

## Pharmacogenomics and pharmacogenetics of thiazolidinediones: role in diabetes and cardiovascular risk factors

The most important goal in the treatment of patients with diabetes is to prevent the risk of cardiovascular disease (CVD), the first cause of mortality in these subjects. Thiazolidinediones (TZDs), a class of antidiabetic drugs, act as insulin sensitizers increasing insulin-dependent glucose disposal and reducing hepatic glucose output. TZDs including pioglitazone, rosiglitazone and troglitazone, by activating PPAR- $\gamma$  have shown pleiotropic effects in reducing vascular risk factors and atherosclerosis. However, troglitazone was removed from the market due to its hepatotoxicity, and rosiglitazone and pioglitazone both have particular warnings due to being associated with heart diseases. Specific genetic variations in genes involved in the pathways regulated by TZDs have demonstrated to modify the variability in treatment with these drugs, especially in their side effects. Therefore, pharmacogenomics and pharmacogenetics are an important tool in further understand intersubject variability *per se* but also to assess the therapeutic potential of such variability in drug individualization and therapeutic optimization.

**Keywords:** cardiovascular disease • diabetes • pharmacogenetics • pharmacogenomics • single nucleotide polymorphisms • thiazolidinediones • vascular risk factors

### Rosiglitazone, pioglitazone & troglitazone

Diabetes is a pandemic disease particularly in the industrialized countries. The major cause of death in these patients (>65%) are related to cardiovascular disease (CVD). Therefore, given the significance of morbidity and mortality associated with diabetes, and its complications, identification and optimization of an appropriate treatment of the pathological condition is crucial. Over the last few decades, there have been a significant number of new agents developed for the treatment of Type 2 diabetes (T2D) with the final goal to regulate the blood level of glucose and therefore to prevent the risk for CVD. These drugs include sulfonylureas, biguanides,  $\alpha$ -glucosidase inhibitors and dipeptidyl peptidase IV inhibitors (DPP-IV) [1]. Among those, thiazolidinediones (TZDs) are a class of antidiabetic drugs that act as insulin sensitizers increasing insulin-dependent glucose

disposal, and reducing hepatic glucose output. Importantly, TZDs have been demonstrated to reduce insulin resistance not only in a state of T2D but also in nondiabetic conditions such as obesity, which is another important risk factor for CVD [2,3]. Further, it has been demonstrated that TZDs have a capacity to lower glycated hemoglobin (A1c) by approximately 0.5–1.4% [4]. Since their ability in decreasing hyperglycemia and their pleiotropic effects in preventing vascular diseases is substantial, the TZDs have received particular attention of the scientific community and are discussed below in the present review.

To prepare the present manuscript, three investigators sequentially selected the articles and evaluated study quality independently. Selected articles were searched on the PubMed, and Cochrane electronic databases. The search was limited to studies published in English. The principal key words used for the

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research were: pharmacogenetics, pharmacogenomics, genetics, single nucleotide polymorphisms, genetics, diabetes, metabolic syndrome, cardiovascular disease, thiazolidinediones (rosiglitazone, pioglitazone and troglitazone), side effects, vascular risk factors, atherosclerosis, human studies, animal studies and obesity, in combination between them. We excluded abstracts presented to conferences, case reports and case series articles.

As seen with the majority of other drugs, interindividual variability in response to the treatment with TZDs has been reported to be pronounced. The observed variability defines the importance of investigating the impact of individual genetic makeup on the pharmacological response to these drugs, especially to their side effects [5]. The three drugs in this hypoglycemic class introduced in the market are rosiglitazone, pioglitazone and troglitazone. Troglitazone was removed from the US market in 2000 due to its hepatotoxicity. However, besides troglitazone also rosiglitazone and pioglitazone both have particular warnings due to being associated with congestive heart failure [6]. Recently, rosiglitazone is being withdrawn in Europe and in New Zealand due to the increase of myocardial infarction [7] and bone fractures incidence [8], while it still used in US together with metformin. However, according to the American Heart Association/American Diabetes Association consensus statement on TZDs use, their beneficial effects on vascular risk factors support their prescription to diabetic subjects at high risk for CVD [9].

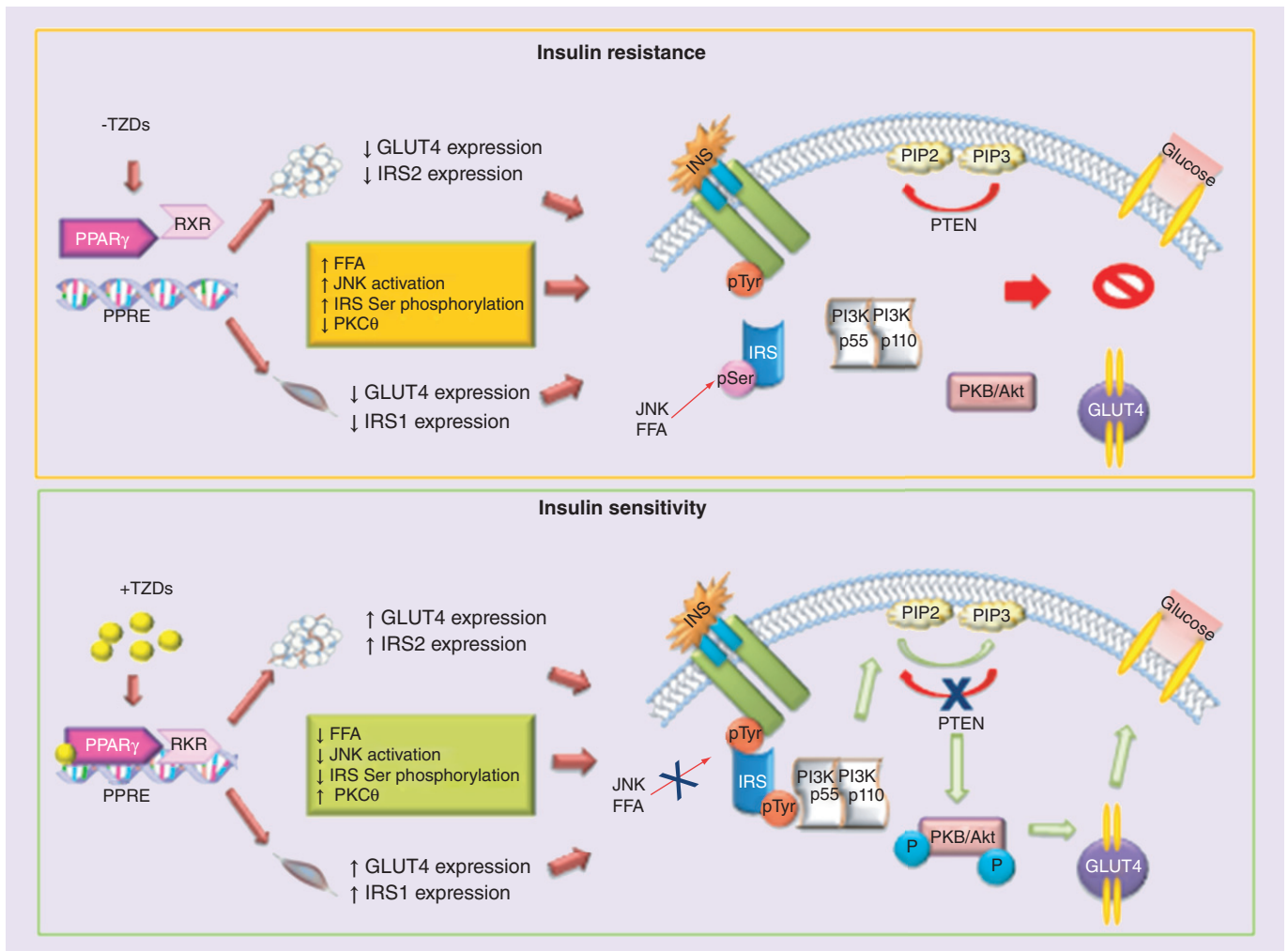
Unfortunately, it remains unclear whether side effects such as hepatotoxicity, myocardial infarct and heart failure are related to the characteristic of these drugs as the unique tocopherol side chain of troglitazone, or are related with other unknown mechanisms [10]. Therefore, the argument has been debated in scientific community. Several clinical trials such as the PROactive Study [11], and further meta-analyses [12] clearly established that therapy with TZDs reduces mortality for cardiovascular events, especially myocardial infarction and stroke. However, although these studies highlighted the positive effects of TZDs against both hyperglycemia and CVD, other recent studies raised concerns on possible augmented incidence of important diseases such as bladder tumors after long use of these drugs, especially in certain individuals [13]. For this reason, the field of pharmacogenetics and pharmacogenomics may improve the understanding in the beneficial properties of TZDs, and the management of CVD risk prevention in diabetic patients.

### Mechanism of action: role of PPARs & PPAR- $\gamma$

TZDs act mainly through the PPAR- $\gamma$  [3,14]. The PPAR- $\gamma$  is a member of the PPAR nuclear receptor

superfamily of ligand-inducible transcription factors. In mammals, there are three PPARs: PPAR $\alpha$ , PPAR $\beta/\delta$  and PPAR- $\gamma$  [15]. By binding to PPAR responsive regulatory elements of a target gene (PPRE, a direct repeat of 'AGGTCA' gapped by a nucleotide) as obligate heterodimers with RXR, activated by the endogenous agonist 9-cis retinoic acid, the PPARs control the expression of genetic networks involved in adipogenesis, lipid metabolism, inflammation, and maintenance of metabolic homeostasis [15]. Ligand binding as TZDs induces a conformational change in the receptor that allows for differential recruitment of cofactors and subsequent modulation of PPAR- $\gamma$  activity [15]. In absence of a ligand, the PPAR- $\gamma$ :RXR complex can recruit corepressor complexes and bind to PPRE, suppressing the transcription of target genes [15]. Adipose tissue is the major mediator of PPAR- $\gamma$  action on insulin sensitivity. PPAR- $\gamma$  activation in mature adipocytes induces the expression of a number of genes involved in the insulin signaling cascade such as *GLUT4* and *CAP*, thereby improving insulin sensitivity in patients with T2D [16]. Although no complete PPRE has been found in the *GLUT4* promoter, PPAR- $\gamma$  and its heterodimers partner RXR have been found to bind and repress the promoter activity of *GLUT4*. The repression is augmented in the presence of the natural ligand, 15D-prostaglandin J<sub>2</sub>, but completely alleviated by rosiglitazone [17]. This may be a novel mechanism by which a PPAR- $\gamma$  ligand exerts antidiabetic effect by detaching the PPAR- $\gamma$  transcription complex from the promoter, thereby increasing the expression of target genes. PPAR- $\gamma$  agonists potentiate insulin signaling and improve insulin sensitivity at various cellular steps [18], mainly by activation of PI3K, PIP3, and serine/threonine kinases, including Akt (Akt1 and 2) pathway [18]. In Figure 1 is schematically reported the mechanism by which TZDs induce insulin sensitivity. Treatment with troglitazone increases insulin-stimulated IRS-1-associated PI3K and Akt activity in skeletal muscle biopsies from T2D patients, and enhanced Akt phosphorylation in skeletal muscle from glucose-tolerant, insulin-resistant, first-degree relatives of T2D patients [19]. Moreover, PPAR- $\gamma$  activation controls the expression of numerous factors secreted from adipose tissue that greatly influence insulin sensitivity: levels of circulating adipocytokines or adipokines, TNF- $\alpha$ , leptin, resistin, 11 beta-hydroxysteroid dehydrogenase 1 and adiponectin [2,19].

