# Modulation of ABC Transporters by Nuclear Receptors. Physiological, Pathological and Pharmacological Aspects

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***Running title***

ABC transporter modulation by nuclear receptors

***Abstract***

ABC transporters are membrane proteins mediating the efflux of endo- and xenobiotics. Transporter expression is not static but instead is subject to a dynamic modulation aiming at responding to changes in the internal environment and thus at maintaining homeostatic conditions. Nuclear receptors are ligand modulated transcription factors that get activated upon changes in the intracellular concentrations of the respective agonists and bind to response elements within the promoter of ABC transporters, thus modulating their expression and, consequently, their activity. This review compiles information about transporter regulation by nuclear receptors classified according to the perpetrator compounds and the biological effects resulting from the regulation. Modulation by hormone receptors is involved in maintaining endocrine homeostasis and may also lead to an altered efflux of other substrates in cases of altered hormonal levels. Xenobiotic receptors play a key role in limiting the accumulation of potentially harmful compounds. In addition, their frequent activation by therapeutic agents makes them common molecular elements mediating drug-drug interactions and cancer multidrug resistance. Finally, lipid and retinoid receptors are usually activated by endogenous molecules, thus sensing metabolic changes and inducing ABC transporters to counteract potential alterations. Furthermore, the axis nuclear receptor-ABC transporter constitutes a promising therapeutic target for the treatment of several disease states like cancer, atherosclerosis and dyslipidemia. In the current work, we summarize the information available on the pharmacological potential of nuclear receptor modulators and discuss their applicability in the clinical practice.

***Keywords***

ABC transporters, drug transporters, nuclear receptors, drug-drug interactions, hormone receptors, P-glycoprotein, multidrug resistance-associated proteins, pregnane x receptor

## *1. Introduction*

Transporters of the ABC (ATP binding cassette) superfamily play a key role in the homeostasis of endobiotics like hormones and in the protection of the organism from potentially harmful xenobiotics. This challenging function is subject to a tight modulation, being the first step of this process, the sensing of alterations in the internal environment. In this regard, nuclear receptors are transcription factors which localize in the cell cytoplasm and nucleus and can be bound mainly by lipophilic compounds. Ligand binding usually leads to an increase in the transcriptional activity of the receptor and, consequently, in the expression of its target genes, which include ABC transporters. The final consequence of this regulatory mechanism is an increase in the transporter activity and thus a higher efflux of its substrates, which in physiological conditions results in recovery of homeostasis. Furthermore, nuclear receptor activation by therapeutic agents is a common mechanism underlying drug-drug interactions. In this regard, activated receptors may trigger an undesired induction of particular drug transporters, affecting the bioavailability of coadministered drugs. In addition, the regulation of ABC transporters by nuclear receptors has also been associated to cancer multidrug resistance due to their inducing role on the efflux of chemotherapeutic agents. In the current review, we will summarize the most relevant data on the nuclear receptor-mediated regulation of ABC transporters by hormones, xenobiotics, bile acids, lipids and retinoids, as well as the clinical relevance and potential therapeutic targets within these regulatory mechanisms.

## *2. General features of nuclear receptors*

### *2.1. Classification and nomenclature*

Nuclear receptor research initiated in the early 60s`of the last century with the discovery of the estrogen receptor α (ERα). Since then, more than 50 transcription factors functioning as nuclear receptors were described. In an attempt to facilitate their nomenclature and classification, the Nuclear Receptors Nomenclature Committee delimited a superfamily of nuclear receptors and proposed a classification system based on phylogenetic associations consisting of subfamilies and groups. Here,

49 nuclear receptors are divided into 6 subfamilies indicated by Arabic numerals. An additional subfamily 0 was added to allocate atypical receptors showing only one of the conserved domains usually found in nuclear receptors. Within each subfamily, groups are indicated with capital letters and individual members within each group are indicated by Arabic numerals [1]. For the sake of clarity, this review adopts the nomenclature recommendation of the pertaining Committee consisting of a first mention of each receptor with both the trivial and the systematic name. Subsequently, receptors are indicated only with their trivial name, as this is the way they usually appear in the original literature. For ABC transporters the systematic nomenclature is preferred. Genes encoding the transporters are indicated in italics. Protein names are completely written in upper case irrespective of the species of origin. In addition to the classical nuclear receptors, we will also review the information available on the aryl hydrocarbon receptor (AhR), a transcription factor displaying different structural properties than the classical receptors, but also playing an important role in the modulation of ABC transporters, particularly by xenobiotics [2].

