Oxidative Enzymopathies and Vascular Disease

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Abstract—In the vasculature, reactive oxygen species (ROS) generated by both mitochondrial respiration and enzymatic sources serve as integral components of cellular signaling and homeostatic mechanisms. Because ROS are highly reactive biomolecules, the cellular redox milieu is carefully maintained by small-molecule antioxidants and antioxidant enzymes to prevent the deleterious consequences of ROS excess. When this redox balance is perturbed, because of either increased ROS production or decreased antioxidant capacity, oxidant stress is increased in the vessel wall and, if not offset, vascular dysfunction ensues. A number of heritable polymorphisms of pro-oxidant enzymes, including 5-lipoxygenase, cyclooxygenase-2, nitric oxide synthase-3, and NAD(P)H oxidase, have been identified and found to modulate ROS production and, thereby, the risk of atherothrombotic cardiovascular disease in individuals with these genetic polymorphisms. Similarly, heritable deficiency of the antioxidant enzymes catalase, glutathione peroxidases, glutathione-S-transferases, heme oxygenase, and glucose-6-phosphate dehydrogenase favors ROS accumulation, and has been associated with an increased risk of vascular disease. Individually, each of these polymorphisms imposes a state of uncompensated oxidant stress on the vasculature and collectively comprise the oxidative enzymopathies. 

Vascular Sources of ROS

Under basal conditions, superoxide anion, a 1-electron reduction product of oxygen, is the primary source from which
most other reactive oxidants are derived in the vasculature and is generated by both metabolic and enzymatic sources, as summarized in Figure 1. These sources include mitochondrial respiration,2 xanthine oxidase,3 cyclooxygenases and lipoxygenases,4 NAD(P)H oxidases,5 and, when substrate or cofactors are not replete, uncoupled nitric oxide synthase(s).6,7 Although these sources are primarily responsible for ambient ROS production and basal cellular homeostatic function, when vascular disease states ensue, redox balance in the vessel wall is compromised because of increased ROS production by these sources. When this is coupled with decreased antioxidant defense, as may occur in the setting of an oxidative enzymopathy, the net result is an accumulation of superoxide anions in the vessel wall, where they are free to react and form a number of pathophysiologically relevant reactive species. These species include hydrogen and lipid peroxides, peroxynitrates and peroxynitrous acids, and hypochlorite and hypochlorous acid (Figure 2), which, in turn, may have deleterious consequences for vascular function.

Vascular Antioxidant Enzymes

To balance basal ROS production, the cellular redox milieu is maintained by a number of key antioxidant enzymes that limit intracellular and extracellular accumulation of deleterious reactive oxygen metabolites. These antioxidant defense enzymes include the superoxide dismutases, which convert superoxide anion into hydrogen peroxide; catalase, which reduces hydrogen peroxide to water; the glutathione peroxidases, which reduce hydrogen peroxide and lipid peroxides to water and lipid alcohols, respectively; glutathione reductase, which reduces glutathione disulfide to reduced glutathione; the glutathione-S-transferases, which detoxify oxidants by glutathiolation; the thiol-disulfide oxidoreductases and peroxiredoxins, which maintain protein thiol redox state; heme
globin, which converts hypoxanthine and xanthine to uric acid, is an additional source of ROS. As xanthine is converted to uric acid, 2 electrons are donated to molybdenum (Mo) at the active site of the enzyme, thereby reducing it from Mo(VI) to Mo(IV). Finally, endothelial nitric oxide synthase (eNOS), when substrate or cofactors are not replete, uncouples to generate superoxide in preference to NO. Q indicates coenzyme Q; C, cytochrome C; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; FE, heme iron; BH4, tetrahydrobiopterin.

