Mitochondrial DNA Damage Can Promote Atherosclerosis Independently of Reactive Oxygen Species Through Effects on Smooth Muscle Cells and Monocytes and Correlates With Higher-Risk Plaques in Humans

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**Background**—Mitochondrial DNA (mtDNA) damage occurs in both circulating cells and the vessel wall in human atherosclerosis. However, it is unclear whether mtDNA damage directly promotes atherogenesis or is a consequence of tissue damage, which cell types are involved, and whether its effects are mediated only through reactive oxygen species.

**Methods and Results**—mtDNA damage occurred early in the vessel wall in apolipoprotein E-null (ApoE−/−) mice, before significant atherosclerosis developed. mtDNA defects were also identified in circulating monocytes and liver and were associated with mitochondrial dysfunction. To determine whether mtDNA damage directly promotes atherosclerosis, we studied ApoE−/− mice deficient for mitochondrial polymerase-γ proofreading activity (polG−/−/ApoE−/−). polG−/−/ApoE−/− mice showed extensive mtDNA damage and defects in oxidative phosphorylation but no increase in reactive oxygen species. polG−/−/ApoE−/− mice showed increased atherosclerosis, associated with impaired proliferation and apoptosis of vascular smooth muscle cells, and hyperlipidemia. Transplantation with polG−/−/ApoE−/− bone marrow increased the features of plaque vulnerability, and polG−/−/ApoE−/− monocytes showed increased apoptosis and inflammatory cytokine release. To examine mtDNA damage in human atherosclerosis, we assessed mtDNA adducts in plaques and in leukocytes from patients who had undergone virtual histology intravascular ultrasound characterization of coronary plaques. Human atherosclerotic plaques showed increased mtDNA damage compared with normal vessels; in contrast, leukocyte mtDNA damage was associated with higher-risk plaques but not plaque burden.

**Conclusions**—We show that mtDNA damage in vessel wall and circulating cells is widespread and causative and indicates higher risk in atherosclerosis. Protection against mtDNA damage and improvement of mitochondrial function are potential areas for new therapeutics. (Circulation. 2013;128:702-712.)

**Key Words:** apoptosis ■ atherosclerosis ■ free radicals

Mitochondria are often regarded as the cellular powerhouses through their ability to generate ATP, the universal fuel for metabolic processes. These protobacteria-related organelles are vital for eukaryotic function, yet they retain some independence, with their own genome and timing of replication. Mitochondria are also recognized as critical regulators of cell death and calcium signaling and are the major cellular source of reactive oxygen species (ROS). Mitochondrial damage can therefore have a significant impact on cellular function and has been implicated in the pathogenesis of both normal aging and disease.

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**Clinical Perspective on p 712**

Human mitochondrial DNA (mtDNA) is a 16,569-bp circular double-stranded molecule attached to the mitochondrial inner membrane. mtDNA encodes proteins of the electron transport chain (subunits of complexes I, III, and IV and the ATP synthase [complex V]), including 13 essential oxidative phosphorylation genes, and ribosomal and transfer RNAs for protein synthesis. During mitochondrial respiration, electrons at complex I and III can react with molecular oxygen to form free radicals. Protection against mtDNA damage and improvement of mitochondrial function are potential areas for new therapeutics.
Mitochondrial DNA Damage in Atherosclerosis

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Mitochondrial DNA defects lead to mitochondrial dysfunction, promoting inflammation, apoptosis, and cell senescence. These processes are proatherogenic, indeed, mtDNA damage is associated with atherosclerosis. Human atherosclerotic aortas have increased mtDNA oxidative lesions compared with normal controls, and patients with coronary artery disease have increased abundance of the common mtDNA 4977-bp deletion in the heart and circulating leukocytes. Furthermore, mtDNA damage is seen early in atherosclerosis in mice deficient for apolipoprotein E (ApoE−/−), and impaired mitochondrial antioxidant activity can promote atherosclerosis. Although suggestive, it is not clear from these data whether mtDNA damage is a cause or consequence of tissue damage in atherosclerosis and whether early mtDNA damage occurs only in the vessel wall. It is also unknown whether the observed mtDNA damage is sufficient to induce mitochondrial dysfunction, whether effects of mtDNA damage on atherosclerosis are mediated entirely by ROS, and whether mtDNA damage promotes primarily atherosclerosis or affects plaque composition.

We studied ApoE−/− mice to confirm the early presence of mtDNA damage. To then determine whether mtDNA damage promotes atherosclerosis, we studied ApoE−/− mice with deficient polymerase-γ proofreading activity (polG−/−/ApoE−/−). mtDNA damage promoted atherosclerosis and affected plaque composition independently of ROS in polG−/−/ApoE−/− mice through effects on vascular smooth muscle cells (VSMCs), monocytes, and hyperlipidemia. Importantly, human atherosclerotic plaques showed increased mtDNA damage, whereas leukocyte mtDNA damage was associated with higher-risk plaques but not plaque burden. Overall, our results show that mtDNA damage is present and causative and indicates higher risk in atherosclerosis with effects that can occur independently of ROS.

Methods

Mice

All animal experiments were performed under UK Home Office licensing and conformed to animal ethics committee approval. Both male and female C57Bl6/J ApoE−/− mice (Jackson Laboratory, Bar Harbor, ME) were fed high-fat diet (21% fat; Special Diet Services No. 829100) or normal chow diet (3.4% fat; Special Diet Services No. 801730) for 0, 7, or 14 weeks. Mice deficient for both polymerase-γ proofreading activity and ApoE were generated by crossing C57BL6/polG−/− (from Dr Nils Larsson) with C57BL6/ApoE−/− mice. Double heterozygotes were then crossed with ApoE−/− mice to generate polG−/−/ApoE−/− breeders. Breeding of these mice created both control (polG+/+/ApoE+/+) and experimental (polG−/−/ApoE−/−) mice. Genotyping of the mice was done as described in the online-only Data Supplement. Both male and female littermate control (polG+/+/ApoE+/+) and experimental (polG−/−/ApoE−/−) mice were fed fat from 6 to 20 weeks. Feeding, bone marrow transplantation, lipid analysis, glucose and insulin tolerance testing, and blood pressure measures were performed as previously described.

Body Composition Analysis

To assess body fat and lean mass composition, experimental mice were scanned in a Minispec TDNMR analyzer (LF50; Bruker Optics, Billerica, MA) at 17 weeks of age.

