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The Genetics of Vascular Complications in Diabetes Mellitus

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Abstract

Prospective identification of which individuals with diabetes mellitus (DM) are at greatest risk of developing cardiovascular (CVD) complications would have considerable public health importance by allowing the allocation of limited resources to be focused on those individuals who would most benefit from aggressive intervention. Over the past 20 years genetic disease association studies have demonstrated that polymorphisms at specific genetic loci may identify those individuals at greatest risk of developing CVD in the setting of DM. This article reviews the evidence accumulated to date on four polymorphic loci with the aim of explaining how these polymorphisms modify the risk of CVD in DM by modifying the functional activity of a specific gene. Utilization of the knowledge of these genetic differences among individuals in targeting drug therapy (pharmacogenomics) is also discussed.

Keywords

Diabetes; CVD; polymorphism; genetic variance; genotype; phenotype

1. Introduction

Due to increasing prevalence rates in the past decade, Diabetes Mellitus (DM) has become a major public health issue. Both macrovascular and microvascular complications are common long term sequelae of the disease. Cardiovascular disease is the single most major cause of death among DM patients, 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 due to 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 as it would allow for a more efficient allocation of resources in order to alleviate the burden of disease[2]. Beginning over 20 years ago hundreds of polymorphisms in genes that are involved in the pathogenesis of CVD and DM have been

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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. Figure 1 provides a functional categorization of these polymorphic genes. Table 1 provides a comprehensive list of the SNPs and polymorphisms that have been assessed to date in the search for loci which 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 will be reviewed in this paper.

2. Paraoxonase

2.1 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. While *PON1* and *PON3* are expressed mainly in the liver and associate with high density lipoprotein (HDL) in the circulation[41,42], *PON2* is expressed in a variety of tissues and is found on the endoplasmic reticulum (ER) and the nuclear membrane[43]. While only *PON1* exhibits the ability to hydrolyze organophosphates, the three enzymes share the ability to metabolize different lactones[44], some of which are the product of phospholipid peroxidation[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 both 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[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[47].

2.2 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* was shown to diminish LDL oxidation[48] and prevent the pro-inflammatory response elicited by oxidized LDL (OxLDL), the latter probably resulting from the metabolism of lipid peroxides[49]. Moreover, *PON1* was shown to be critical for preventing the oxidation of HDL, allowing it to maintain its function[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[51]. *PON1* knockout mice were also more susceptible to the development of atherosclerosis in dietary or genetic models[51, 52]. Demonstrating a therapeutic potential for *PON1* elevation, mice overexpressing *PON1* were more resistant to atherosclerosis compared to wild-type mice[53].

2.3 Paraoxonase genotype and relation to CVD

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, both 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. Due to differences in the affinity of the transcription factor Sp1[54], this polymorphism has a dramatic effect on gene transcription, the -107C allele having significantly increased transcriptional activity compared to the -107T

allele. This variation is reflected in higher plasma PON1 concentrations and activity in carriers of the -107C allele[54,55]. However, although significantly decreasing enzyme concentration and activity, this polymorphism has not consistently been shown to be associated with CVD [56-59]. Interestingly, 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[58].

Of the polymorphisms in the coding region, the Glu192Arg polymorphism has gained the most interest [60]. This polymorphism results in a decrease in serum Paraoxonase activity and concentration[61], possibly due to decreased affinity of the Arg192 polymorphism to the HDL, which leads to decreased protein stability and activity[62]. 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 due to the lack of a significant association between this polymorphism and CVD in large trials and due to possible publication bias[63].

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[64,65]. A recent large trial in which both PON1 activity and genotype were tested has shown that not only do both PON1 phenotype and Glu192Arg genotype determine the risk for CVD, but also that the PON1 genotype is a predictor of its activity and concentration [61].

2.4 Paraoxonase and Diabetes Mellitus

The relationship between PON1 and DM appears to be bidirectional with DM significantly decreasing PON1 concentration[66] and activity[66-70], and in turn, PON1 genotype modulating the risk for type 2 DM[68,71]. 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[72].

