Arteriosclerosis, Thrombosis, and Vascular Biology
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Arterioscler Thromb Vasc Biol 2011;31;253-260; originally published online Nov 18, 2010;
DOI: 10.1161/ATVBAHA.110.218545
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Telomerase Deficiency in Bone Marrow–Derived Cells Attenuates Angiotensin II–Induced Abdominal Aortic Aneurysm Formation

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Objective—Abdominal aortic aneurysms (AAA) are an age-related vascular disease and an important cause of morbidity and mortality. In this study, we sought to determine whether the catalytic component of telomerase, telomerase reverse transcriptase (TERT), modulates angiotensin (Ang) II–induced AAA formation.

Methods and Results—Low-density lipoprotein receptor–deficient (LDLr−/−) mice were lethally irradiated and reconstituted with bone marrow–derived cells from TERT-deficient (TERT−/−) mice or littermate wild-type mice. Mice were placed on a diet enriched in cholesterol, and AAA formation was quantified after 4 weeks of Ang II infusion. Repopulation of LDLr−/− mice with TERT−/− bone marrow–derived cells attenuated Ang II–induced AAA formation. TERT-deficient recipient mice revealed modest telomere attrition in circulating leukocytes at the study end point without any overt effect of the donor genotype on white blood cell counts. In mice repopulated with TERT−/− bone marrow, aortic matrix metalloproteinase-2 (MMP-2) activity was reduced, and TERT−/− macrophages exhibited decreased expression and activity of MMP-2 in response to stimulation with Ang II. Finally, we demonstrated in transient transfection studies that TERT overexpression activates the MMP-2 promoter in macrophages.

Conclusion—TERT deficiency in bone marrow–derived macrophages attenuates Ang II–induced AAA formation in LDLr−/− mice and decreases MMP-2 expression. These results point to a previously unrecognized role of TERT in the pathogenesis of AAA. **(*Arterioscler Thromb Vasc Biol*. 2011;31:253-260.)**

Key Words: aneurysms ■ angiotensin II ■ gene expression ■ macrophages ■ matrix metalloproteinase ■ telomerase

Abdominal aortic aneurysms (AAA) represent a disease of an aging population with increasing incidence and considerable consequences on morbidity and mortality.[1] Although remarkable progress has been made in recent years in understanding the pathogenetic mechanisms of AAA formation, there is currently no validated medical treatment option for this vascular disease.[2,3] AAA are characterized as a chronic inflammatory and degenerative disease driven by wall proteolysis, inflammation, and oxidative stress.[2] In particular, infiltrating macrophages in the aortic wall are a major source of matrix metalloproteinases (MMPs) and cytokines, leading to matrix degradation, further attraction of inflammatory cells, and induction of vascular smooth muscle cell apoptosis.[4] For example, in the murine model of angiotensin (Ang) II–induced AAA formation, we have previously demonstrated that medial accumulation of macrophages in areas of elastin degradation represents one of the earliest events in AAA formation.[3]

AAA are often diagnosed only at the time of rupture, leading to a dramatic increase of morbidity and mortality.[1] Therefore, early detection of AAA constitutes the mainstream of therapy, and the development of biomarkers may represent a possible solution for widespread screening.[5] Although often confounded by lack of specificity, one biomarker that has been suggested to predict AAA formation is leukocyte telomere length.[6,7] Telomeres, the DNA-protein complexes at the ends of eukaryotic chromosomes, are stabilized by the catalytic activity of the telomerase reverse transcriptase (TERT) to serve as protective capping.[8] However, in the majority of adult somatic cells, there is insufficient telomerase activity for telomere maintenance, resulting in telomere attrition and impaired self-renewal.[9] In contrast, stem cells and most cancer cells constitutively overexpress TERT, manifesting high levels of telomerase activity, which confers an apparently indefinite lifespan.[9] In addition to this well-established function of telomerase to elongate telomeres, recent evidence has identified a novel and unappreciated role for TERT in multiple developmental processes, including cell proliferation.[10–12] Although still in its infancy, this novel function of TERT occurs independently of its

Received on: April 28, 2010; final version accepted on: November 4, 2010.

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catalytic activity to elongate telomeres and may involve a direct regulation of chromatin remodeling and gene transcription.[10–12]

Although early studies suggested that TERT expression undergoes silencing in most organ systems, more recent work has revealed that TERT is stringently regulated and induced in response to certain environmental cues.[13] In vascular diseases, telomerase activity is induced[14,15] and reactivation of telomerase occurs in myeloid and lymphoid cells at critical stages of their activation, which has been suggested to enhance their function during inflammatory responses.[16,17] In the present study, we investigated the causal role of TERT in inflammatory remodeling underlying AAA formation. Using bone marrow transplantation (BMT) experiments, we demonstrate that TERT deficiency attenuates AAA formation and decreases MMP-2 activity. These experiments establish a previously unrecognized causal role for TERT in the formation of AAA.

Materials and Methods

Animal Studies

BMT studies were performed by repopulating lethally irradiated male low-density lipoprotein receptor–deficient mice (LDLr−/−, The Jackson Laboratory) with bone marrow–derived cells of male first-generation TERT-deficient mice (TERT−/−, The Jackson Laboratory, B6.129S-Tert^{tm1Yjc}/J, stock number 005423) or their wild-type littermates (TERT+/+) as described.[18] Briefly, mice were maintained on water containing antibiotics (sulfamethoxazole/trimethoprim) for 1 week before BMT until 4 weeks after BMT. Recipient mice were irradiated with a total of 900 Rads from a cesium source that was delivered in 2 doses within 3 to 4 hours. Bone marrow–derived cells of male TERT+/+ or TERT−/− mice were obtained from the tibias and femurs of donor mice and were injected into the tail vein of 8-week-old irradiated male LDLr−/− recipient mice (1.2×10^7 cells per mouse, n=10 with TERT+/+ and n=7 with TERT−/−). After 4 weeks of recovery, a saturated fat-enriched diet was fed until the end of the study (Harlan Teklad TD.88137; Supplemental Table I, available online at http://atvb.ahajournals.org). One week after initiation of this diet feeding, Ang II (1,000 ng/kg per minute) was administered subcutaneously via Alzet osmotic minipumps (model 2004) for 28 days. All studies were performed with the approval of the University of Kentucky Institutional Animal Care and Use Committee.