Pleiotropic beneficial effects of TZDs as PPAR- $\gamma$  agonists have been also demonstrated in the vasculature and against vascular risk factors [15]. PPAR- $\gamma$  agonists have been shown to lower blood pressure (BP) in animals and humans by suppressing the renin-angiotensin (Ang)-aldosterone system (RAAS) includ-



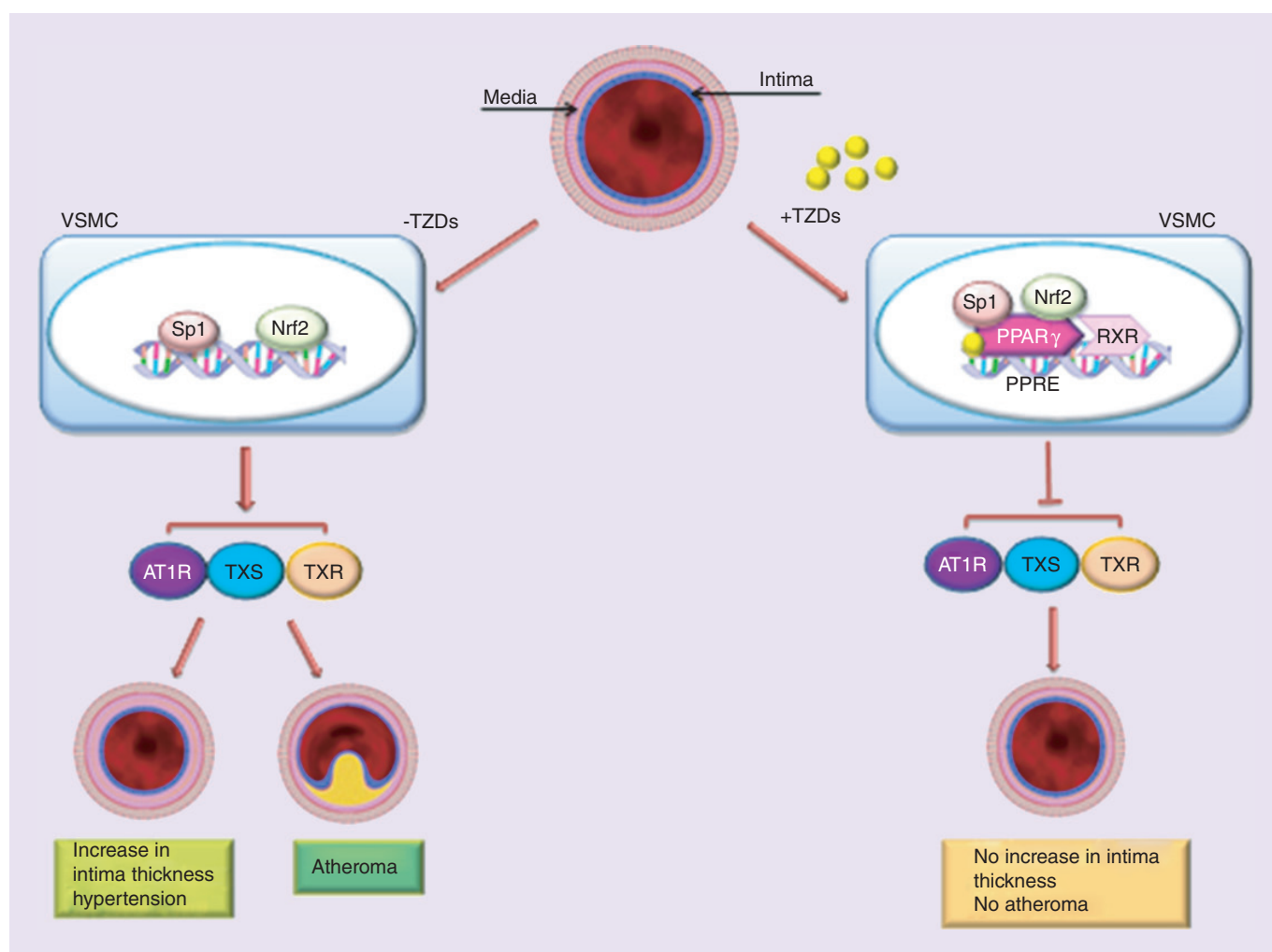
**Figure 1. Schematic representation of thiazolidinediones mechanism of action.** TZDs acting mainly through PPAR- $\gamma$  by binding to PPRE as obligate heterodimers with RXR, control the expression of several genetic networks. In particular, TZDs increase insulin-stimulated IRS-1/2-associated PI3K and Akt activity in skeletal muscle and adipose tissue, enhancing Akt phosphorylation and subsequently GLUT4 translocation.

FFA: Free fatty acids; Ins: Insulin; pTyr: phospho tyrosine; TZD: Thiazolidinedione.

ing the inhibition of *AT1R* expression, Ang-II-mediated signaling pathways and Ang-II-induced adrenal aldosterone synthesis/secretion [20]. PPAR- $\gamma$  agonists also inhibit the progression of atherosclerosis in animals through a pathway involving the suppression of RAAS and the thromboxane A2 system [20]. Interestingly, TZDs treatment, dose-dependently decrease the expression of *AT1R* gene [21]. Recently, it was also shown that PPAR- $\gamma$  agonists exert an inhibitory effect on Ang-II-induced aldosterone synthase expression and aldosterone secretion [22]. Moreover, pharmacological activation of PPAR- $\gamma$  receptors is noted to suppress the activity of TXS and its receptor TXR expression. These biochemical changes are noted to be involved in the development of atherosclerosis [23,24]. TZDs positively affect the vasculature by decreasing the intimal medial thickness (IMT) and inhibiting

the transendothelial migration of monocytes in the vascular smooth muscle cells (VSMC) [25]. Figure 2 represents the protective mechanisms of TZDs against increase of IMT and vascular atherosclerosis.

Another mechanism linking TZDs with prevention of atherosclerosis is their ability, as PPAR- $\gamma$  agonists, to decrease serum levels of oxLDL-cholesterol and triglycerides and increased serum levels of HDL-cholesterol, especially in T2D [26]. Increase in oxLDL has been identified as one of the main risk factor for atherosclerosis and CVD [27]. Specifically, among the TZDs, the most powerful in controlling the blood lipid profile has been shown to be pioglitazone, which compared with rosiglitazone strongly reduce the level of oxLDL [26]. Supporting these findings, an *in vitro* study conducted in human umbilical vein endothelial cells demonstrated as pioglitazone inhibited inflam-



**Figure 2. Thiazolidinediones action on vasculature.** TZDs positively affect the vasculature by decreasing the intimal medial thickness and development of atherosclerosis in the vascular smooth muscle cells. TZDs exert their protective role by inhibiting the gene expression of AT1R, TXS and TXR, involved in mechanism linked with intimal medial thickness and atherosclerosis. TZD: Thiazolidinedione; VSMC: Vascular smooth muscle cell.

matory response which inducing atherosclerosis by the increase of oxLDL [28].

In fact, TZDs prevent atherosclerosis also by decreasing oxidative stress and inflammation [29], which are under control of previously discussed pathways. After an acute insult like ischemia, administration of TZDs significantly increases the production of antioxidant enzymes such as catalase and SOD, increasing free radical scavenging in the periinfarct area [30]. Pretreatment of rats with pioglitazone for 7 days prior to coronary ligation significantly decreased the expression levels of inflammatory markers and the number of infiltrating macrophages in the ischemic region [31].

Animal models have clearly demonstrated that TZD by PPAR- $\gamma$  activation improve insulin release by preserving pancreatic  $\beta$ -cell function, and reduce vascular risk factors [32]. However, these findings have not been clearly established in humans [2]. Confirming these

findings, by using a quantitative real-time polymerase chain reaction (rt-PCR) Wang *et al.* [33] evaluated gene expression profiling to assess cardiorenal safety in the heart and kidney tissue samples in adult male myocardial infarction-induced heart failure and Zucker diabetic fatty rats treated with rosiglitazone. Although variations in the expression of some genes such as *ACE*, *PPAR- $\gamma$* , *IL-6* and *TNF- $\alpha$*  were observed, authors themselves address serious doubts about the transferability of these results obtained in animal models to clinical settings [34].

### Pharmacogenomics & pharmacogenetics of rosiglitazone in diabetes

The antidiabetic effect of rosiglitazone ((RS)-5-[4-(2-[methyl (pyridin-2-yl) amino] ethoxy) benzyl] thiazolidine-2, 4-dione) is associated with reduction in blood glucose level mainly by its pleiotropic effects in adipose

tissue [35]. It is also noted to modulate adipogenesis and affect regulation of the expression of adipocyte factors: TNF- $\alpha$ , IL-6, adiponectin and resistin [36]. Reports on such adipocyte factor genes have revealed that SNPs in such genetic elements are principally responsible for the interindividual and interethnic variability of the hypoglycemic efficacy of rosiglitazone [37]. The relevance of the genetic background is also amply demonstrated by studies in animal models. Using new mouse models of T2D, Pan *et al.* [38,39], demonstrated that the genetic background can affect adverse hepatic and cardiac response to rosiglitazone. Differential pharmacogenetics responses in the same new experimental model were identified by Leiter *et al.* [40]. Genetic variation in drug-metabolizing protein (CYP) genes: *CYP2C8* (*CYP2C8\*3*) and *CYP2C9* (*CYP2C9\*3*), is demonstrated to substantially contribute towards intersubject heterogeneity in terms of the pharmacokinetic behavior of rosiglitazone [41,42]. Clinical data from the Chinese diabetic population has shown that the glucose-lowering effects of rosiglitazone vary in subpopulations representing different *ADIPOQ* gene polymorphisms [43]. Among T2D patients treated with rosiglitazone, subjects possessing the SNP-11377 CC in the *ADIPOQ* responded with a greater reduction in fasting plasma glucose levels in comparison to the CG and GG genotypes [43]. Reports have shown that alleles of leptin G-2548A and TNF- $\alpha$  G-308A, important adipocytokines involved in mediating insulin sensitivity and glucose homeostasis, are linked with higher levels of insulin resistance in Chinese and Caucasian subjects suffering from T2D, respectively [44,45]. Liu *et al.* [37] observed that rosiglitazone showed a markedly increased effect in patients with the AA genotype of leptin G-2548A in terms of fasting serum insulin and postprandial serum insulin compared with the GG+GA genotype. Furthermore, they have shown a reduced efficacy in patients on rosiglitazone with GA+AA genotype of TNF- $\alpha$  G-308A when compared with its GG in terms of fasting serum insulin. Gene expression background-specific effects on lipid deposition and insulin sensitivity of rosiglitazone administration has also been demonstrated by studies in rodents [46]. Furthermore, Yu *et al.* [47] have shown that SNPs in *KCNQ1* are associated with the therapeutic response of rosiglitazone. Kang *et al.* [48,49] have shown that genetic variants in adiponectin are intrinsically associated with the modulation of pharmacodynamics of rosiglitazone in diabetic subjects from different populations. Besides evincing their hypoglycemic effects, TZDs also favorably modulate the lipid profile of blood, thus leading to a reduction in the progression of atherosclerosis and associated cardiovascular risk factors among diabetic patients [7,11]. Rosiglitazone treatment in populations represent-

