



Pharmacogenomics and the prevention of vascular complications in diabetes mellitus

While the majority of patients benefit from front-line therapies for diabetes and dyslipidemia, others present with diminished responses. These differences in drug efficacy appear to be due, in large part, to genetic polymorphisms affecting pharmacokinetics and drug-target interactions. Many studies have been conducted in an attempt to elucidate the pharmacogenetic relationship between a wide range of polymorphisms and their effects on responsiveness to statins, hypoglycemics, insulin sensitizers, antihypertensives and antioxidants. Regarding most polymorphisms, the results of association studies have not been validated consistently across different population groups. This review will present some of these pharmacogenetic relationships, focusing on the haptoglobin polymorphism and the responsiveness to vitamin E supplementation.

KEYWORDS: ACE • ARB • diabetes • haptoglobin • metformin • pharmacogenetics • pharmacogenomics • statins • thiazolidinediones • vitamin E

With prevalence rates constantly rising, diabetes mellitus has become a major public health issue. Long-term sequelae of the disease include both microvascular and macrovascular complications, damaging virtually every vascular bed, and particularly damaging the kidneys, eyes and heart. Diabetes mellitus is the leading cause of blindness in the Western world. Over 30% of all individuals with diabetes mellitus will eventually develop end-stage renal disease. Over 35% of all hospitalizations for cardiovascular disease (CVD) occur in individuals with diabetes mellitus. Current paradigms of pharmacoprevention of diabetic vascular complications have focused on not only reducing hyperglycemia, but also on reducing other contributing factors known to contribute to vascular disease, but which are not necessarily specific for diabetes mellitus (i.e., lipid-lowering therapies, see FIGURE 1) [1].

Pharmacogenetics and pharmacogenomics are based on the hypothesis that an individual's unique genetic fingerprint will lead to unique responses to therapeutic agents. These differences stem from genetic polymorphisms associated with modified activity, or expression of enzymes that may modulate the response to a certain drug. These polymorphisms may result in differences in the pharmacokinetics of a given drug affecting absorption, secretion, excretion or first-pass metabolism. Additionally, these polymorphisms may confer differences in the activities of enzymes that are the drug's molecular target or members in the affected molecular pathway [1]. Differential responses to therapy

have been noted in several of the most prominent pharmacotherapies for diabetes mellitus and dyslipidemia. This report will review some of the diabetes mellitus-associated pharmacogenetic relationships, with a particular focus on the association between haptoglobin genotype and the response to vitamin E.

Statins

Statins, inhibitors of the enzyme HMG-CoA reductase (HMGCR) that catalyzes the rate-limiting step of cellular cholesterol synthesis, are the most commonly used cholesterol-lowering drugs. While numerous polymorphisms have been tested, only a few have been consistently correlated with response to statin therapy. Perhaps the most studied pharmacogenetic relationship to statin therapy is that of the ApoE polymorphisms. The different isoforms of ApoE seem to have different affinities to the low-density lipoprotein (LDL) receptor, thus affecting the clearance of ApoE-presenting lipoproteins. High clearance rates lead to stronger inhibition of HMGCR, thus making statin therapy less effective. In line with this theory, several studies have shown favorable outcomes of statin therapy in carriers of the *E2* allele, which has the lowest affinity for the LDL receptor, while efficacy in carriers of the *E4* allele was decreased [2,3]. However, this relationship was not observed in all studies, as some studies found either no correlation between ApoE genotype and response to statin therapy [3], or increased response among carriers of the *E4* allele [4].

Dan Farbstein & Andrew P Levy[†]

[†]Author for correspondence
Technion-Israel Institute of
Technology, Department of
Anatomy and Cell Biology,
Bruce Rappaport Faculty of
Medicine, POB 9649, Haifa,
Israel