Similar to what is seen in non-DM individuals, PON1 concentration and activity were found to be negatively associated with CVD[73-76]. In the settings of Type 2 DM only a single study has focused on the C(-107)T polymorphism, indicating an increased risk for CVD in carriers of the TT allele compared to carriers of the CT or CC alleles[77]. This genotype was also associated with decreased PON1 concentration and activity[67], and increased plasma OxLDL/Apolipoprotein B (ApoB) ratio among DM individuals[78]. The Glu192Arg polymorphism was extensively studied in the settings of DM. In these settings, HDL from 192Arg allele homozygotes was demonstrated to be less efficient in the metabolism of oxidized phospholipids [69], although these results were contested by others showing either no difference in plasma oxidized LDL[79], or decreased oxidation in the aforementioned genotype[80]. In most epidemiological studies, the 192Arg allele was shown to be significantly correlated with CVD [81-84], although several studies have presented contradicting results[74]. Several studies have highlighted the importance of interaction between the PON1 Glu192Arg polymorphism and DM, arguing that this polymorphism most significantly increases risk for CVD in the presence of DM[85,86].

3. Methyltetrahydrofolate Reductase & Homocysteine Metabolism

3.1 Metabolic pathways of Homocysteine

Homocysteine (Hcy) is positioned at the crossroads of several metabolic pathways. Hcy is synthesized from methionine in a 3-steps 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 (BHMT) in the liver or by Methionine Synthase (MS) in most bodily tissues, the latter depending on methyltetrahydrofolate as a methyl donor and vitamin B₁₂ as a cofactor. Synthesis of methyltetrahydrofolate is catalyzed by the enzyme methylenetetrahydrofolate reductase (MTHFR), in the presence of the vitamin B₂. In a state of methionine excess, Hcy is irreversibly transsulfurated by CBS and vitamin B₆ to cystathionine, which can be converted to cysteine[87]. A third elimination pathway of Hcy is the pathological synthesis of the Hcy-Thiolactone. This reaction is carried out by methionyl-tRNA synthetase (MetRS) when Hcy is mistakenly recognized as methionine[88].

3.2 Atherogenic effects of Homocysteine

Homocysteine and its metabolites were shown to 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-homocysteinylation 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 anti-atherogenic activity of HDL[88]. Hyperhomocysteinemia has also been associated with decreased expression of HDL-associated proteins such as LCAT and Apo-A1, 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 ApoB 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 (H₂O₂) and other reactive oxygen species (ROS). Although Hcy promotes the synthesis of glutathione and Nitrous Oxide (NO), the former scavenging H₂O₂ 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 H₂O₂ and ROS[91].

The prothrombotic effects of Hcy and its metabolites are a result of increased procoagulatory properties of the endothelium and decreased fibrinolysis. Treatment of endothelial cells with Hcy increased the synthesis and activation of procoagulatory 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 (TPA) activation by the endothelium[91].

Disruption of normal endothelial function by Hcy and its metabolites is not restricted to its role in coagulation and thrombolysis. Treatment with Hcy or Hcy thiolactone resulted in increased apoptosis[92] and decreased proliferation[93-95] of cultured endothelial cells. Impaired endothelial dependent vasodilation, most likely resulting from both endothelial injury, increased oxidative stress and decreased NO bioavailability, is another manifestation of hyperhomocysteinemia[90,91].

Increased proliferation of vascular smooth muscle cells (VSMCs) has been shown to occur following exposure to Hcy[96,97]. Thickening of the vessel wall is also the result of the increased collagen production exerted by Hcy[91].

3.3 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 Cystathionine β -Synthase (CBS) presenting with homocysteinuria 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 towards a significant association between cardiovascular disease and elevated Hcy level which is independent of other known cardiovascular risk factors[99-103]. Consequently, Hcy-reducing treatments, generally including folate, vitamin B₆ and vitamin B₁₂, 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 of 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 associated with increased CVD among DM patients as well[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].

3.4 The MTHFR 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 been shown to have a frequency of 30%-40% with 10% of all individuals homozygous for the T allele. TT homozygotes have been shown to have marked hyperhomocysteinemia in states of folate deficiency[91]. Although initial studies demonstrated a strong relationship between the 677CT polymorphism and CVD[119], these results have not been replicated[120] leading to the notion that this polymorphism may only be a minor risk factor for CVD[121,122]. The relationship between the 677 CT polymorphism and CVD in DM is complicated as well. While significant evidence exist linking the 677 CT polymorphism to diabetic retinopathy[123-125] and nephropathy[126-128], evidence is disputed regarding its role in stroke and peripheral and coronary artery disease[129-136].

