The study of Mercer et al,1 published in this issue of Circulation Research, reports new evidence linking oxidative DNA damage, atherosclerosis, and the metabolic syndrome. Although these relationships have been long proposed,2–4 many have criticized previous reports asking the rhetorical question: Which came first, the chicken or the egg? Another question, more specific to the topic of the study by Mercer et al, is: Does oxidative DNA damage actively promote atherosclerosis (and/or metabolic syndrome), or is DNA damage a result of these abnormalities?

Conceptually, the theory is attractive. DNA damage occurs often. Every time you walk outside from your office or laboratory to another building, your skin is bombarded by UV irradiation. Were it not for the presence of robust and often redundant DNA repair systems, multiple layers of cells in your skin would be damaged. In some cases, the cells apoptosis. The causation between induced DNA damage and cellular apoptosis has been established for many different types of cells.5 In other cases, genomic DNA might be altered in such a way as to promote malignant transformation, or mitochondrial DNA (mtDNA) could be damaged such that the cellular burden of reactive oxygen species (ROS) results in further oxidative DNA damage to nuclear DNA (nDNA).6,7

Why does the same paradigm not fit for oxidative DNA damage as a cause of atherosclerosis? First and foremost, the vasculature is not the skin, and the connection between ROS and oxidative DNA damage (much less the connection to atherosclerosis and metabolic syndrome) is less straightforward to study than the effect of UV irradiation on keratinocytes and melanocytes. Directly measuring the impact of ROS in the vasculature, or on the function of organs responsible for the cluster of metabolic abnormalities commonly referred to as the metabolic syndrome (liver, pancreas, and adipose tissue), is not possible in the same way in which it is for the skin.

The notion that oxidative DNA damage contributes to atherosclerosis and its complications is far from new. In 1992, Wallace was among the first to suggest that mtDNA mutations and/or damage correlate with human disease.8 Since that time, one consistent theme from the many laboratories studying oxidative DNA damage and atherosclerosis has been a focus on mtDNA damage. MtDNA lacks histone protection, and mechanisms for repair of mtDNA damage are far less comprehensive than those that exist for nDNA damage.9 A teleologic argument for this difference is that most cells have multiple mitochondria, and damage to a small percentage of mitochondria is unlikely to adversely affect the cell in any major way. This is probably true, because major phenotypes emerge only when mitochondrial function is dramatically altered. Although a link between mtDNA damage and atherosclerosis has been established since the 1990s,3,8,10 the causality was not proven.

Enter Mercer et al,1 who chose to use the ataxia telangiectasia mutated (ATM) protein defect as a model for studying whether oxidative mtDNA damage causes atherosclerosis and the metabolic syndrome. The rationale for these experiments was based on 2 different types of findings. First, some patients with ataxia telangiectasia have insulin resistance and presumably the metabolic syndrome,11 and various studies implicate ATM function in atherosclerosis.12 Secondly, ATM is a serine/threonine kinase that plays a role in DNA repair, mtDNA content, mitochondrial biogenesis, and glucose homeostasis.13,14

For the present study, Mercer et al1 used mice that were apolipoprotein (Apo)E null and either ATM haplodeficient or normal in ATM function. In the ApoE−/− background, ATM haploinsufficiency was associated with hyperlipidemia, hypertension, weight gain, increased numbers of adipocytes, and inflammatory changes in the liver, as well as other features consistent with the metabolic syndrome. These mice also displayed mtDNA damage and mitochondrial dysfunction in multiple organs.

An initial impression is that this study hardly overcomes the burden of proof of causality between oxidative DNA damage and the metabolic syndrome and atherosclerosis. Indeed, a significant limitation of the present study is that the authors did not address mtDNA damage and mitochondrial dysfunction in the aortas from ApoE−/−/ATM−/− mice. These studies could have provided additional insight into the relative roles of nDNA and mtDNA damage in vascular dysfunction.