ing *UCP2*-866G/A and *ADRB3* Trp64Arg genotypes substantially inhibit triglyceride, LDL-cholesterol and adiponectin levels in the blood [50]. Several other variants have been reported. Genetic variants of adipocytokines modulate the therapeutic efficacy of rosiglitazone in T2D patients [51]. In an earlier study Chen *et al.* [52] evaluated the glucose lowering effect of rosiglitazone in newly diagnosed Chinese T2D patients. They observed that rosiglitazone treated group with rs6467136 GA+AA variant of *PAX4* gene showed greater glucose lowering effects compared with GG homozygotes suggesting that rosiglitazone is more likely to achieve target fasting as well as 2-h glucose levels in GA+AA carriers compared with GG homozygotes. On the other hand another study investigating the effect of rosiglitazone in newly diagnosed T2D, observed that R219K variant of *ABCA1* gene had poor rosiglitazone response compared with RR homozygotes [53]. They also reported that neither M883I nor R1587K variant of the *ABCA1* gene had any effect of rosiglitazone response. Furthermore, the *UCP2* -866G/A genotype is known to regulate rosiglitazone-based control of glucose homeostasis [50], and the G allele of *UCP2*-866G/A gene is noted to enhance the risk of obesity among diabetic individuals [54]. The *ADRB3* Trp64Arg genotype is associated with rosiglitazone-related modulation of blood lipid profile [50]. Zhang *et al.* [55] observed that Thr394Thr and Gly482Ser SNPs in *PGC-1 $\alpha$*  genes alter the rosiglitazone-induced beneficial effect on plasma lipid profile in T2D subjects. Rosiglitazone also inhibits the pathogenesis of obesity by modulating mitochondrial remodeling [56]. In addition, rosiglitazone markedly ameliorates endothelial dysfunction associated with T2D [57]. However, investigations are required to validate the relevance of existing forms of genetic polymorphisms associated with rosiglitazone-induced glucose control in terms of quantifying its effect on diabetes-associated cardiovascular complications.

### Pharmacogenomics & pharmacogenetics of pioglitazone in diabetes

Pioglitazone ((RS)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxy] benzyl) thiazolidine-2, 4-dione) is one of the most widely used drugs for the treatment of T2D, and is the only TZDs available in the market in several countries [58]. However, a critical understanding of the clinical outcome associated with pioglitazone-based treatment of T2D and related cardiovascular complications has revealed that variability among subjects, rooted in environmental and physiological factors, is an important reason for the submaximal success of this drug [59]. Pharmacogenomics promises to delineate the mechanistic basis of the variability in pioglitazone efficacy. The principal biotransformation products of piogli-

tazone produced in various phases of drug metabolism are M-I, M-II, M-III, M-IV, M-V and M-VI [60]. M-III and M-IV are two metabolites documented to exert a rather sustained hypoglycemic effect and are thus accentuate the overall efficacy of pioglitazone *per se* [60]. CYP2C8 and CYP3A4 are the principal cytochrome P450 enzymes responsible for the breakdown of pioglitazone in the body into the above-mentioned metabolites [61]. Clinical data have shown that genetic polymorphisms associated with CYP2C8:\*1 homozygotes and the \*3 variant allele carriers, are responsible for variability in bioavailability of pioglitazone among various treatment populations [62,63]. Kadam *et al.* [64] have recently reported that oral clearance of pioglitazone is substantially higher in CYP2C8\*3 carriers and is one of the main factors for the observed variability in pharmacokinetic efficacy of pioglitazone. Moreover, pharmacological inhibition of CYP2C8 enhances bioavailability of pioglitazone in CYP2C8\*3 and CYP2C8\*1 carriers, thus underlining the clinical significance of pharmacogenomics in pioglitazone pharmacotherapy of diabetes [62,63].

Polymorphism in *PPAR-γ* encoding gene *PPARG* p.Pro12Ala (rs1801282) is an important genetic polymorphism established to play a critical role in mediating glucose metabolism [65]. *In vitro* experiments demonstrated that P12A mutation in *PPAR-γ2* gene is responsible for the variation in pioglitazone-induced attenuation of transcriptional activation of a reporter gene in a construct containing the PPRE [66]. This study also indicated that P12A mutation might also be linked to adipogenesis [66]. Furthermore, various research groups have shown that biological variability linked with the glucose-lowering effect of pioglitazone is also partly ascribed to the polymorphism associated with *PPARG* gene [67–69]. The *P12A* SNP rs17584499, located in intron 10, has been identified in Chinese populations as another important factor leading to pharmacodynamic variability in the glucose-lowering effect of pioglitazone [69]. A substantial alteration of pioglitazone-induced reduction in HbA1c was observed in *ADIPOQ* C-11377G genotype and *ADIPOR2* G795A genotype in Chinese and Iranian T2D populations [43,70]. *Retn* expression is induced by adipocyte differentiation and is inhibited by *PPAR-γ* agonists in isolated 3T3-L1 cell cultures [71]. *In vivo* studies demonstrated that systemic release of *retn* from adipocytes is substantially enhanced during diabetes in obese mice. *PPAR-γ* agonists like TZDs attenuate this increase in serum *retn* levels in laboratory animals as well as in clinical subjects. Mice overexpressing the *retn* in the liver have greater serum *retn* and insulin resistance, whereas *retn*<sup>(-/-)</sup> mice have reduced fasting blood glucose [72,73]. Therefore, it can be concluded that elevated serum *retn* levels cause insulin resistance in rodents. A study

in a Japanese population revealed that *retn* C-420G (rs1862513) polymorphism in the GG genotype, an adipokines known to antagonize insulin, is linked with a stronger glucose-lowering effect of pioglitazone [74].

In addition to the glucose-lowering effect, recent clinical studies report that pioglitazone prevent dyslipidemia-based progression of atherosclerotic macrovascular changes in the carotid and coronary arteries of diabetic subjects [7,11,75]. Certain animal studies have shown that pioglitazone attenuates myocardial ischemia/reperfusion injury in rodents via endothelial NOS-, COX-2- and ERK-1/2-dependent pathways [43,76–78]. A genetic study by Saitou *et al.* [79] has shown that the ameliorative potential of pioglitazone on diabetic aggravation of atherosclerosis is independent of the tested genotypes. Polymorphism (S447X) in the *LPL* gene influences the beneficial effects of pioglitazone on cardiovascular complications of diabetes mellitus [80]. Such SNP mediates pioglitazone-linked increase in the synthesis and secretion of *LPL* in multiple types of parenchymal cells [81,82]. Studies have further shown that the S447X variant is intrinsically associated with the pathogenesis of dyslipoproteinemia and coronary artery disease and thus markedly reduces the efficacy of pioglitazone in the *LPL* S447X genotype when compared with subjects with the S447S genotype [82].

### Pharmacogenomics & pharmacogenetics of troglitazone in diabetes

Troglitazone ((RS)-5-(4-[(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl) methoxy] benzyl) thiazolidine-2, 4-dione) was introduced for the first time on the pharmaceutical market in March 1997 as a novel therapy for the treatment of T2D [83]. After a short period of surveillance several cases of troglitazone-induced hepatotoxicity were reported [84]. Therefore, in 2000 the drug was abruptly removed from the market [83]. The symptoms observed in patients were related to an idiosyncratic hepatocellular injury-type liver toxicity determined by the specific metabolism of the troglitazone defined as ‘metabolic idiosyncrasy’ [85]. After removal of the drug from the market, a few studies have assessed whether specific genetic variants could predispose to the hepatotoxicity caused by troglitazone. Watanabe *et al.* [86], in a study performed on 25 Japanese T2D patients with abnormal increase in levels of ALT or AST during troglitazone treatment, analyzed 51 different genes related to drug metabolism, apoptosis, production and elimination of reactive oxygen species, including signal transduction pathways of *PPAR-γ2* and insulin. The genotyping analysis demonstrated that the presence of the combined *GSTT1* and *GSTM1* null genotype [87,88] was a

specific risk factor for the enhanced susceptibility to transaminase increase associated with troglitazone. Oniki *et al.* [89], in a subsequent study performed on 215 participants from the health screening program, reviewed the results of previous study suggesting that the combined *GSTM1* and *GSTT1* null genotypes might also be a risk factor for alcoholic mild liver dysfunction. Kumashiro *et al.* [90], in 2003 examined retrospectively the polymorphism of *CYP450* CYP2D6 and CYP2C19 in eight T2D patients with, and 31 patients without troglitazone-induced liver injury. The frequency of *CYP2D6* SNP was identical for both groups while homozygous or compound heterozygous polymorphisms of *CYP2C19* (2\*/2\*, 2\*/3\*, 3\*/3\*: poor metabolizers genotypes) were significantly more frequent in patients with liver injury ( $p=0.0401$ ).

In the TRIPOD study [91], performed on 93 Hispanic women with a history of gestational diabetes treated with troglitazone for 3 months, no correlation were found between *PPARG* common polymorphism P12A (rs1801282) and response to troglitazone, suggesting that PPAR- $\gamma$  is the main target of the TZDs but its genetic variants may even not modify the response to the therapy. To clarify this issue, Wolford *et al.* [92], screened by direct Sanger sequencing 40kb corresponding to all exons, introns and flanking regions of *PPARG* in 93 non diabetic women with previous gestational diabetes who had participated in the TRIPOD study. Among the 131 identified variants, 8 (not including P12A) were associated with the variation to troglitazone monotherapy response ( $p < 0.05$ ). A screening by Florez *et al.* [93], performed in a cohort of 3458 subjects from the DPP study did not find any association between P12A and troglitazone therapy, supporting previous findings from TRIPOD study. In addition the authors genotyped five of the eight SNPs reported in the Wolford study [92] without find any significant associations between these genetic loci and troglitazone 'responders' and troglitazone 'non-responders.' These controversial results have been extensively analyzed and some potential explanations have been found in the differences from the selected study populations especially in term of race-ethnicity, and in the different statistical methodologies employed in these studies. Moreover, it must be considered the different sensitivity among methods used to evaluate insulin levels (TRIPOD – direct measurement, DPP – indirect measurement) [94]. In Table 1 are reported some of the most important studies in this field.

Franks *et al.* [95], tested the impact of *PPARG* Pro-12Ala SNP on modification in the obesity-related traits in 3356 subjects included in the DPP study treated with metformin ( $n = 989$ ), troglitazone ( $n = 363$ ) or lifestyle modification ( $n = 1004$ ) ver-

sus placebo ( $n = 1000$ ). After 1 year of follow-up the results showed that in the metformin and lifestyle arms all individuals lost weight, especially those carrying the Ala12 allele ( $p = 0.01$ ), while in troglitazone arm subjects had trend to gain weight. In particular, this association between troglitazone and the weight gain was greater in the Ala12 allele carriers rather than Pro12 homozygotes. Moree *et al.* [96] performed a genetic wide association study in 3548 subjects at high risk of T2D enrolled in the DPP. One of the aims of the project was to evaluate the genetic predisposition in different respond to the troglitazone therapy. The results showed an improvement in the functionality of the pancreatic  $\beta$ -cell for patients carrying the protective genotype at *CDKN2A/B* SNP rs10811661 at 1 year of troglitazone treatment ( $p = 0.01$ ) and lifestyle modification ( $p = 0.05$ ). Majithia *et al.* [97], genotyped the missense SNP rs13266634 of the zinc transporter gene *SLC30A8* in 3007 participants from DPP and examined the association with fasting proinsulin and insulin at baseline and at 1 year postintervention. The results showed higher proinsulin levels at baseline for carriers of C allele. After 1 year, proinsulin levels decreased significantly in all groups receiving active intervention (lifestyle, metformin or troglitazone) compared with placebo, but no genotype- active intervention correlations were observed.

