

PPARs: Molecular Targets in the Pharmacogenomics Era

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Abstract: The recent global increase in prevalence of diseases like obesity, type 2 diabetes and hypertension in westernized societies is unfortunately not paralleled by our full understanding of the causative mechanisms. It is now firmly established that the interacting genetic and environmental (diet, smoking) components together determine the development and severity of the particular condition, which makes detailed dissection of such complex traits even more complicated. In effect, there is an unmet urgent need for molecular targets so we can directly modulate the causative factors and devise effective preventive and therapeutic algorithms. Among the most promising molecular targets for treatment of metabolic syndrome-related conditions identified so far, the group of three lipid-sensors, the peroxisome proliferator-activated receptors (PPARs) clearly stands out. The review focuses on pharmacogenetic aspects of recent developments in PPAR biology.

Key words: PPAR – Systems biology – Pharmacogenetics – Thiazolidinediones – cd36

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Introduction

Global epidemics of diseases like obesity, type 2 diabetes mellitus, hypertension or dyslipidemia represent a major health issue of today's world. These entities, often clustering under what becomes known as metabolic syndrome [1], challenge the basic and clinical research by the intricate complexity of their underlying biological mechanisms involving wide range of intertwined metabolic and signaling pathways, dynamically changing upon a blueprint of series of gene-gene and gene-environment interactions. The need for finding molecular targets in order to influence the causative factors and devising effective preventive and therapeutic algorithms is met by only a modest advancement of our knowledge in the field of pathogenesis of the most complex traits. The advent of systems biology-based research and medicine may be crucial in this effort as it brings about a more adequate, multi-level perspective of the processes it studies.

Among the most promising molecular targets for treatment of metabolic syndrome-related conditions, the group of three peroxisome proliferator-activated receptors (PPARs) clearly stands out. The first PPAR being identified in 1990, thousands of papers later we are uncovering, step-by-step, their integrative and orchestrating roles in lipid and carbohydrate metabolism, atherogenesis or cancer. PPARs are high on the lists of potential candidate genes for obesity [2], hypertriglyceridemia [3] and other complex metabolic derangements.

As there are several excellent reviews available discussing the role of PPARs in obesity [4], atherogenesis [5] or hypertension [6], in this review we focused mainly on pharmacogenetic aspects of recent advancements in PPAR biology.

Structure and genomic location of PPARs

Peroxisome proliferator-activated receptors are ligand-activated transcription factors belonging to the superfamily of nuclear hormone receptors (Tab.1), together with the receptors for vitamin D, steroid hormones, thyroid hormone, retinoids and many "orphan receptors", i.e. those without any activator or ligand identified so far. Three PPAR isotypes, i.e. PPAR α (NR1C1); PPAR β (NR1C2, PPAR δ , NUC-1, or FAAR); and PPAR γ (NR1C3), have been reported in vertebrates including mammals [7, 8] and form a C group in the subfamily 1 of the superfamily of the nuclear receptors (hence their code NR1C).

Three distinct, single-copy genes for respective PPARs are located at differing genomic locations in all mammalian species with a complete genome sequence available, i.e. man, rat and mouse, as shown in the Tab. 2. Their conserved genomic organization of six coding exons in multiple species implicates the existence of a common ancestral gene. In mammals, alternative promoter usage and differential splicing of the PPAR γ gene transcripts results in two isoforms, PPAR γ 1 and PPAR γ 2 [9]. In human, a PPAR γ 3 mRNA is transcribed from a third promoter, resulting in a protein identical to PPAR γ 1 as neither of the 5' exons (A1 or A2) is translated (Fig. 1). The three PPAR γ isoforms differ also in their

expression profiles, the variants PPAR γ 2 and PPAR γ 3 are highly expressed in adipose tissue whereas the PPAR γ 1 shows widespread expression [10, 11].