Figure 1. Metabolic and enzymatic sources of superoxide in the vasculature. Superoxide anion (O2-) is formed by several metabolic and enzymatic sources within the cell. NADPH oxidase is comprised of multiple membrane-bound and cytoplasmic subunits. The enzyme is activated when the cytoplasmic subunits p67 and p47 and the small G-protein rac assemble with the membrane-bound NOX (vascular homolog of gp91(phox)) and p22(phox). NADPH oxidase uses NADPH as a substrate, and, in vascular cells, is considered an important source of reactive oxygen species (ROS) generation. The lipoxygenases and cyclooxygenases (COX) generate ROS indirectly by promoting formation of inflammatory mediators. Arachidonic acid (AA) that is cleaved from the membrane by phospholipase A2 (PLA2) is then metabolized by 5-lipoxygenase (5-LO) in the presence of its accessory protein (FLAP) to form leukotrienes (LTs). AA is also metabolized by the cyclooxygenases to form members of another family of inflammatory mediators, the prostaglandins (PGs). Mitochondria also generate superoxide as electrons are transferred from complex I to complex IV during normal cellular respiration. Xanthine oxidase (XO), which converts hypoxanthine and xanthine to uric acid, is an additional source of ROS. As xanthine is converted to uric acid, 2 electrons are donated to molybdenum (Mo) at the active site of the enzyme, thereby reducing it from Mo(VI) to Mo(IV). Finally, endothelial nitric oxide synthase (eNOS), when substrate or cofactors are not replete, uncouples to generate superoxide in preference to NO. Q indicates coenzyme Q; C, cytochrome C; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; FE, heme iron; BH4, tetrahydrobiopterin.

Figure 2. Reactive species generated by superoxide in the vessel wall. Once formed, superoxide may react with a number of other species to generate pathophysiologically relevant reactive oxygen and nitrogen species. Superoxide reacts with nitric oxide to yield peroxynitrite (ONOO-), which, in the presence of hydrogen ions, may form peroxynitrous acid (ONOOH). Superoxide may also cause lipid peroxidation (LOOH) or, via the action of superoxide dismutases (SOD), form hydrogen peroxide. Neutrophil myeloperoxidase (MPO), in turn, catalyzes the reaction of hydrogen peroxide and chloride (Cl-) to form hypochlorous acid (HOCl).
oxygenase, which degrades heme to biliverdin and carbon monoxide (and also releases free iron ions); and glucose-6-phosphate dehydrogenase, which provides NADPH to serve as a reducing equivalent and cofactor for other antioxidant enzymes (Table 1).

Once formed, superoxide is dismutated to hydrogen peroxide by the activity of the superoxide dismutase (SOD) family of antioxidant enzymes. Mammalian organisms possess 3 types of SOD: Cu,Zn-SOD, Mn-SOD, and extracellular (EC)-SOD. Cu,Zn-SOD is located primarily in the cytosolic compartment of all cells, although some activity has been detected in lysosomes, peroxisomes, the nucleus, and the intermembrane compartment of the mitochondrion. Mitochondria also contain Mn-SOD, which accounts for approximately one-tenth of total cellular SOD activity, and, in contrast to Cu,Zn-SOD, is pH-sensitive, manifesting decreasing activity with increasing pH. EC-SOD, a copper-zinc-containing SOD that is localized to the extracellular environment, is synthesized by vascular smooth muscle cells, binds to extracellular matrix and heparan sulfate glycosaminoglycans on the endothelial surface, and serves to minimize the superoxide-dependent inactivation of endothelial-derived nitric oxide.

Hydrogen peroxide that results from the action of SODs (or the action of oxidases, such as xanthine oxidase) is reduced to water by catalase and the glutathione peroxidases (GPxs). Catalase exists as a tetramer comprised of 4 identical monomers that each contain a heme group at the active site. Degradation of hydrogen peroxide is accomplished via a conversion between 2 conformations of catalase: ferricatalase (iron coordinated to water) and compound I (iron complexed with an oxygen atom). Catalase also binds NADPH as a reducing equivalent to prevent oxidative inactivation of the enzyme (formation of compound II) by hydrogen peroxide as it is reduced to water.9,10

The GPxs, a family of tetrameric enzymes that contain the unique amino acid selenocysteine within their active sites, use low-molecular-weight thiols such as glutathione (GSH) to reduce hydrogen and lipid peroxides to their corresponding alcohols. At present, 4 isoforms of GPx have been identified and reasonably well-characterized. GPx-1 (cellular GPx) is ubiquitous and reduces hydrogen peroxide as well as fatty acid peroxides, but not esterified peroxyl lipids. Esterified lipids are reduced by membrane-bound GPx-4 (phospholipid hydroperoxide GPx), which can use several different low-molecular-weight thiols as reducing equivalents.9 GPx-2 (gastrointestinal GPx) is localized to gastrointestinal epithelial cells, where it serves to reduce dietary peroxides. GPx-3 (extracellular GPx) is the only member of the GPx family that resides in the extracellular compartment and is believed to be the most important extracellular antioxidant enzyme in mammals. Because of the low concentration of GSH in extracellular fluid, however, the precise nature of the GPx-3 reducing cosubstrate is unknown; glutaredoxin and thioredoxin have been proposed as candidate reductants.11,12