Muraglitazar, a nonthiazolidinedione/nonfibrate PPAR $\alpha/\gamma$  dual agonist, has been shown to present similar side effects of troglitazone. However, even if for these adverse effects this drug has never been introduced in the market, it could be of particular interest for future investigation in the field of pharmacogenomics. Muraglitazar completed different clinical trials where it significantly reduced serum triglycerides, glucose and A1c and increased HDL cholesterol levels [98–100]. In a further clinical trial conducted in 2005, Nissen *et al.* [101] compared muraglitazar based treatment with placebo or pioglitazone. The former was associated with an excess incidence of the composite end point of death, major adverse cardiovascular events and congestive heart failure. Based on these results, they recommended not approving this drug to treat diabetes until safety was documented through a trial designed to assess cardiovascular events. Consequently in 2006 Bristol-Myers Squibb announced that it had discontinued further development. In a subsequent pharmacogenomic trial performed in 730 patients affected by T2D was evaluated the interaction between muraglitazar and 213 SNPs from 63 genes [102]. Results showed that several common polymorphism in *ren* (rs2368564) and *edn-1* (rs5370) genes were associated with reduced risk of edema, while the  $\beta$ -1 adrenergic receptor rs1801253 was associated with increased susceptibility to edema

Table 1. Summary of genetic studies on troglitazone.								
Patients (male/female)	Controls (male/female)	Country	Ethnicity	Gene (*OMIM)	Gene function	Polymorphism	Comment	Ref.
25 (7/18) (abnormal increase in ALT levels)	85 (40/45)	Japan	Asiatic	<i>GSTT1</i> (*600436) <i>GSTM1</i> (*138350)	Catalyze the conjugation and the detoxification of glutathione and reactive toxic compounds, chemicals and metabolites	Wild/null  Wild/null	The combined null genotype of <i>GSTT1</i> and <i>GSTM1</i> is a susceptible genetic factor for troglitazone-associated abnormal increases in liver enzyme levels	[75]
8 patients with Type 2 diabetes with troglitazone-induced liver injury	31 patients unaffected after using troglitazone	Japan	Asiatic	<i>CYP2C19</i> (*124020)  <i>CYP2D6</i> (*124030)	Enzymatic system responsible for metabolism and elimination of endogenous and exogenous molecules and ingested chemicals	<i>CYP2C19</i> *1 <i>CYP2C19</i> *2 <i>CYP2C19</i> *3  <i>CYP2D6</i> *1 <i>CYP2D6</i> *2 <i>CYP2D6</i> *5 <i>CYP2D6</i> *10	The frequency of <i>CYP2C19</i> homozygous polymorphism was higher in patients with troglitazone-induced liver injury  The frequency of <i>CYP2D6</i> polymorphism was identical in both groups	[79]
93 women TRIPOD study	-	USA	Hispanic	<i>PPARG</i> (*601487)	Fat cell differentiation and whole-body insulin sensitivity	Pro12Ala	Pro12Ala variant did not account for the prevalence of troglitazone nonresponders in the TRIPOD cohort	[80]
93 women Troglitazone in TRIPOD study	-	USA	Hispanic			rs13073869 rs880663 rs4135263 rs1152003 rs6806708 rs13065455 rs13088205 rs13088214	8 of 131 <i>PPARG</i> variants may underlie response to thiazolidinedione therapy in women at risk for Type 2 diabetes	[81]
3548 subjects enrolled in DPP study	-	USA	Caucasian African-American Hispanic Asian American American Indian			Pro12Ala rs880663, rs4135263 rs1152003 rs6806708 rs13065455	<i>PPARG</i> P12A and the five variants have little or no effect on the beneficial response to troglitazone	[82]



Table 1. Summary of genetic studies on troglitazone (cont.).

Patients (male/female)	Controls (male/female)	Country	Ethnicity	Gene (*OMIM)	Gene function	Polymorphism	Comment	Ref.
3356 subjects (1116/2240) enrolled in the DPP study	–					Pro12Ala	At 1 year of treatment with troglitazone, Ala12 allele carriers tended to gain more weight than Pro12 allele homozygotes	[84]
3548 subjects enrolled in the DPP study	–			CDKN2A/B (*600160 – *600431)	Influence on cell cycle regulatory pathways	rs10811661	Improvement in the functionality of the pancreatic $\beta$ -cell after 1 year of troglitazone treatment	[85]
3007 subjects (1017/1990) enrolled in DPP study	–			SLC30A8 (*611145)	Zinc transporter and provider in insulin-secreting pancreatic $\beta$ -cells	rs13266634	No genotype-treatment interactions at 1 year of troglitazone treatment	[86]

[102]. These findings may be indicative of how much pharmacogenomic studies may help in selection of therapeutic drugs potentially prone to cause side effects in certain categories of subjects such as diabetic patients [103].

### Pharmacogenomics & pharmacogenetics of TZDs in cardiovascular risk factors

T2D is an established independent risk factor for CVD. As previously reported in the introduction, the American Heart Association/American Diabetes Association consensus statement on the use of TZDs asserts that a therapy based on these drugs should be privileged in patients at high risk for CVD, given that TZDs have antiinflammatory and antiatherogenic properties [9]. Aimed to investigate the genetic impact in the atheroprotective effects of TZDs, Saitou *et al.* [79] analyzed 99 candidate SNPs and their possible associations with conventional vascular risk factors and the progression or regression of carotid atherosclerosis in diabetic patients treated with pioglitazone or only with diet. Among the analyzed genetic variants, the I/D polymorphism in the *ACE* gene and the C677T polymorphism in the *MTHFR* gene showed a significant association with changes in the values of averaged and maximum IMT, a subclinical markers of atherosclerosis, in both group of patients. In particular, the group of patients carrying the *ACE* D-allele and treated with pioglitazone had lower increase in IMT than the group treated with diet alone ( $p = 0.01$ ). In addition, pioglitazone exerts a protective effect attenuating the increase in IMT ( $p = 0.03$ ) in patients carriers the 677T allele in the *MTHFR* gene which is generally associated to higher homocysteine level and progression of atherosclerosis [79]. These findings strongly suggest as TZDs may have a pleiotropic effects exerting their action through PPAR- $\gamma$  at different molecular levels such as renin-angiotensin modulation. However, their pleiotropic actions could be also the reason of the side effects characteristic of this class of drug, such as heart failure and edema. The positive response of the treatment is based on the presence mainly of the beneficial effect that can results by a dif-

ferent individual dosage based on EC50 and different individual genetic profiles. In Table 2 are reported the specific intracellular targets coupled with the beneficial and side effects associated with TZDs therapy.

Genetic variances of CYP450 enzymes have been involved in CVD [104,105]. This association is mediated by CYP-catalyzed release of arachidonic acid metabolites with vasodilatation, antiinflammatory and antiatherogenic properties, such as epoxyeicosatrienic acids, and dihydroxyeicosatrienoic acids (DHETs) [106,107]. *In vitro*, the TZDs are strong inhibitors of CYP2C8, a member of CYP with function in the oxidative system [108]. Moreover, in a pharmacokinetics study, rosiglitazone was found to be a CYP2C8 substrate and its metabolism increased in individuals with CYP2C8\*3 allele [42]. Kichheiner *et al.* [41] analyzed 31 healthy individuals, receiving a daily oral dose of rosiglitazone for two weeks, in order to confirm the impact of *CYP2C8* polymorphisms and rosiglitazone effects on urinary excretion of epoxyeicosatrienic acids and DHETs. One of the most interesting data showed that subjects with CYP2C8\*3 carriers had greater 14–15 DHET formation rates independently by rosiglitazone treatment, showing in both cases a direct correlation between the amount of produced metabolites and the number of alleles CYP2C8\*3 ( $p < 0.05$  and  $p < 0.01$ , respectively). On the contrary, carriers of the slow metabolizer haplotype C, had a trend to have a lower DHET excretion. Overall the effect of rosiglitazone in these subjects was a decrease in DHET excretion by approximately 10% ( $p < 0.02$ ) [41]. Therefore, according to these findings, understanding the physiological role of *CYP2C8* genetic variants in arachidonic acid metabolism and their correlation with TZDs could be pivotal to improve our knowledge in their impact on cardiovascular physiopathology.

Although TZDs have beneficial effects on cardiovascular parameters such as lowering BP and blood lipid levels, as previously discussed in this review, the treatment plan with the goal to reduce risk for CVD must be carefully individualized for each patient, since several side effects may accompany the use of these drugs

Table 2. Specific intracellular targets coupled with beneficial/side effects associated with thiazolidinediones therapy.

Thiazolidinediones	Receptors	Intracellular targets	Beneficial effects	Side effects
Pioglitazone	PPAR- $\gamma$	• Glut4, IRS1/2, PI3K, CAP	• Improved insulin signaling	• Hypoglycemia
Rosiglitazone		• TNF- $\alpha$ , retn	• Reduced inflammation	• Edema
Troglitazone		• AT1R • TXS, TXR • SOD, catalase • PEPCK, CD36, LPL, ACBP, ACS, aP2, GyK • CEBP $\alpha$ , STAT1/5A/5B	• Reduced blood pressure • Reduced atherosclerosis • Reduced oxidative stress • Increased lipid metabolism • Improved adipogenesis	• Weight gain • Cardiovascular risk • Heart failure • Bone loss in older women • Liver failure

(Table 2). The main side effects that can worsen the preexisting pathological situation in a patient with risk for CVD have been identified in fluid retention, edema and weight gain. For this reason, both pioglitazone and rosiglitazone are strongly not recommended in patients with established heart failure NYHA's class III or IV (Prescribing information Actos and Avandia [109]). However, it needs to be acknowledged that although both belonging to the same class of drug, their effect on cardiovascular events may be different. Rosiglitazone was associated with a high risk of CVD and all-cause mortality [7,110,111]. Controversially with previous discusses findings, an overall examination of clinical trials adopting pioglitazone did not show a clear advantage in terms of reducing cardiovascular related morbidity [112]. Mainly, this compound has often proven to be significantly protective against plaque progression and micro- and macrovascular events [11,113]. However, a more comprehensive evaluation of TZDs cardiovascular effect and safety could be achieved considering the contribution of SNPs in genes involved in vascular physiological pathways affecting the etiology of TZDs-induced side effects. With this objective, Ruano *et al.* [114] performed a retrospective cross sectional study on 87 T2D patients treated with rosiglitazone or pioglitazone. Authors used physiogenomics methodology to test for SNP associations across a PG-Array, consisting of 384 SNPs from 222 candidate genes involved in cardiovascular and metabolic pathways. They reported a significant association between body mass index (BMI) and TZDs therapy with genetic variants in *ADORA1* ( $p < 0.0003$ ), *ADIPOR2* ( $p < 0.007$ ), *PKM2* ( $p < 0.002$ ) and *UCP2* ( $p < 0.008$ ). In contrast, edema induced by TZDs therapy was associated with SNPs in genes controlling vascular permeability such as *NPY* ( $p < 0.006$ ) and *CCL2* ( $p < 0.015$ ) [114]. One of the most interesting finding emerging from this study is the strong correlation between risk-associated SNPs and protective SNPs for one or both investigated outcomes, suggesting the importance of monitoring genes involved in different but combined pathways in order to improve TZDs tolerability and safety.