As for other members of the nuclear receptor superfamily, PPAR proteins are organized in main structural and functional domains: the aminoterminal (N)-terminal region (A/B domain) mediates ligand-independent transactivation functions; the C domain contains a highly conserved zinc finger structure forming the DNA-binding domain (DBD) and a dimerization interface; the D domain is a structurally poorly defined, nonconserved hinge region; the ligand-binding domain

Table 1 – Human nuclear receptors

Classical name	Official name	Ligand	Classical name	Official name	Ligand
TR α	NR1A1	Thyroid hormone	RXR γ	NR2B3	9-cis retinoic acid
TR β	NR1A2	Thyroid hormone	TR2	NR2C1	unknown
RAR α	NR1B1	retinoic acid	TR4	NR2C2	unknown
RAR β	NR1B2	retinoic acid	TLX	NR2E1	unknown
RAR γ	NR1B3	retinoic acid	PNR	NR2E3	unknown
PPAR α	NR1C1	fatty acids, leukotrienes	COUP-TFI	NR2F1	unknown
PPAR β	NR1C2	fatty acids	COUP-TFII	NR2F2	unknown
PPAR γ	NR1C3	fatty acids	EAR2	NR2F6	unknown
Rev-erb α	NR1D1	unknown	ER α	NR3A1	Estrogens
Rev-erb β	NR1D2	unknown	ER β	NR3A2	Estrogens
ROR α	NR1F1	unknown	ERR1	NR3B1	unknown
ROR β	NR1F2	unknown	ERR2	NR3B2	unknown
ROR γ	NR1F3	unknown	ERR3	NR3B3	unknown
LXR β	NR1H2	Oxysterols	GR	NR3C1	Glucocorticoids
LXR α	NR1H3	Oxysterols	MR	NR3C2	Mineralocorticoids
FXR α	NR1H4	Bile acids	PR	NR3C3	Progesterone
FXR β	NR1H5	Bile acids	AR	NR3C4	Androgens
VDR	NR1I1	Vitamin D	NGFIB	NR4A1	unknown
PXR	NR1I2	Xenobiotics	NURR1	NR4A2	unknown
CAR	NR1I3	Xenobiotics	NOR1	NR4A3	unknown
HNF4	NR2A1	unknown	SF1	NR5A1	unknown
HNF4 γ	NR2A2	unknown	LRH1	NR5A2	unknown
RXR α	NR2B1	9-cis retinoic acid	GCNF	NR6A1	unknown
RXR β	NR2B2	9-cis retinoic acid	DAX1	NR0B1	unknown
			SHP	NR0B2	unknown

Table 2 – Genomic locations of PPARs in man, rat and mouse

	Man	Rat	Mouse
PPAR α	HSA 22q12-13.1	RNO 7q34	MMU 15 E2 (48.8 cM)
PPAR β	HSA 6p21.2-p21.1	RNO 20p12	MMU 17 A3.3 (13.5 cM)
PPAR γ	HSA 3p25	RNO 4q42	MMU 6 E3-F1 (52.7 cM)

Abbreviations: HSA – human chromosome, RNO – rat chromosome, MMU – mouse chromosome

(LBD or E/F) mediates ligand-dependent transactivation and dimerization. After being activated by their ligands, PPARs form heterodimers with another nuclear receptor, RXR, and together bind to sites with specific sequence motifs in the promoter region of their target genes, to the so-called PPRE (PPAR response elements). PPRE consist of direct repetition of the consensus AGGTCA half site spaced by one (DR1) or two (DR2) nucleotides. In addition, PPARs can also be activated by phosphorylation of the A/B domain, and the PPAR:RXR heterodimer can be activated by RXR ligands as well. These different activation mechanisms, which can act in parallel, illustrate the capacity of fine-tuning that may be coordinated by PPAR actions.

The interaction of PPARs with their ligands, because of the conformational changes that are induced, allows the recruitment of co-activators and the release of co-repressors (reviewed in [12]). The importance of these molecules has been recently boosted by identification of PGC-1 α (peroxisome proliferator activator protein- γ co-activator-1 α) as an orchestrator of coordinated changes in expression of genes involved in mitochondrial oxidative phosphorylation in type 2 diabetes [13] and a regulator of triglyceride metabolism [14].

The recent observations harnessing the availability of the systems biology methods of genomewide analysis of structural, functional and metabolic “neighborhood” of PPARs reveals much more complicated pattern than that of simple ligand – receptor switch (on/off). The hypothesis of PPARs as integrative master nodes of metabolism was put forward and seems to reflect much closer the biological reality regarding these and other nuclear receptors.