Thioredoxin, via a series of coupled reactions catalyzed by thioredoxin reductase and NADPH, also functions as an antioxidant molecule in the cytosol of vascular endothelial cells and smooth muscle cells of medial arteries. The active site of thioredoxin forms a disulfide bond with cysteine groups within proteins. These oxidized cysteine groups, in turn, are reduced by thioredoxin reductase in a reaction that uses NADPH as a reducing equivalent. Peroxiredoxin, a thioredoxin peroxidase, confers antioxidant properties on the system by scavenging hydrogen peroxide and requires sulfhydryl groups as a cosubstrate. A second thioredoxin system has been identified within mitochondria with sequence homology for the catalytic site of cytosolic thioredoxin; however, little is known about its function.13

Common to these antioxidants is the requirement of NADPH as a reducing equivalent. NADPH maintains catalase in the active form and is used as a cofactor by thioredoxin as well as glutathione reductase, which converts oxidized glutathione (GSSG) to GSH, a cosubstrate for the GPxs. Intracellular NADPH, in turn, is generated by the reduction of NADP⁺ by glucose-6-phosphate dehydrogenase (G6PD), the first and rate-limiting enzyme of the pentose phosphate pathway, during the conversion of glucose-6-phosphate to 6-phosphogluconolactone. By generating NADPH, G6PD is a critical determinant of cytosolic glutathione buffering capacity (GSH/GSSG) and, therefore, can be considered an essential, regulatory antioxidant enzyme. The role of G6PD as an antioxidant enzyme has long been recognized in erythrocytes, and we have previously demonstrated that G6PD is essential to maintain redox balance in vascular endothelial and smooth muscle cells.14-17 Table 1 lists these and other key antioxidant enzymes in the vasculature, their biochemical functions, and unique properties.

**Genetic Determinants of ROS Excess and Vascular Disease Risk**

The presence of both ROS-generating and antioxidant enzyme systems suggests that ROS excess, caused by increased production or decreased antioxidant capacity, results in a state of uncompensated oxidant stress that promotes vascular dysfunction. In fact, many heritable polymorphisms of enzyme sources of ROS and of antioxidant enzymes that alter redox balance have been identified and several appear to be associated with an increased risk of atherothrombotic cardiovascular disorders (Table 2).

To date, heritable polymorphisms of the pro-oxidant enzymes 5-lipoxygenase, cyclooxygenase-2, nitric oxide synthase-3, and NAD(P)H oxidase have been identified and shown to confer an increased risk of vascular disease on individuals who carry them. Similarly, heritable deficiencies of the antioxidant enzymes catalase, GPxs, glutathione-S-transferases, heme oxygenase, and G6PD that increase ROS accumulation have also been associated with vascular dysfunction and disease.

The functional consequences of these heritable polymorphisms and enzyme deficiencies have been studied in both animal models and human subjects. Although animal models provide a unique opportunity to examine the effects of altered gene expression on vascular function, results from these studies must be interpreted with caution as the biological microenvironment often represents an extreme form of enzymatic deficiency or dysfunction. Similarly, observations made in patients that link heritable polymorphisms and
enzyme deficiencies to vascular disease risk are subject to scrutiny as they often demonstrate only association and not causality. Furthermore, disease status may be modulated by both environmental factors and therapeutic interventions to obscure the full effects of antioxidant enzyme deficiency. Nevertheless, here we review the results of relevant and complementary studies performed in animal models and human subjects to demonstrate the role of genetic determinants of ROS excess and vascular disease risk.

**Pro-Oxidant Enzymes**

In murine models, Mehrabian et al. demonstrated that 5-lipoxygenase (5-LO), a key regulatory enzyme in the oxidative biosynthesis of pro-inflammatory leukotrienes, is a major determinant of susceptibility to atherosclerosis. In these studies, they found that resistance to atherosclerosis in mice resulted from decreased 5-LO mRNA and protein levels produced by two amino acid substitutions (Ile645Val and Val646Ile) in the 5-LO cDNA sequence. The functional importance of these amino acid substitutions was confirmed by kinetic studies of mutated human 5-LO cDNA; these mutations resulted in a decrease in $V_{max}$ to $< 20\%$ of control and a relative 5-LO activity in vitro of 11% to 19% of control. In vivo, absence of 5-LO in atherosclerotic prone low-density lipoprotein receptor-null mice resulted in a 26-fold reduction in aortic lesion formation compared with mice that expressed 5-LO.