Quantification of AAA and Atherosclerosis

AAAs were quantified in vivo by measurement of maximal lumen diameters of suprarenal abdominal aortas using high-frequency ultrasound (Visualsonics) on anesthetized mice prior and at day 28 of Ang II infusion.[19] AAAs were also quantified ex vivo by measurement of maximal width of suprarenal aortas dissected free from mice at 28 days and with extraneous tissues removed.[20] *En face* atherosclerosis was quantified as lesion area on the intimal surface of aortic arches, as described previously.[20]

Lipids and Lipoprotein Characterization

Serum cholesterol concentrations and lipoprotein cholesterol distributions were analyzed as described previously.[21]

Histology

Mouse abdominal aortas were fixed in formalin and embedded in OCT (Tissue-Tek, Miles Inc.). Serial 10-μm sections were cut surrounding the cross-section of widest diameter. Aortic elastic laminae were stained with Verhoeff elastic staining. Macrophages were detected using adsorbed rabbit anti-mouse macrophage antisera (1:750 dilution; Accurate Chemicals).[3]

Quantification of Leukocytes

Differential leukocyte count was performed on whole blood at study end point using a HEMAVET 950 hematology analyzer (Drew Scientific).

Cell Culture

Mouse bone marrow macrophages (BMM) were isolated from the femurs of mice and differentiated using Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 20% fetal bovine serum and 30% L929 cell-conditioned medium for 12 days. For MMP-2 mRNA analysis and zymography, 8×10^5 cells/well were seeded on 6-well plates. Medium was changed to DMEM without supplements for 24 hours, and cells were incubated with 1 μmol/L Ang II for an additional 24 hours. RAW 264.7 cells were obtained from American Type Culture Collection and maintained in DMEM supplemented with 10% fetal bovine serum.

Proliferation Assays

BMM were seeded at 1.5×10^5 cells/well on a 24-well plate. Cells were kept in DMEM with 20% fetal bovine serum for 24 hours, stimulated with DMEM with 20% fetal bovine serum and 30% L929 cell-conditioned medium, and counted after 48 hours using a hemocytometer. For 5-bromo-2′-deoxyuridine (BrdU) assays (Chemicon Inc.) cells were incubated with BrdU reagent before stimulation with L929 cell-conditioned growth medium. BrdU incorporation was analyzed after 24 hours using a microplate reader according to the manufacturer's instructions. All proliferation experiments were repeated at least 3 times and performed in triplicate.

Telomere Length Assay

BMM were treated with colcemid, and metaphase spreads were prepared. Fluorescence in situ hybridization was performed using the Dako telomere fluorescence in situ hybridization kit according to manufacturer's instructions. For quantification of telomere length, DNA was isolated from BMM or whole blood using the Qiagen DNeasy kit. Real-time polymerase chain reaction (PCR) was performed using the method described by Cawthon.[22] Samples were run in triplicate with 35 ng of DNA per reaction using an iCycler and SYBR Green I system (Bio-Rad). Telomere repeat copy number data were normalized to 36B4 as single-copy gene. The primer sequences were as follows: tel 1, 5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3′; tel 2, 5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCC-CTA-3′; 36B4 forward, 5′-CAACCCAGCTCTGGAGAAAC-3′; reverse, 5′-AAGCCTGGAAGAAGGAGGTC-3′.

RNA Isolation and Real-Time Reverse Transcription–PCR

RNA was isolated and reverse transcribed as described previously.[23] Quantitative real-time PCR analysis of target gene expression was performed using an iCycler and SYBR Green I system (Bio-Rad). Each sample was analyzed in triplicate and normalized to mRNA expression of the housekeeping gene transcription factor II B (TFIIB).

The following primer sequences were used: MMP-2 forward, 5′-TGGGGGAGATTCTCACTTTG-3′; reverse, 5′-ATCACTGCG-ACCAGTGTCTG-3′; TFIIB forward, 5′-CTCTCCCAAGAGTCACA-TGTCC-3′; reverse, 5′-CAATAACTCGGTCCCCTACAAC-3′.

Zymography

Equal amounts of BMM supernatant or aortic protein were resolved in 7.5% SDS-PAGE containing 1 mg/mL gelatin (Sigma-Aldrich). Subsequently, SDS was removed from the gels by 2 washes (15 minutes) with 2.5% Triton X-100 (Sigma-Aldrich). Gels were incubated for 24 hours (37°C) in zymography buffer (50 mmol/L Tris-HCL, 50 mmol/L Tris-Base, 5 mmol/L $CaCl_2$) and stained with Coomassie Brilliant Blue. Recombinant MMP-2 was used as positive control (R&D Systems).

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Transient Transfections

RAW 264.7 cells were cotransfected with a MMP-2 luciferase reporter construct[24] and a TERT expression vector[25] or a pcDNA3 vector as control using Lipofectamine 2000 (Invitrogen) as described.[23] Transfection efficiency was normalized to *Renilla* luciferase activities generated by cotransfection with pGL4.74[hRluc/TK] (Promega). Luciferase activity was assayed 24 hours after transfection using a Dual Luciferase Reporter Assay System (Promega).

Statistics

To compare 2 groups on a continuous response variable, we used a 2-sample Student *t* test or Mann–Whitney *U* test as appropriate. One-way ANOVA was used to compare multiple groups, followed by Bonferroni's post hoc analysis. All data are presented as mean±SEM.