In Vivo Measurement of Oxygen Consumption and Physical Activity

Oxygen consumption and activity assessment was performed as described in the online-only Data Supplement.

In Vivo Mitochondrial Peroxide Assessment

MitoB, a mitochondria-targeted ratiometric probe, was used to assess in vivo mitochondrial H2O2 as previously described.

Histological Analysis

Analysis of plaque morphometry and composition was performed as described previously.

Quantitative Polymerase Chain Reaction

mtDNA damage was assessed as described previously and in the online-only Data Supplement. The 4977-bp mutation was assayed as described previously.

Mitochondrial Extraction and Activity

Mitochondria were extracted from liver tissue and assessed for complex I and citrate synthase activities as described previously.

Fluorescent Quantitative Western Blotting

Western blotting was performed as described previously. Primary antibodies included citrate synthase (1:500; ab96600; Abcam, UK) and tubulin (1:500; 2148s; Cell Signaling Technology).

Aortic Respiration

Respirometry was performed in permeabilized aortas, as described for other tissues and in the online-only Data Supplement.

Cell Isolation and Culture

Leukocytes were pelleted from whole blood after erythrocyte lysis and separated into CD11b+ monocytes by MACS segregation (Miltenyi Biotec). Isolation purity was determined by Hoescht 33342 staining and CD11b immunocytochemistry of cytopsins. VSMCs were cultured as previously described.

Cellular ATP Content and ROS Assays

Cellular ATP content and ROS were determined as described previously and in the online-only Data Supplement.

Cytokine Assays

Monocytes were incubated with 1 μg/mL lipopolysaccharide for 16 hours. Culture supernatants were analyzed for tumor necrosis factor-α, interleukin-1β, monocyte chemoattractant protein-1, and interleukin-6 as previously described.
Time-Lapse Videomicroscopy
Cells were seeded into 24-well plates in medium containing 10% FBS at 37°C and incubated with 50 μmol/L tert-Butyl hydroperoxide for 30 minutes. Cell proliferation and apoptosis were determined as described.18

Virtual Histology in Vulnerable Atherosclerosis Study
Patients with coronary artery disease were recruited from the Virtual Histology in Vulnerable Atherosclerosis (VIVA) study.21 DNA samples for mitochondrial lesions and serum 8-oxo-G were compared against virtual histology intravascular ultrasound (VH-IVUS)–defined plaque classification.

Statistical Analysis
Data were tested for a normal distribution by examination of the histogram and normal probability plot and by use of the Shapiro-Wilk test. We used the Student’s t test for pairwise comparison or ANOVA with Bonferroni-Holm post hoc analysis for multiple comparisons for data following a normal distribution. The Mann-Whitney U test or Kruskal-Wallis test was used for nonparametric data. In conjunction with the Kruskal-Wallis test, Mann-Whitney tests adjusted for multiple comparisons with the Bonferroni-Holm procedure were performed when indicated. Clinical and VH-IVUS parameters were assessed for association with mtDNA adducts using univariable and multivariable linear regressions. Parameters with values of P<0.1 on univariable analysis were deleted mtDNA.23 mtDNA damage is associated with reduced mitochondrial respiratory complex activity and ATP production despite a slight compensatory increase in mitochondrial mass.23 In other studies, polG−/− mice showed premature aging without elevated mitochondrial oxidative damage.23,24

Results
Mitochondrial DNA Damage Occurs Early in Atherogenesis
Previous studies have shown that aortas of ApoE−/− mice develop mtDNA damage early in atherogenesis.15 However, it is unclear whether such lesions occur only in vessels or whether they are also present in other cells or organs associated with atherosclerosis or lipid deposition. Whether the observed mtDNA damage results in mitochondrial dysfunction is also unknown. ApoE−/− mice were fed either chow or high-fat diet with plaque development analyzed at 0, 7, and 14 weeks; at 7 and 14 weeks, chow-fed mice had either no significant atherosclerosis or established plaques, respectively (Figure 1A and Figure 1 in the online-only Data Supplement). Baseline serum cholesterol was 6.6±0.23 mmol/L, but increased with high-fat feeding (Figure 2 in the online-only Data Supplement).

We used quantitative polymerase chain reaction analysis for mtDNA adducts to assess mtDNA damage. Oxidized bases, single- and double-strand breaks, and abasic sites can accumulate through oxidative damage and stop polymerase progression during polymerase chain reaction. Global damage can be assessed by comparing the amplification of a large segment of mtDNA with amplification of a short target to control for mtDNA copy number.15 Aortic mtDNA adducts increased in both groups but were increased in chow-fed mice at 7 weeks (Figure 1B), even when no significant atherosclerosis was present. We also assessed mtDNA damage in peripheral monocytes and the liver. In these tissues, mtDNA adducts were significantly increased after 7 weeks of high-fat diet and by 14 weeks with both diets (Figure 1C and 1D). We examined respiratory complex subunit expression and complex activity to assess the functional consequences of mtDNA damage. There were similar levels of complex I, II, and V subunit expression (normalized to the nuclear-encoded mitochondrial protein MnSOD) for all time points and both diets (Figure 1E and Figure III in the online-only Data Supplement). In contrast, complex I activity (normalized to the nuclear-encoded protein citrate synthase, a recognized marker of mitochondrial mass)22 was reduced after 14 weeks of chow feeding and by 7 weeks of high-fat diet, correlating with the presence of significant atherosclerosis (Figure 1F).

polG−/−/ApoE−/− Mice Show Increased mtDNA Damage, No Change in ROS, and Reduced Expression and Respiration of Mitochondrial Complexes
We used polG−/−/ApoE−/− mice to determine whether mtDNA defects promote atherosclerosis. polG−/− mice have a defect in exonuclease activity of a nuclear-encoded mtDNA polymerase and accumulate widespread mtDNA point mutations and deleted mtDNA.23 mtDNA damage is associated with reduced mitochondrial respiratory complex activity and ATP production despite a slight compensatory increase in mitochondrial mass.23 In other studies, polG−/− mice showed premature aging without elevated mitochondrial oxidative damage.23,24