PPAR α – the fasting switch

PPAR α was the first PPAR to be identified in early 1990s [7]. PPAR α is predominantly expressed in tissues with high rates of fatty acid oxidation, mostly liver and heart, less in muscle, kidney or brown adipose tissue. One of its major physiological functions is a “metabolic switch” during overnight or prolonged fasting, when the adipose tissue-derived fatty acids enter the liver, stimulating PPAR α expression, which in turn promotes fatty acid oxidation in order to provide an alternative energy source for peripheral tissues [15, 4], a process that is thoroughly disrupted in PPAR α -null mice [15].

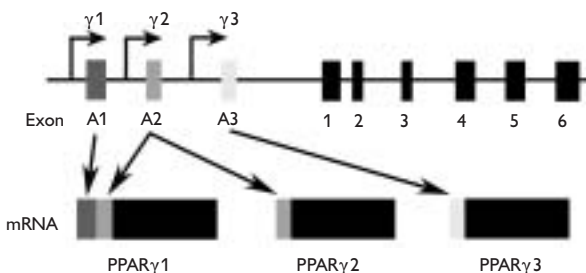


Fig. 1 – Schematic representation of human PPAR γ gene and its alternative transcript processing. Alternative promoter usage results in three different transcripts. Promoters for PPAR $\gamma 1$, 2 and 3 are upstream from A1, B and A2 exons, respectively. As neither of the 5' exons (A1 or A2) is translated, PPAR $\gamma 1$ and PPAR $\gamma 3$ encode the same protein.

PPAR α is a target of several groups of natural (fatty acids, eicosanoids) as well as synthetic compounds, clinically the most prominent being the fibric acid derivatives (e.g. fenofibrate, ciprofibrate, etofibrate, beclofibrate, clofibrate, bezafibrate, gemfibrozil – (Fig. 2) used in treatment of hyperlipoproteinemias. Apart from their lipid-lowering effect, fibrates are known to produce hepatomegaly and peroxisome proliferator-induced carcinogenesis in rodents, an effect not described in humans. Despite large degree of the PPAR α homology between mammalian species (Fig. 3), the distinct expression profile and subtle, but important sequence variations are probably responsible for the discordant effects. A precedent example of this concept can be found in the promoter of apolipoprotein A-I, one of the PPAR α target genes. The three-nucleotide difference between rat and human sequence of ApoA-I gene promoter results in non-responsiveness of the rat ApoA-I promoter to fibrates.

In most rodent models, PPAR α agonists decrease the triglyceride levels and improve insulin sensitivity [16, 17]. However, we have recently reported fenofibrate-induced hyperinsulinemia and increased adiposity and glucose intolerance in a newly established genetic model of metabolic syndrome, the polydactylous inbred rat strain (PD/Cub) [18, 19, 20]. As the effects of fibrates on insulin sensitivity has not been rigorously examined in human subjects [4] and a specific reaction to PPAR γ ligands was also observed in this strain [21], this distinct metabolic response may qualify the PD/Cub rat as an interesting pharmacogenetic model for PPAR ligands.

PPAR α as a mediator of glucocorticoid-induced insulin resistance

An unexpected and intriguing aspect of PPAR α involvement in insulin resistance was discovered recently in an elegant study showing that in Ppar α -deficient mice chronically treated with dexamethasone (a glucocorticoid known to induce insulin resistance and hypertension), none of the hyperglycemia, hyperinsulinemia and hypertension occurred, contrasting with the carriers of the wild-type allele. The

adenoviral reconstitution of Ppar α in the livers of nondiabetic, normotensive, dexamethasone-treated Ppar α -null mice induced hyperglycemia, hyperinsulinemia and increased gluconeogenic gene expression. It also increased blood pressure, renin activity, sympathetic nervous activity and renal sodium retention. The hepatic activation of PPAR α was thus proposed as a mechanism underlying glucocorticoid-induced insulin resistance [22].