### *2.2. Structure*

Nuclear receptors from the subfamilies 1 to 6 usually exhibit 5 to 6 conserved domains with a similar structure and function among the different receptors (Fig. 1). The A/B domain occupies the N-terminal extreme of the nuclear receptor and bears the activation function 1 (AF-1), usually involved in a ligand-independent transcriptional activity [3,4]. In addition, the A/B domain contains target sites for post-translational modifications that can also modulate the nuclear receptor activity [5]. Also, the A/B region has been reported to contain nuclear localization signals, important for the shuttling between the cytoplasm and the nucleus [6]. Following, the C domain of nuclear receptors functions as DNA binding domain (DBD). It is the most conserved region among all members of the superfamily. Its sequence mediates the contact with the nuclear receptor response elements in the promoter or enhancer regions of the target genes. Response elements are usually characterized by direct, inverted or complementary inverted repetitions separated by a particular number of nucleotides. Single amino acid changes in the nuclear receptor may be responsible for the differential affinity for different promoters [3,7]. The D domain or hinge follows the DBD and contributes to the structural flexibility

of the nuclear receptor and may also include nuclear localization signals, as demonstrated for thyroid hormone receptors [6]. The E domain or ligand binding domain (LBD) is notably less conserved than the DBD between different receptors and even between the same receptor of different species [8]. It harbors the dimerization surface contributing to the formation of homo- or heterodimers, the ligand binding pocket, the activation function 2 (AF-2), responsible for the ligand-dependent transcriptional modulation and a cofactor binding surface that links the DNA-bound nuclear receptor with histone modifying enzymes as well as with the transcriptional machinery [3]. The LBD may also contain nuclear export signals and thus contribute to nuclear-cytoplasmic shuttling [6]. The F domain is not present in all the nuclear receptors and its relevance is poorly studied. In the case of ERα, modulation of the dimerization by different C-terminal F domain sequences has been described [9].

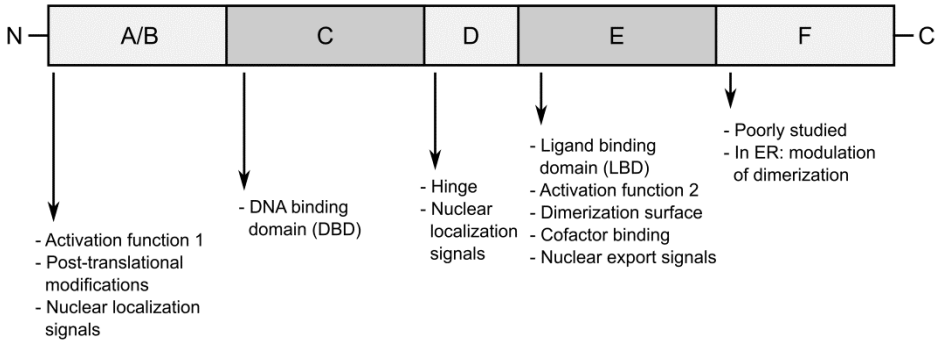


**Fig. 1. Structure of a typical nuclear receptor and the main functions of each region.** C: C-terminal end; ER: estrogen receptor; N: N-terminal end.

### *2.3. Mechanism of action*

Nuclear receptor interaction with coactivator and corepressor proteins, also known as cofactors, is a keystone in the ligand-mediated activation process. Coactivator proteins can exhibit histone acetyltransferase or histone methyltransferase activity, thereby promoting the decondensation of the chromatin around the nuclear receptor target gene. On the contrary, corepressors exhibit or associate with proteins exhibiting histone deacetylase activity, favoring chromatin condensation. An

extensive presentation of the different nuclear receptor cofactors as well as the molecular basis of their interaction with nuclear receptors are beyond the aim of this article and were already reviewed elsewhere [10,11].

In the absence of a ligand, nuclear receptors like the thyroid hormone receptors (TRs, NR1A), the retinoid acid receptors (RARs, NR1B) and the human pregnane X receptor (PXR, NR1I2) associate to the DNA together with corepressor proteins [3,12]. Upon ligand binding, for example due to increased intracellular concentration, different conformational changes take place within the receptor structure and lead to a release of bound corepressors and binding of coactivators finally resulting in chromatin decondensation. Subsequently, different adapter proteins mediate the recruitment of the general transcription machinery, resulting in an up-regulation of the gene expression (Fig. 2).