Tel.: +972 4829 5202

Fax: +972 4851 4103

alevy@tx.technion.ac.il

future
medicine part of fsg

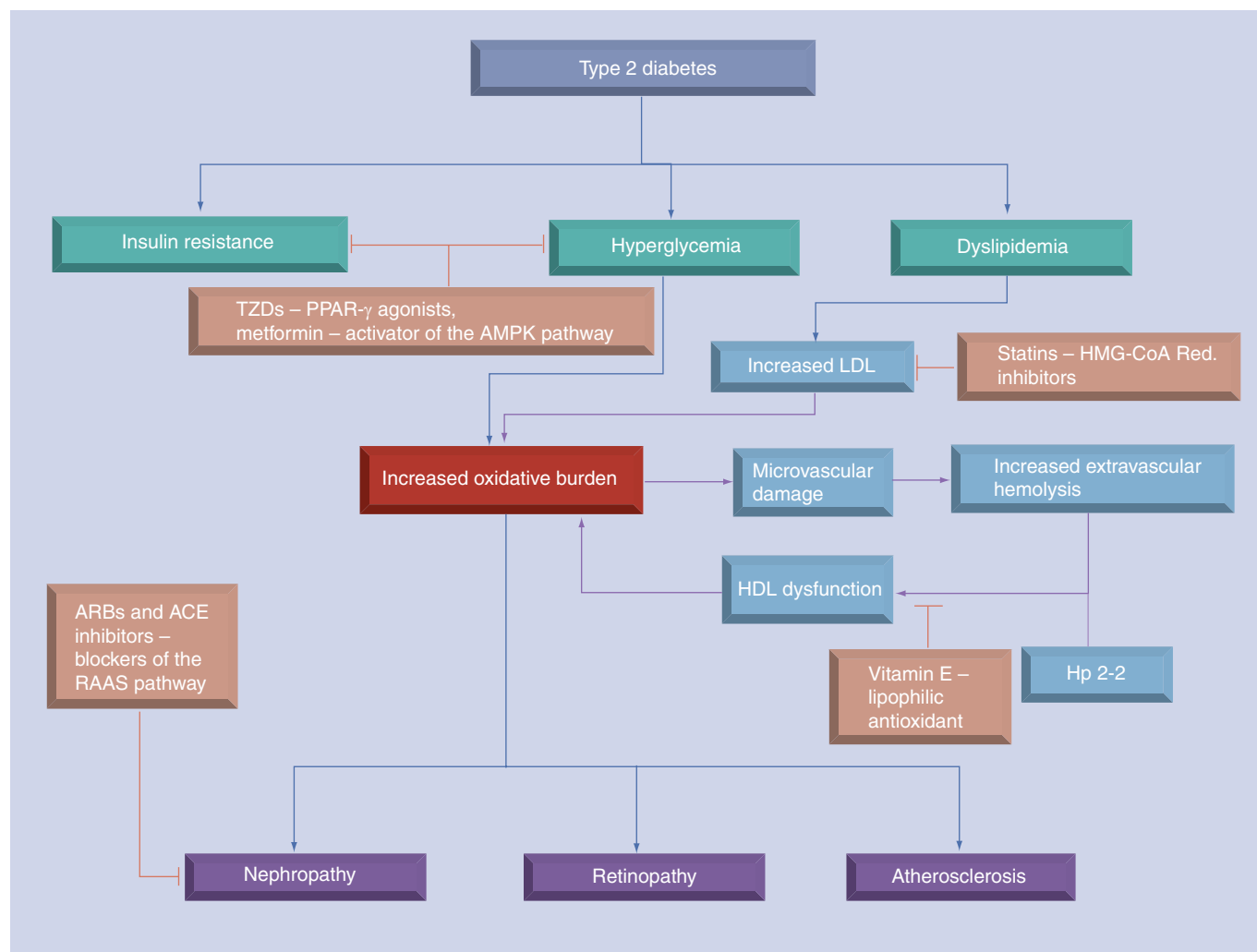


Figure 1. Type 2 diabetes mellitus complications and treatments. ACE: Angiotensin-converting enzyme; ARB: Angiotensin receptor blocker; HDL: High-density lipoprotein; HMG-CoA Red.: HMG-CoA reductase; Hp: Haptoglobin; LDL: Low-density lipoprotein; PPAR- γ : Peroxisome proliferator-activated receptor- γ ; RAAS: Renin-angiotensin-aldosterone system; TZD: Thiazolidinedione.

Polymorphisms of the cholesteryl ester transfer protein (CETP) enzyme, which exchanges triglycerides and cholesteryl esters between different lipoproteins and takes part in the metabolism of high-density lipoprotein (HDL), also seem to confer a differential response to statin treatment. Diabetes mellitus carriers of the *B1* allele, which is found in strong linkage disequilibrium with the *A269C* polymorphism of the promoter of *CETP*, presented with lower baseline HDL levels and higher baseline triglyceride levels, but showed better HDL and triglyceride responses to atorvastatin therapy [5]. Clinically, statin treatment was most beneficial to carriers of the *B1* allele, in terms of attenuating the progression of coronary atherosclerosis [6].

Thiazolidinediones

The family of thiazolidinediones (TZDs), which includes rosiglitazone and pioglitazone, act as

agonists of the peroxisome proliferator-activated receptor- γ (PPAR- γ). PPAR- γ is an important transcription factor that modulates the expression of several genes involved in glucose and fat metabolism, and is also involved in adipogenesis, adipocyte differentiation and cytokine production by adipocytes. TZDs were shown to have many therapeutic effects in diabetics and prediabetics, amongst which are insulin sensitization, reduction of fasting glucose and glycated hemoglobin A_{1c} (HbA_{1c}) levels, anti-inflammatory and anti-thrombotic effects and improvement of lipid profile [7]. However, studies have shown that a high percentage of patients are nonresponsive to TZD therapy, leading to extensive research on the pharmacogenetics of these drugs.

The Pro12Ala nonsynonymous polymorphism of *PPAR- γ 2*, which is mainly expressed in adipose tissue, was extensively studied in relation

to the risk of diabetes mellitus and the response to TZD treatment in preventing the onset of diabetes mellitus. This allele was shown to have decreased transcriptional activity following activation by PPAR- γ agonists [8]. Although the 12Ala allele seems to be associated, in certain populations, with higher risk for Type 2 diabetes mellitus [9] and with prediabetic phenotypes [10], most trials have demonstrated that it does not modulate the response to TZD therapy [11–13].