As we previously mentioned, edema is one of the most common adverse side effects associated to rosiglitazone treatment [115]. Bailey *et al.* [116], in a genetic study from the multiethnic DREAM trial, tested 32,088 SNPs in 4197 patients treated with rosiglitazone. One SNP, rs6123045 in the *NFATC2* was significantly associated with edema ( $p = 7.68 \times 10^{-3}$ ). Specifically, European individuals homozygous for the risk allele (CC) showed higher rate of TZDs-induced edema compared with the individuals heterozygous (CT) or homozygous (TT) for the protective allele. In contrast, the rs6123045 SNP did not show a significant

association with edema onset in Latin Americans under similar treatment. However, a haplotype defined by six SNPs in *NFATC2* was associated with edema both in Europeans and Latin Americans ( $p = 2.26 \times 10^{-5}$  and  $p = 1.47 \times 10^{-2}$ , respectively) [116]. These data further support a variation in response to therapy and its side effects across race-ethnicities characterized by different genetic patterns.

To better understand mechanisms involved in TZDs therapy related-edema, Chang *et al.* [117], genotyped 28 tag SNPs from five candidate genes encoding for water channel proteins (*AQP2*), sodium transport proteins (*NHE3*, *SLC12A1*, *SCNN1G*) and for the TZDs target (*PPAR-γ*) in 168 T2D patients receiving TZDs treatment (rosiglitazone or pioglitazone). They found association between early edema onset and rs296766 T allele variant of *AQP2* ( $p = 0.0059$ ), and rs12904216 G allele variant of the *SLC12A1* ( $p = 0.049$ ). In regards to *PPAR-γ*, the Pro12Ala variant, a *PPAR-γ* SNP associated with induction of fluid retention in T2D Caucasian patients [118], was not evaluated in this study since its low minor allelic frequency in the analysed Asian population. However, other 8 *PPAR-γ* SNPs were considered for haplotype analysis and statistically significant association was found between *PPAR-γ* haplotype and edema induced by TZD after adjusting for sex and age [117].

Identifying the interaction between genetic factors controlling fat metabolism and response to TZDs treatment is pivotal in preventing cardiovascular accidents. Weight gain emerged as another common adverse drug reaction after TZDs therapy, especially in patients with already vascular complications [119]. With the aim to investigate association between adipocytokines involved in regulation of insulin activity and rosiglitazone response, Liu and colleagues [37], analysed *leptin* G-2548A and *TNF-α* G-308A SNPs in 245 T2D patients before and after 12 weeks of rosiglitazone treatment. Patients carrier of *leptin* G allelic variant had smaller basal values of BMI ( $p = 0.038$ ) respect to the one carried AA genotype. After rosiglitazone monotherapy, all T2D patients showed a decrease in fasting and postprandial plasma glucose, in A1c and leptin levels, and a significant increase in HDL levels, suggesting that rosiglitazone can reverse insulin resistance also by decreasing serum leptin level. Particularly, T2D patients' carrier *leptin* AA genotypes had better response to treatment, showing higher differential values of fasting and postprandial serum insulin. Moreover, patients with GA+AA *TNF-α* genotype had lower value of fasting serum insulin than those carrying the GG genotype, indicating that these particular SNPs may be associated with a better response to rosiglitazone monotherapy [37].

Table 3. Summary of genetic studies on thiazolidinediones.

Patients (male/female)	Healthy controls (male/female)	Country	Race/ethnicity	Gene (*OMIM)	Gene function	Polymorphisms	Comment	Ref.
87 (57/30)	nd	USA	African-American Caucasian Hispanic	<i>ADORA1</i> (*102775) <i>PKM2</i> (*179050) <i>ADIPOR2</i> (*607946)	Adipocyte energy homeostasis Intracellular glucose regulation Mediate increased AMPK and PPAR- $\alpha$ ligand activities Mediate fatty acid oxidation and glucose uptake	rs903361 rs2856929 rs7975375	Results from these comparative physiogenomic analysis associations suggest mechanistic links between adenosine signaling and BMI, and between vascular permeability and drug-induced edema	[32]
				<i>UCP2</i> (*601693)	Mitochondrial protein involved in the control of the metabolism of fatty acids	rs660339		
				<i>APOH</i> (*138700)	Lipoprotein metabolism	rs8178847		
				<i>NPY</i> (*162640)	Long-lasting constrictor agent Enhancer of endothelial permeability Participates in pulmonary and larynx edema	rs1468271		
				<i>GYS1</i> (*138570)	Glycogen molecule synthesis	rs2287754		
				<i>CCL2</i> (+158105)	Increases the permeability of the blood-brain barrier Participates in vasogenic brain edema	rs3760396		
				<i>OLR1</i> (*602601)	Participates in capillary formation	rs2742115		
				<i>GHRH</i> (*139190)	Stimulates GH secretion	rs6032470		

nd: Not determined.

Table 3. Summary of genetic studies on thiazolidinediones (cont.).

Patients (male/female)	Healthy controls (male/female)	Country	Race/ethnicity	Gene (*OMIM)	Gene function	Polymorphisms	Comment	Ref.
4197	Rosiglitazone (1750) Ramipril Placebo (1761)	North America South America Europe India Australia	Europeans Latin Americans South Asians African-American Native North American East Asian Other	<i>MFATC2</i> (*600490)	Activated T-cell transcription factor	rs6123045	A significant interaction was detected between the SNP rs6123045 of the ( <i>MFATC2</i> ) gene and edema among Europeans receiving rosiglitazone treatment	[33]
268 (126/142)		Asia	Chinese	<i>AQP2</i> (*107777) <i>SLC12A1</i> (*600839)	Renal AQP2 water channel Transport of Na <sup>+</sup> , K <sup>+</sup> and Cl <sup>-</sup> across the plasma membrane	rs296766 rs12904216	A model based on combination of genetic scores from <i>AQP2</i> and <i>SLC12A1</i> and nongenetic risk factors was developed in order to clinically predict TZD-related edema	[96]
3356 (1116/2240)	Placebo (29.8%) Metformin (29.5%) Lifestyle intervention (29.9%) Troglitazone (10.8%)	USA	European African-American American Hispanic Asian/Pacific Islander American Indian	<i>PPARG</i> (*601487) <i>FTO</i> (*610966) <i>INSIG2</i> (*608660)	Binding of chemicals that induce proliferation of peroxisome, organelles that contribute to the oxidation of fatty acids Regulation of food intake Control of cholesterol and fatty acid metabolism via regulation of SREBFs	Pro12Ala rs9939609 rs7566605	Weight gain, induced by troglitazone treatment, tended to be significantly greater in <i>PPARG</i> Ala12 allelic carriers A nominal association exist between common variants in <i>FTO</i> and <i>INSIG2</i> and obesity-related traits, by interacting with metformin or lifestyle intervention, but not with troglitazone	[84] [105]
nd	31 (15/16)	nd	nd	<i>CYP2C8</i> (+601129)	Drug-metabolizing CYP450 isoform	rs2275622 rs7909236 rs1058930 rs113129 rs1572080	Genetic variants of <i>CYP2C8</i> gene may affect cardiovascular functions modulating epoxyeicosatrienoic acid-formation	[68]

nd: Not determined.

Table 3. Summary of genetic studies on thiazolidinediones (cont.).								
Patients (male/female)	Healthy controls (male/female)	Country	Race/ethnicity	Gene (*OMIM)	Gene function	Polymorphisms	Comment	Ref.
222 (144/78)	160 – diet only 62 – pioglitazone	Japan	Japanese	<i>MTHFR</i> (*607093)	Catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate for homocysteine remethylation to methionine	rs1801133 (C677T)	Pioglitazone exerts an antiatherosclerotic effect on Type 2 diabetic patients carrying the <i>ACE</i> -D allele and the <i>MTHFR</i> 677T allele	[98]
245 (132/113)		China	Chinese	<i>ACE</i> (+106180)	Blood pressure regulation and electrolyte balance by hydrolyzing angiotensin I into angiotensin II	rs1799752 (I/D)		
		China	Chinese	<i>Leptin</i> (*164160)	Regulation of body weight by inhibiting food intake and stimulating energy expenditure	G2548A	<i>Leptin</i> G- 2548A and <i>TNF-<math>\alpha</math></i> G-308A polymorphisms are associated with therapeutic efficacy of rosiglitazone treatment of Type 2 diabetes patients	[99]
				<i>TNF-<math>\alpha</math></i> (*191160)	Multifunctional proinflammatory cytokine Inhibition of lipogenesis and stimulation of lipolysis Impairment of insulin signal pathway	G-308A		

nd: Not determined.

Moreover, one of major risk factors for CVD and stroke is the excess in adiposity localized in the visceral compartment [120]. *PPAR-γ* Pro12Ala SNP, since its role in adipocyte differentiation and its significant association with BMI, has been one of the most studied genetic variant in association with risk for obesity and CVD risk [121,122]. Franks *et al.* [95], tested the possible association between the *PPAR-γ* Pro12Ala variant and the risk for weight gain and change in adipose distribution in 3356 T2D patients participating to the DPP (Diabetes Prevention Program), in which the effects of metformin, troglitazone treatment and/or intensive lifestyle intervention in diabetes were investigated. At baseline, patients carrying Ala12 allele had larger waist

circumference (WC) ( $p < 0.001$ ). After treatment with troglitazone, the Ala12 allele was associated with a trend in development greater weight gain respect to the *PPAR-γ* Pro12 variant [95]. By using the same database, same authors further demonstrated as adjusting for age, sex and race-ethnicity, the minor A allele at *FTO* rs9939609 SNP showed a significant association with heavier weight ( $p = 0.03$ ) and higher BMI ( $p = 0.003$ ) at baseline, while after treatment it appeared to be associated to a greater increase in subcutaneous adipose tissue in the placebo group with respect to metformin and intensive lifestyle intervention after 1 year of treatment [123]. Interaction with troglitazone treatment was analysed only for the allelic variant of *INSIG2*

## Executive summary

### Introduction: rosiglitazone, pioglitazone & troglitazone

- Thiazolidinediones (TZDs) are a class of antidiabetic drugs that act as insulin sensitizers increasing insulin-dependent glucose disposal and reducing hepatic glucose output.
- The three drugs in this hypoglycemic class introduced in the market are rosiglitazone, pioglitazone and troglitazone.
- TZDs have shown pleiotropic effects against vascular risk factors and atherosclerosis.
- Troglitazone was removed from the market due to its hepatotoxicity, and rosiglitazone and pioglitazone both have particular warnings due to being associated with congestive heart failure.