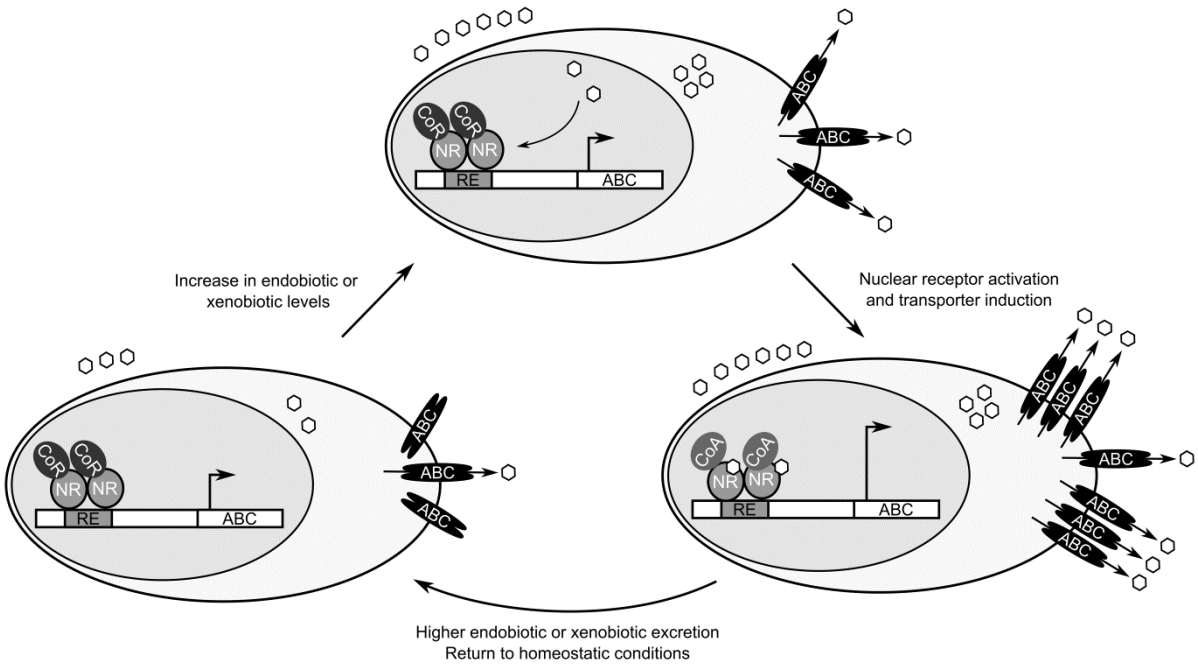


**Fig. 2. Mechanism of action of nuclear receptors with predominant nuclear localization.** Left panel: in basal conditions (i.e. low levels of ligand) the nuclear receptor is mainly bound to corepressor proteins and the expression of ABC transporters is kept at low levels. Upper panel: upon an increase in the endobiotic- or xenobiotic levels, basal levels of ABC transporters may not be enough to maintain homeostasis related intracellular concentrations. Consequently, a rise in the intracellular and

nuclear agonist levels and with that an increased binding to the nuclear receptor take place. Right panel: binding of the ligand triggers the dissociation of corepressors and the binding of coactivators resulting in an increase in the transcriptional activity of the nuclear receptor and thus in the expression of target ABC transporters. Then, higher proteins levels of the ABC transporters result in an increased substrate (ligand) efflux and thus in a return to the homeostatic (basal) conditions. ABC: ABC transporter; CoA: coactivator protein; CoR: corepressor protein; NR: nuclear receptor; RE: response element.

Alternatively, for other receptors like the estrogen receptors and the progesterone receptors, a predominantly cytosolic localization has been described. Upon ligand binding, the receptor translocates into the nucleus, homodimerizes and binds to coactivator proteins, also leading to an increase in the transcription of the target genes [13,14]. Induction of ABC transporters results in an enhanced efflux of their substrates and thus in the return to the homeostatic conditions (Fig. 3). In the case of ligands with antagonistic function, an increase in the corepressor binding and chromatin condensation take place, thus leading to a decrease in the gene expression [15].