4. Endothelial Nitric Oxide Synthase and Nitric Oxide metabolism

4.1 Role of NO and eNOS 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 anti-coagulatory and anti-thrombogenic properties of the endothelium[137], 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 [138]. Acting directly on VSMCs, NO is a potent vasodilator[138] and a regulator of cell proliferation[139]. NO protects the endothelium and the underlying intima from inflammatory

processes, inhibiting the expression of chemoattractants such as MCP-1 and of adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1)[138]. NO is also capable of acting as an anti-oxidant, inhibiting pro-oxidative reactions catalyzed by H₂O₂[140].

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[141]. 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[137]. It acts as a membrane bound homodimer[142] 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 (NADPH)[139]. eNOS is a constitutively expressed enzyme that 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[143].

4.2 Polymorphisms of the NOS3 gene and their relation to CVD

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 due to protein cleavage[144,145], but these results were contradicted by others[146,147]. 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[147,148]. 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[149,150], increased coronary vascular resistance[150] and increased vasoconstriction in response to stimuli[151]. However, these results were not observed in all trials, some finding no relation between the 298Asp allele and endothelial function[152-154]. This polymorphism has also been identified as an important determinant of collateral vessels development[155,156].

In the promoter region, the T(-786)C polymorphism was identified as a predictor of eNOS expression, the -786C allele associated with decreased mRNA levels, which translate to decreased NO production[157] and decreased endothelial function[153,158,159]. The variability in mRNA expression most likely results from differential binding of an inhibitory transcription factor to the -786C allele[158], perhaps the replication protein A1[157].

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 4 tandem repeats and the second allele, denoted b, presents with 5 tandem repeats. Sparse evidence exists regarding the effect of this polymorphism on eNOS expression and activity. While the b allele has been linked to protein expression[160] and increased plasma NO concentrations in some[161] but not all trials [162-164], the a allele was associated with increased specific activity[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-786C polymorphism[160,162].

The effect of the aforementioned polymorphisms on CVD has been evaluated in a recent meta-analysis. When all studies were taken into account, all three polymorphisms were found to be mild risk factors for CHD. However, when only the larger studies were examined, this association was either lost or was no longer significant[165]. In this meta-analysis, no

significant association was found between these polymorphisms and other CVD outcomes such as stroke and carotid stenosis.

4.3 NOS3 polymorphisms and Diabetes Mellitus

Endothelial dysfunction and decreased NO bioavailability is a prominent feature of T2DM, and may even appear before the onset of the disease[166]. Decreased NO release by endothelial cells may be the result of several mechanisms, including a deficiency in L-arginine, deficiencies in the various co-factors 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[167]. Similar to the observations in non-DM patients, the evidence regarding the impact of *NOS3* polymorphisms on NO production and endothelial function is inconclusive[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[171]. A recent meta-analysis also investigated the relationship between *NOS3* polymorphism and diabetic retinopathy, failing to find a significant association between the two[172]. The relationship between the Glu298Asp polymorphism and other cardiovascular diseases in the setting of DM was identified by several[131,173] but not all[174,175] studies. However, these were mostly retrospective studies, with only one prospective study demonstrating such a relationship in patients with Type 1 DM[176].

5. Haptoglobin and hemoglobin-mediated oxidative stress

5.1 Haptoglobin Metabolism

Haptoglobin (Hp) is an acute-phase plasma born glycoprotein produced mainly by hepatocytes, most widely known for its ability to strongly bind free hemoglobin (Hb) following its release from erythrocytes[177]. The concentrations of Hp in the plasma are high, ranging from 0.3mg/ml to 3mg/ml, producing an Hp:Hb molar ratio of 400:1. This allows effective scavenging of free Hb, even in the scenario of hemolysis when its levels are sharply increased[178]. In fact, Hp has a major role in iron preservation during hemolysis, as it prevents Hb filtration in the glomeruli[179] and renal damage[180]. The Hp-Hb complex is transported to the liver and other tissues to be degraded by Hp-Hb scavenger receptors, such as the CD163 receptor present on macrophages and liver Kupfer cells[181,182]. Another important aspect of Hb scavenging by Hp is the reduction in oxidative stress. Extracorporeal Hb can initiate a free radical reaction by releasing heme iron, which acts as a potent Fenton reagent. This reaction results in the production of ROS that cause oxidative damage to their surroundings[183]. Hp binding to Hb prevents this cascade by shielding the heme iron from its aqueous surrounding[184, 185]. Moreover, Hp maintains Hb integrity by preventing oxidation of the globin by heme iron. This allows effective clearance of Hb by the CD163 receptor[186].