Results

TERT Deficiency in Bone Marrow–Derived Cells Prevents Ang II–Induced AAA Formation

To define whether telomerase in hematopoietic bone marrow cells affects AAA formation, LDLr−/− mice were lethally irradiated and reconstituted with bone marrow–derived cells isolated from TERT−/− mice or their wild-type littermates. As a model for AAA formation, mice were infused with Ang II for 28 days, and suprarenal abdominal aortic diameters and areas were quantified in vivo by ultrasound and ex vivo using morphometric analysis.[21] As depicted in Figure 1A to 1C, repopulation of LDLr−/− mice with TERT−/− bone marrow cells attenuated Ang II–induced AAA formation. The mean maximal suprarenal aortic diameters assessed by in vivo ultrasound were 1.17 and 1.00 mm in mice repopulated with TERT+/+ or TERT−/− bone marrow–derived cells, respectively ($P<0.01$, Figure 1A). Similarly, the mean aortic area was decreased in mice repopulated with TERT−/− bone marrow–derived cells compared with the wild-type littermates (0.78 versus 1.10 mm^2, respectively, $P<0.01$, Figure 1B). In contrast to mice expressing TERT in bone marrow–derived cells, Ang II infusion did not increase the aortic diameter or area in recipients of TERT−/− bone marrow–derived cells compared with baseline (Supplemental Figure I). Ex vivo measurements of mean maximal aortic width confirmed an even greater difference between the donor genotypes (1.20 mm in mice repopulated with TERT+/+ bone marrow–derived cells versus 0.95 mm in mice repopulated with TERT−/− bone marrow–derived cells, $P<0.001$, Figure 1C). Finally, TERT deficiency in bone marrow–derived cells did not affect atherosclerosis formation in this model (Supplemental Figure II).

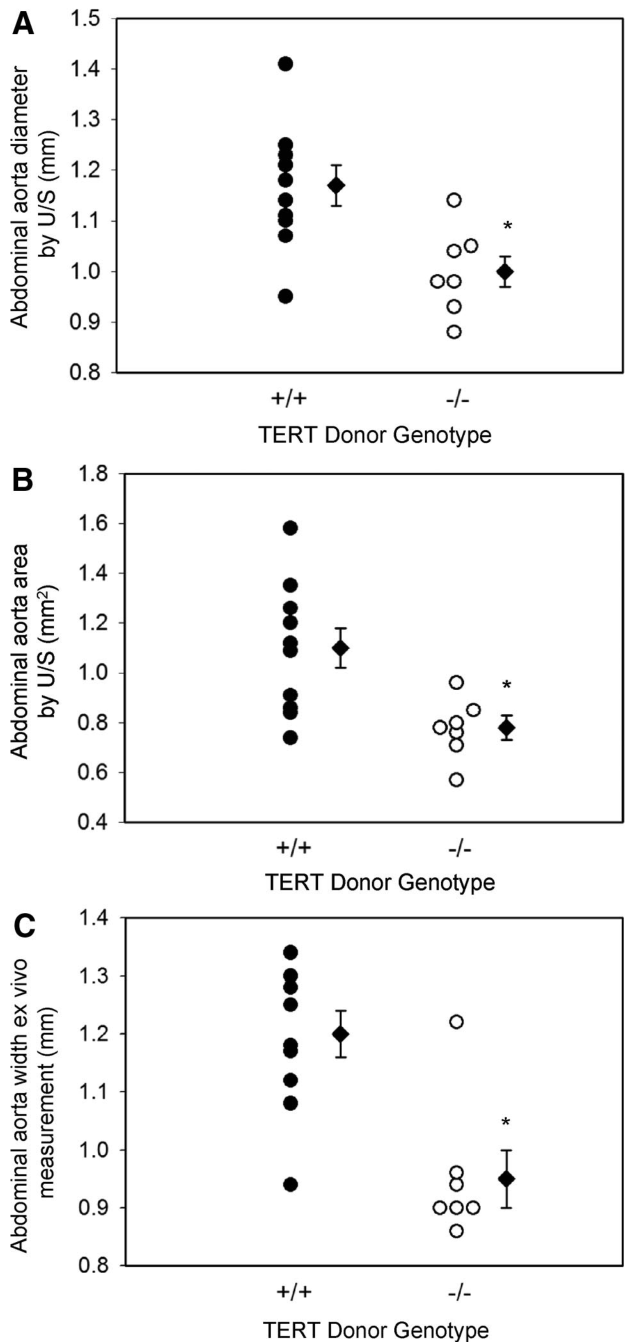

Figure 1. Repopulation of LDLr−/− mice with TERT-deficient bone marrow–derived cells attenuated Ang II–induced aneurysm formation. A and B, Ultrasound measurements of abdominal aorta diameter (A) and area (B) after 28 days of Ang II infusion (*$P<0.01$). C, Ex vivo measurements of maximal abdominal aorta widths (*$P<0.001$). Black circles (TERT+/+ donors) and white circles (TERT−/− donors) represent measurements in individual mice, diamonds represent means, and bars indicate SEM.

TERT Deficiency in Bone Marrow Cells Decreases Elastin Degradation and Macrophage Infiltration in the Abdominal Aorta

Ang II–induced aneurysm formation is associated with medial infiltration of macrophages and degradation of the aortic elastic laminae.[3] To assess these histopathologic parameters in LDLr−/− mice repopulated with TERT−/− bone marrow–derived cells, we performed Verhoeff's elastin staining and immunohistochemical analysis of macrophage infiltration. As depicted in Figure 2A and 2B, mice repopulated with TERT+/+ bone marrow revealed aortic dilatation and frequent elastin breaks in the media. At these sites of elastin breaks, macrophages were abundantly identified. In contrast, mice reconstituted with TERT−/− bone marrow revealed decreased frequency of elastin breaks and accumulation of macrophages at these sites.

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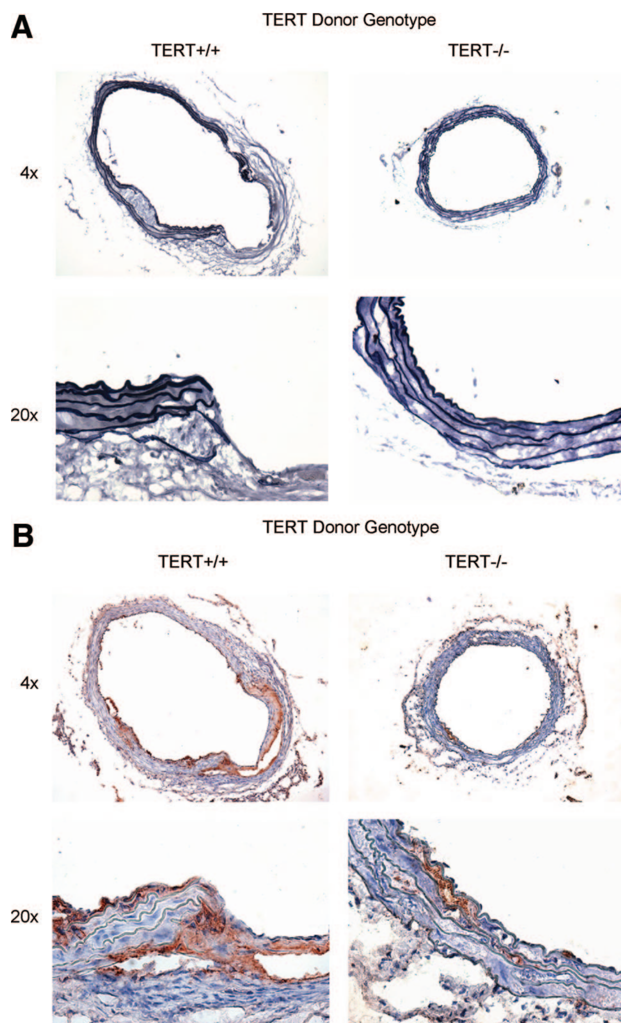