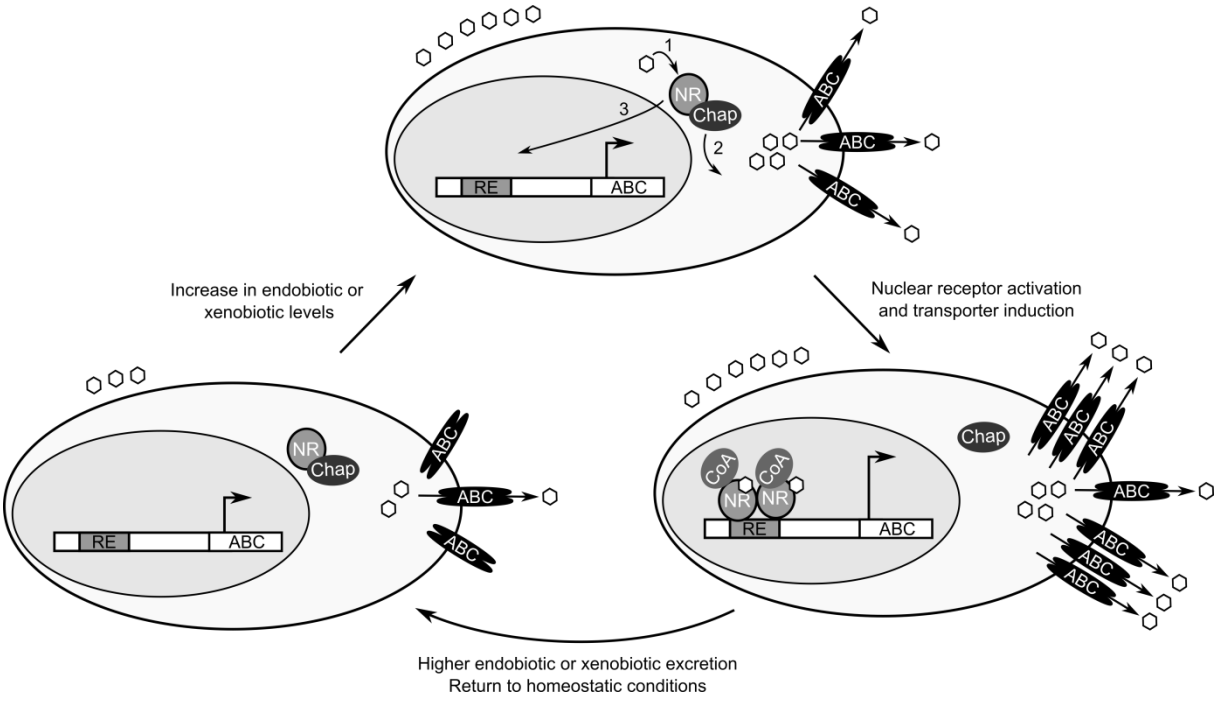


**Fig. 3. Mechanism of action of nuclear receptors with predominant cytosolic localization.** Left panel: in basal conditions (i.e. low levels of ligand) the nuclear receptor is localized in the cytosol and bound to chaperone proteins that prevent its nuclear translocation. The expression of ABC transporters is kept at low levels. Upper panel: upon an increase in the endobiotic- or xenobiotic levels, basal levels of ABC transporters may not be enough to maintain homeostasis related intracellular concentrations. Consequently, a rise in the intracellular agonist levels and with that an increased binding to the

nuclear receptor take place. Subsequently, conformational changes triggered by ligand binding lead to the dissociation of the chaperone proteins and the receptor translocation into the nucleus. Right panel: once in the nucleus, the activated nuclear receptor binds together with coactivator proteins to response elements in the promoters of ABC transporter genes, thus resulting in an increase in their expression. Then, higher proteins levels of the ABC transporters result in an increased substrate (ligand) efflux and thus in a return to the homeostatic (basal) conditions. ABC: ABC transporter; CoA: coactivator protein; CoR: corepressor protein; NR: nuclear receptor; RE: response element.

Although particular nuclear receptors usually display a conserved mechanism of action based on their localization, dimerization pattern and response elements, the final effect on the target gene transcription depends not only on the agonist or antagonist function of the ligand. In this regard, different phosphorylation states of a given receptor may lead to a differential recruitment of coactivators or corepressors and thus to an increase or decrease of the transcriptional activity [16]. Furthermore, changes in the expression of cofactors may lead to an altered activity of the nuclear receptor both in the presence and in the absence of a ligand [17]. The differential expression profile of coactivators and corepressors between different cell types was suggested as a mechanism underlying tissue specific responses to a given ligand [3]. In the following sections, we will summarize the major reports on ABC transporter regulation by nuclear receptors based on a functional classification of the receptors in hormone receptors, xenobiotic receptors, lipid and bile acid receptors and retinoid receptors.

## *3. Modulation of ABC transporters by hormone receptors*

### *3.1. Thyroid hormone receptors (TRα/NR1A1 and TRβ/NR1A2)*

Thyroid hormones T3 and T4 exert most of their biological actions through binding to the receptors TRα and TRβ. T3 and T4 levels can be altered in different pathological states [18]. The drug transporter ABCB1 (P-glycoprotein/Mdr1) can be induced in intestinal cell lines by both T3 and T4 [19]. This effect is mediated by TRβ binding to a response element in the *ABCB1* promoter. In relation with this observation, other authors reported an increase in ABCB1 expression in white blood cells from patients with Graves-Basedow disease, characterized by higher T3 and T4 levels in plasma, in

comparison to healthy subjects [20]. In addition, patients with hypothyroidism exhibited a reduced renal clearance of the ABCB1 substrate digoxin compared with the clearance after hormone supplementation treatment. This effect was partially attributed to a decreased renal secretion of digoxin via ABCB1. Moreover, TRα expression exhibited a significant correlation with ABCB1 basal expression, further suggesting a role of thyroid hormones and the associated nuclear receptors in the regulation of the basal gene expression [21]. These reports highlight the importance of dose optimization in patients with thyroid disorders when using ABCB1 substrates, especially for drugs showing a narrow therapeutic window.

The bile salt export pump (BSEP/ABCB11) is one of the main transporters contributing to bile salt dependent bile flow. Alterations in the transporter activity may lead to changes in the bile composition. In this regard, the modulation of murine *Abcb11* expression by TRβ in a ligand-independent fashion has been described [22]. The effect was characterized by the interaction of TRβ with the nuclear repressor corepressor 1 (NCoR1), with changes in the degree of this interaction leading to alterations in *Abcb11* expression and thus in the bile salt secretion, bile hydrophobicity and finally, in the absorption of cholesterol from the diet.