In human subjects, histological examination of pathological specimens from patients with atherosclerotic vascular disease confirmed abundant 5-LO expression in plaques that localized to macrophages and inflammatory cells. These studies, in turn, led investigators to identify 5-LO polymorphisms associated with an increased risk of atherosclerosis. For example, Dwyer et al. showed that variant 5-LO genotypes—tandem promoter repeats of Sp-1 binding motifs—identified a subpopulation of individuals with increased carotid intima-media thickness, as a marker of atherosclerosis, and that diets rich in n-6 fatty acids interact positively with these genetic variants to promote leukotriene-mediated inflammatory responses. These investigators also found that

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**TABLE 1. Key Vascular Antioxidant Enzymes**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SODs</td>
<td>Dismutation of $\cdot O_2^-$ into $H_2O_2$ and $O_2$</td>
<td>Cytosolic, nuclear, and lysosomal localization</td>
</tr>
<tr>
<td>Cu,Zn-SOD</td>
<td>Dismutation of $\cdot O_2^-$ into $H_2O_2$ and $O_2$</td>
<td>Mitochondrial localization</td>
</tr>
<tr>
<td>Mn-SOD</td>
<td>Dismutation of $\cdot O_2^-$ into $H_2O_2$ and $O_2$</td>
<td>Synthesized by VSMC; bound to matrix and EC proteoglycans</td>
</tr>
<tr>
<td>EC-SOD</td>
<td>Dismutation of $\cdot O_2^-$ into $H_2O_2$ and $O_2$</td>
<td>Principal peroxisome localization</td>
</tr>
<tr>
<td>Catalase$^a$</td>
<td>Reduction of $H_2O_2$ into $H_2O$ and $O_2$</td>
<td>Principal peroxisome localization</td>
</tr>
<tr>
<td>GPxs$^{11, 12}$</td>
<td>Reduction of $H_2O_2$ to $H_2O$ and LOOH to LOH</td>
<td>Ubiquitous GSH is obligatory co-substrate</td>
</tr>
<tr>
<td>GPx-1</td>
<td>Reduction of $H_2O_2$ to $H_2O$ and LOOH to LOH</td>
<td>Essential extracellular antioxidant enzyme; glutaredoxin and thioredoxin can serve as co-substrates</td>
</tr>
<tr>
<td>GPx-4</td>
<td>Reduction of $H_2O_2$ to $H_2O$ and PLOOH to PLOH and Chol-DOH to Chol-DOH</td>
<td>Utilizes several different low-molecular-weight thiols as reducing co-substrates</td>
</tr>
<tr>
<td>GSSG reductase$^{68}$</td>
<td>Reduction of GSSG to GSH</td>
<td>NADPH is essential co-substrate; co-localizes with peroxisomes</td>
</tr>
<tr>
<td>GSH-S-transferase$^{69}$</td>
<td>Metabolism of xenobiotics by GSH conjugation</td>
<td>Extensive enzyme family</td>
</tr>
<tr>
<td>Thiol-Disulfide Oxidoreductases$^{13, 68}$</td>
<td>Catalysis of protein disulfide bond formation</td>
<td>Endoplasmic reticulum and plasma membrane localization</td>
</tr>
<tr>
<td>Protein–disulfide isomerase</td>
<td>Reduction of protein disulfides to thiols; reduction of hydrogen and lipid peroxides; has peroxynitrite reductase activity</td>
<td>Cytosolic localization</td>
</tr>
<tr>
<td>Thioredoxin</td>
<td>Reduction of oxidized thioredoxin and PDI disulfides to (vicinal) diithiols</td>
<td>Sec-containing enzyme; NADPH as reducing co-substrate</td>
</tr>
<tr>
<td>Glutaredoxin</td>
<td>Catalysis of protein disulfide reduction and glutathiolation of protein cysteinyl groups</td>
<td>GSH required for reduction of active site disulfide to vicinal diithiol</td>
</tr>
<tr>
<td>Peroxiredoxin</td>
<td>Reduction of $H_2O_2$ to $H_2O$ and LOOH to LOH</td>
<td>Three classes of enzyme based on active-site cysteinyl residues; thiol reductant not yet identified, may involve thioredoxin system</td>
</tr>
<tr>
<td>Heme oxygenase$^{70}$</td>
<td>Metabolizes heme to biliverdin, CO, and free iron; the latter is subsequently bound by ferritin</td>
<td>Two isoforms, one of which contains Sec; uses thioredoxin for reducing equivalents</td>
</tr>
<tr>
<td>Methionine sulfoxide reductase$^{71}$</td>
<td>Reduces methionine sulfoxide to methionine</td>
<td>Ubiquitous; important for antioxidant defense in all cells</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase$^{14–16, 64}$</td>
<td>Exclusive source of cytosolic NADPH</td>
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levels of the inflammatory marker C-reactive protein were increased 2-fold in patients with the variant genotype. More recently, Helgadottir et al22 showed that a promoter haplotype comprised of 4 linked polymorphisms in the 5-LO activating peptide, an accessory protein that facilitates presentation of substrate arachidonate to 5-LO, conferred significantly less in individuals carrying the C allele than those with the wild type promoter.