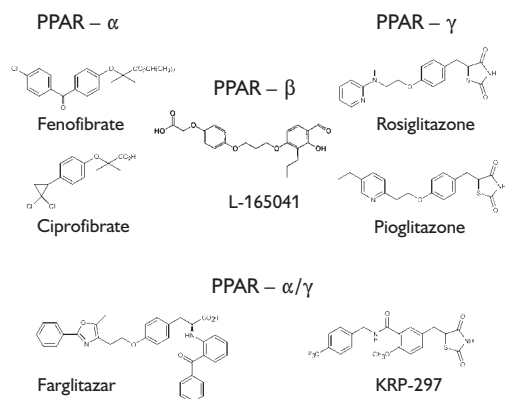


Fig. 2 – Chemical structures of selected PPAR agonists.

PPAR β – an unfolding story

PPAR β (also known as PPAR δ) is ubiquitously expressed with relatively higher levels in brain, colon, and skin. Although there have been extensive studies on PPAR α and PPAR γ , much less is known about the function of PPAR δ . When activated in adipose tissue, PPAR δ induces transcription of genes involved in fatty acid catabolism and energy dissipation [23], counter-balancing PPAR γ -driven lipid storage. Other studies have suggested PPAR δ might play a role in preadipocyte proliferation [24], epidermal maturation and wound healing [25] or in colon cancer [26, 27, 28]. A selective PPAR δ agonist, GW501516, has been developed (Fig. 2). When administered to insulin-resistant obese rhesus monkeys, GW501516 caused a dramatic dose-dependent rise in serum high density lipoprotein cholesterol while lowering the levels of small-dense low density lipoprotein, fasting triglycerides, and fasting insulin [29]. Altogether, though almost neglected for a long time, PPAR δ seems to be very promising target for treatment of metabolic syndrome [30]. Clinical studies of this compound are currently underway.

PPAR γ – metabolic syndrome and more

PPAR γ , originally identified for its crucial role in differentiation and gene expression regulation in adipocytes, was subsequently found to be expressed in other tissues and plethora of additional functions was ascribed to it [31].

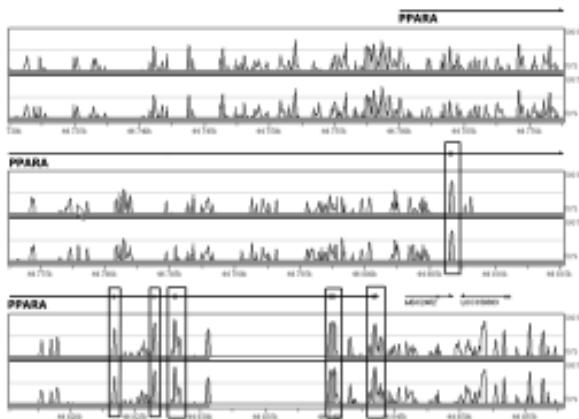


Fig. 3 – Comparative alignment of PPAR α gene sequence in human (template), rat (upper track) and mouse (lower track) using VISTA (<http://gsd.lbl.gov/vista/index.shtml>). The regions surrounding the six exons (boxes) are highlighted. The “peaks and valleys” graphs represent percent conservation between aligned sequences at a given coordinate on the base sequence. The Vista curve is calculated as a windowed-average identity score for the alignment and a score is calculated at each base in the coordinate sequence [70]. Only 50–100% section of the comparative alignment is shown.

TZD class of drugs is a potent PPAR γ agonists used in the therapy of insulin resistant states [32]. The thiazolidinedione class of drugs was shown to improve insulin sensitivity of peripheral tissues in model organisms and human subjects [33]. Their efficacy in improving glycemic control is attributed to the enhancement of insulin-stimulated glucose disposal in liver, adipose and muscle tissues. However, the underlying mechanism for TZD action remains unclear, the main issues raised by current findings [34] include the mode of TZD effect on muscle (direct vs. adipokine-mediated) and the dissociation of PPAR γ downstream events (dependence on type of agonist