Other polymorphisms suspected to affect the response to TZDs are listed in TABLE 1.

Antihypertensive treatment & the angiotensin-converting enzyme insertion/deletion polymorphism

The insertion/deletion (I/D) polymorphism of angiotensin-converting enzyme (ACE) seems to modify plasma ACE concentrations [14], the *D* allele correlating with higher concentrations of the enzyme. Studies concerning the ability of ACE inhibitors to prevent proteinuria and albuminuria, surrogate markers of diabetic nephropathy, have yielded conflicting results, the majority finding that the *D* allele is associated with decreased renal protection [15–18], but some claiming the opposite [19–21]. Acting directly on the angiotensin 1 receptor (AT1), angiotensin receptor blockers (ARBs) represent an alternative treatment for diabetic nephropathy that may not be influenced by the *ACE I/D* polymorphism [22,23].

Metformin & the organic cation transporter family

Metformin, a biguanide compound, is considered to be one of the most successful antidiabetic drugs due to its ability to increase insulin sensitivity and to decrease blood glucose levels via mechanisms that include decreasing gut absorption of glucose, decreasing glucose production by the liver and improving glucose utilization. Besides its hypoglycemic effects, metformin is also known to improve lipid metabolism and vascular function [24]. Although the precise molecular mechanism of metformin action is unknown, it is believed to activate AMPK [25]

in the liver and enhance tyrosine kinase activity of insulin receptors in skeletal muscle and fat tissue [26].

The pharmacokinetic properties of metformin are dependent on the organic cation transporter (OCT) family of proteins. OCT1 is integral for metformin uptake by the liver [27], probably accounting for normal distribution of the drug [28]. OCT2 mediates the secretion of metformin in the renal tubular system, a function that does not demand its extensive metabolism [26].

The *SLC22A1* gene, which encodes the OCT1 protein, and the *SLC22A2* gene, which encodes the OCT2 protein, have been shown to have numerous polymorphic loci. A study on different nonsynonymous polymorphisms of the *SLC22A1* gene demonstrated that seven of the 12 alleles known to exist in humans are associated with the production of an OCT1 protein that is either partially functional or nonfunctional, thus decreasing metformin influx into the cells. A common characteristic of all reduced or nonfunctional alleles for OCT is a decrease in metformin-induced AMPK activation. Healthy volunteers carrying these alleles do not respond to metformin in an oral glucose tolerance test [29]. Of note, these findings were contradicted in a small-scale retrospective study where OCT1 polymorphisms were found to only slightly modulate the response to metformin [30].

As expected from the expression patterns of the OCT1 and OCT2 proteins in different tissues, OCT1 reduced-activity alleles were associated with higher area under the curve (AUC) and C_{max} values, and lower oral values of distribution (V_d/F), probably stemming from the decreased hepatic uptake of metformin [31]. Activity of the different *SLC22A2* alleles was studied mostly using 1-methyl-4-phenylpyridinium (MPP⁺) [26]. While being a known substrate of the OCT family, MPP⁺ may not accurately reflect the influence of nonsynonymous polymorphisms on metformin transport, since some of the nonsynonymous polymorphisms of *SLC22A1* were associated with decreased metformin transport while maintaining normal

Table 1. Polymorphisms possibly affecting response to thiazolidinedione therapy.

Protein	Variant/polymorphism	Results	Ref.
Perilipin	11482G>A	11482A associated with decreased weight gain	[53]
Adiponectin	SNP45 T/G SNP276 G/T	45 G/G and 276 G/G associated with smaller reduction in fasting plasma glucose, HbA _{1c} and smaller increase in adiponectin	[54]
Lipoprotein lipase	S447X	S/S genotype is associated with greater reduction in fasting plasma glucose, but also with greater weight gain	[55]

HbA_{1c}: Glycated hemoglobin; SNP: Single nucleotide polymorphism.

MPP⁺ transport, and *vice versa* [26]. However, a recent study demonstrated the ability of three different *SLC22A2* alleles to decrease metformin transport in an *in vitro* model. Healthy carriers of these alleles were characterized by increased metformin AUC and C_{max}, and decreased renal clearance (Cl_{renal}), probably due to decreased secretion of the drug (Cl_{sec}) [31].

Although evidence for the possible modulation of response to metformin by *SLC22A1* and *SLC22A2* polymorphisms is mounting, clinical studies showing differential response of Type 2 diabetics to metformin that correlates to OCT1/OCT2 activity have not yet been reported.

Vitamin E & haptoglobin

Haptoglobin (Hp), a liver-produced plasma glycoprotein, is mostly known for its ability to sequester free hemoglobin (Hb), once the latter is released from red blood cells [32]. Extracorporeal Hb can act as a biological Fenton reagent by releasing heme iron, initiating production of highly reactive oxygen species (ROS) and causing oxidative damage to surrounding tissue [33]. However, Hp binding to Hb prevents ROS production by Hb, thereby decreasing the oxidative potential of Hb [34,35]. The concentration of Hp in the blood is much higher than that of free Hb, the molar ratio reaching 400:1, thus allowing rapid scavenging of free Hb [36]. The Hp-Hb complex is cleared both from the intravascular and extravascular compartment by the CD163 Hp-Hb receptor expressed on monocytes/macrophages [37].