### *3.2. Vitamin D receptor (VDR/NR1I1)*

1α,25-dihydroxyvitamin $D_3$ (synonym: 1α,25-dihydroxycholecalciferol; calcitriol) is a hormone playing a major role in calcium and phosphate metabolism and bone homeostasis. Besides these well-known effects, VDR has been described to bind to response elements in the promoter of *ABCB1* and mediate its up-regulation by vitamin D in the intestinal cell lines Caco-2 and LS174T [23–25]. A role for VDR has also been demonstrated in the *ABCB1* induction by the secondary bile acid lithocholic acid. Here, the transporter induction may lead to prevention of the intestinal toxicity by this bile acid [24]. Besides these examples of modulation at the intestinal level, ABCB1 up-regulation by vitamin D has also been reported in isolated rat brain capillaries and brain endothelial cell lines from rat and human origin. This effect was associated with a reduced accumulation of the neurotoxic protein amyloid beta, a potential ABCB1 substrate [26]. Furthermore, an up-regulation of ABCB1 in mouse kidney by vitamin D was observed in association with a reduced area under the curve (AUC) of the

ABCB1 substrate digoxin [27]. In addition, up-regulation of ABCC2 (multidrug resistance associated protein 2, MRP2) expression and activity by vitamin D in Caco-2 cells has been described [25]. These observations were reproduced in an everted rat intestinal sac model [28]. Taking into account that ABCB1 and ABCC2 regulation by VDR also leads to a modulation in transporter activity, alterations in the transport of endogenous compounds or therapeutic drugs substrates of these transporters could be expected as a consequence of alterations in the vitamin D levels.

### *3.3. Estrogen receptors (ERα/NR3A1 and ERβ/NR3A2)*

Estrogens are steroid hormones mediating several physiological processes related to the development and reproduction. Their effects result mostly from binding of physiological estrogens like 17β-estradiol to ERα and ERβ [14]. In addition, ERs can be activated by drugs like 17α-ethinylestradiol, a frequent component of contraceptive pills, and by several endocrine disrupting chemicals. As a consequence of their ubiquitous expression, ERs have been described to modulate several ABC transporters in diverse cell types and were associated with physiological and pathological states. For instance, estrogens and their receptors play a major role in the pathogenesis of hormone associated cancers like breast cancer [14]. Furthermore, estrogen signaling involves a wide range of transcription factors and cofactors interacting with the classical ERs [3,14]. The different availability of these factors exhibited by different cell types or tissues is likely to contribute to the differential response that may be observed after exposure to a given ER agonist. A summary of cases involving ABC transporter modulation via ER and other hormone nuclear receptors can be found in Table 1.

#### *3.3.1. Contribution to epithelial and endothelial barriers*

Epithelial barriers rely on the activity of efflux transporters to exert their protective function. The contraceptive agent 17α-ethinylestradiol as well as the phytoestrogen genistein, one of the major isoflavones present in soy beans and red clover, were described to up-regulate ABCB1 and ABCC2 expression and activity in differentiated Caco-2 cells, a model of the intestinal epithelial barrier [29]. ERβ, the most abundant ER in the intestine, was demonstrated as the participating molecular mediator as coincubation with the antagonist ICI 182,780 totally prevented the inducing effect. As a

consequence of this enhanced ABCB1 and ABCC2 dependent transport, a reduced bioavailability of orally administered drugs can be expected. In this regard, an increase in the oral clearance of the ABCB1 substrate talinolol in healthy volunteers receiving genistein has been reported [30]. Concomitantly, the transporter induction may exert a protective effect against diet associated toxicants.

Similarly, the blood-brain barrier plays a key role in limiting the penetration of toxic compounds into the central nervous system. In a study using primary human brain endothelial cells and contrarily to the previous findings in an intestinal model, ERβ was reported to mediate ABCB1 down-regulation by 5α-androstane-3β,17β-diol, a metabolite of dihydrotestosterone [31]. In a similar way, an ERβ-mediated decrease of ABCG2 (breast cancer resistance protein, BCRP) protein and mRNA levels was reported after exposition of rat brain capillaries to 17β-estradiol [32]. Likewise, a decrease in ABCG2 protein expression by 17α-ethinylestradiol has also been described [33]. Furthermore, decreased ABCG2 protein levels has been observed after exposure to the highly selective ERβ agonist diarylpropionitrile [32]. Besides transcriptional down-regulation, stimulation of ABCG2 proteasomal degradation was demonstrated as a mechanism underlying ERβ-mediated decrease in ABCG2 expression [Hartz et al., JPET, 2010->now reference Nr. 34]. Transporter down-regulation correlated with a decreased efflux of their substrates in all the cases. Considering that ABCG2 transports therapeutic agents and potentially toxic compounds, these findings suggest a higher penetration of these compounds into the brain upon activation of the receptor, for instance by drugs or by phytoestrogens. Alternatively, ABCG2 down-regulation by ERβ might be useful as a therapeutic strategy in those cases aiming at increasing the delivery of drugs into the brain.

