in the range of 48% to 110%. The abluminal incubation of calcium chloride also led to structural disruption of the medial layer and inflammatory responses. This inflammation occurred on the luminal and medial aspect of the artery. This model leads to the development of luminal dilation without the preceding mechanical effects that are noted in the elastase-infused model.[31]

The elastase-induced model of AAA has been used to define the role of MMPs in the disease process. Deficiency of either MMP-2 or MMP-9 also led to an attenuation of calcium chloride-induced aortic dilation.[31] Studies were also performed in which elicited peritoneal macrophages from wild-type mice were introduced via an intravenous injection into MMP-9−/− or MMP-2−/− mice. This procedure was performed 1 day before abluminal incubation of calcium chloride and repeated 1 week after the procedure. Introduction of wild-type macrophages into MMP-9−/− mice was sufficient to facilitate the development of calcium chloride-induced AAA. However, the presence of macrophages from wild-type mice failed to promote AAAs in MMP-2−/− mice.[31]

Angiotensin-Associated AAAs

Infusion of AngII into either LDL receptor−/− or apoE−/− mice leads to the production of AAAs.[32,33] Delivery of AngII at doses of 500 to 1000 ng/kg per minute, via subcutaneously implanted osmotic mini-pumps, leads to AAAs in the suprarenal region within the 28-day infusion period.

Several features of these AngII-induced AAAs have been defined. The initial publication noted that arterial blood pressure was not altered by the AngII infusion, although this measurement was recorded in anesthetized mice.[33] Subsequent studies in conscious mice using a computerized tail-cuff inflation technique have also demonstrated that the lower doses do not increase blood pressure, although the highest dose may promote modest increases in the range of 25 mm Hg.[34,35] However, increases in blood pressure do not appear to account for the development of AAA, because interventions have been demonstrated to eliminate AAA formation independent of effects on blood pressure.[36] Another important characteristic of these mice that mimics human AAA is the enhanced propensity for the development of AAAs in male mice, with the incidence being approximately twice that of females.[35]

Although the majority of AngII-infusion studies have used hyperlipidemic mice, recently it was reported that AAAs develop during AngII-infusion into wild-type C57BL/6 mice.[37] This is the background strain of hyperlipidemic mice used in other studies. The incidence of AAAs was much lower in C57BL/6 mice compared with studies in hyperlipidemic mice, although the AAAs that developed were of equivalent size. Therefore, although hyperlipidemia is not required for the development of AngII-induced AAA, its presence augments the incidence.

The sequence of cellular events during AAA initiation and maturation has recently been determined by the characterization of infrarenal aortic segments of apoE−/− mice after infusion of AngII for 1 to 56 days.[38] The precipitating event that occurs within days of AngII infusion appears to be medial macrophage accumulation in the region that is prone to AAA formation, associated with elastin degradation. It is not clear whether the macrophage accumulation acts as a stimulus for elastin degradation or vice versa. By 3 to 10 days, there were gross dissections of the aortas leading to prominent vascular hematomas. This form of thrombus is distinct from the laminated form that is present in mature human disease. Inflammatory responses were provoked that were likely caused by the presence of thrombi. The inflammatory response was largely restricted to a pronounced infiltration of macrophages. Subsequently, there was en-

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hanced extracellular matrix deposition and infiltration of other leukocyte populations, primarily B and T lymphocytes. The dilated region of the aorta gradually regained circumferential elastin fibers and completely re-endothelialized. Prominent neovascularization was present throughout the lumen of the remodeled tissue. At late stages of the disease evolution, large atherosclerotic lesions were apparent, as defined by lipid-laden foam cells.

The characteristics of AngII-induced AAAs are consistent with an activation of an inflammatory response and the stimulation of a proteolytic cascade. In agreement with this premise, there was increased expression of NF-kappaB and urokinase in the aneurysmal tissue of AngII-infused apoE−/− mice.[39,40] The role of the urokinase pathway in the development of AAAs was confirmed by the reduced incidence of AAAs in C57BL/6 and apoE−/− mice that were deficient in urokinase.[37] Furthermore, the broad-spectrum MMP inhibitor, doxycycline, significantly reduced the incidence and severity of AngII-induced AAA formation.[36]