■ Hp genotype & relation to oxidative stress: *in vitro* studies

The *Hp* gene is located at chromosomal locus 16q22, and has two common alleles, *Hp1* and *Hp2*, the prevalence of the *Hp1* allele being 0.4 and that of the *Hp2* allele being 0.6. In most Western populations, the prevalence of the *Hp 1-1* genotype is 16%, *Hp 2-2* is 36% and *Hp 2-1* is 48% [32]. The *Hp2* allele represents a duplication of exons 3 and 4 of the *Hp1* allele,

which probably occurred early in human evolution. This duplication is responsible for the differences in shape and structure of the Hp proteins found in individuals with the three different *Hp* genotypes. Individuals with the *Hp 2-2* genotype have cyclic multimeric Hp, those with the *Hp1-1* genotype have linear dimeric Hp, while in *Hp 2-1* genotype individuals, heteromeric linear polymers of Hp are found [38].

The different *Hp* genotypes confer different degrees of protection from Hb-mediated oxidation. *Hp 1-1* acts as a more potent antioxidant than *Hp 2-2* [35], not as a result of different binding affinities to Hb, but rather due to the increased ability of the Hp 1-1 complex to shield heme iron contained in the Hb molecule from its aqueous surrounding. The higher accessibility of iron within the Hp 2-2-Hb complex compared with the Hp 1-1-Hb complex leads to increased production of ROS. Oxidative modification or glycosylation of Hb, which occurs in diabetes mellitus, further increases the difference in the accessibility of heme iron in the Hp complexes [39]. Oxidative stress and hyperglycemic conditions are also known to downregulate the expression of the CD163 receptor on macrophages, either by shedding of the receptor or by decreasing mRNA transcription [40,41]. Furthermore, the Hb-Hp 2-2 clearance rate by CD163 is slower than for Hb-Hp 1-1 [42], resulting in prolonged presence of the redox active Hp 2-2-Hb complex in both the intravascular and extravascular compartment in Hp 2-2 individuals.

Another novel proatherogenic effect of the impaired Hb-Hp clearance in Hp 2-2 diabetes mellitus individuals has recently been elucidated. Hp can compete with lecithin cholesterol acyltransferase (LCAT) for the binding site on ApoA1, thus decreasing the activity of LCAT and preventing HDL maturation [43]. Furthermore, when bound to HDL, the Hb-Hp complex may oxidize, via its redox active heme iron, HDL lipids and proteins, compromising HDL functions [44].

Table 2. Risk of cardiovascular disease conferred by the *Hp 2-2* genotype.

Study	Outcome	Ref.
Strong Heart Study	Three- to five-fold increased CVD in <i>Hp 2-2</i> vs non- <i>Hp 2-2</i>	[56]
Rambam	Eightfold increased death or CHF following MI in <i>Hp 2-2</i> vs <i>Hp 1-1</i>	[57]
Munich	Twofold increased MI in <i>Hp 2-2</i> vs non- <i>Hp 2-2</i>	[58]
EDC	Twofold increased CAD in <i>Hp 2-2</i> vs <i>Hp 1-1</i> ; intermediate risk in <i>Hp 2-1</i>	[59]
ICARE	Twofold increased MI, stroke or cardiovascular death in <i>Hp 2-2</i> vs non- <i>Hp 2-2</i>	[51]

CAD: Coronary artery disease; CHF: Congestive heart failure; CVD: Cardiovascular disease; EDC: Pittsburgh Epidemiology of Diabetes Complications Study; ICARE: Israel Cardiovascular Events Reduction with Vitamin E Study; MI: Myocardial infarction.

■ Effect of Hp genotype on oxidative stress *in vivo*

The *in vitro* studies described above have also been examined *in vivo*. In the settings of diabetes mellitus, levels of redox active heme-iron are increased in Hp 2-2 compared with Hp 1-1 mice and humans [1]. Similarly, mouse peritoneal macrophages (MPMs) isolated from Hp 2-2 diabetes mellitus mice present with greater oxidative stress than those isolated from Hp 1-1 diabetes mellitus mice [39]. Humans and mice with the Hp 2-2 genotype and diabetes mellitus have increased levels of redox active iron in their blood and increased levels of redox active iron deposited in various tissues [39]. In humans, Hp 2-2 individuals have lower vitamin C levels as compared with Hp 1-1 individuals [38]. HDL of both Hp 2-2 mice and Hp 2-2 humans suffering from diabetes mellitus was shown to contain higher levels of lipid peroxides compared with Hp 1-1 diabetes mellitus individuals [44]. Additionally, cholesterol efflux from injected ³H-cholesterol-loaded macrophages was reduced in Hp 2-2 mice [45].

