Because of increasing prevalence rates in the past decade, diabetes mellitus (DM) has become a major public health issue. Macrovascular and microvascular complications are common long-term sequelae of the disease. Cardiovascular disease (CVD) is the single most major cause of death among patients with DM, accounting for approximately 65% of mortality in DM.1 End stage renal disease is also a major complication of DM with nearly a third of all individuals with DM eventually requiring treatment with dialysis. Overall, DM accounts for 35% of hospitalizations caused by CVD and is the leading cause of blindness in the Western world. Identification of those individuals with DM who are at greatest risk for the development of CVD would have considerable public health importance because it would allow for a more efficient allocation of resources to alleviate the burden of disease.2 Beginning more than 20 years ago, hundreds of polymorphisms in genes that are involved in the pathogenesis of CVD and DM have been examined for their ability to predict which individuals with DM will develop CVD. These polymorphisms may modify the activity of proteins, making them more or less active, or alter their expression and stability, thus modulating their ability to retain normal vascular physiology and metabolism. Fig. 1 provides a functional categorization of these polymorphic genes. Table 1 provides a comprehensive list of the single-nucleotide polymorphisms (SNPs) and polymorphisms that have been assessed to date in the search for loci that may predict CVD in DM. However, only a handful of these polymorphisms have been shown reproducibly to predict diabetic CVD across various populations and ethnic groups. These polymorphisms are reviewed in this article.

**PARAOXONASE**

*The Paraoxonase Family*

Located at locus 7q21.3, the cluster of the paraoxonase (PON) gene family is comprised of three members: *PON1*, *PON2*, and *PON3*. The three members are highly homogenous, presenting with 70% similarity at the nucleotide level and 60% similarity of the amino acid sequence.40 However, their expression patterns are varied. Although *PON1* and *PON3* are expressed mainly in the liver and associate with high density lipoprotein (HDL) in the circulation,47,42 *PON2* is expressed in a variety of tissues and is found on the endoplasmic reticulum and the nuclear membrane.43 Although only *PON1* exhibits the

This work was supported by grants from the United States-Israel Binational Science Foundation, Israel Science Foundation, Juvenile Diabetes Research Foundation, the Kennedy Leigh Charitable Trust, and grant R01KD085226 from the NIH to Andrew P. Levy.

Financial Disclosure Information: Dr Levy has served in the past as a consultant for Synvista Therapeutics.

a Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel

b Department of Cell Biology and Anatomy, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel

* Corresponding author.

E-mail address: dfarb@tx.technion.ac.il

Cardiol Clin 28 (2010) 477–496
doi:10.1016/j.ccl.2010.04.005
0733-8651/10/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
ability to hydrolyze organophosphates, the three enzymes share the ability to metabolize different lactones,\textsuperscript{44} some of which are the product of phospholipid peroxidation.\textsuperscript{45} It has been suggested that this enzymatic activity has a pivotal role in preserving the integrity of cell membranes, protecting it from a wide variety of endogenous and exogenous toxins. Supporting this hypothesis is the localization of PON1 to the same HDL subfraction as clusterin, which is also suspected to have a role in cell membrane protection.\textsuperscript{46} Recently, it has been suggested that the PON enzymes have a role in innate immunity, being able to hydrolyze the quorum sensing signal molecule \(N\)-acyl-homoserine-lactone.\textsuperscript{47}

**Antiatherogenic Properties of Paraoxonase**

HDL is known to attenuate the development of atherosclerosis by a variety of mechanisms, including removal of excess cholesterol from cells of the vessel wall (reverse cholesterol transport) and limiting low density lipoprotein (LDL) oxidation. These antiatherogenic activities are catalyzed by the many proteins associated with the HDL particle. PON1 and PON3, which are associated with HDL in the plasma, take part in the antioxidant activity of HDL. PON1 diminishes LDL oxidation\textsuperscript{48} and prevents the proinflammatory response elicited by oxidized LDL (OxLDL), the latter probably resulting from the metabolism of lipid peroxides.\textsuperscript{49} Moreover, PON1 is critical for preventing the oxidation of HDL, allowing it to maintain its function.\textsuperscript{50} Similar results were obtained in a study of PON1 knockout mice, where the ability of HDL isolated from these mice to limit lipid peroxidation and LDL-induced inflammation was decreased.\textsuperscript{51} PON1 knockout mice were also more susceptible to the development of atherosclerosis in dietary or genetic models.\textsuperscript{51,52} Demonstrating a therapeutic potential for PON1 elevation, mice overexpressing PON1 were more resistant to atherosclerosis compared with wild-type mice.\textsuperscript{53}