Conclusions

In the area of providing mechanistic insight, a shortcoming of the ability to assess the usefulness of mouse models of AAAs is our current lack of knowledge of the biochemical and cellular characteristics of initiating and perpetrating factors in the human disease. This may be partially overcome by the initiation of a study analogous to the Pathobiological Determinants in Youth Trial (PDAY).[41] The PDAY study acquired tissues from individuals that died under traumatic circumstances. This tissue acquisition in individuals aged 15 to 30 was used to define the cellular sequence of atherosclerotic lesion development and has been a major contributor to the delineation of mechanisms of the disease.[42,43] The current definition of cellular and biochemical characteristics of human AAAs are limited to descriptions of advanced disease from tissues acquired at the time of surgical repair. The ability to extrapolate the results from animal models will require a systematic study of the mode by which human AAA are initiated and mature.

Even in the absence of detailed knowledge of the human disease, there may be intriguing insights provided by animal models of AAAs that will offer mechanistic insight into the human disease. One fascinating aspect of the AAAs developed in hyperlipidemic and AngII-infused mice is the localization to the suprarenal segment of the aorta. This presumably reflects an inherent property of the arterial wall that promotes formation of aneurysms at this location. Development of AAAs at this locus may be caused by heterogeneity of responses in different regions of aorta that may be attributable to the differing origins of the medial cells.[44] Responses or gene expression that are specific to this region may provide a mechanistic basis for the localization of AAA formation.

A common hypothesis in the development of AAAs in humans is the involvement of specific MMPs, particularly MMP-2 and MMP-9.[45,46] Evidence has included the increased abundance of MMP-2 and MMP-9 mRNA and protein in human AAA tissue, and an enhanced presence of the active protein. In agreement with this mode of AAA production, there has been general agreement that broad-spectrum inhibition of MMPs or deletion of specific elastolytic members of this class attenuate the production of AAA in several mouse models. Therefore, the consistency of responses points to a common mechanism of extracellular matrix destruction, despite the wide range of insults that are used to provoke the disease.

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References

1. Bengtsson H, Sonesson B, Bergqvist D. Incidence and prevalence of abdominal aortic aneurysms, estimated by necropsy studies and population screening by ultrasound. *Ann NY Acad Sci.* 1996;800:1–24.
2. Johnston KW, Rutherford RB, Tilson MD, Shah DM, Hollier L, Stanley JC. Suggested standards for reporting on arterial aneurysms. Subcommittee on Reporting Standards for Arterial Aneurysms, Ad Hoc Committee on Reporting Standards, Society for Vascular Surgery and North Am Chapter, International Society for Cardiovascular Surgery. *J Vasc Surg.* 1991;13:452–458.
3. Dobrin PB. Animal models of aneurysms. *Ann Vasc Surg.* 1999;13:641–648.
4. Carrell TW, Smith A, Burnand KG. Experimental techniques and models in the study of the development and treatment of abdominal aortic aneurysm. *Br J Surg.* 1999;86:305–312.
5. Brophy CM, Tilson JE, Braverman IM, Tilson MD. Age of onset, pattern of distribution, and histology of aneurysm development in a genetically predisposed mouse model. *J Vasc Surg.* 1988;8:45–48.
6. Reilly JM, Savage EB, Brophy CM, Tilson MD. Hydrocortisone rapidly induces aortic rupture in a genetically susceptible mouse. *Arch Surg.* 1990;125:707–709.
7. Maki JM, Rasanen J, Tikkanen H, Sormunen R, Makikallio K, Kivirikko KI, Soininen R. Inactivation of the lysyl oxidase gene Lox leads to aortic aneurysms, cardiovascular dysfunction, and perinatal death in mice. *Circulation.* 2002;106:2503–2509.
8. Silence J, Lupu F, Collen D, Lijnen HR. Persistence of atherosclerotic plaque but reduced aneurysm formation in mice with stromelysin-1 (MMP-3) gene inactivation. *Arterioscler Thromb Vasc Biol.* 2001;21:1440–1445.
9. Silence J, Collen D, Lijnen HR. Reduced atherosclerotic plaque but enhanced aneurysm formation in mice with inactivation of the tissue inhibitor of metalloproteinase-1 (TIMP-1) gene. *Circ Res.* 2002;90:897–903.
10. Lemaitre V, Soloway PD, D'Armiento J. Increased medial degradation with pseudo-aneurysm formation in apolipoprotein E-knockout mice deficient in tissue inhibitor of metalloproteinases-1. *Circulation.* 2003;107:333–338.
11. Zhang SH, Reddick RL, Piedrahita JA, Maeda N. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science.* 1992;258:468–471.
12. Plump AS, Smith JD, Hayek T, Aaltosetala K, Walsh A, Verstuyft JG, Rubin EM, Breslow JL. Severe hypercholesterolemia and atherosclerosis in apolipoprotein-E-deficient mice created by homologous recombination in ES cells. *Cell.* 1992;71:343–353.
13. Ishibashi S, Goldstein JL, Brown MS, Herz J, Burns DK. Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice. *J Clin Invest.* 1994;93:1885–1893.
14. Tangirala RK, Rubin EM, Palinski W. Quantitation of atherosclerosis in murine models: Correlation between lesions in the aortic origin and in the entire aorta, and differences in the extent of lesions between sexes in LDL receptor-deficient and apolipoprotein E-deficient mice. *J Lipid Res.* 1995;36:2320–2328.
15. Carmeliet P, Moons L, Lijnen R, Baes M, Lemaitre V, Tipping P, Drew A, Eeckhout Y, Shapiro S, Lupu F, Collen D. Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. *Nature Genet.* 1997;17:439–444.
16. Prescott MF, Sawyer WK, Von Linden-Reed J, Jeune M, Chou M, Caplan SL, Jeng AY. Effect of matrix metalloproteinase inhibition on progression of atherosclerosis and aneurysm in LDL receptor-deficient mice