At the blood-testis barrier, protecting germinal cells from potentially genotoxic agents, ABC transporters also play a major role in limiting the toxicant and drug penetration. The mycotoxin zearalenone, a frequent contaminant of cereals, down-regulated *Abcb1a* und *Abcb1b* at the mRNA level in rat testes but concomitantly resulted in an increase in the protein levels. The opposite effect (mRNA decrease, protein increase) was observed in the Sertoli cell derived cell line SerW3. The prevention of the effect by ICI 182,780 suggests also an ER-dependent mechanism. In addition, *Abcc4* (multidrug resistance associated protein 4, MRP4) and *Abcc5* (multidrug resistance associated protein

5, MRP5) exhibited an induction at the mRNA level in testes from exposed animals, an effect also associated with ER [34]. Nevertheless, the authors also described a dissociation between mRNA and protein levels, probably as a consequence of a translational regulation, leading to a decreased protein expression in the tissue of animals exposed to particular doses of the toxin. This could result in an increase in the penetration of drugs or environmental toxicants to the testes potentially disturbing the spermatogenesis [34].

The placenta constitutes a barrier protecting the fetus from toxic compounds present in the maternal circulation. ABCG2 is expressed in the apical membrane of syncytiotrophoblast cells and contributes to this protective function [35]. In this regard, a down-regulation of ABCG2 expression by pregnancy-associated 17β-estradiol concentrations has been described in the human placental cell line BeWo [36]. The effect was associated with a down-regulation of ERβ by the estrogen. A correlation between the transcriptional activity of the *ABCG2* promoter and the ER protein expression in BeWo cells has already been established, however only for ERα [37]. A similar down-regulation by 17β-estradiol was observed in human term placenta explant cultures, being prevented by ICI 182,780 [38]. However, the physiological relevance of these findings is doubtful, since the hormonal profile during pregnancy is characterized by the increase in the levels not only of 17β-estradiol but also of progesterone. Concomitant exposure of BeWo cells to both hormones resulted in an increase of both ABCG2 expression and activity, thus suggesting an enhanced protection of the fetus [36]. Besides, physiological estrogens, xenoestrogens like para-nonylphenol and bisphenol A have also been described to down-regulate placental ABCG2 expression in term placenta explant cultures. Prevention of the effect by ICI 182,780 indicates an ER mediated mechanism. This observation suggests exposure to endocrine disrupting compounds as a factor increasing the penetration of ABCG2 substrates into the fetal blood [38].

### *3.3.2. Hepatic transport of endo and xenobiotics*

In hepatocytes, ABCC3 (multidrug resistance associated protein 3, MRP3) is expressed in the basolateral membrane thereby mediating the active transport of substrates into the sinusoidal blood. Although ABCC3 basal expression is low, it can be induced upon drug exposure or in pathological

states, for instance in case of an impairment of the biliary secretion. The synthetic estrogen 17α-ethinylestradiol has been described to modulate ABCC3 expression in rat liver, rat primary hepatocytes [39] and also in the human hepatic cell line HepG2 [40]. The effects were prevented by ICI 182,780, thus indicating mediation by ERα, the only ER isoform present in the hepatocytes. However, human *ABCC3* promoter does not bear estrogen response elements capable of binding ER, nor does the rat promoter. Indeed, the effect was mediated by up-regulation of the transcription factor c-Jun by 17α-ethinylestradiol. This leads to an enhanced interaction between c-Jun and ERα and thus to an increased binding to an AP-1 binding site within the *ABCC3* promoter [40]. These observations could help explain previous reports demonstrating an increased urinary excretion of the ABCC3 substrate acetaminophen-glucuronide in women taking oral contraceptives [41].

Intrahepatic cholestasis of pregnancy takes place during the last trimester of pregnancy and is characterized by increased levels of serum bile acids. To date, the mechanisms underlying this disease are not fully understood. Since ABCB11 mediates the rate limiting step in the bile salt canalicular secretion, its down-regulation may be associated to the impaired bile salt transport observed in pregnancy. Indeed, in mice treated with 17β-estradiol, mimicking the peak in the estrogen concentration reached before delivery, ABCB11 expression was down-regulated [42]. The effect was ERα-mediated, however independent of the binding of the receptor to estrogen response elements in the *Abcb11* promoter. On the contrary, 17β-estradiol binding favored the interaction between ERα and the farnesoid X receptor α (FXRα/NR1H4), a well-known *Abcb11* positive regulator. This receptor-receptor interaction impaired the recruitment of the coactivator PGC1α (peroxisome proliferator-activated receptor-γ coactivator 1) and increased the recruitment of the corepressor NCoR (nuclear receptor corepressor), with the final repressive effect on ABCB11 expression [43]. A similar inhibitory effect was described after exposure to 17α-ethinylestradiol, where treatment of mice with the estrogen leads to a down-regulation of *Abcb11* in wild type mice but not in *ERα*$^{-/-}$ mice. This finding might contribute to explain the intrahepatic cholestasis associated with the intake of contraceptives or during hormonal replacement therapy [44].