5.2 The Hp 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 Hp 1-1, Hp 2-1 and Hp 2-2 genotypes being 16%, 48% and 36% respectively[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 Hp 1 allele. Exon 3 contains a cysteine residue that can form a disulfide bridge between Hp monomers. Therefore, its duplication in the Hp 2 allele makes the Hp2 protein monomer bivalent, while the Hp1 protein monomer is

monovalent. This 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 Hp 1-1 genotype. The Hp2 monomer, conversely, binds two other Hp molecules and forms cyclic polymers in individuals with the Hp 2-2 genotype. In the Hp 2-1 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].

5.3 Hp genotype and diabetic CVD -a specific gene-disease interaction

As opposed to the other CVD-related genes discussed above, the Hp polymorphism appears to have a unique interaction with DM. In the setting of DM, it has been shown by several groups that the Hp 2-2 polymorphism confers a 2-5 fold increased risk for CVD compared to the Hp 1-1 and Hp 2-1 genotype[188-192]. This was also seen in *in vivo* studies, where Hp 2-2 DM mice were more prone to develop retinopathy[193], nephropathy[194] and atherosclerosis [195,196]. Interestingly in the absence of DM, the Hp 1-1 genotype may be associated with increased CVD[197,198].

5.4 Hp polymorphism and Oxidative Stress – a mechanism for the gene-disease interaction

The underlying mechanism for the specific interaction between the Hp 2-2 genotype and DM appears to be the result of its interaction with Hb. In DM individuals, extravascular and intravascular hemolysis occur in higher rates compared to non-DM individuals, thus increasing the amount of Hp-Hb molecules in plasma and tissues. As already mentioned, Hp is considered an antioxidant due to 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]. While the affinity for Hb is similar for Hp 1-1 and Hp 2-2[199], the ability to seclude the heme-iron from its aqueous surrounding 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, Hp 2-Hb, as compared to Hp 1-Hb evoked increased oxidative stress in cultured CD163-transfected CHO cells[200]. *In vivo*, the ability of Hp 1-1 to better shield Hb is translated to a decrease in redox active heme iron in Hp 1-1 DM compared to Hp 2-2 DM mice and humans, both in blood and tissues[200,201]. The increase in plasma oxidative stress in Hp 2-2 individuals is also indicated by the decrease in antioxidants, such as vitamin C[187] in the serum of Hp 2-2 individuals. 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 to 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. It was shown that Hp, either free or Hb-bound, is a member of the HDL proteome [205]. Since the Hp 2-2 polymers contain more Hp monomers than the Hp 1-1 dimers, Hp is more abundant in the HDL of Hp 2-2 individuals[205]. In Hp 2-2 DM individuals, 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 Hp 2-2 DM individuals to increased oxidative stress, expressed by an increase in HDL associated lipid peroxides. This oxidative modification of HDL in Hp 2-2 DM individuals 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 Hp 2-2 DM individuals into a proatherogenic species. This pathway may form the basis for the pharmacogenetic relationship between Vitamin E and the Hp 2-2 genotype whereby vitamin E can markedly reduce CVD in Hp 2-2 DM individuals, which was recently reviewed[206, 207].

6. Conclusion 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. This has been due to the nature of the polymorphisms being tested, which are usually SNPs (single nucleotide polymorphisms) that have no established effect on protein activity or expression. Such polymorphisms are likely in linkage disequilibrium with other genetic markers which 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 pathophysiology of the disease, the Hp and PON polymorphisms have been shown to be risk factors for CVD in diverse populations.

Genetic testing certainly portends to be an important component of personalized medicine, but only when if it can be accompanied by a treatment plan that would match the genetic profile of the patient. While 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 [206,207], these genetic markers might be useful in the identification of which individuals may benefit from specific drug treatments.