Common promoter variants in the cyclooxygenase 2 (COX-2) gene have also been shown to influence the risk for cardiovascular disease in human subjects. This is not surprising because COX-2 generates prostanoids, which modulate macrophage matrix metalloproteinase-2 and matrix metalloproteinase-9, which have functional significance, either for gene expression or enzyme activity. Papapetri et al23 demonstrated a novel promoter polymorphism in the COX-2 gene (–765G→C) that localizes to the putative binding site for Sp1 and reduces promoter activity by 30% compared with the –765G allele. In patients undergoing elective coronary artery bypass grafting surgery, a greater increase in C-reactive protein was seen postoperatively in patients with the –765G allele. Cipollone et al24 studied the association between this polymorphism and first myocardial infarction or ischemic stroke in 864 patients and 864 hospitalized controls. These investigators found that the prevalence ratios for myocardial infarction or stroke were 0.48 (95% CI, 0.36 to 0.68) and 0.33 (95% CI, 0.24 to 0.55) for the heterozygous (–756 GC) and homozygous (–756 CC) polymorphisms, respectively. They also noted that expression of both COX-2 and matrix metalloproteinases in atherosclerotic plaques obtained by carotid endarterectomy was significantly less in individuals carrying the C allele than those carrying the G allele. Thus, this polymorphism appears to confer protection from ischemic atherosclerotic events compared with the wild type promoter.

The endothelial nitric oxide synthase gene is highly polymorphic, and some of these polymorphisms appear to have functional significance, either for gene expression or enzyme activity. Several human studies have investigated the potential association between these polymorphisms and the risk of atherothrombotic vascular disease, but offer conflicting results because of the low population prevalence of the individual polymorphisms and the small sample sizes examined. Recently, Casas et al26 performed a meta-analysis of 26 case-control studies involving >23 000 subjects focusing on the Glu298Asp, the –786T→G promoter polymorphism, and the intron-4 variable nucleotide tandem repeat (4, minor allele; 5, major allele) polymorphisms in the endothelial nitric oxide synthase gene. They found that both the Glu298Asp and intron-4 variable nucleotide tandem repeat polymorphisms conferred a significant, albeit moderate, increase in the risk of ischemic heart disease (odds ratio = 1.31, 95% CI, 1.13 to 1.51; and odds ratio = 1.34, 95% CI, 1.03 to 1.75, respectively). This increased risk may occur because the 298Asp isoform of the enzyme appears to have a shorter half-life than the 298Glu

### Table 2. Enzyme Genotypes and Vascular Disease

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<th>Disease Risk</th>
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<td>Cyclooxygenase</td>
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</tr>
<tr>
<td>NO synthase</td>
<td>Hypertension</td>
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<tr>
<td>Heme oxygenase-1</td>
<td>Flow-mediated dilation</td>
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<td>NAD(P)H oxidase</td>
<td>Carotid intima-media thickness</td>
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<td>Catalase</td>
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<td>Mn-SOD</td>
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<td>GPx</td>
<td>Cerebrovascular disease</td>
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isoform, and individuals with homozygosity for the intron-4 minor allele have been shown to have a small, but significant, decrease in levels of plasma nitrogen oxides, suggesting that both polymorphisms result in decreased levels of bioavailable nitric oxide.