Figure 2. Decreased aortic elastin degradation and macrophage accumulation in TERT−/− bone marrow recipient mice. A, Representative Verhoeff's staining for elastin fibers in cross-sections of the suprarenal aorta. B, Representative immunostaining of cross-sections of the suprarenal aorta with rabbit antiserum for mouse macrophages demonstrates macrophage infiltration at sites of elastin degradation.

The Donor Genotype Has No Significant Effect on Serum Cholesterol Concentrations or Circulating Leukocyte Numbers and Telomere Lengths

Compared with wild-type donors, repopulation of LDLr−/− mice with TERT−/− bone marrow cells revealed no significant effect on serum cholesterol concentrations or distributions (Supplemental Figure III and IV). Furthermore, no differences were observed in white blood cell counts 28 days after Ang II infusion (Table), confirming a sufficient repopulation of LDLr−/− mice with TERT−/− hematopoietic stem cells. Quantification of telomere lengths in circulating leukocytes in vivo revealed modest telomere shortening in mice repopulated with TERT−/− bone marrow, although this difference was not statistically significant (Figure 3A). Similarly, in vitro analysis of telomere lengths by fluorescence in situ hybridization and quantification using real-time PCR demonstrated unaltered telomere lengths in TERT−/− bone marrow–derived macrophages (Figure 3B and C). To finally confirm a sufficient replicative capacity of TERT−/− bone marrow–derived macrophages in vitro, we next analyzed proliferation rates by means of cell counting and DNA synthesis. Consistent with the competent repopulation ability of TERT−/− hematopoietic cells in vivo, we observed no significant difference in proliferation rates between macrophages isolated from TERT-deficient mice and their wild-type littermates (Figure 3D and 3E).

Table. Differential Leukocyte Count at Study End Point

Donor Genotype	TERT+/+ (n=14)	TERT−/− (n=16)	P Value
White blood cells, $\times 10^3/\mu L$	3.97±0.78	3.59±0.52	0.69
Monocytes, $\times 10^3/\mu L$	0.32±0.06	0.25±0.04	0.34
Neutrophils, $\times 10^3/\mu L$	1.38±0.26	1.26±0.13	0.69
Lymphocytes, $\times 10^3/\mu L$	1.96±0.38	1.72±0.28	0.61

Data are presented as mean±SEM.

TERT−/− Deficiency Is Associated With Reduced MMP-2 Expression and Activity

Because TERT deficiency did not affect the proliferative capacity of hematopoietic stem cells or the number of circulating monocytes, the decreased AAA formation was likely due to altered leukocyte function rather than quantity. Among the mechanisms that promote AAA formation, MMP-2 function is critical because of its proteolytic activity to degrade extracellular matrix.[26–28] Therefore, we next analyzed MMP-2 activity in aortic tissues isolated from LDLr−/− mice repopulated with TERT+/+ or TERT−/− bone marrow–derived cells and infused with Ang II for 28 days. As depicted in Figure 4A and 4B, MMP-2 activity was significantly reduced in TERT−/− recipient mice. In vitro, incubation of wild-type bone marrow–derived macrophages with Ang II significantly increased MMP-2 mRNA abundance. In contrast, this inducible MMP-2 expression was decreased in TERT-deficient macrophages (Figure 4C). Furthermore, the altered MMP-2 expression in TERT-deficient macrophages resulted in a corresponding decrease in MMP-2 activity as analyzed by zymography (Figure 4D), demonstrating that TERT deficiency in macrophages decreases MMP-2 activity. To finally address the mechanisms underlying the regulation of MMP-2 transcription by TERT, we performed transient transfection assays using a luciferase reporter construct driven by a 1686-kb MMP-2 promoter fragment. Consistent with the requirement of TERT for MMP-2 mRNA expression, overexpression of TERT significantly induced MMP-2 promoter activity (Figure 4E).

Discussion

AAA is a common and frequently lethal age-related vascular disease.[2,3] Understanding the molecular mechanisms underlying this disease constitutes a critical step toward the development of a medical treatment option.[2] In the present study, we demonstrated that deletion of TERT in bone marrow cells attenuates AAA development in response to Ang II infusion of LDLr−/− mice. This decrease of AAA formation in mice lacking TERT expression occurred in the absence of a replicative defect of hematopoietic stem cells but