### *3.3.3. Multidrug resistance*

Over-expression of ABC transporters is a common mechanism underlying multidrug resistance exhibited by cancer cells. In this regard, ERs have been reported to mediate transporter up-regulation in multidrug resistant breast cancer. For instance, a correlation has been reported between ERα and ABCB1 expression levels in MCF-7 and ZR-75-1 cells, models of $ER\alpha^+$ breast cancer. Moreover, over-expression or knock-down of $ER\alpha^+$ were associated with an increased and decreased ABCB1 expression, respectively. Furthermore, treatment with ICI 182,780 exerted a chemosensitizing effect in a model of breast cancer xenotransplantation [45]. ABCB1 modulation by ERα was reported to be mediated by binding of the nuclear receptor to a half estrogen response element in the *ABCB1* promoter and required the presence of the transcription factor Sp1 [45]. In a similar way, an analysis of breast cancer specimens exhibited a correlation between $ER\alpha^+$ expression and ABCC11 (multidrug resistance associated protein 8, MRP8) levels, an ABC transporter mediating the efflux of fluoropyrimidines and methotrexate [46]. Furthermore, the analysis of differentially expressed genes between patients with and without recurrences pointed out *ABCC11* as an over-expressed gene in non-responder patients and thus as a potential candidate responsible for the poorer outcome [Ma XJ et al., Cancer Cell, 2004, now->48]. Since the *ABCC11* promoter harbors an estrogen response element, a direct control of transporter expression by ERα seems feasible [46].

Besides the above-mentioned transporters, ABCG2 has also been described to mediate the multidrug resistant phenotype in breast cancer. Treatment of the cell line MCF-7 with 17β-estradiol resulted in ABCG2 up-regulation at the transcriptional level. The prevention of the effect by the ER antagonist tamoxifen, the presence of estrogen response elements in *ABCG2* promoter as well as the absence of an effect in the $ER\alpha^-$ cell line MDA-MB-231 indicate a mediation by this nuclear receptor [47]. In addition, the treatment with the estrogen receptor antagonist toremifene alone was enough to down-regulate ABCG2 expression in MCF-7 cells, also confirming an association between ER and this transporter [48]. Furthermore, a synergistic effect of ERα activation by 17β-estradiol and proinflammatory cytokines on *ABCG2* expression has been described. In particular, binding of activated ERα to the *ABCG2* promoter allows the recruitment of the NFκB component p65 to a response element downstream of the estrogen response element. p65 binding leads to stabilization of

the ERα binding to the promoter and thus to an enhanced *ABCG2* transcription. Therefore, higher ABCG2 expression levels may be expected in tumors where proinflammatory cytokines are locally produced [49].

Although ERα represents the most studied ER in breast cancer, ERβ was also reported to mediate *ABCG2* expression and thus chemoresistance. In this regard, a clinical study demonstrated a positive correlation between ERβ and ABCG2 expression levels. Moreover, over-expression of ERβ in the $ERα^-/ERβ^-$ cell line MDA-MB-453 was sufficient to render ABCG2 expression inducible by 17β-estradiol. Also, the prevention of ABCG2 induction by tamoxifen and the binding of ERβ to *ABCG2* promoter further confirm a role of ERβ in the regulation of this transporter [50], as previously described for ERα [47]. This observation points out the importance of classifying breast tumors not only according to ERα expression but also according to ERβ presence.

In addition to ERα-mediated transcriptional activation of *ABCG2*, inhibition of the protein synthesis by 17β-estradiol in MCF-7 without changes in mRNA levels has been described [51]. Prevention by tamoxifen also points out an ER-dependent mechanism. The reason of the discrepancies between these results and those obtained by others [e.g. [47]] might rely on different culture conditions leading to activation of a translational mechanism in the first case that was not present in the second study. In this regard, microRNAs (miRNAs) were already described to mediate differences in the ABC transporter modulation in a same model after a particular treatment. For instance, in HepG2 cells, both an increase in *ABCC2* transcription [52] and an inhibition in the translation of the same protein [53] have been reported. The authors suggested differentially expressed miRNAs and a differential sensitivity to these miRNAs between cells lines cultured under different conditions as a possible mechanism underlying this observation [53].

### *3.3.4. Cholesterol efflux*

ABC transporters, in particular ABCA1 and ABCG1 mediate active cholesterol efflux from the cell and are involved in the pathogenesis of atherosclerosis [54]. In addition to the well-known role of macrophages in the foam cell formation, vascular smooth muscle cells (VSMCs) have been reported to contribute to atherosclerotic plaque development. Treatment of mouse primary VSMCs with 17β-

estradiol resulted in an induction of *ABCA1* and *ABCG1* at the mRNA and protein levels. The effect was dependent on the ERβ activation, although the induced transporters did not appear to be target genes of this nuclear receptor. Indeed, activation of ERβ leads to the induction of the liver X receptor α (LXRα/NR1H3), which is then responsible for the induction of the transporter expression and cholesterol efflux. Although evidence in this field remains contradicting, these results support an association of ERβ and ABC transporters in the protective role of estrogens against atherosclerosis development [55].

### *3.4. Glucocorticoid receptor (GR/NR3C1)*

Glucocorticoid receptors exist as two main isoforms GRα and GRβ, resulting from alternative splicing of the *GR* gene [56]. GRα is the isoform with most ubiquitous expression. For instance, its expression has been demonstrated in the placenta as well as in placental cell lines [57]. Furthermore, in the same study a GR-mediated up-regulation of *ABCB1* by the synthetic glucocorticoid dexamethasone has been observed. These findings suggest a potential alteration in the drug efflux across the placental barrier in the case of corticosteroid administration during pregnancy [57]. Similar observations were obtained in the hepatic and intestinal cell lines HepG2 and Caco-2, respectively [58].