### Mechanism of action: role of PPARs & PPAR-γ

- TZDs act mainly through the PPAR-γ.
- In absence of a ligand, the PPAR-γ:RXR complex can recruit corepressor complexes and bind to PPRE, suppressing the transcription of target genes.
- Among these genes, PPAR-γ activation induces the expression of genes involved in the insulin signaling cascade such as *GLUT4* and *CAP*, thereby improving insulin sensitivity in patients with diabetes.

### Pharmacogenomics & pharmacogenetics of rosiglitazone in diabetes

- Genetic variation in drug-metabolizing protein (*CYP450*) genes: *CYP2C8* (*CYP2C8\*3*) and *CYP2C9* (*CYP2C9\*3*) contribute towards intersubject heterogeneity in pharmacokinetic behavior of rosiglitazone.
- Glucose-lowering effects of rosiglitazone vary in subpopulations representing different *ADIPOQ* gene polymorphisms.
- Rosiglitazone shows a markedly increased effect in patients with the AA genotype of *leptin* G-2548A.

### Pharmacogenomics & pharmacogenetics of pioglitazone in diabetes

- SNPs associated with *CYP2C8*:\*1 homozygotes and the \*3 variant allele carriers are responsible for variability in bioavailability of pioglitazone among various study populations.
- Glucose-lowering effect of pioglitazone is partly ascribed to the polymorphisms associated with *PPARG* gene.
- *retn* C-420G (rs1862513) polymorphism in the GG genotype is linked with a stronger glucose-lowering effect of pioglitazone.

### Pharmacogenomics & pharmacogenetics of troglitazone in diabetes

- Presence of the combined *GSTT1* and *GSTM1* null genotype is a specific risk factor for the enhanced susceptibility to hepatotoxicity associated with troglitazone.
- TRIPOD study found no correlation between *PPARG* common polymorphism P12A and response to troglitazone.
- Improvement in the pancreatic β-cell functionality for patients carrying the protective genotype at *CDKN2A/B* SNP rs10811661 at 1 year of troglitazone treatment.

### Pharmacogenomics & pharmacogenetics of TZDs in cardiovascular risk factors

- Patients carrying the *ACE*-D allele or *MTHFR* 677T allele and treated with pioglitazone have lower risk for atherosclerosis than the group treated with diet alone.
- *CYP2C8* genetic variants in arachidonic acid metabolism and their correlation with TZDs are pivotal in the variability for risk in cardiovascular disease.
- Edema induced by TZDs is associated with SNPs in genes controlling vascular permeability such as *NPY* and *CCL2*.
- *Leptin* G-2548A and *TNF-α* G-308A SNPs are associated with variability in vascular risk.

rs7566605 SNP but no significant association was found with variation in increase of adipose tissue in T2D patients [123]. In Table 3 are reported some of the most important studies in this field.

### Conclusion & future perspective

Combining the predictive power of SNPs of genes affecting the etiology of TZDs-induced side effects could be important in order to improve the rational clinical application of these drugs. According to results from previous discussed studies, a model incorporating higher weighted genetic score based on these genetic variants, combined with specific nongenetic risk factors, such as female sex and older age, could help to improving the clinical prediction of TZD-related side effects in diabetic subjects with high risk for CVD.

The potential of TZDs as the basis of therapeutic strategies in treating cardiovascular complications during diabetes has been affirmed by preclinical data, which seem to hold promise and therefore should be used as an adjunct therapy to provide maximal relief to the cardiovascular system. However, an exhaustive pharmacogenomic assessment of TZDs has highlighted the significance of studying the importance of population-specific genetic variability and the manner in which this variability influences the therapeutic effect of TZDs. This observation attains a heightened significance when assessed in the context of life-threatening acute cardiovascular complications

during diabetes, as even a minor genetic polymorphism-based variation may snowball into a significant cardiovascular problem. Therefore, pharmacogenomics is an important tool in not only validating the clinical relevance of intersubject variability *per se* but also to assess the therapeutic potential of such variability in drug individualization and therapeutic optimization. Furthermore, among the wide arrays of genetic variations proposed in the literature and discussed above, it is also important to define first, the bigger picture of interrelationship between the genetic variations and therapeutic efficacy of these drugs; second, the comparisons between the relative quantitative significance of various forms of genetic polymorphism in mediating the pharmacological effect of TZDs on the pathogenesis of diabetes-related cardiovascular complications; and third, the identification of the principal sources of therapeutically relevant fluctuations in drug effect that may be effectively regulated by appropriate methods.

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### References

Papers of special note have been highlighted as:

- of interest; •• of considerable interest
- 1 Meerza D, Naseem I, Ahmed J. Pharmacology of signaling pathways: in Type 2 diabetes. *Diab. Metabol. Syndr.* 7(3), 180–185 (2013).
  - 2 Yki-Jarvinen H. Thiazolidinediones. *N. Engl. J. Med.* 351(11), 1106–1118 (2004).
  - **A very remarkable review describing the mechanisms, indications and limitations of thiazolidinedione therapy.**
  - 3 Willson TM, Lambert MH, Kliewer SA. Peroxisome proliferator-activated receptor gamma and metabolic disease. *Annu. Rev. Biochem.* 70, 341–367 (2001).
  - 4 Martens FM, Visseren FL, Lemay J, De Koning EJ, Rabelink TJ. Metabolic and additional vascular effects of thiazolidinediones. *Drugs* 62(10), 1463–1480 (2002).
  - 5 Aquilante CL. Pharmacogenetics of thiazolidinedione therapy. *Pharmacogenomics* 8(8), 917–931 (2007).
  - 6 Filion KB, Joseph L, Boivin JF, Suissa S, Brophy JM. Thiazolidinediones and the risk of incident congestive heart failure among patients with Type 2 diabetes mellitus. *Pharmacoeconomol. Drug Saf.* 20(8), 785–796 (2011).
  - 7 Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N. Engl. J. Med.* 356(24), 2457–2471 (2007).
  - **A major study on the effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes.**
  - 8 Kumar S, Hoffman SJ, Samadfam R *et al.* The effect of rosiglitazone on bone mass and fragility is reversible and can be attenuated with alendronate. *J. Bone Miner Res.* 28(7), 1653–1665 (2013).
  - 9 Nathan DM, Buse JB, Davidson MB *et al.* Management of hyperglycemia in Type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 29(8), 1963–1972 (2006).
  - 10 Tolman KG. Thiazolidinedione hepatotoxicity: a class effect? *Int. J. Clin. Pract. Suppl.* (113), 29–34 (2000).
  - 11 Dormandy JA, Charbonnel B, Eckland DJ *et al.* Secondary prevention of macrovascular events in patients with Type 2 diabetes in the PROactive Study (PROspective pioglitAZone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 366(9493), 1279–1289 (2005).



- An important clinical study that demonstrated that the pioglitazone reduces the composite of all-cause mortality, nonfatal myocardial infarction and stroke in patients with Type 2 diabetes who have a high risk of macrovascular events.
- 12 Lincoff AM, Wolski K, Nicholls SJ, Nissen SE. Pioglitazone and risk of cardiovascular events in patients with Type 2 diabetes mellitus: a meta-analysis of randomized trials. *JAMA* 298(10), 1180–1188 (2007).
- 13 Neumann A, Weill A, Ricordeau P, Fagot JP, Alla F, Allemand H. Pioglitazone and risk of bladder cancer: clarification of the design of the French study. *Diabetologia* 56(1), 228–229 (2013).
- 14 Saltiel AR, Olefsky JM. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes* 45(12), 1661–1669 (1996).
- 15 Berger J, Moller DE. The mechanisms of action of PPARs. *Annu. Rev. Med.* 53, 409–435 (2002).
- 16 Furuta M, Yano Y, Gabazza EC *et al.* Troglitazone improves GLUT4 expression in adipose tissue in an animal model of obese Type 2 diabetes mellitus. *Diabetes Res. Clin. Pract.* 56(3), 159–171 (2002).
- 17 Armoni M, Kritiz N, Harel C *et al.* Peroxisome proliferator-activated receptor-gamma represses GLUT4 promoter activity in primary adipocytes, and rosiglitazone alleviates this effect. *J. Biol. Chem.* 278(33), 30614–30623 (2003).
- 18 Jiang G, Dallas-Yang Q, Li Z *et al.* Potentiation of insulin signaling in tissues of Zucker obese rats after acute and long-term treatment with PPARgamma agonists. *Diabetes* 51(8), 2412–2419 (2002).
- 19 Leonardini A, Laviola L, Perrini S, Natalicchio A, Giorgino F. Cross-Talk between PPARgamma and Insulin Signaling and Modulation of Insulin Sensitivity. *PPAR Res.* 2009, 818945 (2009).
- 20 Sugawara A, Uruno A, Kudo M, Matsuda K, Yang CW, Ito S. PPARgamma agonist beyond glucose lowering effect. *Korean J. Intern. Med.* 26(1), 19–24 (2011).
- 21 Sugawara A, Takeuchi K, Uruno A *et al.* Differential effects among thiazolidinediones on the transcription of thromboxane receptor and angiotensin II type 1 receptor genes. *Hypertens. Res.* 24(3), 229–233 (2001).
- 22 Uruno A, Matsuda K, Noguchi N *et al.* Peroxisome proliferator-activated receptor-gamma suppresses CYP11B2 expression and aldosterone production. *J. Mol. Endocrinol.* 46(1), 37–49 (2011).
- 23 Ikeda Y, Sugawara A, Taniyama Y *et al.* Suppression of rat thromboxane synthase gene transcription by peroxisome proliferator-activated receptor gamma in macrophages via an interaction with NRF2. *J. Biol. Chem.* 275(42), 33142–33150 (2000).
- 24 Sugawara A, Uruno A, Kudo M *et al.* Transcription suppression of thromboxane receptor gene by peroxisome proliferator-activated receptor-gamma via an interaction with Sp1 in vascular smooth muscle cells. *J. Biol. Chem.* 277(12), 9676–9683 (2002).
- 25 Kudzma DJ. Effects of thiazolidinediones for early treatment of Type 2 diabetes mellitus. *Am. J. Manag. Care* 8(16 Suppl.), S472–S482 (2002).
- 26 Olansky L, Marchetti A, Lau H. Multicenter retrospective assessment of thiazolidinedione monotherapy and combination therapy in patients with Type 2 diabetes: comparative subgroup analyses of glycemic control and blood lipid levels. *Clin. Ther.* 25(Suppl. B), B64–B80 (2003).
- 27 Pirillo A, Norata GD, Catapano AL. LOX-1, OxLDL, and atherosclerosis. *Mediators Inflamm.* 2013, 152786 (2013).
- 28 Jiang DQ, Chu LX, Liu ZY, Zhang SB. Pioglitazone decreased CD40/CD40L expression on human umbilical vein endothelial cells induced by oxidized low-density lipoprotein. *Clin. Chim. Acta* 370(1–2), 94–99 (2006).
- 29 Kapadia R, Yi JH, Vemuganti R. Mechanisms of anti-inflammatory and neuroprotective actions of PPAR-gamma agonists. *Front. Biosci.* 13, 1813–1826 (2008).
- 30 Tureyen K, Kapadia R, Bowen KK *et al.* Peroxisome proliferator-activated receptor-gamma agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents. *J. NeuroChem.* 101(1), 41–56 (2007).
- 31 Ito H, Nakano A, Kinoshita M, Matsumori A. Pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, attenuates myocardial ischemia/reperfusion injury in a rat model. *Lab. Invest.* 83(12), 1715–1721 (2003).
- 32 Finegood DT, McArthur MD, Kojwang D *et al.* Beta-cell mass dynamics in Zucker diabetic fatty rats. Rosiglitazone prevents the rise in net cell death. *Diabetes* 50(5), 1021–1029 (2001).
- 33 Wang X, Liu X, Zhan Y *et al.* Pharmacogenomic, physiological, and biochemical investigations on safety and efficacy biomarkers associated with the peroxisome proliferator-activated receptor-gamma activator rosiglitazone in rodents: a translational medicine investigation. *J. Pharmacol. Exp. Ther.* 334(3), 820–829 (2010).
- 34 Wang X. Assessment of cardiac safety for PPARgamma agonists in rodent models of heart failure: a translational medicine perspective. *Curr. Mol. Pharmacol.* 5(2), 264–271 (2012).
- 35 Hammarstedt A, Andersson CX, Rotter Sopasakis V, Smith U. The effect of PPARgamma ligands on the adipose tissue in insulin resistance. *Prostaglandins Leukot. Essent. Fatty Acids* 73(1), 65–75 (2005).
- 36 Berger JP, Akiyama TE, Meinke PT. PPARs: therapeutic targets for metabolic disease. *Trends Pharmacol. Sci.* 26(5), 244–251 (2005).
- 37 Liu HL, Lin YG, Wu J *et al.* Impact of genetic polymorphisms of leptin and TNF-alpha on rosiglitazone response in Chinese patients with Type 2 diabetes. *Eur. J. Clin. Pharmacol.* 64(7), 663–671 (2008).
- 38 Pan HJ, Reifsnnyder P, Vance DE, Xiao Q, Leiter EH. Pharmacogenetic analysis of rosiglitazone-induced hepatosteatosis in new mouse models of Type 2 diabetes. *Diabetes* 54(6), 1854–1862 (2005).
- 39 Pan HJ, Lin Y, Chen YE, Vance DE, Leiter EH. Adverse hepatic and cardiac responses to rosiglitazone in a new mouse model of Type 2 diabetes: relation to dysregulated phosphatidylcholine metabolism. *Vasc. Pharmacol.* 45(1), 65–71 (2006).