and the elicited biological response). The two most commonly used TZDs in treatment of type 2 diabetes are rosiglitazone (RSG) and pioglitazone. TZDs are thought to shift the imbalanced free fatty acids (FFA) partitioning between muscle, liver and adipose tissues towards a greater FFA uptake in adipocytes, with concomitant boost in new adipocyte differentiation, further enhancing the FFA storage capacity [35]. This mechanism is also proposed to underlie one of the often described side-effect of TZD administration, the increased adiposity. Two identified PPAR γ targets are likely effectors for this action, CD36/FAT (fatty acid translocase) and FATP (fatty acid transport protein). Apart from the increase in adiposity, several other more severe side-effects have been also reported, mainly edema [36], idiosyncratic acute hepatotoxicity [37] and heart failure [38, 39]. Actually, the first TZD introduced to human medicine, troglitazone, has been withdrawn from the market due to cases of hepatotoxicity-driven liver failure. This only documents the fact that despite frequent use of TZDs in therapy, the molecular basis of their action is far from being fully understood, and especially their pharmacogenetic profile is yet to be determined in order to identify individuals potentially susceptible to exacerbation of unwanted side effects or simply with a diminished response to TZD therapy. The evidence for a relatively strong pharmacogenetic component arises mostly from the study of genetically defined rodent models of insulin resistance.

One such example has been recently described in models of metabolic syndrome, the spontaneously hypertensive (SHR) and BN.SHR4 inbred rat strains, both carrying the defective allele of the abovementioned fatty acid translocase *Cd36/Fat*, one of the key target genes of PPAR γ . The administration of rosiglitazone failed to improve glucose tolerance and hypertriglyceridemia in sucrose-fed BN.SHR4 rats [40]. In congenic and transgenic SHR with wild type *Cd36*, administration of pioglitazone (PIO) was associated with significantly lower circulating levels of fatty acids, triglycerides, and insulin as well as lower hepatic triglyceride levels and epididymal fat pad weights than in SHR harboring mutant *Cd36*. That led to identification of *Cd36* as a key determinant of the insulin sensitizing actions of these TZD ligands of PPAR γ [41], an observation with possible direct implications for therapeutic considerations in human population, as CD36 deficiency is rather frequent in type 2 diabetic patients in some ethnic groups.

Of course, not only TZDs are able to activate PPAR γ . Among the non-TZD PPAR γ agonists, following a recent observation of structural resemblance of PIO and telmisartan, an angiotensin II type 1 receptor antagonist, the latter was found to act as a partial agonist of PPAR γ . Given the known inhibitory effects of AngII receptor blockade on renal sodium reabsorption, authors propose the use of telmisartan as a template for development of antidiabetic PPAR γ ligands without the adverse effects of fluid retention, peripheral edema and heart failure associated with conventional agonists of PPAR γ [42].

Surprisingly, the inhibition of PPAR γ or modest reduction in its activity (observed in carriers of the Pro12Ala variant – see below) leads also to amelioration of metabolic

profile of treated individuals, even more complicating any simple mechanistic explanation of the mode of PPAR γ effect on metabolic pathways. One of the possible hypotheses is that resembling a sine-shaped curve of dependency on PPAR γ action, where starting with relatively large impairment of PPAR γ in carriers of loss-of-function mutations, who develop lipodystrophy and severe insulin resistance, going through a region of modest reduction in PPAR γ activity where an improvement in insulin sensitivity occurs, reaching region of obesity associated-insulin resistance, where activation of PPAR γ precipitates yet again in insulin resistance, only to change course again, when super-stimulated by TZDs or other agonists [43].

PPAR γ and osteoporosis

Lately, an interesting observation was made in respect of relation of PPAR γ and osteoformation as it was shown that PPAR γ deficient ES cells spontaneously differentiate into osteoblasts and PPAR γ haploinsufficiency enhances bone mass in mice, possibly through osteoblastogenesis [44]. If confirmed by further studies, this fact may bring a completely new paradigm to osteoporosis treatment, once bone-selective PPAR γ antagonists are developed [45].

