Oxidative Enzymopathies and Vascular Disease

Jane A. Leopold, Joseph Loscalzo

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.

Key Words: antioxidants ■ atherosclerosis ■ genetic polymorphism ■ reactive oxygen species

Cellular respiration in an oxygen-rich environment generates abundant derivatives of partially reduced oxygen, collectively termed reactive oxygen species (ROS). Under basal conditions, ROS serve as an integral component of cellular signaling pathways; however, when these highly reactive metabolic products are in excess, they impose an oxidant stress on the cellular environment, which, in turn, modifies biomolecules to modulate cell and organism phenotype.1 To protect against cellular oxidant stress and its adverse sequelae, adaptive enzymatic mechanisms have evolved to metabolize ROS into less reactive forms and minimize their potential to produce oxidative damage and cellular dysfunction.

The importance of maintaining a balance between ROS production and metabolism is highlighted when there is deficiency or dysfunction of vascular antioxidant enzymes. This results in a net accumulation of ROS because of decreased antioxidant capacity in the setting of ambient ROS production by vascular sources. Recent interest has focused on heritable polymorphisms of vascular antioxidant enzymes as one mechanism by which antioxidant capacity is diminished. These polymorphisms have been associated with an increased risk of vascular disease in both animal models and human studies and collectively may be recognized as 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, xanthine oxidase, cyclooxygenases and lipoxygenases, NAD(P)H oxidases, and, when substrate or cofactors are not replete, uncoupled nitric oxide synthase(s). 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, peroxynitrites 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.
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 (GPx). 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; thioredoxin 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.\textsuperscript{18} 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\textsubscript{max} to \textless 20\% of control and a relative 5-LO activity in vitro of 11\% to 19\% of control.\textsuperscript{19} 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.\textsuperscript{20} These studies, in turn, led investigators to identify 5-LO polymorphisms associated with an increased risk of atherosclerosis. For example, Dwyer et al.\textsuperscript{21} 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 \textit{n}-6 fatty acids interact positively with these genetic variants to promote leukotriene-mediated inflammatory responses. These investigators also found that