**Paraoxonase Genotype and Relation to Cardiovascular Disease**

Genetic polymorphisms have been discovered in all the three members of the paraoxonase gene family. In PON1, which has gained the most
attention concerning its relationship with CVD, several polymorphisms have been identified in coding regions, affecting the amino acid sequence, and in the promoter region. Of the polymorphisms of the promoter, the C(-107)T polymorphism has been most widely studied. Because of the differences in the affinity of the transcription factor Sp1, this polymorphism has a dramatic effect on gene transcription, the -107C allele having significantly increased transcriptional activity compared with the -107T allele. This variation is reflected in higher plasma PON1 concentrations and activity in carriers of the -107C allele. However, although significantly decreasing enzyme concentration and activity, this polymorphism has not consistently been shown to be associated with CVD. A recent trial has shown that although the promoter polymorphisms do not affect the risk for CVD, they do influence the extent of the disease as measured by the number of coronary vessels undergoing stenosis.

Of the polymorphisms in the coding region, the Glu192Arg polymorphism has gained the most interest. This polymorphism results in a decrease in serum paraoxonase activity and concentration, possibly caused by decreased affinity of the Arg192 polymorphism to the HDL, which leads to decreased protein stability and activity. Of the many polymorphisms of the PON enzymes, the Glu192Arg polymorphism alone was found to be associated with CVD in a large meta-analysis, although the external validity of this association was questioned because of the lack of a significant association between this polymorphism and CVD in large trials and because of possible publication bias. Following the many failures to associate specific polymorphisms of the PON1 gene with vascular disease, it has been suggested that the relationship between PON1 phenotype expressed by serum concentration and activity, rather than genotype, and CVD should be explored. Indeed, it has been found that PON1 activity is a predictor of CVD, regardless of PON1 genotype. A recent large trial in which PON1 activity and genotype were tested has shown that not only do PON1 phenotype and Glu192Arg genotype determine the risk for CVD but also that the PON1 genotype is a predictor of its activity and concentration.

**Paraoxonase and Diabetes Mellitus**

The relationship between PON1 and DM appears to be bidirectional with DM significantly decreasing PON1 concentration and activity, and in turn, PON1 genotype modulating the risk for type 2 DM. The importance of paraoxonase activity in the prevention of DM was also demonstrated in an in vivo model, where increased PON1 expression in mice has impeded the development of streptozotocin-induced DM. Similar to what is seen in non-DM individuals, PON1 concentration and activity were found to be negatively associated with CVD. In the settings of Type 2 DM, only a single study has focused on the C-107T polymorphism, indicating an increased risk for CVD in carriers of the TT allele compared with carriers of the CT or CC alleles. This genotype was also associated with decreased PON1 concentration and activity, and increased plasma OxLDL/apolipoprotein B (apo B) ratio among individuals with DM. The Glu192Arg polymorphism was extensively studied in the settings of DM. In these settings, HDL from 192Arg allele homozygotes was less efficient in the metabolism of oxidized phospholipids, although these results were contested by others showing either no difference in plasma oxidized LDL or decreased oxidation in the aforementioned genotype. In most epidemiologic studies, the 192Arg allele was significantly correlated with CVD, although several studies have presented contradicting results. Several studies have highlighted the importance of interaction between the PON1 Glu192Arg polymorphism and DM, arguing that this polymorphism most significantly increases the risk for CVD in the presence of DM.