However, in the breadth of their studies, the authors do, in our opinion, provide a level of evidence consistent with mtDNA damage causing the metabolic syndrome and atherosclerosis, well surpassing that of prior studies. The authors were able to define a complex phenotype consisting of...
histological change in the aorta, advanced atherosclerosis, metabolic changes with abnormal function of liver and pancreas, and mtDNA damage. Furthermore, they show that accelerated atherosclerosis in ApoE<sup>−/−</sup>/ATM<sup>−/−</sup> mice compared with ApoE<sup>−/−</sup> mice was partially reversed in bone marrow transplant experiments, indicating that ATM deficiency enhances atherosclerosis by stimulating stress-activated signaling pathways in macrophages (Figure).<sup>12</sup> Incidentally, activation of these signaling pathways and impairment of phosphoinositide 3-kinase/Akt pathway have been implicated in the development of insulin resistance in ApoE<sup>−/−</sup>/ATM<sup>−/−</sup> mice. <sup>12</sup>

Plaque macrophages in ApoE<sup>−/−</sup>/ATM<sup>−/−</sup> mice demonstrated increased apoptosis, consistent with the findings of mtDNA damage in these cells. Macrophage apoptosis has been demonstrated to lead to necrotic core formation in the plaque and ultimately plaque instability. It should be noted, however, that although the ApoE<sup>−/−</sup>/ATM<sup>−/−</sup> genotype had accelerated atherosclerosis, there was also an increase in proliferating cells and reduction in apoptotic cells in the plaque area. The impact of the bone marrow transplant experiments on this phenotype was unclear.

The authors also extensively characterized metabolic and metabolomic changes related to ATM haploinsufficiency in these mice. Their studies on tissues and cells isolated from these mice included demonstration of the impact of ATM haploinsufficiency on relevant signaling pathways involved in DNA repair. Additionally, the increased mtDNA damage observed in the insulin-sensitive tissues such as liver, skeletal muscle, and pancreas of ApoE<sup>−/−</sup>/ATM<sup>−/−</sup> mice relative to ApoE<sup>−/−</sup>/ATM<sup>−/+</sup> mice may have contributed to the metabolic syndrome and atherosclerosis (Figure). Impaired glucose tolerance in ApoE<sup>−/−</sup>/ATM<sup>−/−</sup> mice, relative to ApoE<sup>−/−</sup>/ATM<sup>−/+</sup> mice (with no difference in serum insulin levels and insulin-stimulated glucose clearance), could indicate impaired liver and/or pancreatic function resulting from mitochondrial deletions or attenuation of signaling pathways involved in membrane translocation of glucose transporter 4 involved in glucose uptake (Figure).<sup>15</sup> Furthermore, decreased mitochondrial complex I activity in the liver of ApoE<sup>−/−</sup>/ATM<sup>−/−</sup> mice suggests mitochondrial dysfunction and a feed-forward increase in mitochondrial ROS. The authors propose, with reasonable evidence, that these events lead to impaired lipid metabolism (β-hydroxybutyrate and lipid accumulation) and reduced glycolysis and eventually to development of insulin intolerance and signs of the metabolic syndrome.

**Non-standard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>ApoE</td>
<td>apolipoprotein E</td>
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<tr>
<td>ATM</td>
<td>ataxia telangiectasia mutated</td>
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<tr>
<td>mtDNA</td>
<td>mitochondrial DNA</td>
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<tr>
<td>nDNA</td>
<td>nuclear DNA</td>
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<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
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![Figure](http://circres.ahajournals.org/) Schematic diagram depicting molecular pathways that regulate atherosclerosis and the metabolic syndrome in ATM haploinsufficient ApoE<sup>−/−</sup> mice. Thick up and down arrows indicate increase and decrease, respectively, whereas ↓ indicates inhibition. VSMC indicates vascular smooth muscle cell.
Despite these very convincing experiments, it is important to consider a number of important questions.

1. Are the phenotypic changes described in the ATM+/−/ApoE−/− mice attributable solely to mtDNA damage (and hence mitochondrial dysfunction) or are these changes due at least in part to genomic DNA damage causing yet to be delineated molecular changes?

2. There is also increasing evidence that ATM plays a role in signaling pathways other than those involving direct DNA damage. Are there hormones or cytokines present in the oxidative milieu that may activate or inhibit ATM?

3. Because both genomic and mtDNA damage are present in ATM+/− mice, is genomic DNA damage inducing mtDNA damage and/or dysfunction and is this important in the metabolic abnormalities described by Mercer et al?

4. Finally, although H2O2 production is important in this model, as evidenced by the measurement of 2′,7′-dichlorohydro fluorescein diacetate (H2DCFDA), what role does superoxide play in cellular dysfunction.

For all these reasons, one must accept that even with the strength of the findings of Mercer et al, many questions remain regarding the causative role of mtDNA damage in atherosclerosis and metabolic syndrome. This is, however, the hallmark of a well done study. It generates a host of additional questions that can only be answered by further experimentation. The study of Mercer et al fits our criteria for an important, innovative and well done study and we look forward to more information on this important topic in the future.

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**Disclosures**

None.

**References**


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