■ Translation of the increased oxidative stress to increased morbidity & mortality in mice & humans with the Hp 2-2 genotype & diabetes mellitus

Hp 2-2 diabetes mellitus mice have been shown to be at a higher risk for developing nephropathy [46], retinopathy [47] and atherosclerosis [45,48] compared with Hp 1-1 diabetes mellitus mice. Occlusion of the left anterior descending artery resulted in a larger infarct in Hp 2-2 mice, and following myocardial ischemia and reperfusion, levels of lipid oxidation products are increased in Hp 2-2 mice [49].

In humans, several longitudinal studies from distinct ethnic groups and geographic areas comprising over 30,000 individuals have clearly identified Hp genotype as a risk factor for the development of cardiovascular diseases in the presence of diabetes mellitus. Individuals with the Hp 2-2 genotype appear to have a two- to five-fold increase in the risk of CVD (see TABLE 2).

■ Vitamin E & the Hp 2-2 genotype

Vitamin E is a group of eight lipophilic antioxidants, which are produced only in plants. Being lipophilic molecules, these antioxidants are localized mostly to hydrophobic environments, such as cell membranes and lipoproteins where they can easily scavenge lipid peroxides, thus attenuating the oxidation cascade and

preventing tissue damage. It has been previously demonstrated that levels of vitamin E, along with several other antioxidants, are decreased in diabetes mellitus [50].

While it was expected that vitamin E supplementation to diabetics would be beneficial in terms of decreasing cardiovascular complications by decreasing the oxidative stress that is associated with the disease, several meta-analyses have demonstrated that the indiscriminate use of vitamin E is not associated with any discernible clinical benefit. However, among Hp 2-2 diabetes mellitus participants, vitamin E supplementation has recently been shown to significantly decrease the risk for CVD [51,52]. The ability of vitamin E to improve HDL function and reduce the levels of HDL-associated lipid peroxides among Hp 2-2, but not among Hp 1-1 diabetes mellitus individuals [44], provides a plausible explanation for this pharmacogenetic relationship between the Hp genotype and vitamin E supplementation.

Future perspective

Genetic factors clearly influence drug responsiveness. Pharmacogenomics is the cornerstone of personalized medicine and promises to reduce healthcare costs and improve standards. However, the promise of pharmacogenomics has yet to be realized. One reason for this lack of success in finding genetic markers that consistently and reliably predict responsiveness to drug therapy is the nature of the genetic markers that have been used in studies performed to date. Nearly all pharmacogenomic studies have searched for an association between a single nucleotide polymorphism that is only indirectly linked to a disease pathway via linkage and responsiveness to a drug. However, population stratification may limit the ability to extrapolate from one population to another based on such genetic markers, and explains why many apparent pharmacogenomic interactions are frequently not confirmed in populations from distinct ethnic groups or geographic areas. The Hp polymorphism described here does not suffer from this pitfall, as the gene itself is directly linked to the pathophysiology of the disease, and the drug therapy (vitamin E) directly intervenes on the allele-disease interaction. Finally, it is critical that any pharmacogenomic interaction that is first observed in a retrospective analysis of a clinical trial be confirmed in a prospective study, due to concerns for potential bias in patient ascertainment and in survivorship bias. Another aspect of pharmacogenomic studies that

should be taken into account is that a patient may carry several polymorphisms. In the face of this possibility, it is important to understand not only the effect of isolated polymorphisms, but the interaction between them as well. Pharmacogenomics is clearly the key for optimized treatment and prevention of diabetes mellitus-related complications, but further research is needed to fulfill its potential.

Financial & competing interests disclosure

Dr Levy is a consultant for Synviva Therapeutics. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Pharmacogenetics of statin therapy

- There are numerous candidate genes.
- ApoE polymorphisms were extensively studied, yielding tentative results.
- No long-term large-scale clinical trials have been undertaken to date.

Pharmacogenetics of thiazolidinediones therapy

- There are numerous candidate genes.
- The Pro12Ala polymorphism, which has been demonstrated to decrease the risk of diabetes mellitus, does not modulate response to therapy.
- Other candidate genes were shown to have an effect only in small-scale trials.

Pharmacogenetics of ACE inhibitors & ARBs

- Represents how pharmacogenetics may assist in choosing adequate therapy.
- The effect of the angiotensin-converting enzyme (ACE) DD polymorphism on response to ACE inhibitors is debated.
- The effect of the ACE DD polymorphism on response to angiotensin receptor blocker (ARB) treatment awaits large-scale trials.

Pharmacogenetics of metformin

- The pharmacogenetics of metformin are based on the organic cation transporter (OCT) family that governs metformin distribution and clearance.
- OCT1 polymorphisms have been implicated in both responsiveness to treatment and pharmacokinetics.
- OCT2 polymorphisms have been implicated in pharmacokinetics.
- There are currently no large-scale clinical trials showing either short-term or long-term efficiency.