Association studies of PPAR polymorphisms

Among the polymorphisms occurring in the PPAR genes, the common Pro12Ala polymorphism in the exon 2 of the PPAR γ 2 gene is by far the most prevalent (frequency of α 15% in Caucasians) and intensively studied one. Over 40 papers report its associations to varied traits ranging from glucose homeostasis [46], diabetic nephropathy [47], and polycystic ovary syndrome [48] to essential hypertension [49] and lifelong control of body weight [50]. Several lines of evidence now support the notion of the Ala variant is associated with reduced risk of type 2-diabetes, but this effect seems to disappear once the diabetes manifests itself, leaving the issue unresolved [51]. As for PPAR α , its most common C \rightarrow G polymorphism in exon 5 (L162V) was found to be associated with a decreased level of fasting serum triglyceride in glucose tolerant white subjects [52], lower body mass index in patients with non-insulin-dependent diabetes mellitus [53] and suggested as a risk factor for Alzheimer disease [54]. Though the currently available studies analyzing PPAR polymorphisms provide interesting information, they seem to be rather sketchy snapshots of a vast field. If we are to reach deeper understanding of relationship between variations in PPAR genes and human disease, a more systematic approach is needed. It can be envisaged that systematic thorough analyses of the SNP and haplotype characteristics of PPAR genes in relation to their pleiotropic roles in metabolism will follow shortly thanks to the ongoing efforts of international consortia generating the human and model haplotype and SNP maps [55]. Such studies may provide crucial and comprehensive information for pharmacogenetic considerations in treatment with PPAR agonists.

Dual PPAR α / γ agonists – double benefit or double trouble?

Though the effect of commonly used TZD, i.e. RSG and PIO on glycemic control is well documented, controversial results are available concerning their action on lipid levels. A summary analysis of 19 double-blind, placebo-controlled studies using either RSG or PIO was recently published [56]. The authors concluded that PIO seems to be more beneficial than RSG, though the results may have been shifted by a) stronger residual activation of PPAR α by PIO and b) differing population characteristics (the dyslipidemia in people included in PIO studies was more pronounced). Altogether, as expected, no spectacular lipopenic effect of either PIO or RSG similar to that of fibrates was found. Such observations lead to attempts to derive novel compounds that would combine the insulin sensitizing effect of TZD and lipid-lowering action of fibrates. Several such dual PPAR α / γ agonists have been derived and are summarized in the Tab. 3, the most recently derived classes include also alpha-aryloxy-alpha-methylhydrocinnamic acids [57] or aryloxazolidine-2,4-diones [58]. However, as the first reported results seem promising, thorough testing and validation are necessary to prevent potential mishaps down the road similar to those concerning troglitazone and clofibrate. Incidentally, all current clinical trials involving of one of the first compounds of this new class, ragaglitazar, were stopped worldwide and all new planned trials were postponed due to findings of urinary bladder tumors in mice and rats treated with ragaglitazar.

With the increasing amount of data supporting an important metabolic role of the so far least analyzed member of the PPAR family – PPAR β/δ – a PPAR $\alpha/\beta/\delta$ triple agonist with equal potency and efficacy on all three receptors has been derived [59] as a potential tailored drug for the metabolic syndrome. It should be noted though that several naturally occurring compounds exhibit also such pan-agonistic actions, namely the unsaturated fatty acids.

In summary, an account of recent development in the field of PPAR biology is given with the emphasis on novel medications targeting either single or multiple members of this nuclear receptor family, including pharmacogenetic considerations. The perspective of PPARs as molecular targets for treatment of

Table 3 – Dual PPAR α / γ ligands

Ragaglitazar (NNC-610029, DRF 2725)	Ebdrup et al., 2003	[60]
Farglitazar (GI-262570)	Agostini et al., 2004	[61]
Tesaglitazar (AZ-242, AR-H039242)	Ljung et al., 2002	[62]
DRF 2519	Chakrabarti et al., 2004	[63]
MK -0767	Doebber et al., 2004	[64]
TZD18	Guo et al., 2004	[65]
(S)-9d	Takamura et al., 2004	[66]
LY-465608	Etgen et al., 2002	[67]
NC-2100	Fukui et al., 2000	[68]
KRP-297	Murakami et al., 1998	[69]

metabolic syndrome and other prevalent human diseases has been validated by numerous studies, yet their real potential will probably be appreciated only after systems biology methods are fully integrated in basic and clinical research.

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