We first characterized the mtDNA damage and dysfunction in polG+/−/ApoE−/− mice and their polG+/−/ApoE−/− littermates. Quantitative polymerase chain reaction showed increased mtDNA adducts in polG+/−/ApoE−/− aortas and monocytes (Figure 2A and 2B). Although polG+/− mice have been reported to have normal ROS levels,24,25 ApoE−/− mice have increased ROS, and it was important to determine ROS in polG+/−/ApoE−/− mice. In vivo mitochondrial H2O2 was assayed with the targeted probe MitoB.17 MitoB accumulates within mitochondria where it reacts with H2O2 to form Mitop. Quantification of the MitoP/MitoB ratio enables measurement of mitochondrial H2O2. There were no significant differences in MitoP/MitoB ratio in aortic, heart, and liver extracts between polG+/−/ApoE−/− and polG+/−/ApoE−/− mice (Figure 2C).

polG+/−/ApoE−/− aortas had reduced complex I and complex IV subunit expression, but there were no significant differences in the abundance of complexes II and III and citrate synthase (Figure 2D and Figure IV in the online-only Data Supplement). We performed respirometry on aortic tissue to identify functional consequences of mtDNA damage and reduced complex expression. The aortas were permeabilized to allow substrate entry, and the respiration of different complexes was assessed by using a specific sequence of substrates and inhibitors.19 polG+/−/ApoE−/− aortas had reduced complex I– and complex IV–supported respiration compared with polG+/−/ApoE−/− control aortas (Figure 2E and 2G). No difference was observed in complex II–supported respiration (Figure 2F).

polG−/−/ApoE−/− Mice Have Increased Atherosclerosis
To determine the effect of mtDNA and dysfunction on atherosclerosis, polG−/−/ApoE−/− and their polG+/−/ApoE−/− littermates
were fat fed from 6 to 20 weeks, and atherosclerosis was examined in 3 different vascular beds. Atherosclerosis in the aorta, brachiocephalic artery, and descending aorta was increased in polG−/−/ApoE−/− mice compared with controls (Figure 3A and 3B). Although there was no difference in overall plaque composition, the percentage of cells undergoing apoptosis was increased and the percentage of proliferating cells was decreased in polG−/−/ApoE−/− plaques (Table 1).

We have demonstrated that mtDNA damage occurs in both vessel wall and circulating monocytes in ApoE−/− mice, both of which might promote atherosclerosis. To determine the relative contribution of vessel wall and circulating bone marrow–derived cells, we transplanted ApoE−/− mice with polG−/−/ApoE−/− or control polG+/+/ApoE−/− marrow and fat fed the mice from 6 to 20 weeks. Transplantation with polG−/−/ApoE−/− marrow did not affect the extent of atherosclerosis in any vascular bed (Figure 3C and 3D). However, polG−/−/ApoE−/− marrow transplantation increased the necrotic core area and decreased the relative fibrous cap area, features of increased plaque vulnerability (Figure 3E).26

Effects of Reduced polG Proofreading Activity on VSMCs and Monocytes

Our data suggest that the increased atherosclerosis seen in polG−/−/ApoE−/− mice and the changes in plaque composition seen after polG−/−/ApoE−/− transplantation represent changes in vessel wall cells and monocytes. We therefore examined the in vitro characteristics of cells derived from polG−/−/ApoE−/− mice. We initially assessed in vitro ROS with dichlorodihydrofluorescein diacetate (DCFDA), which fluoresces on reaction with different oxidative species, including H2O2, peroxynitrite, and hydroxyl radicals. Although DCFDA is not specific for mitochondrial ROS, there was no difference between polG−/−/ApoE−/− and polG+/+/ApoE−/− VSMCs (Figure 4 in the online-only Data Supplement), consistent with the in vivo MitoP/B data. In contrast, ATP content assessed with a luciferin-luciferase assay was significantly reduced in polG−/−/ApoE−/− VSMCs and monocytes (Figure 4A and 4B).

To assess whether polG−/− cells have defects that might promote atherosclerosis, we used time-lapse videomicroscopy to examine cell proliferation and apoptosis. polG−/−/ApoE−/− VSMCs had markedly reduced cell proliferation, both basally and after treatment with the ROS-generating agent tert-Butyl hydroperoxide (Figure 4C). Indeed, under basal conditions, polG−/−/ApoE−/− VSMCs proliferated poorly and senesced early (passage 7±2 versus 19±2 [mean±SEM]). Both polG−/−/ApoE−/− VSMCs and monocytes showed increased apoptosis compared with polG+/−/ApoE−/− controls (Figure 4D and 4E).

To assess whether mtDNA defects could promote an inflammatory phenotype, we examined lipopolysaccharide-induced
monocyte cytokine release. polG−/−/ApoE−/− monocytes had increased release of interleukin-1β and tumor necrosis factor-α (Figure 4F), suggesting that increased proinflammatory cytokine release could contribute to the plaque vulnerability observed with polG−/− bone marrow transplantation.

polG−/−/ApoE−/− Mice Show Reduced Adiposity, Activity, and V02 but Have Hyperlipidemia

Atherosclerosis is promoted not only by local effects on cells making up the plaque but also by systemic metabolic effects, including increased serum cholesterol, hypertension, and diabetes mellitus. The deficiency of DNA polymerase-γ activity in polG−/−/ApoE−/− mice was systemic; we therefore examined polG−/−/ApoE−/− mice for metabolic changes using a combination of dynamic testing and a comprehensive laboratory animal monitoring system. After 14 weeks of high-fat feeding, female and male polG−/−/ApoE−/− mice had reduced total body weight compared with control polG+/+/ApoE−/− mice (Figure 5A). Time-domain nuclear magnetic resonance imaging showed that this was attributable to reduced percent body fat (Figure 5B), which was confirmed on organ weight analysis. Both brown and white adipose tissue mass was reduced relative to total body weight, with no changes in other organs (Figure 5C). The comprehensive laboratory animal monitoring system was used to assay food and water consumption, physical activity, and oxygen consumption. Although there were no differences in food and water consumption (data not shown), both physical activity and oxygen consumption (V02) were reduced in polG−/−/ApoE−/− mice (Figure 5D and 5E).