**METHYLTETRAHYDROFOLATE REDUCTASE AND HOMOCYSTEINE METABOLISM**

**Metabolic Pathways of Homocysteine**

Homocysteine (Hcy) is positioned at the cross-roads of several metabolic pathways. Hcy is synthesized from methionine in a three-step reaction, which includes activation of methionine by ATP, loss of a methyl group, and enzymatic hydrolysis. Remethylation of Hcy to methionine is catalyzed by betaine-homocysteine methyltransferase in the liver or by methionine synthase (MS) in most bodily tissues, the latter depending on methylenetetrahydrofolate as a methyl donor and vitamin B12 as a cofactor. Synthesis of methylenetetrahydrofolate is catalyzed by the enzyme methylenetetrahydrofolate reductase (MTHFR) in the presence of vitamin B2. In a state of methionine excess, Hcy is irreversibly trans-sulfurated by cystathionine β-synthase (CBS) and vitamin B6 to cystathionine, which can be converted to cysteine. A third elimination pathway of Hcy is the pathologic synthesis of the Hcy-thiolactone. This reaction is performed by methionyl-tRNA synthetase (MetRS) when Hcy is mistakenly recognized as methionine.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Polymorphism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>16p3</td>
<td>—</td>
<td>—</td>
<td>Bowden et al (^3)</td>
</tr>
<tr>
<td>9p21</td>
<td>—</td>
<td>rs2383206</td>
<td>Doria et al (^4)</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
<td>Insertion/deletion</td>
<td>Grammer et al (^5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Burdon et al (^6)</td>
</tr>
<tr>
<td>ADIPQ/ACDC</td>
<td>Adiponectin</td>
<td>–11391G&gt;A</td>
<td>Gable et al (^7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–11377C&gt;G</td>
<td>Gable et al (^7), Prior et al (^8), Lacquemant et al (^9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–11365C&gt;G</td>
<td>Qi et al (^10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-4041 A&gt;C</td>
<td>Lacquemant et al (^9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-4034A&gt;C</td>
<td>Qi et al (^10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3964A&gt;G</td>
<td>Qi et al (^10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45T&gt;G</td>
<td>Gable et al (^7), Lacquemant et al (^9), Qi et al (^10), Vendramini et al (^11), Kim et al (^12), Bacci et al (^13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>276G&gt;T</td>
<td>Gable et al (^7), Lacquemant et al (^9), Qi et al (^10), Vendramini et al (^11), Kim et al (^12), Bacci et al (^13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A349G</td>
<td>Vendramini et al (^11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+2019 delA</td>
<td>Lacquemant et al (^9)</td>
</tr>
<tr>
<td>ADIPOR1</td>
<td>Adiponectin receptor 1</td>
<td>rs2232853</td>
<td>Soccio et al (^14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs12733285</td>
<td>Soccio et al (^14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1342387</td>
<td>Soccio et al (^14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs7539542</td>
<td>Soccio et al (^14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs10920531</td>
<td>Soccio et al (^14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs4950894</td>
<td>Soccio et al (^14)</td>
</tr>
<tr>
<td>AGT</td>
<td>Angiotensinogen</td>
<td>M235T</td>
<td>Burdon et al (^6), Lin et al (^15)</td>
</tr>
<tr>
<td>AGTR1</td>
<td>Angiotensin-II receptor 1</td>
<td>T573C</td>
<td>Lin et al (^15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A1166C</td>
<td>Lin et al (^15)</td>
</tr>
<tr>
<td>AGTR1</td>
<td>Angiotensin receptor 1</td>
<td>rs5186</td>
<td>Burdon et al (^6)</td>
</tr>
<tr>
<td>aP2</td>
<td>Fatty-acid binding protein</td>
<td>T-78C</td>
<td>Tuncman et al (^16)</td>
</tr>
<tr>
<td>APOA4</td>
<td>Apolipoprotein A-IV</td>
<td>Gln360His</td>
<td>Kretowski et al (^17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thr347Ser</td>
<td>Kretowski et al (^17)</td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
<td>ε2/ε3/ε4</td>
<td>Winkler et al (^18)</td>
</tr>
<tr>
<td>Gene</td>
<td>Protein</td>
<td>SNP(s)</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>CD40</td>
<td>CD-40</td>
<td>rs1535045, rs3765459</td>
<td>Burdon et al(^{19})</td>
</tr>
<tr>
<td>CD40L</td>
<td>CD-40 Ligand</td>
<td>rs3092948, rs3092929, rs3092923, rs3092920</td>
<td>Burdon et al(^{19})</td>
</tr>
<tr>
<td>CETP</td>
<td>Cholesteryl ester transfer protein</td>
<td>rs289714, I405V, R451Q</td>
<td>Burdon et al(^{20})</td>
</tr>
<tr>
<td>COX2</td>
<td>Cyclooxygenase-2</td>
<td>rs689466, rs20417, rs2745557, rs5277, rs20432, rs2066826, rs5275, rs10911902</td>
<td>Rudock et al(^{21})</td>
</tr>
<tr>
<td>CX3CR1</td>
<td>Fractalkine receptor</td>
<td>T280M</td>
<td>Boger et al(^{22})</td>
</tr>
<tr>
<td>ENPP1</td>
<td>Ectoenzyme nucleotide pyrophosphate phosphodiesterase 1</td>
<td>K121Q</td>
<td>Chen et al(^{23})</td>
</tr>
<tr>
<td>EPHX2</td>
<td>Epoxide hydrolase 2</td>
<td>rs7003694, rs7837347, R287Q, rs721619, rs747276</td>
<td>Burdon et al(^{24})</td>
</tr>
<tr>
<td>HL</td>
<td>Hepatic Lipase</td>
<td>C-480T</td>
<td>Burdon et al(^{20})</td>
</tr>
<tr>
<td>HSP70-2</td>
<td>Heat Shock Protein 70-2</td>
<td>A1267G (A/B)</td>
<td>Giacconi et al(^{25})</td>
</tr>
<tr>
<td>GCH1</td>
<td>GTP cyclohydrolase 1</td>
<td>C59038T</td>
<td>Liao et al(^{26})</td>
</tr>
<tr>
<td>GSTT1</td>
<td>Glutathione S transferase theta-1</td>
<td>1/0</td>
<td>Hayek et al(^{27}), Doney et al(^{28})</td>
</tr>
</tbody>
</table>