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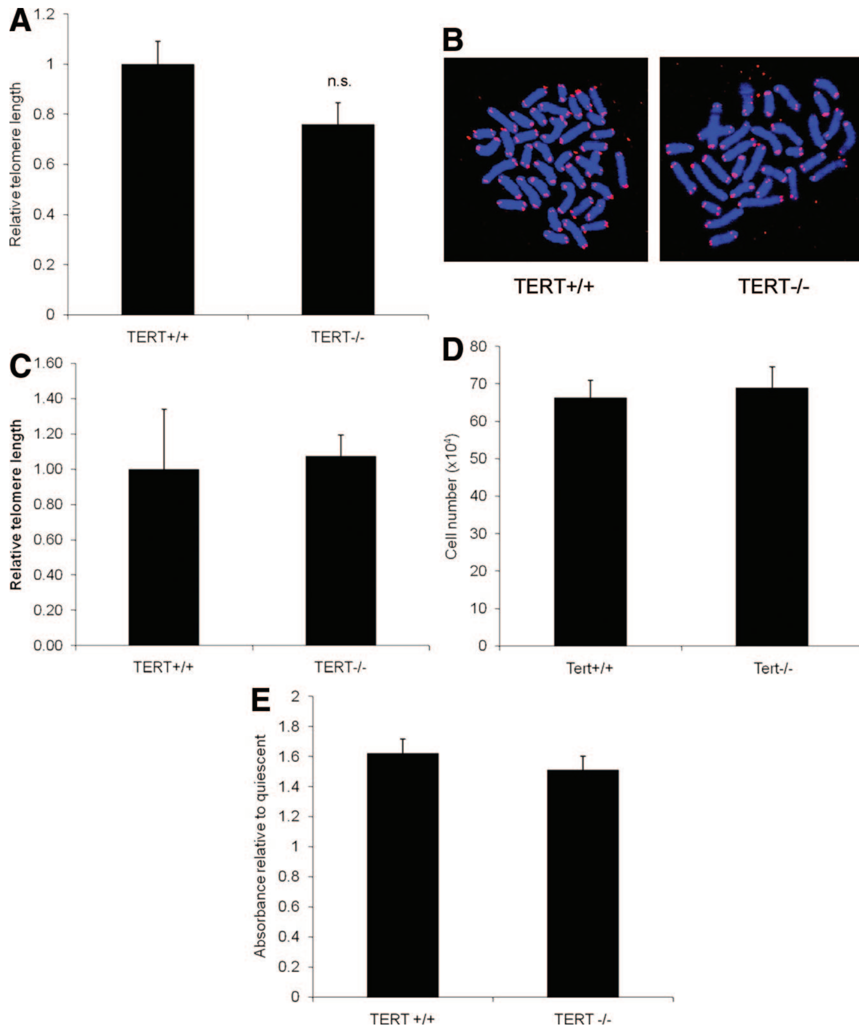

Figure 3. Telomere length and proliferative capacity are preserved in TERT-deficient macrophages. A, Telomere lengths were measured in blood leukocytes after 28 days of Ang II infusion using real-time PCR. Data are presented as fold change relative to TERT+/+ donor genotype (mean±SEM; TERT+/+, n=16; TERT−/−, n=14; *P*=0.07). B, Telomeric fluorescence in situ hybridization assay on metaphase chromosomes of TERT+/+ and TERT−/− BMM. C, Quantification of telomere lengths in TERT+/+ and TERT−/− BMM using real-time PCR (n=3 per group). Data are presented as fold change relative to TERT+/+ (mean±SEM). D, BMM were serum starved for 24 hours and stimulated with growth medium. Cells were counted after 48 hours using a hemocytometer (mean±SEM, n=3 per group). E, BMM were serum starved for 24 hours, incubated with BrdU reagent, and stimulated with growth medium. BrdU incorporation was analyzed after 24 hours. Data are presented as fold change relative to quiescent cells (mean±SEM, n=3 per group).

was associated with altered MMP-2 expression in macrophages. These data reveal an unanticipated causal role for TERT in the development of AAA.

Multiple human population studies have correlated decreased telomere length in peripheral blood leukocytes with an increased risk for atherosclerotic cardiovascular disease.[29,30] Similarly, 2 recent studies have demonstrated decreased telomere length in leukocytes of patients with AAA.[6,7] However, a causal relationship between loss of telomerase or telomere attrition and cardiovascular disease has never been established because of the lack of prospective studies.[31] In fact, 2 recent large cohort studies found no correlation between telomere length and overall mortality, further challenging a causative link between telomere attrition and cardiovascular disease.[32,33] Therefore, decreased telomere length in circulating leukocytes of patients with vascular disease may merely reflect an increased replicative history of hematopoietic stem cells necessary to mount the chronic inflammatory response in the arterial wall, particularly because leukocyte telomere shortening is observed in many other chronic inflammatory diseases[34,35] and occurs even during acute infections.[36]

Meanwhile, animal models may provide initial insight into the causal role of telomerase and telomere attrition in vascular diseases. Using a TERT-deficient mouse model, we demonstrated in the present study that deletion of TERT in bone marrow cells reduces AAA formation. Mice deficient for the TERT or the telomerase RNA component (TERC) retain their telomeres in early generation offsprings, which has been attributed to the very long telomeres in mice.[37–39] However, interbreeding of telomerase-deficient mice to yield successive generations results in progressive telomere attrition and ultimately degeneration of proliferative tissues in late-generation mice.[38,39] Therefore, hematopoietic stem cells from early-generation telomerase-deficient mice display normal proliferation, whereas critically short telomeres in late-generation mice impair progenitor cell proliferation and limit self-renewal capacity.[38–41] This observation has led to the conclusion that telomerase may be dispensable for stem cell proliferation if telomeres are not limiting.[38,40,41] Similarly, in our study using first-generation TERT−/− mice, deficiency for the locus did not reduce telomere lengths or impair the proliferative capacity of bone marrow–derived macrophages in vitro. Moreover, hematopoietic stem cells from first-generation TERT−/− mice effectively reconstituted irradiated mice in vivo, as evidenced by normal circulating leukocyte numbers and subpopulations. However, circulating leukocytes of mice repopulated with TERT−/− stem cells