Several polymorphisms in the gene coding for the p22phox subunit of NAD(P)H oxidase with functional consequences have been described in humans. The −930A→G promoter polymorphism, which has been associated with hypertension, increases p22phox expression, NADPH oxidase activity, and ROS formation. The C242T polymorphism, which results in a His72Tyr substitution at the putative heme binding site, appears to confer an increased risk of ischemic stroke (odds ratio = 1.81, 95% CI, 1.15 to 2.86) in a Japanese population and progression of coronary atherosclerosis in a Western population. Notwithstanding a statistically significant association between this coding region polymorphism and atherothrombotic disease risk, functional studies suggest that this amino acid substitution actually reduces the superoxide-generating activity of NADPH oxidase. Interestingly, when the effects of the C242T, A640G, and −930A→G polymorphisms on neutrophil ROS production were examined, superoxide production was decreased by the C242T polymorphism only; the A640G, and −930A→G polymorphisms had no effect on respiratory burst. Despite these findings, other studies have failed to relate this polymorphism to vascular disease; the prevalence was similar in patients with or without angiographic evidence of atherosclerosis and was not associated with impaired endothelial-dependent vasodilator responses.

With the recent cloning of unique vascular isoforms of this enzyme (NOXs) and identification of NOX isoforms in vascular endothelial and smooth muscle cells, analysis of these related genes for variants that may be associated with an increased risk atherothrombotic disease awaits study.

Antioxidant Enzymes

Inherited catalase deficiency is a rare autosomal recessive disorder that has been characterized uniquely in families of Hungarian, Swiss, and Japanese origin. The phenotypes of these individuals are generally normal with one reported exception: Japanese subjects with profound deficiency (acatalasemia) have oral ulcerations. A recent report identified a series of mutations in a Hungarian kindred with catalase deficiency (GA insertion in exon 2, G insertion in exon 2, and T→G substitution in intron 7) associated with adverse lipid profiles and a high incidence (12.7%) of familial diabetes mellitus. In addition, Hungarian hypocatalasemic patients were found to have higher plasma levels of homocysteine and lower levels of folate, suggesting that these patients are at greater risk for cardiovascular disease. Common functional polymorphisms in the catalase promoter have been identified in a Swedish population, although their relationship to vascular disease risk has not yet been determined. Interestingly, in an isolated Chinese population, a variant within the catalase promoter region has been associated with essential hypertension; however, this study did not measure catalase activity and, therefore, the significance of this finding is unknown.

No convincing relationship has been reported between Cu,Zn-SOD or EC-SOD deficiency and atherothrombotic disease risk, either in animal models or in human population studies. In fact, eliminating EC-SOD in atherosclerosis-prone apolipoprotein E-null mice does not appear to influence atheroma burden at all. In contrast, a potentially functional polymorphism in the signal sequence of Mn-SOD (Ala16→Val) has been identified in humans and appears to be a minor determinant of carotid atherosclerosis as demonstrated by increased carotid intima-media thickness.

Glutathione peroxidase-1 (GPx-1) is a ubiquitous antioxidant enzyme whose deficiency has been shown to promote endothelial dysfunction, heart failure, and abnormal structural changes in the vasculature and myocardium. Interestingly, hyperhomocystinemia appears to enhance vascular oxidant stress and atherothrombosis, in part by suppressing expression of the GPx-1 gene; overexpressing GPx-1 in an animal model of hyperhomocystinemia rescues this adverse phenotype. Recently, Blankenberg et al have shown a strong dose-dependence for erythrocyte GPx-1 activity as an independent determinant of risk for cardiovascular events: patients in the highest quartile of enzyme activity had a hazard ratio of 0.29 (95% CI, 0.15 to 0.58) compared with those in the lowest quartile. Importantly, no genotyping was performed in this trial. Another recent trial by Wintert et al analyzed the association between GPx-1 genotypes and the risk of angiographically significant coronary artery disease. These investigators focused on a polyalanine sequence polymorphism in exon 1 of the GPx-1 gene, which produces 3 alleles with 5, 6, or 7 alanine repeats. They studied 207 men with angiographic evidence of coronary artery disease compared with 146 controls and found that after adjusting for age, body mass index, and tobacco use, men with at least one 6-alanine allele had a significantly increased risk of coronary artery disease with an odds ratio of 2.07 (95% CI, 1.08 to 3.99). In a study of diabetic Japanese patients, investigators identified an additional polymorphism, Pro198Leu, which was associated with increased carotid intima-media thickness, prevalence of cardiovascular disease, and peripheral vascular disease. They also found that this polymorphism, when associated with the 6-alanine allele, resulted in a 40% decrease in GPx activity.