(continued on next page)
<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Polymorphism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1</td>
<td>Glutathione S transferase mu-1</td>
<td>1/0</td>
<td>Hayek et al\textsuperscript{27} Doney et al\textsuperscript{28}</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Glutathione S transferase pi-1</td>
<td>Ile105Val</td>
<td>Doney et al\textsuperscript{28}</td>
</tr>
<tr>
<td>IL6</td>
<td>Interleukin 6</td>
<td>-174G/C</td>
<td>Danielsson et al\textsuperscript{29}</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
<td>rs285 rs320</td>
<td>Burdon et al\textsuperscript{20} Burdon et al\textsuperscript{20}</td>
</tr>
<tr>
<td>MT1A</td>
<td>Metallothionein 1A</td>
<td>A647C A1245G</td>
<td>Giacconi et al\textsuperscript{30} Giacconi et al\textsuperscript{30}</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemoattractant protein 1</td>
<td>A-2518G</td>
<td>Boger et al\textsuperscript{22}</td>
</tr>
<tr>
<td>NFKBIA</td>
<td>Inhibitor of NFκB</td>
<td>A/G in the 3'-UTR</td>
<td>Romzova et al\textsuperscript{31}</td>
</tr>
<tr>
<td>NFKB1</td>
<td>Nuclear factor κ B</td>
<td>CA repeat</td>
<td>Romzova et al\textsuperscript{31}</td>
</tr>
<tr>
<td>NQO1</td>
<td>Nicotinamide adenine dinucleotide phosphate: quinone oxidoreductase 1</td>
<td>C609T (rs1800566)</td>
<td>Han et al\textsuperscript{32}</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor 1</td>
<td>–675 4G/5G insertion/deletion</td>
<td>Saely et al\textsuperscript{33}</td>
</tr>
<tr>
<td>PON2</td>
<td>Paraoxonase 2</td>
<td>S311C</td>
<td>Burdon et al\textsuperscript{20}</td>
</tr>
<tr>
<td>PPARA</td>
<td>Peroxisome proliferator activated receptor α</td>
<td>L162V C2528G</td>
<td>Tai et al\textsuperscript{34} Doney et al\textsuperscript{35} Doney et al\textsuperscript{35}</td>
</tr>
<tr>
<td>P-selectin</td>
<td>P-selectin</td>
<td>Tyr715Pro Asn562Asp Ser290Asn</td>
<td>Zalewski et al\textsuperscript{36} Zalewski et al\textsuperscript{36} Zalewski et al\textsuperscript{36}</td>
</tr>
<tr>
<td>Prognostic Genes</td>
<td>Gene</td>
<td>Allele</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------</td>
<td>------</td>
<td>--------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>PTPN1</strong></td>
<td>Protein tyrosine phosphatase 1B</td>
<td>rs2904268</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs803742</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1967439</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs718630</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs4811078</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2206656</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs932420</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs3787335</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2426158</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2904269</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs941798</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1570179</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs3787345</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1885177</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs754118</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs3215684</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs968701</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2282147</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs718049</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs718050</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs3787348</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1484insG</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs914458</td>
<td>Burdon et al&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>RANTES</strong></td>
<td>Regulated on activation, normal T cell expressed and secreted</td>
<td>A(−403)G</td>
<td>Boger et al&lt;sup&gt;22&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-28G</td>
<td>Boger et al&lt;sup&gt;22&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ln1.1C/T</td>
<td>Boger et al&lt;sup&gt;22&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>VEGF</strong></td>
<td>Vascular endothelial growth factor</td>
<td>Insertion/Deletion</td>
<td>Buraczynska et al&lt;sup&gt;38&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G405C</td>
<td>Buraczynska et al&lt;sup&gt;38&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T(-1498)C</td>
<td>Suganthalakshmi et al&lt;sup&gt;39&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G(-1190)A</td>
<td>Suganthalakshmi et al&lt;sup&gt;39&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C(-634)G</td>
<td>Suganthalakshmi et al&lt;sup&gt;39&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C(-7)T</td>
<td>Suganthalakshmi et al&lt;sup&gt;39&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Atherogenic Effects of Homocysteine