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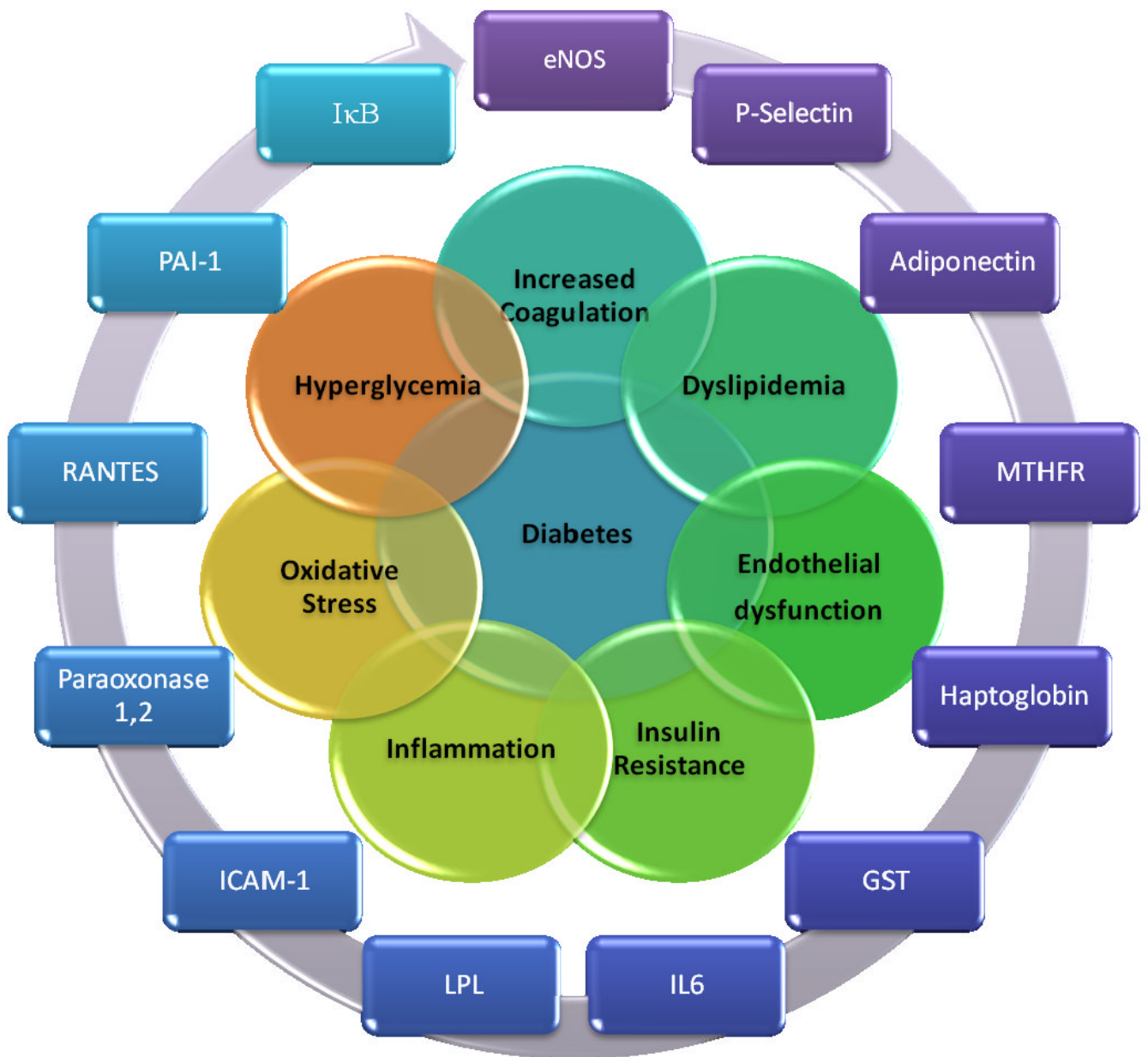


Figure 1. Candidate genes for diabetic CVD. eNOS: endothelial nitric oxide synthase, MTHFR: methylenetetrahydrofolate reductase, GST: glutathione S transferase, IL6: interleukin 6, LPL: lipoprotein lipase, PON: paraoxonase, RANTES: regulated on activation, normal T cell expressed and secreted, PAI-1: plasminogen activator inhibitor 1, IκB: inhibitor of NFκB.