Deficiency of glutathione peroxidase-3 (GPx-3), an essential extracellular peroxidase, has been associated with decreased nitric oxide bioavailability and increased platelet-dependent thrombosis. Early work by our group linked a reduction in plasma GPx-3 activity with increased platelet activation and cerebrovascular arterial thrombosis. More recently, we identified a promoter haplotype consisting of 7 tightly linked polymorphisms that reduce promoter activity, an effect that was more pronounced under hypoxic conditions. This promoter haplotype was associated with an increased risk of arterial thrombotic stroke in the young with an odds ratio of 2.1 (95% CI, 1.1 to 3.9) and with an odds ratio of 4.6 (95% CI, 1.6 to 13.1) for the risk of cerebral venous thrombosis.

The glutathione-S-transferases (GST) are xenobiotic-metabolizing enzymes that are involved in the detoxification of reactive electrophiles, including those found in tobacco
smoke. Although the relationship between polymorphic genotypes of these enzymes and cancer risk has been appreciated for some time, only recently has their association with coronary artery disease been recognized. In particular, patients with the GSTM1*0 null allele were found to have the highest levels of the inflammatory markers, C-reactive protein, fibrinogen, von Willebrand factor, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, as well as greater levels of DNA damage present in atherosclerotic plaques.

A recent meta-analysis found that the GSTM1*0 null allele and the GSTT1*1 functional allele are associated with an increased risk of coronary heart disease in smokers (odds ratio = 2.30, 95% CI, 1.40 to 9.00; and odds ratio = 2.50, 95% CI, 1.30 to 4.80, respectively), and the GSTT1*1 functional allele is associated with an increased risk of peripheral arterial disease (odds ratio = 3.60, 95% CI, 1.40 to 9.00). Because of its ability to detoxify heme and generate vasoprotective levels of carbon monoxide, heme oxygenase has been studied as a potential atheroprotective gene. Recent data from a Japanese population suggest that the −413T→A promoter polymorphism in the heme oxygenase-1 gene is associated with an increased atherothrombotic risk. The A allele appears to enhance gene expression and protect against ischemic heart disease, whereas in contrast the T allele increases the risk of myocardial infarction (odds ratio = 1.42, 95% CI, 1.01 to 1.77) and angina pectoris (odds ratio = 1.86, 95% CI, 1.08 to 2.41). Recent studies have shown that short tandem repeats (<25 GT) in the promoter were associated with a reduced risk of restenosis after femoropopliteal angioplasty (relative risk = 0.43, 95% CI, 0.24 to 0.71), whereas long tandem repeats (>25 GT) conferred an increased risk of restenosis after coronary stenting (odds ratio = 3.74, 95% CI, 1.61 to 8.70) and adverse cardiac events during 6 months of follow-up (odds ratio = 3.26, 95% CI, 1.58 to 6.72). This promoter polymorphism has also been associated with hypertension in a population of Japanese women, but not men; women with the AA genotype had an increased frequency of hypertension (odds ratio = 1.59, 95% CI, 1.14 to 2.20) and use of antihypertensive agents compared with those with the AT or TT genotype.

Glucose-6-phosphate dehydrogenase (G6PD), the principal source of cytosolic reducing equivalents necessary for thiol redox balance in all cells, is a highly polymorphic gene with many functional variants. G6PD deficiency is the most common enzymopathy in humans, with >400 million cases worldwide. To date, 30% to 40% reductions in enzyme activity: Val68Met, Arg227Leu, and Leu323Pro. Because blacks have an increased prevalence of cardiovascular disease, these data suggest that G6PD deficiency may contribute to endothelial dysfunction, vascular injury, and myocardial dysfunction.

Conclusions

Maintenance of redox balance in the cardiovascular system is of paramount importance as uncompensated oxidant stress contributes to endothelial dysfunction and vascular disease. Risk factor-mediated injury, inflammation, and thrombosis all promote oxidant stress and, thus, this intermediate molecular phenotype appears to be an essential link among the determinants of atherothrombotic disease. Many genetically determined heritable differences in pro-oxidant and antioxidant enzyme activities have been identified, and some have been found to modulate atherothrombotic risk. How these genetic variants interact with one another and with environmental risk factors to modulate the resulting oxidative enzmopathies are the subjects of ongoing studies of the mechanisms of this complex disease process.

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