Pharmacogenetics of haptoglobin

- The pharmacogenetics of haptoglobin are based on the differences in antioxidant activity, which is higher in Hp 1-1 and Hp 2-1 genotypes compared with the Hp 2-2 genotype.
- The efficacy of vitamin E in Hp 2-2 individuals has been shown in the retrospective analysis of one trial and one prospective study.

Future perspective

- Functional polymorphisms with relevance to the pathophysiology of the disease are more likely to predict responsiveness to therapy in different populations.
- Large-scale clinical trials are needed to distinguish between significant and nonsignificant pharmacogenetic effects.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

- 1 Levy AP: Application of pharmacogenomics in the prevention of diabetic cardiovascular disease: mechanistic basis and clinical evidence for utilization of the haptoglobin genotype in determining benefit from antioxidant therapy. *Pharmacol. Ther.* 112, 501–512 (2006).
- 2 Donnelly LA, Palmer CN, Whitley AL *et al.*: Apolipoprotein E genotypes are associated with lipid-lowering responses to statin treatment in diabetes: a Go-DARTS study. *Pharmacogenet. Genomics* 18, 279–287 (2008).
- 3 Schmitz G, Langmann T: Pharmacogenomics of cholesterol-lowering therapy. *Vascul. Pharmacol.* 44, 75–89 (2006).
- 4 Comprehensive review of the pharmacogenetics of statin therapy.
- 4 Tavintharan S, Lim SC, Chan YH, Sum CF: Apolipoprotein E genotype affects the response to lipid-lowering therapy in Chinese patients with Type 2 diabetes mellitus. *Diabetes Obes. Metab.* 9, 81–86 (2007).
- 5 van Venrooij FV, Stolk RP, Banga JD *et al.*: Common cholesteryl ester transfer protein gene polymorphisms and the effect of atorvastatin therapy in Type 2 diabetes. *Diabetes Care* 26, 1216–1223 (2003).
- 6 Kuivenhoven JA, Jukema JW, Zwinderman AH *et al.*: The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. *N. Engl. J. Med.* 338, 86–93 (1998).
- 7 Aquilante CL: Pharmacogenetics of thiazolidinedione therapy. *Pharmacogenomics* 8, 917–931 (2007).
- 8 Gurnell M: PPAR γ and metabolism: insights from the study of human genetic variants. *Clin. Endocrinol. (Oxf)* 59, 267–277 (2003).
- 9 Ludovico O, Pellegrini F, Di Paola R *et al.*: Heterogeneous effect of peroxisome proliferator-activated receptor γ 2 Ala12 variant on Type 2 diabetes risk. *Obesity (Silver Spring)* 15, 1076–1081 (2007).
- 10 Tonjes A, Scholz M, Loeffler M, Stumvoll M: Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor γ with Pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals. *Diabetes Care* 29, 2489–2497 (2006).