Table 1
SNPs and genetic variations implicated in the risk of cardiovascular disease, in the settings of Diabetes Mellitus

Gene	Protein	Polymorphism	References
16p3	-		[3]
9p21	-	rs2383206	[4]
ACE	Angiotensin Converting Enzyme	Insertion/Deletion	[5,6]
ADIPOQ/ACDC	Adiponectin	-11391G>A	[7]
		-11377C>G	[7-9]
		-11365C>G	[10]
		-4041 A>C	[9]
		-4034A>C	[10]
		-3964A>G	[10]
		45T>G	[7,9-13]
		276G>T	[7,9-13]
		A349G	[11]
		+2019 delA	[9]
ADIPOR1	Adiponectin Receptor 1	rs2232853	[14]
		rs12733285	[14]
		rs1342387	[14]
		rs7539542	[14]
		rs10920531	[14]
		rs4950894	[14]
AGT	Angiotensinogen	M235T	[6,15]
AGT1R	Angiotensin-II Receptor 1	T573C	[15]
		A1166C	[15]
AT1R	Angiotensin Receptor 1	rs5186	[6]
aP2	Fatty-acid binding protein	T-78C	[16]
APOA4	Apolipoprotein A-IV	Gln360His	[17]
		Thr347Ser	[17]

Gene	Protein	Polymorphism	References
APOE	Apolipoprotein E	ε2/ε3/ε4	[18]
CD40	CD-40	rs1535045	[19]
		rs3765459	[19]
CD40L	CD-40 Ligand	rs3092948	[19]
		rs3092929	[19]
		rs3092923	[19]
		rs3092920	[19]
CETP	Cholesteryl ester transfer protein	rs289714	[20]
		I405V	[20]
		R451Q	[20]
COX2	Cyclooxygenase-2	rs689466	[21]
		rs20417	[21]
		rs2745557	[21]
		rs5277	[21]
		rs20432	[21]
		rs2066826	[21]
		rs5275	[21]
		rs10911902	[21]
CX3CR1	Fractalkine receptor	T280M	[22]
ENPP1	Ectoenzyme nucleotide pyrophosphate phosphodiesterase 1	K121Q	[23]
EPHX2	Epoxide hydrolase 2	rs7003694	[24]
		rs7837347	[24]
		R287Q	[24]
		rs721619	[24]
		rs747276	[24]
HL	Hepatic Lipase	C-480T	[20]
HSP70-2	Heat Shock Protein 70-2	A1267G (A/B)	[25]
GCHI	GTP cyclohydrolase 1	C59038T	[26]

Gene	Protein	Polymorphism	References
GSTT1	Glutathione S transferase theta-1	I/0	[27,28]
GSTM1	Glutathione S transferase mu-1	I/0	[27,28]
GSTP1	Glutathione S transferase pi-1	Ile105Val	[28]
IL6	Interleukin 6	-174G/C	[29]
LPL	Lipoprotein Lipase	rs285	[20]
		rs320	[20]
MT1A	Metallothioneins	A647C	[30]
		A1245G	[30]
MCP-1	Monocyte Chemoattractant Protein 1	A-2518G	[22]
NFKBIA	Inhibitor of NFκB	A/G in the 3'-UTR	[31]
NFKB1	Nuclear factor κ B	CA repeat	[31]
NQO1	Nicotinamide adenine dinucleotide phosphate: Quinone oxidoreductase I	C609T (rs1800566)	[32]
PAI-1	Plasminogen Activator Inhibitor 1	-675 4G/5G insertion/deletion	[33]
PON2	Paraoxonase 2	S311C	[20]
PPARA	Peroxisome proliferator activated receptor α	L162V	[34,35]
		C2528G	[35]
P-Selectin	P-Selectin	Tyr715Pro	[36]
		Asn562Asp	[36]
		Ser290Asn	[36]
PTPN1	Protein Tyrosine Phosphatase 1B	rs2904268	[37]
		rs803742	[37]
		rs1967439	[37]
		rs718630	[37]
		rs4811078	[37]
		rs2206656	[37]
		rs932420	[37]
		rs3787335	[37]
rs2426158	[37]		

Gene	Protein	Polymorphism	References
		rs2904269	[37]
		rs941798	[37]
		rs1570179	[37]
		rs3787345	[37]
		rs1885177	[37]
		rs754118	[37]
		rs3215684	[37]
		rs968701	[37]
		rs2282147	[37]
		rs718049	[37]
		rs718050	[37]
		rs3787348	[37]
		1484insG	[37]
		rs914458	[37]
RANTES	regulated on activation, normal T cell expressed and secreted	A(-403)G	[22]
		C-28G	[22]
		In1.1C/T	[22]
VEGF	Vascular Endothelial Growth Factor	Insertion / Deletion	[38]
		G405C	[38]
		T(-1498)C	[39]
		G(-1190)A	[39]
		C(-634)G	[39]
		C(-7)T	[39]