The alterations in body adiposity that we observed (reduced weight, reduced fat percent) can be associated with altered serum lipid levels and insulin sensitivity. Surprisingly, although there were no significant differences in serum lipids before and at 7 weeks of fat feeding (data not shown), polG−/−/ApoE−/− mice had increased serum total and low-density lipoprotein cholesterol after 12 weeks compared with polG+/+/ApoE−/− mice (Figure 5F). This was not seen in ApoE−/− mice transplanted with polG−/−/ApoE−/− marrow (Figure VI in the online-only Data Supplement). In contrast, there were no significant differences in glucose or insulin tolerance testing (Figure 5G and 5H) or blood pressure (Figure VII in the online-only Data Supplement). We also examined whether the observed increased atherosclerosis in polG−/−/ApoE−/− mice was attributable to increased inflammatory cell indexes. In contrast, we found a reduction in total leukocyte count with reduced lymphocyte fraction in both polG−/−/ApoE−/− mice and mice receiving polG−/−/ApoE−/− mice bone marrow and reduced monocyte count in polG−/−/ApoE−/− mice (Figure VIII in the online-only Data Supplement).

Human Plaques Show Increased mtDNA Damage, and mtDNA Damage Is Associated With Higher-Risk Plaques

Our data show that mtDNA defects are present and widespread, reduce mitochondrial function in ApoE−/− mice, and promote atherosclerosis in polG−/−/ApoE−/− mice; however, the relevance of these findings to human disease is unclear. In particular, the bone marrow transplantation data suggest that circulating cells with mtDNA damage could promote plaque
vulnerability, potentially via increased local cytokine release. We therefore examined mtDNA adducts and the 4977-bp mitochondrial deletion in normal human ascending aorta and age- and sex-matched carotid endarterectomy plaques. Human plaques showed a 2-fold increase in mtDNA adducts and a 1.7-fold increase in the 4977-bp deletion compared with normal vessels (Figure 6A and 6B).

To examine whether leukocyte mtDNA damage correlates with atherosclerosis extent or plaque vulnerability in humans, we assessed mtDNA adducts in patients from the VIVA trial. VIVA used 3-vessel VH-IVUS to examine 1096 plaques in 170 patients undergoing intracoronary stenting for stable angina (n=100) or acute coronary syndromes (n=70).21 VH-IVUS can classify plaques by their composition because different plaque components may be identified by their backscatter spectrum (Figure 6C); it also can measure plaque burden accurately. VIVA and the recent Providing Regional Observations to Study Predictors of Events in the Coronary Tree (PROSPECT) study showed that VH-IVUS–defined thin-capped fibroatheromas have the highest risk of cardiovascular events on subsequent follow-up.21,28 We examined the association between patient demographics, VH-IVUS–defined plaque classification, and plaque burden with mtDNA adducts.

On multivariable analysis, mtDNA adducts were not associated with patient demographics, including family history or previous history of myocardial infarction, smoking, age, sex, hypertension, or drug therapy, although they were positively associated with diabetes mellitus (sB=0.33, P=0.012) and negatively associated with serum cholesterol (Table 2). mtDNA adducts did not change significantly 3 months after percutaneous coronary intervention in stable angina patients but were reduced after treatment in patients with acute coronary syndromes (Figure IX in the online-only Data Supplement). Although there was an association between serum 8-oxo-G, a DNA marker of oxidation, and mtDNA adducts on univariable analysis, this was not statistically significant on multivariable analysis (Table 2). In contrast, although there was no association between leukocyte mtDNA adducts and 3-vessel or culprit vessel plaque volume, 3-vessel and culprit vessel VH-IVUS–defined thin-capped fibroatheromas number was strongly associated with both mtDNA adducts (Table 2) and the 4977 mtDNA deletion (Table I in the online-only Data Supplement). Importantly, other VH-IVUS–identified plaque types were not positively associated with mtDNA adducts, and VH-IVUS–identified thick-cap fibroatheromas were negatively associated with the 4977-bp mtDNA deletion.

Table 1. Apoptosis Is Increased and Cell Proliferation Is Reduced in polG−/−/ApoE−/− Mouse Plaques

<table>
<thead>
<tr>
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<th>Control</th>
<th>polG−/−/ApoE−/−</th>
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<tbody>
<tr>
<td>α-SMA-positive area, %</td>
<td>25.52±2.41</td>
<td>24.78±2.19</td>
</tr>
<tr>
<td>MAC 3-positive area, %</td>
<td>55.71±5.08</td>
<td>58.42±4.35</td>
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<tr>
<td>Necrotic core area, %</td>
<td>69.15±1.84</td>
<td>71.48±2.36</td>
</tr>
<tr>
<td>Cap area/plaque area</td>
<td>21.9±1.8</td>
<td>22.1±1.5</td>
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| TUNEL-positive cells, % | 0.887 (0.096–1.24) | 1.55 (0.82–2.61) *
| Ki67-positive cells, % | 2.81±0.39 | 1.85±0.2 *

Shown are the percentage of α-SMA, Mac-3, and necrotic core and fibrous cap/plaque areas and percentage of cells undergoing apoptosis (TUNEL) or proliferation (Ki67) in plaques from control mice (polG+/+/ApoE−/−) and polG−/−/ApoE−/− mice. Data are mean±SEM for parametric data and medians (1st and 3rd quartiles) for nonparametric data. ApoE indicates apolipoprotein E; α-SMA, α-smooth muscle cell actin; polG, polymerase-γ proofreading activity; and TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.

*P<0.05, n=12.
mtDNA damage is implicated in a variety of aging-related phenotypes, including atherosclerosis. mtDNA defects lead to impaired mitochondrial function, resulting in increased ROS, which in turn damage mitochondria further. Oxidative stress is seen in atherosclerosis, and ROS are seen as a major effector pathway by which mtDNA damage might promote aging and age-related disease. In contrast, mtDNA damage can also directly promote inflammation, apoptosis, and senescence, all processes involved in both atherogenesis and the transition to an unstable plaque phenotype. Furthermore, plaques comprise cells from both the vessel wall and the bone marrow, and monocyte invasion of the vessel wall is one of the earliest events in atherosclerosis. It is therefore unclear whether the vessel wall is the only target of mtDNA damage in atherosclerosis and whether the effect is mediated only through ROS.