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overexpressing MMP-3, MMP-12, and MMP-13 and on restenosis in rats after balloon injury. *Ann NY Acad Sci.* 1999;878:179–190.
17. Kuhlencordt PJ, Gyurko R, Han F, Scherrer Crosbie M, Aretz TH, Hajjar R, Picard MH, Huang PL. Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice. *Circulation.* 2001;104: 448–454.
18. Chen J, Kuhlencordt PJ, Astern J, Gyurko R, Huang PL. Hypertension does not account for the accelerated atherosclerosis and development of aneurysms in male apolipoprotein e/endothelial nitric oxide synthase double knockout mice. *Circulation.* 2001;104:2391–2394.
19. Knowles JW, Reddick RL, Jennette JC, Shesely EG, Smithies O, Maeda N. Enhanced atherosclerosis and kidney dysfunction in eNOS(−)/(−)Apo(−)/(−) mice are ameliorated by enalapril treatment. *J Clin Invest.* 2000;105:451–458.
20. Boucher P, Gotthardt M, Li WP, Anderson RGW, Herz J. LRP: Role in vascular wall integrity and protection from atherosclerosis. *Science.* 2003; 300:329–332.
21. Herz J, Strickland DK. LRP: a multifunctional scavenger and signaling receptor. *J Clin Invest.* 2001;108:779–784.
22. Fukamizu A, Sugimura K, Takimoto E, Sugiyama F, Seo MS, Takahashi S, Hatae T, Kajiwara N, Yagami K, Murakami K. Chimeric renin-angiotensin system demonstrates sustained increase in blood pressure of transgenic mice carrying both human renin and human angiotensinogen genes. *J Biol Chem.* 1993;268:11617–11621.
23. Nishijo N, Sugiyama F, Kimoto K, Taniguchi K, Murakami K, Suzuki S, Fukamizu A, Yagami K. Salt-sensitive aortic aneurysm and rupture in hypertensive transgenic mice that overproduce angiotensin II. *Lab Invest.* 1998;78:1059–1066.
24. Anidjar S, Salzmann JL, Gentric D, Lagneau P, Camilleri JP, Michel JB. Elastase-induced experimental aneurysms in rats. *Circulation.* 1990;82: 973–981.
25. Pyo R, Lee JK, Shipley JM, Curci JA, Mao D, Ziporin SJ, Ennis TL, Shapiro SD, Senior RM, Thompson RW. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. *J Clin Invest.* 2000;105: 1641–1649.
26. Curci JA, Thompson RW. Variable induction of experimental abdominal aortic aneurysms with different preparations of porcine pancreatic elastase. *J Vasc Surg.* 1999;29:385.
27. Lee JK, Borhani M, Ennis TL, Upchurch GR Jr., Thompson RW. Experimental abdominal aortic aneurysms in mice lacking expression of inducible nitric oxide synthase. *Arterioscler Thromb Vasc Biol.* 2001;21: 1393–1401.
28. Gertz SD, Kurgan A, Eisenberg D. Aneurysm of the rabbit common carotid artery induced by periarterial application of calcium chloride in vivo. *J Clin Invest.* 1988;81:649–656.
29. Freestone T, Turner RJ, Higman DJ, Lever MJ, Powell JT. Influence of hypercholesterolemia and adventitial inflammation on the development of aortic aneurysm in rabbits. *Arterioscler Thromb Vasc Biol.* 1997;17: 10–17.
30. Chiou AC, Chiu B, Pearce WH. Murine aortic aneurysm produced by periarterial application of calcium chloride. *J Surg Res.* 2001;99:371–376.
31. Longo GM, Xiong W, Greiner TC, Zhao Y, Fiotti N, Baxter BT. Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. *J Clin Invest.* 2002;110:625–632.