- 40 Leiter EH, Reifsnnyder PC, Zhang W, Pan HJ, Xiao Q, Mistry J. Differential endocrine responses to rosiglitazone therapy in new mouse models of Type 2 diabetes. *Endocrinology* 147(2), 919–926 (2006).
- 41 Kirchheiner J, Meineke I, Fuhr U, Rodriguez-Antona C, Lebedeva E, Brockmoller J. Impact of genetic polymorphisms in CYP2C8 and rosiglitazone intake on the urinary excretion of dihydroxycoicostrienoic acids. *Pharmacogenomics* 9(3), 277–288 (2008).
- 42 Kirchheiner J, Thomas S, Bauer S *et al.* Pharmacokinetics and pharmacodynamics of rosiglitazone in relation to CYP2C8 genotype. *Clin. Pharmacol. Ther.* 80(6), 657–667 (2006).
- 43 Li Z, Peng X, Wu Y, Xia Y, Liu X, Zhang Q. The influence of adiponectin gene polymorphism on the pioglitazone response in the Chinese with Type 2 diabetes. *Diabetes Obes. Metab.* 10(9), 794–802 (2008).
- 44 Ren W, Zhang SH, Wu J, Ni YX. Polymorphism of the leptin gene promoter in pedigrees of Type 2 diabetes mellitus in Chongqing, China. *Chin. Med. J. (Engl.)* 117(4), 558–561 (2004).
- 45 Sookoian SC, Gonzalez C, Pirola CJ. Meta-analysis on the G-308A tumor necrosis factor alpha gene variant and phenotypes associated with the metabolic syndrome. *Obes. Res.* 13(12), 2122–2131 (2005).
- 46 Seda O, Sedova L, Oliyarnyk O *et al.* Pharmacogenomics of metabolic effects of rosiglitazone. *Pharmacogenomics* 9(2), 141–155 (2008).
- 47 Yu W, Hu C, Zhang R *et al.* Effects of KCNQ1 polymorphisms on the therapeutic efficacy of oral antidiabetic drugs in Chinese patients with Type 2 diabetes. *Clin. Pharmacol. Ther.* 89(3), 437–442 (2011).
- 48 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(5), 1139–1144 (2005).
- 49 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(6), 1320–1324 (2006).
- 50 Yang M, Huang Q, Wu J *et al.* Effects of UCP2 -866 G/A and ADRB3 Trp64Arg on rosiglitazone response in Chinese patients with Type 2 diabetes. *Br. J. Clin. Pharmacol.* 68(1), 14–22 (2009).
- 51 Jazet IM, Pijl H, Meinders AE. Adipose tissue as an endocrine organ: impact on insulin resistance. *Neth. J. Med.* 61(6), 194–212 (2003).
- 52 Chen M, Hu C, Zhang R *et al.* Association of PAX4 genetic variants with oral antidiabetic drugs efficacy in Chinese Type 2 diabetes patients. *Pharmacogenomics J.* 14(5), 488–492 (2014).
- 53 Wang J, Bao YQ, Hu C *et al.* Effects of ABCA1 variants on rosiglitazone monotherapy in newly diagnosed Type 2 diabetes patients. *Acta Pharmacol. Sin.* 29(2), 252–258 (2008).
- 54 Krempler F, Esterbauer H, Weitgasser R *et al.* A functional polymorphism in the promoter of UCP2 enhances obesity risk but reduces Type 2 diabetes risk in obese middle-aged humans. *Diabetes* 51(11), 3331–3335 (2002).
- 55 Zhang KH, Huang Q, Dai XP *et al.* Effects of the peroxisome proliferator activated receptor-gamma coactivator-1alpha (PGC-1alpha) Thr394Thr and Gly482Ser polymorphisms on rosiglitazone response in Chinese patients with Type 2 diabetes mellitus. *J. Clin. Pharmacol.* 50(9), 1022–1030 (2010).
- 56 Wilson-Fritch L, Nicoloso S, Chouinard M *et al.* Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. *J. Clin. Invest.* 114(9), 1281–1289 (2004).
- 57 Pistrosch F, Passauer J, Fischer S, Fuecker K, Hanefeld M, Gross P. In Type 2 diabetes, rosiglitazone therapy for insulin resistance ameliorates endothelial dysfunction independent of glucose control. *Diabetes Care* 27(2), 484–490 (2004).
- 58 Kung J, Henry RR. Thiazolidinedione safety. *Expert Opin. Drug Saf.* 11(4), 565–579 (2012).
- 59 Scheen AJ. Outcomes and lessons from the PROactive study. *Diabetes Res. Clin. Pract.* 98(2), 175–186 (2012).
- 60 Hanefeld M. Pharmacokinetics and clinical efficacy of pioglitazone. *Int. J. Clin. Pract. Suppl.* (121), 19–25 (2001).
- 61 Scheen AJ. Pharmacokinetic interactions with thiazolidinediones. *Clin. Pharmacokinet.* 46(1), 1–12 (2007).
- 62 Aquilante CL, Kosmiski LA, Bourne DW *et al.* Impact of the CYP2C8 \*3 polymorphism on the drug-drug interaction between gemfibrozil and pioglitazone. *Br. J. Clin. Pharmacol.* 75(1), 217–226 (2013).
- 63 Tornio A, Niemi M, Neuvonen PJ, Backman JT. Trimethoprim and the CYP2C8\*3 allele have opposite effects on the pharmacokinetics of pioglitazone. *Drug Metab. Dispos.* 36(1), 73–80 (2008).
- 64 Kadam R, Bourne D, Kompella U, Aquilante C. Effect of cytochrome P450 2C8\*3 on the population pharmacokinetics of pioglitazone in healthy Caucasian volunteers. *Biol. Pharm. Bull.* 36(2), 245–251 (2013).
- 65 Auwerx J. PPARgamma, the ultimate thrifty gene. *Diabetologia* 42(9), 1033–1049 (1999).
- 66 Masugi J, Tamori Y, Mori H, Koike T, Kasuga M. Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator-activated receptor-gamma 2 on thiazolidinedione-induced adipogenesis. *BioChem. Biophys. Res. Commun.* 268(1), 178–182 (2000).
- 67 Ramirez-Salazar M, Perez-Luque E, Fajardo-Araujo M, Garza SM, Malacara JM. Effect of the Pro12Ala polymorphism of the PPAR gamma 2 gene on response to pioglitazone treatment in menopausal women. *Menopause* 15(6), 1151–1156 (2008).
- 68 Hsieh MC, Lin KD, Tien KJ *et al.* Common polymorphisms of the peroxisome proliferator-activated receptor-gamma (Pro12Ala) and peroxisome proliferator-activated receptor-gamma coactivator-1 (Gly482Ser) and the response to pioglitazone in Chinese patients with Type 2 diabetes mellitus. *Metabolism* 59(8), 1139–1144 (2010).
- 69 Pei Q, Huang Q, Yang GP *et al.* PPAR-gamma2 and PTPRD gene polymorphisms influence Type 2 diabetes patients' response to pioglitazone in China. *Acta Pharmacol. Sin.* 34(2), 255–261 (2013).
- 70 Namvaran F, Azarpira N, Rahimi-Moghaddam P, Dabbaghmanesh MH. Polymorphism of peroxisome