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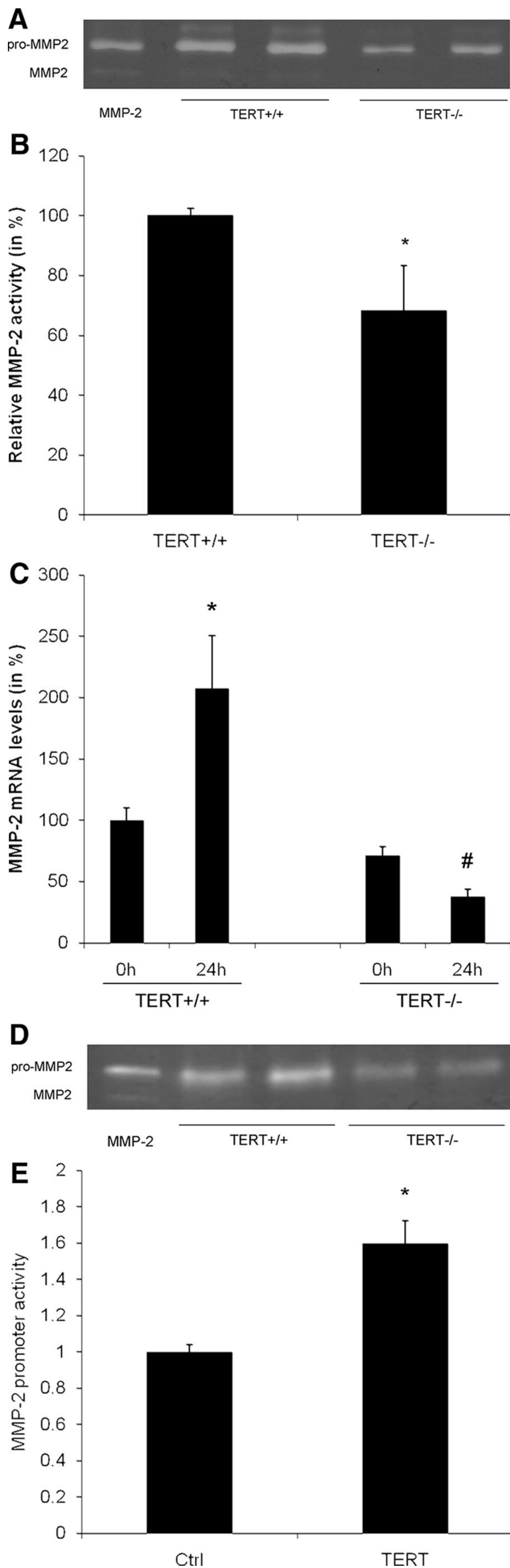

displayed mild telomere shortening, most likely as a result of the increased proliferation required to regenerate the stem cell compartment. Consistent with our studies, Allsopp et al have demonstrated that stem cells from early-generation TERT−/− mice exhibit normal telomere lengths and provide efficient hematopoietic recovery for 2 rounds of serial BMT, despite modest decreases in telomere lengths after the first transplantation.[41] Collectively, from these observations we would infer that the protection in AAA formation in mice reconstituted with TERT−/− bone marrow is likely the result of TERT deficiency affecting leukocyte function rather than proliferation or quantity. Although the loss of telomere length in leukocytes of LDLr−/− mice reconstituted with TERT-deficient bone marrow was not statistically significant, we cannot exclude the possibility that leukocyte telomere attrition might have affected AAA formation in these mice. However, if this were the case, telomere attrition would rather protect against AAA formation as opposed to causing the disease. Our study was not designed to address the contribution of telomere length to AAA formation but rather to investigate the role of TERT itself in the disease process. Considering this limitation, our data warrant further study specifically investigating the contribution of progressive telomere attrition to AAA formation.

Alternatively and more likely, TERT may affect AAA formation through a mechanism that is distinct from its catalytic activity to extend telomeres. There is accumulating appreciation of a noncanonical telomere-independent function of TERT.[42] Support for this novel function of TERT derives from recent experiments showing that TERT modulates the chromatin state,[10,12] interacts with DNA-modifying enzymes,[12,43] and activates gene expression,[11,12,44] effects that do not require catalytic activity and occur independently of telomere maintenance.[11,12,42] Considering this evidence, we reasoned that TERT deficiency might modulate macrophage function and analyzed MMP expression. Degradation of extracellular matrix and elastolysis through the activities of MMPs constitute the best-described mechanisms underlying AAA formation. MMP-2 deficiency has been demonstrated to protect mice from AAA formation.[28] Moreover, MMP-2 is the dominant metalloproteinase in the early stages of AAA formation causing the initial elastolysis,[4,45] a step driven

Figure 4. TERT−/− deficiency is associated with reduced MMP-2 expression and activity. A and B, Representative zymography and densitometric analysis of MMP-2 activity in the aorta of LDLr−/− mice repopulated with TERT-deficient bone marrow–derived cells (densitometry: n=4 per group, *$P<0.05$ versus TERT+/+). Recombinant MMP-2 was used as positive control for calibration. C, TERT+/+ and TERT−/− mouse BMM were serum deprived for 24 hours and stimulated with 1 μmol/L Ang II for 24 hours. MMP-2 mRNA expression was analyzed at the indicated time points using real-time reverse transcription–PCR. Transcript levels were normalized to TFIIB expression (*$P<0.05$ versus 0 hours, #$P<0.05$ versus TERT+/+ at 24 hours). D, TERT+/+ and TERT−/− BMM were serum deprived for 24 hours and stimulated with 1 μmol/L Ang II for 24 hours. Supernatant was used for zymography. E, RAW 264.7 cells were cotransfected with a MMP-2 luciferase reporter construct and a TERT expression vector or a pcDNA3 vector as control. Luciferase activity was assayed 24 hours after transfection (*$P<0.05$ versus control).

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primarily by macrophages in the Ang II–infusion model.[3] In our studies, TERT deficiency prevented Ang II–induced MMP-2 expression and resulted in reduced activity of secreted MMP-2. Conversely, TERT overexpression activated the MMP-2 promoter pointing to a transcriptional mechanism underlying the positive regulation of MMP-2 by TERT. Our observation that TERT is not only necessary for MMP-2 expression but also sufficient to activate MMP-2 transcription is consistent with a previous gain-of-function study demonstrating that overexpression of TERT increases MMP-2 levels in human fibroblasts.[46]

An intriguing question that arises from the observation that TERT induces MMP-2 promoter activity relates to the transcriptional mechanisms governing this regulation. The silencing of Ang II–induced MMP-2 expression in TERT-deficient macrophages and transactivation of the MMP-2 promoter occurred in cells with normal telomere length, supporting the concept of a noncanonical telomere length–independent mechanism. Several previously reported telomere-independent functions of TERT are conceivable to explain this observation. TERT has been shown to physically interact with nuclear factor-κB,[47] which may positively regulate downstream MMP-2 expression. Alternatively, TERT may induce MMP-2 promoter activity through its recently described chromatin remodeling activity,[10,12,48] particularly because an epigenetic regulation of the MMP-2 promoter has been well documented.[49] Finally, TERT has recently been characterized as a transcriptional modulator of the Wnt/β-catenin signaling pathway.[12] Interestingly, this study established that TERT interacts with the Brahma-related gene 1, the central catalytic subunit of numerous chromatin-modifying enzymatic complexes, resulting in the activation of Wnt-dependent promoters. Considering that MMP-2 constitutes a target gene of the Wnt-signaling pathway,[50] TERT may induce MMP-2 transcription through a Wnt-dependent transcriptional activation. Our findings in the context of these intriguing studies provide justification for further defining the mechanism governing the transcriptional regulation of MMP-2 by TERT. In particular, additional studies will be required to investigate telomere-dependent activities of TERT in vascular biology and functions that are mediated through noncanonical mechanisms independently of its reverse transcriptase function.