- 11 Florez JC, Jablonski KA, Sun MW *et al.*: Effects of the Type 2 diabetes-associated PPAR γ P12A polymorphism on progression to diabetes and response to troglitazone. *J. Clin. Endocrinol. Metab.* 92, 1502–1509 (2007).
- 12 Blüher M, Lubben G, Paschke R: Analysis of the relationship between the Pro12Ala variant in the PPAR- γ 2 gene and the response rate to therapy with pioglitazone in patients with Type 2 diabetes. *Diabetes Care* 26, 825–831 (2003).
- 13 Snitker S, Watanabe RM, Ani I *et al.*: Changes in insulin sensitivity in response to troglitazone do not differ between subjects with and without the common, functional Pro12Ala peroxisome proliferator-activated receptor- γ 2 gene variant: results from the Troglitazone in Prevention of Diabetes (TRIPOD) study. *Diabetes Care* 27, 1365–1368 (2004).
- 14 Winkelmann BR, Nauck M, Klein B *et al.*: Deletion polymorphism of the angiotensin I-converting enzyme gene is associated with increased plasma angiotensin-converting enzyme activity but not with increased risk for myocardial infarction and coronary artery disease. *Ann. Intern. Med.* 125, 19–25 (1996).
- 15 van Essen GG, Rensma PL, de Zeeuw D *et al.*: Association between angiotensin-converting-enzyme gene polymorphism and failure of renoprotective therapy. *Lancet* 347, 94–95 (1996).
- 16 Parving HH, Jacobsen P, Tarnow L *et al.*: Effect of deletion polymorphism of angiotensin converting enzyme gene on progression of diabetic nephropathy during inhibition of angiotensin converting enzyme: observational follow up study. *BMJ* 313, 591–594 (1996).
- 17 Penno G, Chaturvedi N, Talmud PJ *et al.*: Effect of angiotensin-converting enzyme (ACE) gene polymorphism on progression of renal disease and the influence of ACE inhibition in IDDM patients: findings from the EUCLID Randomized Controlled Trial. EURODIAB Controlled Trial of Lisinopril in IDDM. *Diabetes* 47, 1507–1511 (1998).
- 18 Jacobsen P, Rossing K, Rossing P *et al.*: Angiotensin converting enzyme gene polymorphism and ACE inhibition in diabetic nephropathy. *Kidney Int.* 53, 1002–1006 (1998).
- 19 Yoshida H, Mitarai T, Kawamura T *et al.*: Role of the deletion of polymorphism of the angiotensin converting enzyme gene in the progression and therapeutic responsiveness of IgA nephropathy. *J. Clin. Invest.* 96, 2162–2169 (1995).
- 20 van der Kleij FG, Navis GJ, Gansevoort RT *et al.*: ACE polymorphism does not determine short-term renal response to ACE-inhibition in proteinuric patients. *Nephrol. Dial. Transplant.* 12(Suppl. 2), 42–46 (1997).
- 21 Ha SK, Yong Lee S, Su Park H *et al.*: ACE DD genotype is more susceptible than ACE II and ID genotypes to the antiproteinuric effect of ACE inhibitors in patients with proteinuric non-insulin-dependent diabetes mellitus. *Nephrol. Dial. Transplant.* 15, 1617–1623 (2000).
- 22 Andersen S, Tarnow L, Cambien F *et al.*: Long-term renoprotective effects of losartan in diabetic nephropathy: interaction with ACE insertion/deletion genotype? *Diabetes Care* 26, 1501–1506 (2003).
- **First trial to show that the pharmacogenetics of angiotensin receptor blockers differs from that of angiotensin-converting enzyme inhibitors.**
- 23 Andersen S, Tarnow L, Cambien F *et al.*: Renoprotective effects of losartan in diabetic nephropathy: interaction with ACE insertion/deletion genotype? *Kidney. Int.* 62, 192–198 (2002).
- 24 Kirpichnikov D, McFarlane SI, Sowers JR: Metformin: an update. *Ann. Intern. Med.* 137, 25–33 (2002).
- 25 Zhou G, Myers R, Li Y *et al.*: Role of AMP-activated protein kinase in mechanism of metformin action. *J. Clin. Invest.* 108, 1167–1174 (2001).
- 26 Takane H, Shikata E, Otsubo K, Higuchi S, Ieiri I: Polymorphism in human organic cation transporters and metformin action. *Pharmacogenomics* 9, 415–422 (2008).
- 27 Wang DS, Jonker JW, Kato Y, Kusahara H, Schinkel AH, Sugiyama Y: Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *J. Pharmacol. Exp. Ther.* 302, 510–515 (2002).
- 28 Shu Y, Brown C, Castro RA *et al.*: Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. *Clin. Pharmacol. Ther.* 83, 273–280 (2008).
- 29 Shu Y, Sheardown SA, Brown C *et al.*: Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J. Clin. Invest.* 117, 1422–1431 (2007).
- **Shows the potential pharmacogenetic relationship between metformin and OCT1.**
- 30 Shikata E, Yamamoto R, Takane H *et al.*: Human organic cation transporter (OCT1 and OCT2) gene polymorphisms and therapeutic effects of metformin. *J. Hum. Genet.* 52, 117–122 (2007).
- 31 Song IS, Shin HJ, Shim EJ *et al.*: Genetic variants of the organic cation transporter 2 influence the disposition of metformin. *Clin. Pharmacol. Ther.* 84, 559–562 (2008).
- **Shows the effect of OCT2 on the pharmacokinetics of metformin and not 1-methyl-4-phenylpyridinium (MPP⁺).**
- 32 Bowman BH, Kurosky A: Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. *Adv. Hum. Genet.* 12, 189–261, 453–454 (1982).
- 33 Sadrzadeh SM, Graf E, Panter SS, Hallaway PE, Eaton JW: Hemoglobin. A biologic fenton reagent. *J. Biol. Chem.* 259, 14354–14356 (1984).
- 34 Miller YI, Altamentova SM, Shaklai N: Oxidation of low-density lipoprotein by hemoglobin stems from a heme-initiated globin radical: antioxidant role of haptoglobin. *Biochemistry* 36, 12189–12198 (1997).
- 35 Melamed-Frank M, Lache O, Enav BI *et al.*: Structure-function analysis of the antioxidant properties of haptoglobin. *Blood* 98, 3693–3698 (2001).
- 36 Garby L, Noyes WD: Studies on hemoglobin metabolism. I. The kinetic properties of the plasma hemoglobin pool in normal man. *J. Clin. Invest.* 38, 1479–1483 (1959).
- 37 Kristiansen M, Graversen JH, Jacobsen C *et al.*: Identification of the haemoglobin scavenger receptor. *Nature* 409, 198–201 (2001).
- 38 Langlois MR, Delanghe JR: Biological and clinical significance of haptoglobin polymorphism in humans. *Clin. Chem.* 42, 1589–1600 (1996).
- 39 Asleh R, Guetta J, Kalet-Litman S, Miller-Lotan R, Levy AP: Haptoglobin genotype- and diabetes-dependent differences in iron-mediated oxidative stress *in vitro* and *in vivo*. *Circ. Res.* 96, 435–441 (2005).
- 40 Levy AP, Purushothaman KR, Levy NS *et al.*: Downregulation of the hemoglobin scavenger receptor in individuals with diabetes and the Hp 2–2 genotype: implications for the response to intraplaque hemorrhage and plaque vulnerability. *Circ. Res.* 101, 106–110 (2007).
- 41 Timmermann M, Hogger P: Oxidative stress and 8-iso-prostaglandin F(2 α) induce ectodomain shedding of CD163 and release of tumor necrosis factor- α from human monocytes. *Free Radic. Biol. Med.* 39, 98–107 (2005).
- 42 Asleh R, Marsh S, Shilkrut M *et al.*: Genetically determined heterogeneity in hemoglobin scavenging and susceptibility to diabetic cardiovascular disease. *Circ. Res.* 92, 1193–1200 (2003).