Homocysteine and its metabolites have an atherogenic potential, affecting cell survival, proliferation and apoptosis, thrombosis, and lipid metabolism and peroxidation. Hcy-thiolactone is capable of forming amide bonds with lysine residues, thus creating n-homocysteinylated proteins, altering their activity and solubility. N-homocysteinylation of the HDL-associated enzyme PON1 that hydrolyses Hcy-thiolactone and oxidized phospholipids renders it inactive thus decreasing the antiatherogenic activity of HDL.88 Hyperhomocysteinemia has also been associated with decreased expression of HDL-associated proteins, such as lecithin-cholesterol acyltransferase (LCAT) and apolipoprotein A-1 (apo A-1), and accelerated HDL catabolism, resulting in overall decreased HDL levels and activity.89 Although LDL oxidation by Hcy was not proven to take place in vivo,90 modification of apolipoprotein B-100 (apo B-100) by Hcy-thiolactone leads to its aggregation, making it cytotoxic to endothelial cells.88 Because of the strong relationship between oxidative stress and CVD, Hcy oxidative potential has been extensively studied. A central role in Hcy-mediated oxidative stress has been attributed to its reaction with copper to produce hydrogen peroxide (H$_2$O$_2$) and other reactive oxygen species (ROS). Although Hcy promotes the synthesis of glutathione and nitrous oxide (NO), the former scavenging H$_2$O$_2$ and the latter scavenging Hcy itself, prolonged exposure to high levels of Hcy leads to saturation of these reactions, thus allowing excess Hcy to produce free H$_2$O$_2$ and ROS.91 The pro-thrombotic effects of Hcy and its metabolites are a result of increased pro-coagulatory properties of the endothelium and decreased fibrinolysis. Treatment of endothelial cells with Hcy increased the synthesis and activation of pro-coagulatory molecules, such as tissue factor and factor V, and decreased the synthesis and activation of anticoagulatory molecules, such as heparan sulfate, protein C, NO, and prostacyclins.91 N-homocysteinylation of fibrinogen renders it more resistant to fibrinolysis,88 a process that is attenuated further by decreased tissue plasminogen activator activation by the endothelium.91 Disruption of normal endothelial function by Hcy and its metabolites is not restricted to its role in coagulation and thrombosis. Treatment with Hcy or Hcy thiolactone resulted in increased apoptosis92 and decreased proliferation93–95 of cultured endothelial cells. Impaired endothelial-dependent vasodilation, most likely resulting from endothelial injury, increased oxidative stress, and decreased NO bioavailability, is another manifestation of hyperhomocysteinemia.90,91 Increased proliferation of vascular smooth muscle cells (VSMCs) occurs following exposure to Hcy.96,97 Thickening of the vessel wall is also the result of the increased collagen production exerted by Hcy.91

Hyperhomocysteinemia and Cardiovascular Disease

Hyperhomocysteinemia was first implicated in the pathogenesis of vascular disease in 1969, following an observation that children carrying inherited deficiencies in the enzyme CBS presenting with homocystinuria commonly suffer from vascular diseases.98 This observation was the cornerstone for extensive research regarding the relationship between Hcy and vascular diseases. Over the years, this relationship has been thoroughly studied with considerable evidence pointing toward a significant association between cardiovascular disease and an elevated Hcy level, which is independent of other known cardiovascular risk factors.99–103 Consequently, Hcy-reducing treatments, generally including folic acid, vitamin B$_6$, and vitamin B$_12$, were tested for their ability to reduce the risk for CVD among individuals with hyperhomocysteinemia. Although supplementation decreases homocysteine levels, in most studies this has not been accompanied by a reduction in the risk for CVD.102,104–106 Moreover, the results of two studies suggested a potentially harmful effect of vitamin supplementation.107,108 An exception to these findings is the effect of folate and vitamin supplementation on stroke, where a significant risk reduction was noted.109 It has been suggested that Hcy is only a marker of other pathologic phenomena related to CVD. However, other explanations for the discrepancy between the results of the observational studies and the clinical trials have been offered, amongst which are the duration of Hcy-lowering treatments and the confounding effect of folate fortification of grains.110 Another possibility is that Hcy reduction may only be helpful in early stages of CVD. Similarly to what is seen in the general population, hyperhomocysteinemia is also associated with increased CVD among patients with DM.111–115 Although it is unclear whether hyperhomocysteinemia is one of the manifestations of DM,116–118 in-group studies have found several correlates to Hcy levels, the most prominent being renal function.115,118