Sources of Funding

This work was supported by the American Recovery and Reinvestment Act of 2009 (National Institutes of Health/National Heart, Lung, and Blood Institute Grant 1R01-HL091924-01A1 to D.B.). Drs Gizard and Zhao were supported by Fellowship Grants from the American Heart Association (0725313B and 0815514D, respectively).

Disclosures

None.

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Supplement Material

Supplemental Table I:

Composition of cholesterol-enriched diet

(Harlan Teklad TD.88137)

Formula	g/kg
Casein	195.0
DL-Methionine	3.0
Sucrose	341.46
Corn Starch	150.0
Anhydrous Milkfat	210.0
Cholesterol	1.5
Cellulose	50.0
Mineral Mix	35.0
Calcium Carbonate	4.0
Vitamin Mix	10.0
Ethoxyquin	0.04

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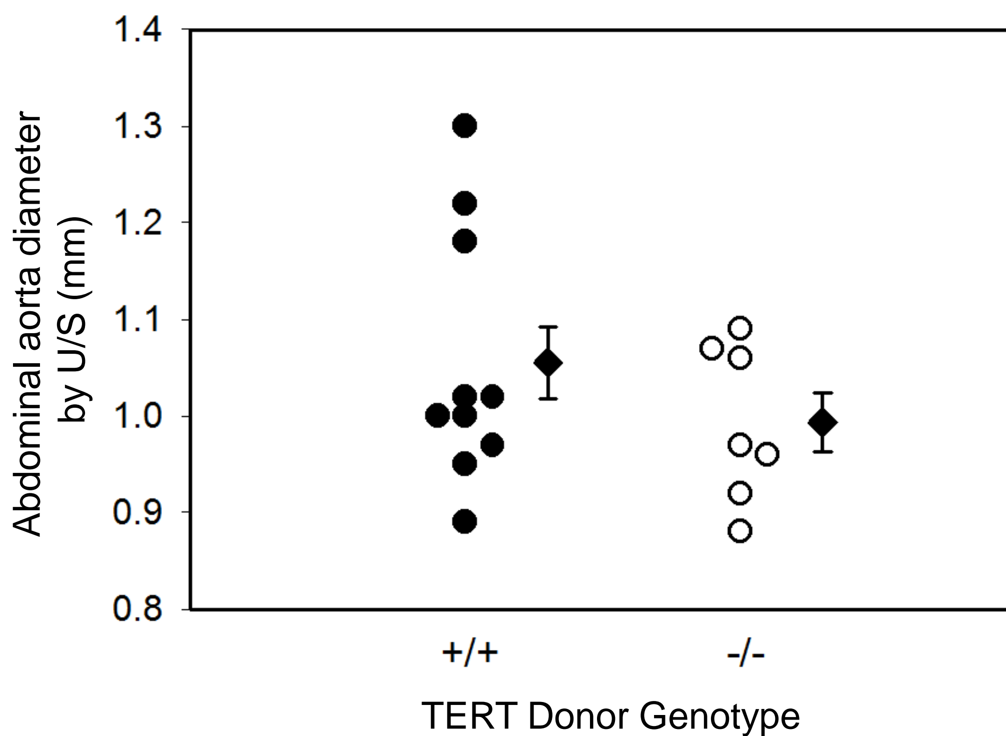
Supplemental Figure I



Supplemental Figure I: Ultrasound measurements of abdominal aorta diameters at day 0 of Ang II infusion. Black circles (TERT+/+ donors) and white circles (TERT-/- donors) represent measurements in individual mice, diamonds represent means, and bars indicate SEM.

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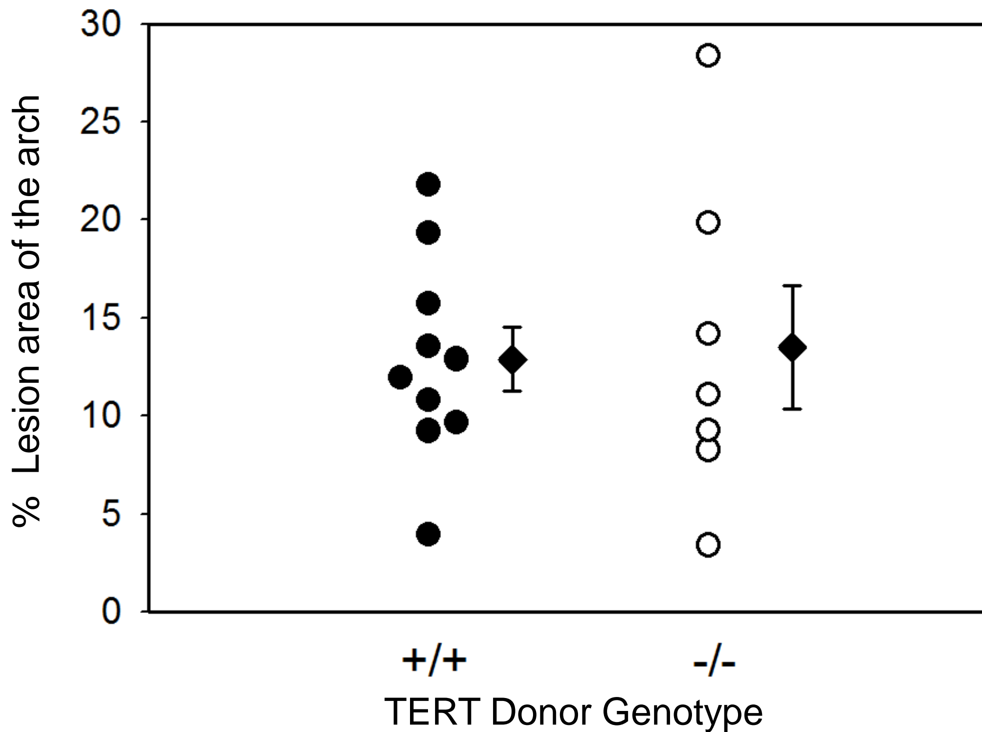
Supplemental Figure II



Supplemental Figure II: Atherosclerotic lesion size was measured on aortic arches of LDLr-/- mice repopulated with TERT+/+ and TERT-/- bone marrow-derived cells and infused with Ang II for 28 days. Black circles (TERT+/+ donors) and white circles (TERT-/- donors) represent measurements in individual mice, diamonds represent means, and bars indicate SEM.