In ApoE−/− mice, we show that mtDNA damage occurs in arteries, circulating cells, and other organs during atherogenesis. Moreover, we confirm that mtDNA damage is an early event in atherogenesis, detectable even before significant atherosclerotic plaque has developed. mtDNA damage generally increases with time in these mice, is increased by a high-fat diet, and is sufficient to reduce mitochondrial function as assayed by complex I activity. This early onset raises the possibility that mtDNA damage has a causative role rather than being just a late consequence of extensive ROS and tissue damage in advanced plaques. We therefore studied mice defective for both proofreading activity of mtDNA polymerase-γ and ApoE. We show that mtDNA damage directly promotes atherogenesis, in part through effects on VSMCs and through increasing serum lipids. Transplantation of polG−/−/ApoE−/− bone marrow reduced the relative fibrous cap area and increased the necrotic core area of plaques, consistent with a more unstable phenotype.

Although the mechanisms inducing mtDNA damage in fat-fed ApoE−/− mice and polG−/− mice are different, the lesions caused have many similarities, and we show that the downstream consequences on complex activity are similar. polG−/− mice accumulate mtDNA mutations in an approximately linear manner as the polymerase defect introduces point mutations and deletions into the mitochondrial genome. Although the quantitative polymerase chain reaction adducts assay detects a variety of lesions, including strand breaks and abasic sites, these lesions can result from base excision repair of mutations. polG−/−/ApoE−/− mice showed increased mtDNA adducts and reduced expression of complexes I and IV, which may be especially vulnerable to mtDNA damage because they have the highest number of mtDNA-encoded subunits. Although the exact mechanisms underlying the decreased abundance are unclear, reduced transcript levels may be involved. The changes in complex expression in polG−/−/ApoE−/− mice led to reduced complex I and IV respiration and reduced cellular ATP content. Although not directly examined in our study, reduced complex activity could also contribute to the decreased respiration.

Oxidative stress is considered to contribute to atherogenesis, and ROS have been suggested to play a key role in the mitochondrial theory of aging. However, despite extensive...
point mutations and deletions of mtDNA, polG−/− mice do not demonstrate increased general levels of mitochondrial ROS in bulk tissues.24,25 In addition, levels of antioxidant enzymes, protein carbonylation, and aconitase activity measurements indicate no or only minor oxidative stress in polG−/− mouse tissues,24 although reduction in mitochondrial ROS can alleviate some of the features in polG−/− mice.34 We assessed ROS with the general probe DCFDA in cultured cells and the targeted probe MitoB in vivo.17 polG−/−/ApoE−/− mice or cells showed no increase in ROS, suggesting for the first time that mtDNA defects may increase atherosclerosis independently of ROS. We might anticipate that complex dysfunction would lead to increased oxidative stress in polG−/− mouse tissues,24 although reduction in mitochondrial ROS can alleviate some of the features in polG−/− mice.34 We assessed ROS with the general probe DCFDA in cultured cells and the targeted probe MitoB in vivo.17 polG−/−/ApoE−/− mice or cells showed no increase in ROS, suggesting for the first time that mtDNA defects may promote atherosclerosis independently of ROS. We might anticipate that complex dysfunction would lead to increased oxidative stress in polG−/− mouse tissues,24 although reduction in mitochondrial ROS can alleviate some of the features in polG−/− mice.34 We assessed ROS with the general probe DCFDA in cultured cells and the targeted probe MitoB in vivo.17 polG−/−/ApoE−/− mice or cells showed no increase in ROS, suggesting for the first time that mtDNA defects may promote atherosclerosis independently of ROS.

Despite no increase in ROS, polG−/−/ApoE−/− VSMCs and monocytes showed increased apoptosis and decreased proliferation, and plaques in polG−/−/ApoE−/− mice showed a similar phenotype. Both VSMC apoptosis and monocyte/macrophage apoptosis have been reported to promote atherosclerosis and can result from mitochondrial dysfunction.30,31,35 Mitochondria are important in regulating apoptosis through the mitochondrial permeability transition pore.38 Opening of the mitochondrial permeability transition pore leads to release of cell death–promoting factors such as cytochrome C and apoptosis-inducing factor.39,40 Decreased levels of ATP favor pore opening38 and therefore increase apoptosis. Decreased ATP also promotes cell senescence through AMP-activated protein kinase, which decreases the expression of proliferative genes such as cyclin A and B.39,41

We also show that mtDNA defects may increase atherosclerosis through systemic effects, polG−/−/ApoE−/− mice showed reduced total body weight with reduced fat mass and increased serum lipids. This picture is suggestive of lipodystrophy, a condition for which mitochondrial dysfunction has been implicated.42 In lipodystrophy, adipocyte stores are compromised by impaired fatty acid oxidation, decreased adipogenesis, and increased adipocyte apoptosis43; the subsequent fat redistribution results in hyperlipidemia.43

Contrasting with our findings in polG−/−/ApoE−/− mice, transplantation with polG−/−/ApoE−/− bone marrow did not alter atherosclerosis extent but increased necrotic core and decreased relative fibrous cap areas, features of increased plaque vulnerability.26 These effects may be attributable to increased mtDNA damage and subsequent cell death because polG−/−/ApoE−/− monocytes showed reduced ATP content and increased apoptosis. Increased macrophage apoptosis promotes necrotic core expansion44 because efferocytosis is impaired and overwhelmed, leading to secondary necrosis and inflammation.42,45 polG−/−/ApoE−/− monocytes also had increased secretion of tumor necrosis factor-α and interleukin-1β. The proinflammatory phenotype may result from increased mitochondrial permeability transition pore opening, which leads to mtDNA release into the cytosol. mtDNA binds to and activates the inflammasome, with subsequent increased expression and release of interleukin-1β and tumor necrosis factor-α.45,48

Having shown that mtDNA defects promote atherosclerosis and plaque vulnerability in a murine model, we examined the relevance of these findings in human disease. Consistent with previous work12 we show that human plaques have increased mtDNA damage compared with age- and sex-matched normal aortas. More important, we show that leukocyte mtDNA damage is associated with higher-risk plaques as assayed by VH-IVUS. The recent VIVA and PROSPECT studies showed that VH-IVUS–defined thin-capped fibroatheromas have the highest risk of cardiovascular events on subsequent