The Methylene tetrahydrofolate Reductase 677 CT Polymorphism

Following the discovery of the relationship between CBS deficiency, increased Hcy, and
CVD, other polymorphisms and mutations that may interfere with Hcy metabolism have been examined. The most documented polymorphism is that of the enzyme MTHFR, where a substitution of C to T occurs at position 677. This substitution results in a missense polymorphism, producing a thermolabile enzyme with decreased reactivity. The 677T allele has a frequency of 30% to 40% with 10% of all individuals homozygous for the T allele. TT homozygotes have marked hyperhomocysteinemia in states of folate deficiency. Although initial studies demonstrated a strong relationship between the 677CT polymorphism and CVD, these results have not been replicated leading to the notion that this polymorphism may only be a minor risk factor for CVD. The relationship between the 677 CT polymorphism and CVD in DM is also complicated. Although significant evidence exists linking the 677 CT polymorphism to diabetic retinopathy and nephropathy, and the -786C allele associated with decreased flow or stimuli-dependent vasodilation, carriers of the allele present with decreased mRNA levels, which translates to decreased NO production and release by the endothelium.

**ENDOTHELIAL NITRIC OXIDE SYNTHASE AND NITRIC OXIDE METABOLISM**

**Role of Nitric Oxide and Endothelial Nitric Oxide Synthase in Cardiovascular Physiology**

NO has been identified as an important factor in maintaining normal cardiovascular function and preserving the integrity of the vascular bed. It inhibits thrombosis and coagulation not only by maintaining anticoagulatory and anti-thrombogenic properties of the endothelium but also by inhibiting platelet activation and aggregation and thereby reducing platelet derived growth factor (PDGF)-induced proliferation of vascular smooth muscle cells in the vessel wall. Acting directly on VSMCs, NO is a potent vasodilator and a regulator of cell proliferation. NO protects the endothelium and the underlying intima from inflammatory processes, inhibiting the expression of chemoattractants, such as monocyte chemotactic protein-1 (MCP-1), and of adhesion molecules, such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). NO is also capable of acting as an antioxidant, inhibiting pro-oxidative reactions catalyzed by H$_2$O$_2$. In vivo, NO is synthesized by the nitric oxide synthase (NOS) family of enzymes. The endothelial nitric oxide synthase (eNOS) is synthesized from the gene NOS3, which is located at chromosomal locus 7q35-36. Despite its name, eNOS expression is not restricted to endothelial cells and can be found in other cell types, such as erythrocytes, leukocytes, and mast cells. It acts as a membrane-bound homodimer catalyzing the synthesis of NO from L-arginine, a reaction that demands the presence of several cofactors, such as heme, tetrahydrobiopterin, and nicotinamide adenine dinucleotide phosphate. The enzyme eNOS is constitutively expressed and is subjected to regulation by a variety of factors, such as calcium and calmodulin, phosphorylation, protein-protein interaction, and fatty-acid modification. Altogether, these factors determine NO production and release by the endothelium.

**Polymorphisms of the NOS3 Gene and Their Relation to Cardiovascular Disease**

As a result of the importance of NO and eNOS in vascular physiology, polymorphisms were examined in the gene NOS3. In the coding region, the Glu298Asp (G894T) polymorphism has been most widely studied. Several studies reported differences in NO synthesis and reduced NO availability in carriers of the 298Asp variant, probably caused by protein cleavage, but these results were contradicted by others. Characteristics of enzymatic activity, such as K_M, V_max, and K_i, for various inhibitors did not differ between the 298Asp and 298Glu proteins. In several independent studies, the 298Asp variant has been associated with poor endothelial and vasomotor function, carriers of the allele presenting with decreased flow or stimuli-dependent vasodilation, increased coronary vascular resistance, and increased vasoconstriction in response to stimuli. However, these results were not observed in all trials, some finding no relation between the 298Asp allele and endothelial function. This polymorphism has also been identified as an important determinant of collateral vessels development.

In the promoter region, the T-786C polymorphism was identified as a predictor of eNOS expression, the -786C allele associated with decreased mRNA levels, which translates to decreased NO production and decreased endothelial function. The variability in mRNA expression most likely results from differential binding of an inhibitory transcription factor to the -786C allele, perhaps the replication protein A1.