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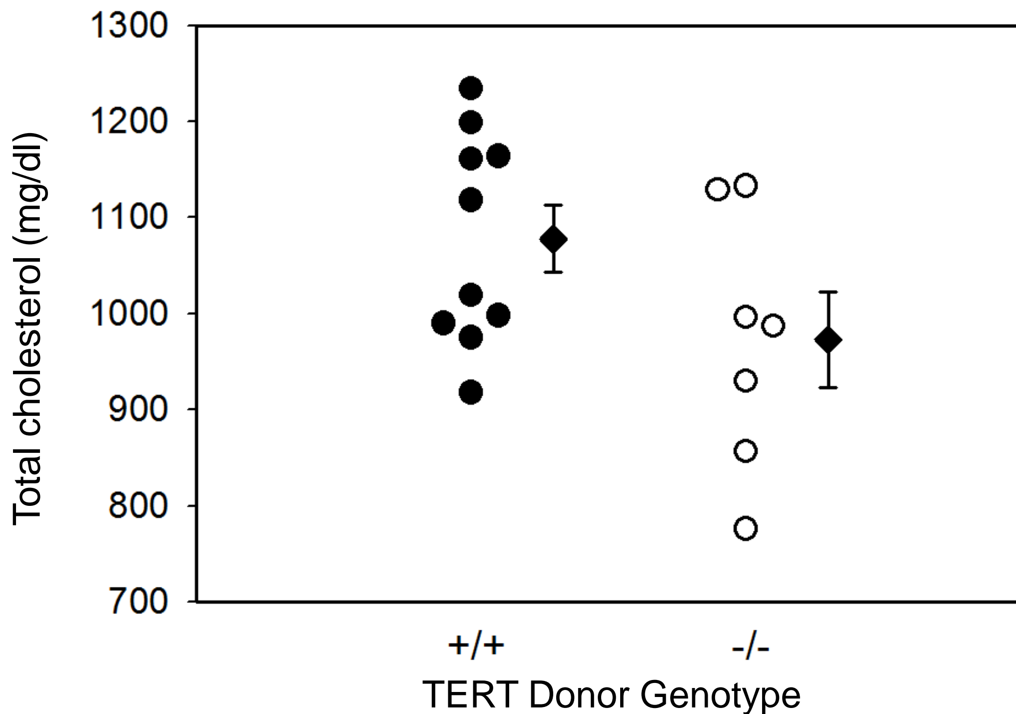
Supplemental Figure III



Supplemental Figures III and IV: The donor genotype has no significant effect on serum cholesterol. Serum cholesterol (III) and plasma lipoprotein distribution (IV) were measured after 28 days of Ang II infusion (black circles, TERT+/+ donors; white circles, TERT-/- donors; diamonds represent means ± SEM, $p = 0.09$).

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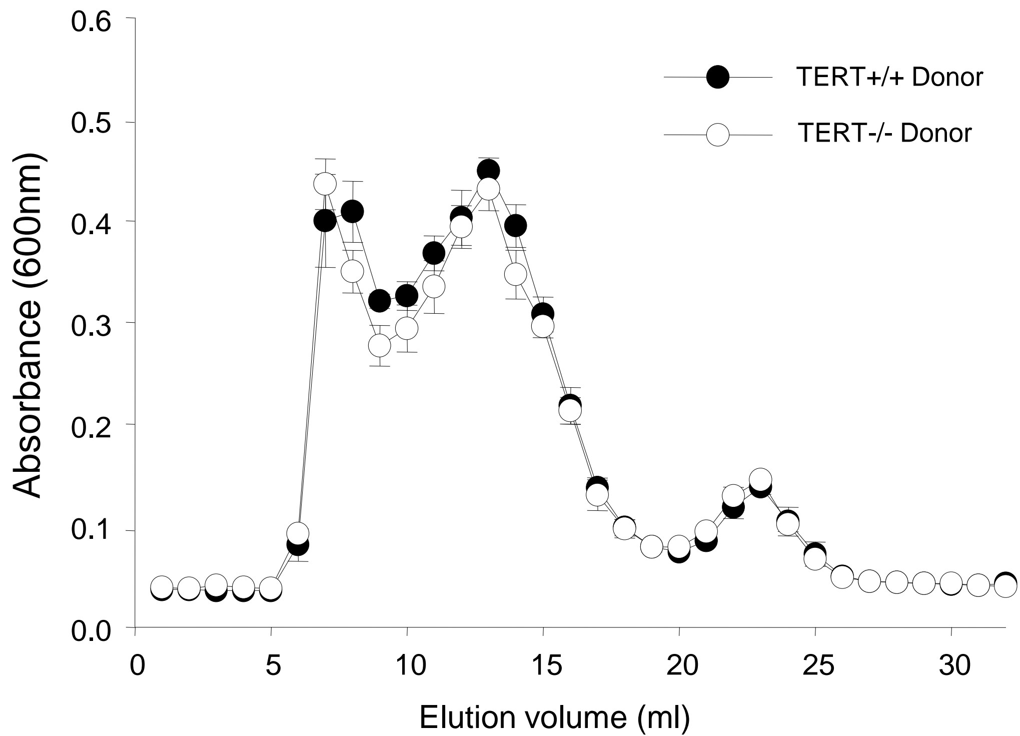
Supplemental Figure IV



Supplemental Figures III and IV: The donor genotype has no significant effect on serum cholesterol. Serum cholesterol (III) and plasma lipoprotein distribution (IV) were measured after 28 days of Ang II infusion (black circles, TERT+/+ donors; white circles, TERT-/- donors; diamonds represent means ± SEM, $p = 0.09$).

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