Figure 5. polG−/−/ApoE−/− mice show reduced adiposity, activity, and V_o2 but have hyperlipidemia. A, Total body weight for polG−/−/ApoE−/− and control polG+/+/ApoE−/− mice after 14 weeks of fat feeding (n=12). B, Percent body fat in polG−/−/ApoE−/− mice and control polG+/+/ApoE−/− mice (n=12) assessed by time-domain nuclear magnetic resonance imaging. C, Organ weight relative to total body weight for the same groups as in A, D. Activity measured as beam breaks in polG−/−/ApoE−/− and control polG+/+/ApoE−/− mice (n=3). E, Oxygen consumption (V_o2) normalized to body mass for the same groups as in D. F, Serum lipids in polG−/−/ApoE−/− and control polG+/+/ApoE−/− mice after 12 weeks of fat feeding (n=12). G, Fasted glucose tolerance test in polG−/−/ApoE−/− and control polG+/+/ApoE−/− mice after 9 weeks of fat feeding (n=12). H, Insulin tolerance test for the same groups as G. BAT indicates brown adipose tissue; Chol, serum cholesterol; Trig, serum triglyceride; and WAT, white adipose tissue. Bars represent medians (F); other data are mean±SEM.
follow-up.21,28 Consistent with our findings that monocyte mtDNA damage promotes features of plaque vulnerability, leukocyte mtDNA damage does not correlate with plaque volume in humans and was associated only with VH-IVUS–defined thin-capped fibroatheromas. The association between mtDNA adducts and 8-oxo-G was consistent with previous findings that ROS can induce mtDNA damage; however, the correlation was not present on multivariable analysis, and the absence of an association between 8-oxo-G and the 4977-bp deletion suggests that mtDNA defects can occur independently of ROS.

Although ROS are undoubtedly important in mtDNA damage and atherosclerosis, our data suggest another mechanism linking mtDNA defects and atherosclerosis. mtDNA defects lead to decreased expression of respiratory complexes and reduced mitochondrial respiration in VSMCs, monocyte/macrophages, and other organs. ATP content is reduced, which promotes apoptosis and inhibits cell proliferation. These processes also compromise adipose tissue stores, promoting hyperlipidemia, which, together with VSMC apoptosis, increases atherosclerosis. mtDNA defects also promote plaque vulnerability through increased monocyte apoptosis and inflammatory cytokine release.

Conclusions
We show that mtDNA damage can promote atherosclerosis and plaque vulnerability and indicates increased risk in patients. Protection against mtDNA damage and improvement of mitochondrial function are potential areas for new therapeutics. Biomarkers of mtDNA damage also show promise to risk stratify coronary artery disease patients.

Sources of Funding
The study was funded by British Heart Foundation grants FS/10/70/28507, PG/11/57/29003, and RG/08/009/25841; a Raymond and Beverly Sackler Studentship; a Medical Research Council Center for Obesity and Related Metabolic Diseases grant; and the Cambridge National Institute of Health Research Biomedical Research Center.
Table 2. mtDNA Adducts Are Associated With Higher-Risk Plaque

<table>
<thead>
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<th>Clinical parameters</th>
<th>Mean (SD), median (Q1–Q3), or Frequency (%)</th>
<th>Association With mtDNA Adducts</th>
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<tr>
<td></td>
<td>(n=159)</td>
<td>Univariable Analysis</td>
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<tr>
<td></td>
<td>β Coefficient</td>
<td>P Value</td>
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<tr>
<td>Age, y</td>
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<td>0.09</td>
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<td>Male, n (%)</td>
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<td>ACS presentation, n (%)</td>
<td>66 (41.5)</td>
<td>−0.01</td>
</tr>
<tr>
<td>Statin treatment for &gt;3 mo, n (%)</td>
<td>65 (40.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.55 (1.31)</td>
<td>−0.31*</td>
</tr>
<tr>
<td>Smoked in the past 3 mo, n (%)</td>
<td>42 (26.4)</td>
<td>0.10</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>83 (52.2)</td>
<td>−0.002</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>17 (10.7)</td>
<td>−0.07</td>
</tr>
<tr>
<td>Family history of MI, n (%)</td>
<td>70 (44.0)</td>
<td>0.05</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>22 (13.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>42 (26.4)</td>
<td>0.10</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>450 (208–622)</td>
<td>0.03</td>
</tr>
<tr>
<td>Smoked in the past 3 mo, n (%)</td>
<td>3–Vessel N of VHFA</td>
<td>0 (0–2)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>3–Vessel N of VHFA</td>
<td>83 (52.2)</td>
</tr>
<tr>
<td>Previous MI, n (%)</td>
<td>3–Vessel N of VHFA</td>
<td>17 (10.7)</td>
</tr>
<tr>
<td>Family history of MI, n (%)</td>
<td>3–Vessel N of VHFA</td>
<td>70 (44.0)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>3–Vessel N of VHFA</td>
<td>22 (13.8)</td>
</tr>
<tr>
<td>Serum 8-oxo-G</td>
<td>2.59 (1.68–5.23)</td>
<td>0.21*</td>
</tr>
</tbody>
</table>

IVUS parameters

| 3-Vessel plaque volume, mm³ | 1102 (803–1405) | 0.03 | 0.72 |
| Culprit vessel plaque volume, mm³ | 450 (208–622) | 0.00 | 1.00 |
| 3-Vessel N of VHFA | 0 (0–1) | −0.01 | 0.86 |
| 3-Vessel N of VHFA | 0 (0–1) | −0.01 | 0.87 |
| 3-Vessel N of VHFA | 1 (0–2) | 0.01 | 0.90 |
| 3-Vessel N of VHFA | 4 (2–6) | 0.20* | 0.013* |
| Culprit vessel N of VHFA | 1 (1–2) | 0.19* | 0.016* |

ACS indicates acute coronary syndrome; IVUS, intravascular ultrasound; MI, myocardial infarction; mtDNA, mitochondrial DNA; Q1–Q3, 1st and 3rd quartiles; VHFA, virtual histology intravascular ultrasound–identified thin-capped fibroatheromas; VHFA, virtual histology intravascular ultrasound–identified fibrocalcific plaque; VHFA, virtual histology intravascular ultrasound–identified pathological intimal thickening; VHFA, virtual histology intravascular ultrasound–identified thin-capped fibroatheromas; and VHFA, virtual histology intravascular ultrasound–identified thick-capped fibroatheroma.

*Significant.

Disclosures

None.