32. Ravisankar P, Cassis. L. A., Szilvassy SJ, Daugherty A. Absence of CCR2 receptors in bone marrow-derived cells decreases angiotensin II induced atherosclerosis and abdominal aortic aneurysms. *Proc Arterioscler Thromb Vasc Biol.* 2002;3:35.
33. Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J Clin Invest.* 2000;105:1605–1612.
34. Daugherty A, Manning MW, Cassis LA. Antagonism of AT2 receptors augments Angiotensin II-induced abdominal aortic aneurysms and atherosclerosis. *Br J Pharmacol.* 2001;134:865–870.
35. Manning MW, Cassis LA, Huang J, Szilvassy SJ, Daugherty A. Abdominal aortic aneurysms: fresh insights from a novel animal model of the disease. *Vasc Med.* 2002;7:45–54.
36. Manning MW, Cassis LA, Daugherty A. Differential effects of doxycycline, a broad-spectrum matrix metalloproteinase inhibitor, on angiotensin II-induced atherosclerosis and abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol.* 2003;23:483–488.
37. Deng GG, Martin-McNulty B, Sukovich DA, Freay A, Halks-Miller M, Thinnes T, Loskutoff DJ, Carmeliet P, Dole WP, Wang YX. Urokinase-type plasminogen activator plays a critical role in angiotensin II-induced abdominal aortic aneurysm. *Circ Res.* 2003;92:510–517.
38. Saraff K, Babamusta F, Cassis LA, Daugherty A. Aortic dissection precedes formation of aneurysms and atherosclerosis in angII-Infused apoE deficient mice. *Arterioscler Thromb Vasc Biol.* 2003;23:1621–1626.
39. Tham DM, Martin McNulty B, Wang YX, Wilson DW, Vergona R, Sullivan ME, Dole W, Rutledge JC. Angiotensin II is associated with activation of NF-kappa B-mediated genes and downregulation of PPARs. *Physiol Genomics.* 2002;11:21–30.
40. Wang YX, Martin McNulty B, Freay AD, Sukovich DA, Halks Miller M, Li WW, Vergona R, Sullivan ME, Morser J, Dole WP, Deng GG. Angiotensin II increases urokinase-type plasminogen activator expression and induces aneurysm in the abdominal aorta of apolipoprotein E-deficient mice. *Am J Pathol.* 2001;159:1455–1464.
41. Wissler RW, Strong JP. Risk factors and progression of atherosclerosis in youth. PDAY Research Group. Pathological Determinants of Atherosclerosis in Youth. *Am J Pathol.* 1998;153:1023–1033.
42. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis—A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, Am Heart Association. *Arterioscler Thromb.* 1994;14:840–856.
43. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, Am Heart Association. *Circulation.* 1995;92:1355–1374.
44. Hungerford JE, Little CD. Developmental biology of the vascular smooth muscle cell: building a multilayered vessel wall. *J Vasc Res.* 1999;36: 22–27.
45. Goodall S, Crowther M, Hemingway DM, Bell PR, Thompson MM. Ubiquitous elevation of matrix metalloproteinase-2 expression in the vasculature of patients with abdominal aneurysms. *Circulation.* 2001; 104:304–309.
46. Thompson RW, Holmes DR, Mertens RA, Liao S, Botney MD, Mecham RP, Welgus HG, Parks WC. Production and localization of 92-kilodalton gelatinase in abdominal aortic aneurysms. An elastolytic metalloproteinase expressed by aneurysm-infiltrating macrophages. *J Clin Invest.* 1995;96:318–326.

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