- proliferator-activated receptor gamma (PPARGgamma) Pro12Ala in the Iranian population: relation with insulin resistance and response to treatment with pioglitazone in Type 2 diabetes. *Eur. J. Pharmacol.* 671(1–3), 1–6 (2011).
- 71 Steppan CM, Bailey ST, Bhat S *et al.* The hormone resistin links obesity to diabetes. *Nature* 409(6818), 307–312 (2001).
- **A significant article that demonstrates that circulating resistin levels are decreased by antidiabetic thiazolidinediones. Resistin has been suggested therefore as a candidate adipocyte derived factor that contributes to insulin resistance *in vivo*.**
- 72 Banerjee RR, Rangwala SM, Shapiro JS *et al.* Regulation of fasted blood glucose by resistin. *Science* 303(5661), 1195–1198 (2004).
- 73 Rangwala SM, Rich AS, Rhoades B *et al.* Abnormal glucose homeostasis due to chronic hyperresistinemia. *Diabetes* 53(8), 1937–1941 (2004).
- 74 Makino H, Shimizu I, Murao S *et al.* A pilot study suggests that the G/G genotype of resistin single nucleotide polymorphism at -420 may be an independent predictor of a reduction in fasting plasma glucose and insulin resistance by pioglitazone in Type 2 diabetes. *Endocr. J.* 56(9), 1049–1058 (2009).
- 75 Mazzone T, Meyer PM, Feinstein SB *et al.* Effect of pioglitazone compared with glimepiride on carotid intima-media thickness in Type 2 diabetes: a randomized trial. *JAMA* 296(21), 2572–2581 (2006).
- **A key clinical trial in patients with Type 2 diabetes mellitus. In this study it was shown that pioglitazone slows progression of carotid artery intima media thickness compared with glimepiride.**
- 76 Honda T, Kaikita K, Tsujita K *et al.* Pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, attenuates myocardial ischemia-reperfusion injury in mice with metabolic disorders. *J. Mol. Cell Cardiol.* 44(5), 915–926 (2008).
- 77 Ye Y, Lin Y, Manickavasagam S, Perez-Polo JR, Tieu BC, Birnbaum Y. Pioglitazone protects the myocardium against ischemia-reperfusion injury in eNOS and iNOS knockout mice. *Am. J. Physiol. Heart Circ. Physiol.* 295(6), H2436–H2446 (2008).
- 78 Wang H, Zhu QW, Ye P *et al.* Pioglitazone attenuates myocardial ischemia-reperfusion injury via up-regulation of ERK and COX-2. *BioSci. Trends* 6(6), 325–332 (2012).
- 79 Saitou M, Osonoi T, Kawamori R *et al.* Genetic risk factors and the anti-atherosclerotic effect of pioglitazone on carotid atherosclerosis of subjects with Type 2 diabetes -- a retrospective study. *J. Atheroscler. Thromb.* 17(4), 386–394 (2010).
- 80 Groenemeijer BE, Hallman MD, Reymer PW *et al.* Genetic variant showing a positive interaction with beta-blocking agents with a beneficial influence on lipoprotein lipase activity, HDL cholesterol, and triglyceride levels in coronary artery disease patients. The Ser447-stop substitution in the lipoprotein lipase gene. REGRESS Study Group. *Circulation* 95(12), 2628–2635 (1997).
- 81 Bogacka I, Xie H, Bray GA, Smith SR. The effect of pioglitazone on peroxisome proliferator-activated receptor-gamma target genes related to lipid storage *in vivo*. *Diabetes Care* 27(7), 1660–1667 (2004).
- 82 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(4), 552–557 (2007).
- 83 Ikeda T. Drug-induced idiosyncratic hepatotoxicity: prevention strategy developed after the troglitazone case. *Drug Metab. Pharmacokinet.* 26(1), 60–70 (2011).
- 84 Watkins PB, Whitcomb RW. Hepatic dysfunction associated with troglitazone. *N. Engl. J. Med.* 338(13), 916–917 (1998).
- 85 He K, Talaat RE, Pool WF *et al.* Metabolic activation of troglitazone: identification of a reactive metabolite and mechanisms involved. *Drug Metab. Dispos.* 32(6), 639–646 (2004).
- 86 Watanabe I, Tomita A, Shimizu M *et al.* A study to survey susceptible genetic factors responsible for troglitazone-associated hepatotoxicity in Japanese patients with Type 2 diabetes mellitus. *Clin. Pharmacol. Ther.* 73(5), 435–455 (2003).
- 87 Pemble S, Schroeder KR, Spencer SR *et al.* Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *BioChem. J.* 300(Pt 1), 271–276 (1994).
- 88 Rebbeck TR. Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. *Cancer Epidemiol. Biomarkers Prev.* 6(9), 733–743 (1997).
- 89 Oniki K, Ueda K, Hori M, Mihara S, Marubayashi T, Nakagawa K. Glutathione-S-transferase (GST) M1 null genotype and combined GSTM1 and GSTT1 null genotypes as a risk factor for alcoholic mild liver dysfunction. *Clin. Pharmacol. Ther.* 81(5), 634–635 (2007).
- 90 Kumashiro R, Kubota T, Koga Y *et al.* Association of troglitazone-induced liver injury with mutation of the cytochrome P450 2C19 gene. *Hepatol. Res.* 26(4), 337–342 (2003).
- 91 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-gamma2 gene variant: results from the Troglitazone in Prevention of Diabetes (TRIPOD) study. *Diabetes Care* 27(6), 1365–1368 (2004).
- 92 Wolford JK, Yeatts KA, Dhanjal SK *et al.* Sequence variation in PPARG may underlie differential response to troglitazone. *Diabetes* 54(11), 3319–3325 (2005).
- 93 Florez JC, Jablonski KA, Sun MW *et al.* Effects of the Type 2 diabetes-associated PPARG P12A polymorphism on progression to diabetes and response to troglitazone. *J. Clin. Endocrinol. Metab.* 92(4), 1502–1509 (2007).
- 94 Distefano JK, Watanabe RM. Pharmacogenetics of anti-diabetes drugs. *Pharmaceuticals (Basel)* 3(8), 2610–2646 (2010).
- 95 Franks PW, Jablonski KA, Delahanty L *et al.* The Pro12Ala variant at the peroxisome proliferator-activated receptor gamma gene and change in obesity-related traits in the Diabetes Prevention Program. *Diabetologia* 50(12), 2451–2460 (2007).

- 96 Moore AF, Jablonski KA, Mcateer JB *et al.* Extension of Type 2 diabetes genome-wide association scan results in the diabetes prevention program. *Diabetes* 57(9), 2503–2510 (2008).
- 97 Majithia AR, Jablonski KA, Mcateer JB *et al.* Association of the SLC30A8 missense polymorphism R325W with proinsulin levels at baseline and after lifestyle, metformin or troglitazone intervention in the Diabetes Prevention Program. *Diabetologia* 54(10), 2570–2574 (2011).
- 98 Buse JB, Rubin CJ, Frederich R *et al.* Muraglitazar, a dual (alpha/gamma) PPAR activator: a randomized, double-blind, placebo-controlled, 24-week monotherapy trial in adult patients with Type 2 diabetes. *Clin. Ther.* 27(8), 1181–1195 (2005).
- 99 Cox SL. Muraglitazar: an agent for the treatment of Type 2 diabetes and associated dyslipidemia. *Drugs Today* 41(9), 579–587 (2005).
- 100 Kendall DM, Rubin CJ, Mohideen P *et al.* Improvement of glycemic control, triglycerides, and HDL cholesterol levels with muraglitazar, a dual (alpha/gamma) peroxisome proliferator-activated receptor activator, in patients with Type 2 diabetes inadequately controlled with metformin monotherapy: a double-blind, randomized, pioglitazone-comparative study. *Diabetes Care* 29(5), 1016–1023 (2006).
- 101 Nissen SE, Wolski K, Topol EJ. Effect of muraglitazar on death and major adverse cardiovascular events in patients with Type 2 diabetes mellitus. *JAMA* 294(20), 2581–2586 (2005).
- 102 Geese WJ, Achanzar W, Rubin C *et al.* Genetic and gene expression studies implicate renin and endothelin-1 in edema caused by peroxisome proliferator-activated receptor gamma agonists. *Pharmacogenet. Genomics* 18(10), 903–910 (2008).
- 103 Panicker GK, Karnad DR, Salvi V, Kothari S. Cardiovascular risk of oral antidiabetic drugs: current evidence and regulatory requirements for new drugs. *J. Assoc. Phys. India* 60, 56–61 (2012).
- 104 Yasar U, Bennet AM, Eliasson E *et al.* Allelic variants of cytochromes P450 2C modify the risk for acute myocardial infarction. *Pharmacogenetics* 13(12), 715–720 (2003).
- 105 Visser LE, Van Schaik RH, Jan Danser AH *et al.* The risk of myocardial infarction in patients with reduced activity of cytochrome P450 2C9. *Pharmacogenet. Genomics* 17(7), 473–479 (2007).
- 106 Node K, Huo Y, Ruan X *et al.* Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 285(5431), 1276–1279 (1999).
- 107 Spiecker M, Liao JK. Vascular protective effects of cytochrome p450 epoxygenase-derived eicosanoids. *Arch. Biochem. Biophys.* 433(2), 413–420 (2005).
- 108 Sahi J, Black CB, Hamilton GA *et al.* Comparative effects of thiazolidinediones on *in vitro* P450 enzyme induction and inhibition. *Drug Metab. Dispos.* 31(4), 439–446 (2003).
- 109 Avandia.  
www.avandia.com
- 110 Richter B, Bandeira-Echtler E, Bergerhoff K, Clar C, Ebrahim SH. Rosiglitazone for Type 2 diabetes mellitus. *Cochrane Database Syst. Rev.* (3), CD006063 (2007).
- 111 Graham DJ, Ouellet-Hellstrom R, Macurdy TE *et al.* Risk of acute myocardial infarction, stroke, heart failure, and death in elderly Medicare patients treated with rosiglitazone or pioglitazone. *JAMA* 304(4), 411–418 (2010).
- 112 Richter B, Bandeira-Echtler E, Bergerhoff K, Clar C, Ebrahim SH. Pioglitazone for Type 2 diabetes mellitus. *Cochrane Database Syst. Rev.* (4), CD006060 (2006).
- 113 Mannucci E, Monami M, Lamanna C, Gensini GF, Marchionni N. Pioglitazone and cardiovascular risk. A comprehensive meta-analysis of randomized clinical trials. *Diabetes Obes. Metab.* 10(12), 1221–1238 (2008).
- 114 Ruano G, Bernene J, Windemuth A *et al.* Physiogenomic comparison of edema and BMI in patients receiving rosiglitazone or pioglitazone. *Clin. Chim. Acta* 400(1–2), 48–55 (2009).
- 115 Cho S, Atwood JE. Peripheral edema. *Am. J. Med.* 113(7), 580–586 (2002).
- 116 Bailey SD, Xie C, Do R *et al.* Variation at the NFATC2 locus increases the risk of thiazolidinedione-induced edema in the Diabetes REDuction Assessment with ramipril and rosiglitazone Medication (DREAM) study. *Diabetes Care* 33(10), 2250–2253 (2010).
- 117 Chang TJ, Liu PH, Liang YC *et al.* Genetic predisposition and nongenetic risk factors of thiazolidinedione-related edema in patients with Type 2 diabetes. *Pharmacogenet. Genomics* 21(12), 829–836 (2011).
- **One major study that by evaluating the association between thiazolidinedione, edema and clinical and genetic variables aimed to develop a simple points system to predict risk of thiazolidinedione edema association.**
- 118 Hansen L, Ekstrom CT, Tabanera YPR, Anant M, Wassermann K, Reinhardt RR. The Pro12Ala variant of the PPARγ gene is a risk factor for peroxisome proliferator-activated receptor-gamma/alpha agonist-induced edema in Type 2 diabetic patients. *J. Clin. Endocrinol. Metab.* 91(9), 3446–3450 (2006).
- 119 Kawaguchi-Suzuki M, Frye RF. Current clinical evidence on pioglitazone pharmacogenomics. *Front. Pharmacol.* 4, 147 (2013).
- 120 Wilson PW, D'agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Arch. Intern. Med.* 162(16), 1867–1872 (2002).
- 121 Masud S, Ye S, Group SaS. Effect of the peroxisome proliferator activated receptor-gamma gene Pro12Ala variant on body mass index: a meta-analysis. *J. Med. Genet.* 40(10), 773–780 (2003).
- 122 Gurnell M. 'Striking the right balance' in targeting PPARγ in the metabolic syndrome: novel insights from human genetic studies. *PPAR Res.* 2007, 83593 (2007).
- 123 Franks PW, Jablonski KA, Delahanty LM *et al.* Assessing gene-treatment interactions at the FTO and INSIG2 loci on obesity-related traits in the Diabetes Prevention Program. *Diabetologia* 51(12), 2214–2223 (2008).