- 43 Spagnuolo MS, Cigliano L, D'Andrea LD, Pedone C, Abrescia P: Assignment of the binding site for haptoglobin on apolipoprotein A-I. *J. Biol. Chem.* 280, 1193–1198 (2005).
- 44 Asleh R, Blum S, Kalet-Litman S *et al.*: Correction of HDL dysfunction in individuals with diabetes and the haptoglobin 2–2 genotype. *Diabetes* 57, 2794–2800 (2008).
- **Demonstration of the ability of vitamin E to correct high-density lipoprotein dysfunction in Hp2–2 mice and humans.**
- 45 Asleh R, Miller-Lotan R, Aviram M *et al.*: Haptoglobin genotype is a regulator of reverse cholesterol transport in diabetes *in vitro* and *in vivo*. *Circ. Res.* 99, 1419–1425 (2006).
- 46 Miller-Lotan R, Herskowitz Y, Kalet-Litman S *et al.*: Increased renal hypertrophy in diabetic mice genetically modified at the haptoglobin locus. *Diabetes. Metab. Res. Rev.* 21, 332–337 (2005).
- 47 Miller-Lotan R, Miller B, Nakhoul F, Aronson D, Asaf R, Levy AP: Retinal capillary basement membrane thickness in diabetic mice genetically modified at the haptoglobin locus. *Diabetes. Metab. Res. Rev.* 23, 152–156 (2007).
- 48 Levy AP, Levy JE, Kalet-Litman S *et al.*: Haptoglobin genotype is a determinant of iron, lipid peroxidation, and macrophage accumulation in the atherosclerotic plaque. *Arterioscler. Thromb. Vasc. Biol.* 27, 134–140 (2007).
- 49 Blum S, Asaf R, Guetta J *et al.*: Haptoglobin genotype determines myocardial infarct size in diabetic mice. *J. Am. Coll. Cardiol.* 49, 82–87 (2007).
- 50 Ceriello A: Oxidative stress and diabetes-associated complications. *Endocr. Pract.* 12(Suppl. 1), 60–62 (2006).
- 51 Milman U, Blum S, Shapira C *et al.*: Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both Type 2 diabetes mellitus and the haptoglobin 2–2 genotype: a prospective double-blinded clinical trial. *Arterioscler. Thromb. Vasc. Biol.* 28, 341–347 (2008).
- **This clinical trial demonstrates the ability of vitamin E to reduce diabetes mellitus-associated cardiovascular disease among Hp 2–2 individuals.**
- 52 Levy AP, Gerstein HC, Miller-Lotan R *et al.*: The effect of vitamin E supplementation on cardiovascular risk in diabetic individuals with different haptoglobin phenotypes. *Diabetes Care* 27, 2767 (2004).
- 53 Kang ES, Cha BS, Kim HJ *et al.*: The 11482G>A polymorphism in the perilipin gene is associated with weight gain with rosiglitazone treatment in Type 2 diabetes. *Diabetes Care* 29, 1320–1324 (2006).
- 54 Kang ES, Park SY, Kim HJ *et al.*: The influence of adiponectin gene polymorphism on the rosiglitazone response in patients with Type 2 diabetes. *Diabetes Care* 28, 1139–1144 (2005).
- 55 Wang G, Wang X, Zhang Q, Ma Z: Response to pioglitazone treatment is associated with the lipoprotein lipase S447X variant in subjects with Type 2 diabetes mellitus. *Int. J. Clin. Pract.* 61, 552–557 (2007).
- 56 Levy AP, Hochberg I, Jablonski K *et al.*: Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: the Strong Heart Study. *J. Am. Coll. Cardiol.* 40, 1984–1990 (2002).
- 57 Suleiman M, Aronson D, Asleh R *et al.*: Haptoglobin polymorphism predicts 30-day mortality and heart failure in patients with diabetes and acute myocardial infarction. *Diabetes* 54, 2802–2806 (2005).
- 58 Roguin A, Koch W, Kastrati A, Aronson D, Schomig A, Levy AP: Haptoglobin genotype is predictive of major adverse cardiac events in the 1-year period after percutaneous transluminal coronary angioplasty in individuals with diabetes. *Diabetes Care* 26, 2628–2631 (2003).
- 59 Costacou T, Ferrell RE, Orchard TJ: Haptoglobin genotype: a determinant of cardiovascular complication risk in Type 1 diabetes. *Diabetes* 57, 1702–1706 (2008).