References


mer length is associated with high-risk plaques on virtual histology intra–


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tosis in atherosclerosis: the importance of lesion stage and phagocytic effi–


33. Brinkman K, Smeitink JA, Romijn JA, Reiss P. Mitochondrial toxicity induced by nuclear–


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**CLINICAL PERSPECTIVE**

Damage to mitochondria, the powerhouse of the cell, has been found in human atherosclerosis and is associated with increased levels of free radicals and oxidative stress. Indeed, to date, it has been thought that free radicals mediate almost all of the effect of mitochondrial DNA (mtDNA) damage in atherosclerosis. We show that mtDNA damage occurs early in atherosclerosis in both the vessel wall and circulating cells and is sufficient to cause mitochondrial dysfunction. Using mice, we show that mtDNA damage directly promotes atherosclerosis and features of unstable plaques independently of oxidative stress. mtDNA damage inhibits cell proliferation, promotes cell death, and increases the proinflammatory activity of monocytes. Using virtual histology intravascular ultrasound, we show that mtDNA damage is associated with higher-risk plaques in patients, is independent of other risk factors, and decreases after treatment in patients with acute coronary syn–
drome. Thus, mtDNA damage in the vessel wall and circulating cells is widespread and causative, indicates higher risk in atherosclerosis, and can promote disease independently of oxidative stress. mtDNA damage may be useful as a biomarker for high-risk atherosclerosis, and protection against mtDNA damage and improvement in mitochondrial function are poten–
tial areas for new therapeutics.
Mitochondrial DNA Damage Can Promote Atherosclerosis Independently of Reactive Oxygen Species Through Effects on Smooth Muscle Cells and Monocytes and Correlates With Higher-Risk Plaques in Humans

Emma Yu, Patrick A. Calvert, John R. Mercer, James Harrison, Lauren Baker, Nichola L. Figg, Sheetal Kumar, Julie C. Wang, Liam A. Hurst, Daniel R. Obaid, Angela Logan, Nick E.J. West, Murray C.H. Clarke, Antonio Vidal-Puig, Michael P. Murphy and Martin R. Bennett

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Supplemental methods

Mice genotyping
Genotyping of ApoE<sup>−/−</sup> mice was performed by PCR using company protocols (Jackson Laboratory). The genotyping of polG<sup>−/−</sup> mice was performed by PCR using the following primers at 5µM: FWD: CTTCGGAAGAGCAGTCGGGTG, REV: GGGCTGCAAAGACTCCGAAG. The cycling parameters were 1 cycle of 94<sup>°</sup>C, 1 min and 30 cycles of 94<sup>°</sup>C, 30 s; 61<sup>°</sup>C, 30s; 72<sup>°</sup>C, 1min. Expected product sizes were polG<sup>+/+</sup> 520 bp, polG<sup>+/−</sup> 520 and 720 bp, polG<sup>−/−</sup> 720 bp.

In vivo measurement of oxygen consumption and physical activity
After 13 weeks of high fat feeding, mice were transferred to individual monitoring cages, kept at 22<sup>°</sup>C, under an alternating 12h:12h light-dark cycle. Following 24 hours of acclimatization, mice were assessed for 48 hours. Water and food consumption, ambulatory activity, and respiratory exchange ratio (VCO<sub>2</sub>/VO<sub>2</sub>) were measured using a continuous laboratory animal monitoring system (CLAMS, Columbus Instruments, OH) fitted with an indirect calorimetry system (miniMOX; University of Cambridge, Cambridge, UK).

QPCR for mitochondrial DNA lesions
QPCR for murine mitochondrial DNA lesions was performed using previously described primers<sup>1</sup>. For assessment of human mtDNA adducts, primers used at 5 µM were as follows:
Long primers: producing a 10235 bp product, FWD: ACATACCCATGGCCAACCT, REV: TATGTTTGCCTTTCTGATGA. Short primers: producing a 113 bp product, FWD:ACATACCCATGGCCAACCT, REV: GGGCCTTTGCGTAGTTGAT.
QPCR was performed in triplicate, on 20 ng of sample DNA in 20 µl reactions on a
Rotor-Gene™ 6000 QPCR thermocycler (Corbett Research, AU). Fast Sybr Mix
(Qiagen, Germany) was used for the short reaction and PFU Ultra II Hotstart PCR
Master Mix (Agilent Technologies, CA, USA) with 1 in 400 dilution of Eva Green
(Biotium, CA, USA) for the long reaction. Cycling parameters for the short reaction
were 95ºC for 5 minutes followed by 45 cycles of 94ºC for 30s, 60ºC for 30s.
Conditions for the long amplification were 94ºC for 2 minutes followed by 45 cycles of
92ºC for 30s, 71ºC for 30s, and 68ºC for 5 minutes. The relative amplification of the
10kb product was calculated by the comparative Ct method\(^2\). DNA lesion frequencies
were calculated using Poisson transformation\(^3\).

QPCR for the common 4977 bp mtDNA deletion was performed as previously
described\(^1\). For human lesions, primers used at 5 µM were as follows: Control
product: FWD: ACATACCCATGGCCAACCT, REV: GGGCCTTTGCGTAGTTGTAT,
producing a 113 bp product. Mutant product primers: FWD:
CTATTGATCCCCACCTCCAA, REV: GCGAGGGCTGTGAGTTTTAG, producing a
332 bp product. Reaction conditions were as described for the short adducts
reaction.

**Cellular ATP content and ROS assays**

Cellular ATP content was determined with a luciferin-luciferase-based
bioluminescence assay (ATP lite: Luminescence ATP detection system, Perkin
Elmer, USA) as per the manufacturer’s instructions. Luminescence was measured on
a Synergy HT Plate Reader (Biotek Instruments, Vermont, USA) with standard
curves generated from known concentrations of ATP standards. Determination of
intracellular ROS with 2′,7′-dichlorodihydrofluorescein diacetate was performed as previously described\textsuperscript{16}.

**Aortic respiration**

To assess mitochondrial respiration, descending aortas were dissected and maintained in preservation solution (BIOPS: 10 mM Ca-EGTA buffer, 0.1 µM free calcium, 20 mM imidazole, 20 mM taurine, 50 mM MES, 0.5 mM dithiothreitol, 6.56 mM MgCl\textsubscript{2}, 5.77 mM ATP, 15 mM phosphocreatine, pH 7.1) at 4°C. Samples were then permeabilized in 50 µg/ml saponin solution before washing three times in respiration buffer (0.5 mM EGTA, 3 mM MgCl\textsubscript{2}·6H\textsubscript{2}O, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH\textsubscript{2}PO\textsubscript{4}, 20 mM HEPES, 110 mM mannitol, 0.3 mM dithiothreitol, 1 mg/ml fatty acid free BSA, pH 7.1). Respirometry was performed at 37°C with a Clarke-type oxygen electrode (Strathkelvin Instruments Ltd, UK).