A third polymorphism that has gained much attention is a 27 base pair tandem repeat in intron 4, where one allele, denoted a, presents with four tandem repeats and the second allele, denoted b, presents with five tandem repeats. Sparse evidence exists regarding the effect of this polymorphism on eNOS expression and activity.
Although the b allele has been linked to protein expression\textsuperscript{160} and increased plasma NO concentrations in some\textsuperscript{161} trials,\textsuperscript{162–164} the allele was associated with increased specific activity.\textsuperscript{160} Lacking a mechanism that links it with protein activity or expression, it has been suggested that this intron polymorphism is found in linkage disequilibrium with other functional polymorphisms. This theory was supported by several studies that found linkage disequilibrium between this polymorphism and the T\textsuperscript{-}786C polymorphism.\textsuperscript{160,162}

The effect of the aforementioned polymorphisms on CVD was evaluated in a recent meta-analysis. When all studies were taken into account, all three polymorphisms were found to be mild risk factors for coronary heart disease (CHD). However, when only the larger studies were examined, this association was either lost or was no longer significant.\textsuperscript{165} In this meta-analysis, no significant association was found between these polymorphisms and other CVD outcomes, such as stroke and carotid stenosis.

**NOS3 Polymorphisms and Diabetes Mellitus**

Endothelial dysfunction and decreased NO bioavailability are prominent features of type 2 DM and may even appear before the onset of the disease.\textsuperscript{166} Decreased NO release by endothelial cells may be the result of several mechanisms, including a deficiency in L-arginine, deficiencies in the various cofactors, and increased NO scavenging by ROS. Aberrant insulin signaling through the PI3K/Akt pathway in endothelial cells prevents eNOS phosphorylation on Serine 1177, thus decreasing its activity.\textsuperscript{167} Similar to the observations in patients without DM, the evidence regarding the impact of NOS3 polymorphisms on NO production and endothelial function is inconclusive.\textsuperscript{168–170} A limited body of data exists regarding the relationship between NOS3 polymorphisms and cardiovascular outcomes in the setting of DM. A recent review that studied the relationship between the different NOS3 polymorphisms and diabetic nephropathy was able to show a significant effect of the Glu298Asp across different populations. A specific interaction between the Glu298Asp and the 4a/b polymorphisms and severe diabetic nephropathy was shown in East Asian populations.\textsuperscript{171} A recent meta-analysis also investigated the relationship between NOS3 polymorphism and diabetic retinopathy, failing to find a significant association between the two.\textsuperscript{172} The relationship between the Glu298Asp polymorphism and other cardiovascular diseases in the setting of DM was identified by several\textsuperscript{131,173} but not all\textsuperscript{174,175} studies. However, these were mostly retrospective studies with only one prospective study demonstrating such a relationship in patients with type 1 DM.\textsuperscript{176}

**Haptoglobin Polymorphism**

The Hp gene has been localized to chromosome 16q22. Two Hp alleles exist in man: Hp1, with an allele frequency of 0.4; and Hp2, with an allele frequency of 0.6 in most Western populations. The alleles are found in a Hardy-Weinberg equilibrium, the frequency of the Hp1\textendash}1, Hp2\textendash}1, and Hp2\textendash}2 genotypes being 16%, 48%, and 36%, respectively.\textsuperscript{177} The Hp2 allele, whose development most likely occurred in early human evolution, has evolved from the Hp1 allele via a duplication of exons 3 and 4 present in the Hp1 allele. Exon 3 contains a cysteine residue that can form a disulfide bridge between Hp monomers. Therefore, its duplication in the Hp2 allele makes the Hp2 protein monomer bivalent, whereas the Hp1 protein monomer is monovalent. This difference has significant implications for the stoichiometry and structure of Hp found in serum. Being monovalent,
the Hp1 monomer can only bind to one other Hp molecule, forming linear dimers in the Hp1-1 genotype. The Hp2 monomer, conversely, binds two other Hp molecules and forms cyclic polymers in individuals with the Hp2-2 genotype. In the Hp2-1 genotype, heteromeric linear polymers are formed, with Hp1 proteins bracketing a chain of linear Hp2 proteins. Hp1-1 dimers may also be found in the Hp2-1 genotype.\(^{187}\)

**Haptoglobin Genotype and Diabetic Cardiovascular Disease: a Specific Gene-Disease Interaction**

As opposed to the other CVD-related genes discussed earlier, the Hp polymorphism appears to have a unique interaction with DM. In the setting of DM, the Hp2-2 polymorphism confers a twofold to fivefold increased risk for CVD compared with the Hp1-1 and Hp2-1 genotypes.\(^{188–192}\) This finding was also seen in in vivo studies where mice with Hp2-2 DM were more prone to develop retinopathy,\(^{193}\) nephropathy,\(^{194}\) and atherosclerosis.\(^{195,196}\) In the absence of DM, the Hp1-1 genotype may be associated with increased CVD.\(^{197,198}\)