### TABLE 1. Key Vascular Antioxidant Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SODs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu,Zn-SOD</td>
<td>Dismutation of $\cdot$O\textsubscript{2} into H\textsubscript{2}O\textsubscript{2} and O\textsubscript{2}</td>
<td>Cytosolic, nuclear, and lysosomal localization</td>
</tr>
<tr>
<td>Mn-SOD</td>
<td>Dismutation of $\cdot$O\textsubscript{2} into H\textsubscript{2}O\textsubscript{2} and O\textsubscript{2}</td>
<td>Mitochondrial localization</td>
</tr>
<tr>
<td>EC-SOD</td>
<td>Dismutation of $\cdot$O\textsubscript{2} into H\textsubscript{2}O\textsubscript{2} and O\textsubscript{2}</td>
<td>Synthesized by VSMC; bound to matrix and EC proteoglycans</td>
</tr>
<tr>
<td>Catalase\textsuperscript{9}</td>
<td>Reduction of H\textsubscript{2}O\textsubscript{2} into H\textsubscript{2}O and O\textsubscript{2}</td>
<td>Principal peroxisome localization</td>
</tr>
<tr>
<td><strong>GPx</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPx-1</td>
<td>Reduction of H\textsubscript{2}O\textsubscript{2} to H\textsubscript{2}O and LOOH to LOH</td>
<td>Ubiquitous GSH is obligate co-substrate</td>
</tr>
<tr>
<td>GPx-3</td>
<td>Reduction of H\textsubscript{2}O\textsubscript{2} to H\textsubscript{2}O 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\textsubscript{2}O\textsubscript{2} to H\textsubscript{2}O and PLOOH to PLOH and Chol-DOH to Chol-CH</td>
<td>Utilizes several different low-molecular-weight thiols as reducing co-substrates</td>
</tr>
<tr>
<td><strong>GSSG reductase</strong>\textsuperscript{68}</td>
<td>Reduction of GSSG to GSH</td>
<td>NADPH is essential co-substrate; co-localizes with peroxidases</td>
</tr>
<tr>
<td><strong>GSH-S-transferase</strong>\textsuperscript{20}</td>
<td>Metabolism of xenobiotics by GSH conjugation</td>
<td>Extensive enzyme family</td>
</tr>
<tr>
<td><strong>Thiol-Disulfide Oxidoreductases</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein–disulfide isomerase</td>
<td>Catalysis of protein disulfide bond formation</td>
<td>Endoplasmic reticulum and plasma membrane localization</td>
</tr>
<tr>
<td>Thioredoxin</td>
<td>Reduction of protein disulfides to thiols; reduction of hydrogen and lipid peroxides; has peroxynitrile reductase activity</td>
<td>Cytosolic localization</td>
</tr>
<tr>
<td>Thioredoxin reductase</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 cysteiny1 groups</td>
<td>GSH required for reduction of active site disulfide to vicinal dihol</td>
</tr>
<tr>
<td>Peroxiredoxin</td>
<td>Reduction of H\textsubscript{2}O\textsubscript{2} to H\textsubscript{2}O and LOOH to LOH</td>
<td>Three classes of enzyme based on active-site cysteiny1 residues; thiol reductant not yet identified, may involve thioredoxin system</td>
</tr>
<tr>
<td>Heme oxygenase\textsuperscript{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\textsuperscript{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\textsuperscript{14–16,64}</td>
<td>Exclusive source of cytosolic NADPH</td>
<td></td>
</tr>
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</table>
levels of the inflammatory marker C-reactive protein were increased 2-fold in patients with the variant genotype. More recently, Helgadottir et al.\(^22\) showed that a promoter haplotype comprised of 4 linked polymorphisms in the \(5\)-LO activating peptide\(^22\) gene (\(376G\)–\(\rightarrow\)C promoter polymorphism, \(-786G\)–\(\rightarrow\)T promoter polymorphism, 27-bp VNTR in intron 4 Glu298Asp polymorphism) conferred a 2-fold increased risk of myocardial infarction or 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 \((-756 G C\)) and homozygous \((-756 C C\)) 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.\(^25\) 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 al.\(^36\) performed a meta-analysis of 26 case-control studies involving >23,000 subjects focusing on the Glu298Asp, the \(-786T\)\(\rightarrow\)C, 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
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 $p22^{phox}$ subunit of NAD(P)H oxidase with functional consequences have been described in humans. The $-930A\rightarrow G$ promoter polymorphism, which has been associated with hypertension, increases $p22^{phox}$ 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, early work by our group linked 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 cerebral venous thrombosis, in part by suppressing expression of the GPx-1 gene; overexpressing GPx-1 in an animal model of hyperhomocysteinemia rescues this adverse phenotype. Recently, Blankenberg et al showed 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 Winter 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, >440 biochemical variants have been identified with >120 different mutations characterized. Most of these mutations are single or double missense mutations or, much less commonly, small deletions in the gene. Selective pressure exists for these mutations, all of which reduce activity, because erythrocytes with reduced G6PD activity are more susceptible to oxidative damage, which renders cells parasitized by plasmodium species more readily cleared by the reticuloendothelial system than cells with a normal complement of enzyme activity.

Until very recently, G6PD deficiency was believed to be an important pro-oxidant mechanism only in erythrocytes. Increasing evidence from several groups, however, supports the view that deficiency of this important antioxidant enzyme leads to increased oxidative cell injury in neurons, pancreatic beta cells, embryonic stem cells, and, importantly, vascular cells, including vascular smooth muscle cells and endothelial cells. Furthermore, G6PD deficiency appears to be related to a decreased potential for angiogenesis and worsening ventricular function with ischemia-reperfusion injury in animal models.

The findings of these in vitro and in vivo studies are supported by one recent study on the risk of hypertension and diabetes in G6PD-deficient individuals, and one study of endothelial dysfunction in young, otherwise healthy G6PD-deficient individuals. G6PD deficiency is very common in the black population, among whom it is found in 11.4% of males and 2.5% of females (compared with <0.1% of whites). The B variant corresponds to the wild-type enzyme, whereas the A variant (which has normal to mildly reduced activity) is found in 20% of blacks and corresponds to an Asn126Asp mutation. A variants comprise 3 additional mutations on an A background, each leading to >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 antioxidan
t 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 enzy
mopathies are the subjects of ongoing studies of the mecha
nisms of this complex disease process.

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References

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