Complex I-supported respiration rates were acquired using 10 mM glutamate and 5 mM malate. State 3 respiration was stimulated by the addition of 5 mM ADP. After the addition of 1 µM rotenone, complex II-supported respiration was assessed with 10 mM succinate. 0.5 mM \textit{N,N,N′,N′-Tetramethyl-p-phenylenediamine} (TMPD) and 2 mM ascorbate were then used to induce complex IV respiration, after inhibition of complex III with 5 µM antimycin. Outer mitochondrial membrane intactness was assessed by the addition of 10 µM cytochrome C. Tissues were removed from the electrode chambers and dried, with oxygen flux expressed as nanomoles O\textsubscript{2} per minute per dry weight.

**Serum 8-oxo-G assays**
Serum samples were filtered through a 0.45 µm filter and assessed for 8-oxo-G using a competitive ELISA assay (OxiSelect™ Oxidative DNA Damage ELISA #STA 320, Cell Biolabs Inc, California, USA) as per the manufacturer’s instructions. Absorbance was measured on a Synergy HT Plate Reader (Biotek Instruments, Vermont, USA) with standard curves generated from known concentrations of 8-oxo-G standards.
<table>
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<tr>
<th>Clinical Parameters</th>
<th>Association with mtDNA 4977 deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean (sd) 62.3 (10.4)</td>
</tr>
<tr>
<td>Male</td>
<td>Median freq (Q1-Q3) 122 (76.7%)</td>
</tr>
<tr>
<td>ACS presentation</td>
<td>Mean (sd) 66 (41.5%)</td>
</tr>
<tr>
<td>Statin treatment for &gt;3 months</td>
<td>Mean (sd) 65 (40.9%)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>Mean (sd) 4.55 (1.31)</td>
</tr>
<tr>
<td>Smoked in the past 3 months</td>
<td>Mean (sd) 42 (26.4%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Mean (sd) 83 (52.2%)</td>
</tr>
<tr>
<td>Previous MI</td>
<td>Mean (sd) 17 (10.7%)</td>
</tr>
<tr>
<td>Family history of MI</td>
<td>Mean (sd) 70 (44.0%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Mean (sd) 22 (13.8%)</td>
</tr>
<tr>
<td>8-oxo-G</td>
<td>Mean (sd) 2.59 [1.68-5.23]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IVUS Parameters</th>
<th>Association mtDNA 4977 deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-vessel plaque vol (mm³)</td>
<td>Mean (sd) 1102 [803-1405]</td>
</tr>
<tr>
<td>Culprit vessel plaque vol (mm³)</td>
<td>Mean (sd) 450 [208-622]</td>
</tr>
<tr>
<td>3-vessel VHPIT</td>
<td>Mean (sd) 0 [0-1]</td>
</tr>
<tr>
<td>3-vessel VHFCa</td>
<td>Mean (sd) 0 [0-1]</td>
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<tr>
<td>3-vessel VHThCFA</td>
<td>Mean (sd) 1 [0-2]</td>
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<tr>
<td>Culprit vessel VHTCFA</td>
<td>Mean (sd) 4 [2-6]</td>
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</tbody>
</table>

**Supplemental Table 1.** Association between clinical and virtual histology intravascular ultrasound factors and mtDNA 4977 deletion

sd (standard deviation), Q1-Q3 (first and third quartiles), Vol (volume), MI (myocardial infarction), LDL (low density lipoprotein), HDL (high density lipoprotein), VH-IVUS (virtual histology intravascular ultrasound), VHPIT (VH-IVUS identified pathological intimal thickening), VHFCa (VH-IVUS identified fibro-calcific plaque), VHThCFA VH-IVUS identified thick-capped fibroatheroma), VHTHCFA VH-IVUS identified thin-capped fibroatheroma), mtDNA (mitochondrial DNA).
Supplemental Figures

Supplemental Figure 1
Representative images of aortic root sections, stained with haematoxylin and eosin. Scale bars represent 500 µm.

Supplemental Figure 2
Serum cholesterol and triglycerides in ApoE\(^{-/-}\) mice fed either chow or a high fat diet from 6w of age for 0, 7 or 14w.
Supplemental Figure 3
Quantitative fluorescent Western blot of aortic mitochondrial respiratory complex (CI-CIV) proteins in ApoE-/- mice fed chow or high fat diet for 0, 7 or 14w. The nuclear encoded manganese superoxide dismutase (MnSOD) was used as the control.

Supplemental Figure 4
Quantitative fluorescent Western blot of aortic mitochondrial respiratory complex (CI-CIV) proteins, and citrate synthase (CS) in control or polG+/- mice. Tubulin (tub) was used as control.

Supplemental Figure 5
DCFDA fluorescence in VSMCs derived from polG+/+/ApoE-/- (control)(n=3) or polG+/-/ApoE-/- mice (n=4).
Supplementary Figure 6. Lipid levels in bone marrow transplanted mice
Serum lipids in ApoE⁻/⁻ mice transplanted with polG⁻/⁻/ApoE⁻/⁻ or control ApoE⁻/⁻ marrow, after fat feeding from 6-20w. n=12. Chol=serum cholesterol, Trig=serum triglyceride

Supplemental Figure 7
Mean systolic blood pressure in polG⁺/+ ApoE⁺⁻ (control) or polG⁻⁻/ApoE⁻⁻ mice undergoing fat feeding from 6-20w. n=12.
Supplemental Figure 8
(A) Blood counts in polG+/ApoE−/− and control ApoE−/− mice after fat feeding from 6-20w. (B) Blood counts in ApoE−/− mice transplanted with polG+/ApoE−/− or control polG+/+/~/ApoE−/− marrow and fat feeding from 6-20w. (n=12).
Supplemental Figure 9

(A-B) mtDNA adducts at baseline (0 months, pre-PCI) and 3m later in patients presenting with stable angina (A) or ACS (B).
References