**Haptoglobin Polymorphism and Oxidative Stress: a Mechanism for the Gene-Disease Interaction**

The underlying mechanism for the specific interaction between the Hp2-2 genotype and DM appears to be the result of its interaction with Hb. In individuals with DM, extravascular and intravascular hemolysis occur in higher rates compared with individuals without DM, thus increasing the amount of Hp-Hb molecules in plasma and tissues. As already mentioned, Hp is considered an antioxidant because of its ability to scavenge Hb and prevent the initiation of radical chain reactions. However, this antioxidant activity varies greatly between the different Hp genotypes.\(^{184}\) Although the affinity for Hb is similar for Hp1-1 and Hp2-2,\(^{199}\) the ability to seclude the heme-iron from its aqueous surroundings is greatly decreased in the latter. This disparity is further magnified by oxidation and glycation of Hb, both common in DM.\(^{200}\) Under hyperglycemic conditions, Hp2-Hb, as compared with Hp1-Hb, evoked increased oxidative stress in cultured CD163-transfected Chinese hamster ovary cells.\(^{200}\) In vivo, the ability of Hp1-1 to better shield Hb is translated to a decrease in redox active heme iron in Hp1-1 DM compared with mice and humans with Hp2-2 DM, both in blood and tissues.\(^{200,201}\) The increase in plasma oxidative stress in individuals with Hp2-2 is also indicated by the decrease in antioxidants, such as vitamin C,\(^{187}\) in the serum of individuals with Hp2-2. Furthermore, increased oxidative stress and hyperglycemia lead to decreased expression of CD163 on macrophages by inducing its shedding and decreasing transcription.\(^{202,203}\) Finally, clearance rate of the Hp-Hb-2-2 complex by the CD163 receptor is decreased compared with Hp-Hb-1-1, prolonging its presence in tissues\(^{199}\) and perhaps decreasing the expression of CD163 itself.\(^{204}\)

Another novel aspect of Hp-Hb-2-2 mediated oxidative stress is related to the association of Hp with HDL. Hp, either free or Hb-bound, is a member of the HDL proteome.\(^{205}\) Because the Hp2-2 polymers contain more Hp monomers than the Hp1-1 dimers, Hp is more abundant in the HDL of individuals with Hp2-2.\(^{205}\) In individuals with Hp2-2 DM, as a result of the impaired clearance of Hp-Hb, there is an increased binding of Hp-Hb to HDL, which along with the decreased antioxidant properties of Hp 2-2, expose HDL particles of individuals with Hp2-2 DM to increased oxidative stress, expressed by an increase in HDL-associated lipid peroxides. This oxidative modification of HDL in individuals with Hp2-2 DM results in a decrease in HDL-related functions, such as reverse cholesterol efflux and cholesterol esterification,\(^{195,205}\) and paradoxically may result in the transformation of the HDL particle in individuals with Hp2-2 DM into a proatherogenic species. This pathway may form the basis for the pharmacogenetic relationship between vitamin E and the Hp2-2 genotype whereby vitamin E can markedly reduce CVD in individuals with Hp2-2 DM, which was recently reviewed.\(^{206,207}\)

**SUMMARY AND FUTURE PERSPECTIVES**

With the advent of genome-wide association studies, hundreds of genetic polymorphisms with a possible impact on diabetic CVD are being investigated. However, most of these polymorphisms have failed to show any significant effect when tested across various populations. These findings are caused by the nature of the polymorphisms being tested, which are usually SNPs that have no established effect on protein activity or expression. Such polymorphisms are likely in linkage disequilibrium with other genetic markers that directly alter disease progression and therefore these SNP-disease associations may not be preserved in all populations and may be subject to population stratification. Polymorphisms in the Hp or PON genes discussed here do not suffer from this setback. Having a direct effect on the
benefit from specific drug treatments.

Genetic testing certainly portends to be an important component of personalized medicine, but only when it can be accompanied by a treatment plan that would match the genetic profile of patients. Although such treatment plans may include more aggressive treatment to at-risk individuals, they may also include more frequent screening and closer monitoring, as is customarily done for carriers of genetic mutations associated with increased risk for cancer. In addition, as illustrated by the pharmacogenomic interaction between the Hp genotype and vitamin E on CVD risk, these genetic markers might be useful in the identification of which individuals may benefit from specific drug treatments.

REFERENCES

44. Draganov DI, Teiber JF, Speelman A, et al. Human paraoxonases (PON1, PON2, and PON3) are
Vascular Complications in Diabetes Mellitus