In addition, an induction by dexamethasone was reported for ABCB1, ABCC2 and ABCG2 expression in rat brain endothelial cells. The effect on ABCB1 and ABCG2 expression was partially prevented by the GR antagonist RU486, thus indicating a participation of this receptor. This observation may gain importance, for instance, in patients with brain tumors cotreated with corticosteroids to reduce edema. In this case, a lower brain penetration of chemotherapeutic agents may compromise the anticancer therapy [59]. Conversely, dexamethasone was reported to down-regulate ABCG2 expression and reduce resistance to mitoxantrone in breast cancer cell lines. This effect might be the consequence of a cross-talk between GR, PR and PXR and could result in a reduced resistance to ABCG2 substrates [60].

### *3.5. Progesterone receptor (PR/NR3C3)*

Progesterone is a steroid hormone exhibiting a major physiological relevance in reproductive functions. Its levels oscillate during the menstrual cycle and markedly increase during pregnancy. Classical progesterone receptors comprise PR-A and PR-B, both being products of the same gene but differing on the expression pattern and promoter affinity [61]. It was reported that ABCB1 protein expression in the endometrium increases during the secretory phase as well as in gestational endometrium concomitantly with rising progesterone concentrations [62]. In this regard, activation of PR was described to trigger the transcriptional activity of a reporter gene under the control of the murine *Abcb1b* promoter, encoding one of the ABCB1 variants in rodents, in breast cancer cells. The effect appeared to be specifically mediated by PR-A and represented the first study associating ABCB1 and a nuclear PR [61]. In another work, progesterone was described to up-regulate ABCB1 expression in granulosa cells, also in a PR-dependent manner, possibly contributing to the efflux of steroid hormones, well-known ABCB1 substrates [63].

ABCG2 was also described to be modulated via PR-dependent mechanisms. For instance, a PR-B-dependent induction of ABCG2 by progesterone in the placental cell line BeWo has been demonstrated [64]. These observations indicate a role of progesterone and the PR in the protection of the fetus from harmful compounds present in the maternal blood. Furthermore, ABCG2 is expressed in several tumors, mediating the efflux of chemotherapeutic agents and thus drug resistance [65]. In this regard, intratumoral ABCG2 expression is also subject to transcriptional modulation by PR. For instance, a down-regulation of ABCG2 by progesterone has been demonstrated in $PR^+$ breast cancer cell lines. Changes in protein expression also correlated with a reduced efflux of the ABCG2 substrate mitoxantrone. Moreover, the absence of effect in a $PR^-$ cell line as well as the prevention by the PR antagonist RU486 further indicate a PR-mediated mechanism [66,67].

### *3.6. Androgen receptor (AR/NR3C4)*

Gender specific transporter expression was reported for renal *Abcc3* and *Abcc4*, where male mice exhibited a lower transporter expression compared to the female counterparts. Moreover, experiments with gonadectomized mice supplemented with 17β-estradiol or dihydrotestosterone (DHT) suggest an

inhibitory effect of the masculine sexual hormone on the transporter expression [68]. Although an androgen response element in the promoter of *Abcc4* has been described [68], other studies pointed out an indirect mechanism of action of the androgens to modulate transporter expression. For instance, an up-regulation of ABCC4 expression by DHT in prostate cancer cells has been described, being independent of the androgen response elements within *ABCC4* promoter [69]. A similar study demonstrated a reduced ABCC4 expression in prostate cancer samples from patients undergoing antiandrogen treatment with bicalutamide. In an attempt to elucidate the molecular mechanisms, the authors tested a reporter gene under the control of an androgen response element without achieving a significant induction by DHT [70]. A cross-talk between the AR and c-Jun transcription factor could allow explaining the use of alternative binding sites in the transporter promoter [69]. As part of the same study, ABCC4 expression in prostate cancer cells was associated with an enhanced resistance to methotrexate, thus highlighting the role of AR and ABCC4 in cancer acquired resistance [69].

Hepatic ABCB1 expression was reported to be higher in female than in male rats, also leading to an enhanced hepatobiliary secretion of the substrate doxorubicin. Treatment of female rats with testosterone decreased ABCB1 expression to levels similar to those of male rats, thus associating the sexual steroid to the transporter expression. However, the specific mediation of these effects by the androgen receptor was not evaluated [71].

Table **1.** ABC transporter modulation by hormone receptors.

| **Receptor** | **Transporter** | **Modulatory compounds** | **Model or tissue** | **Effect reported** | | | **Physiological process or biological effect** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| | | | | **mRNA** | **Protein** | **Activity** | | |
| TR | ABCB1 | T3 and T4 | Human leucocytes | ↑ | | ↑ | Lower substrate bioavailability | [20–21] |
| TR | ABCB1 | T3 and T4 | Intestinal cell lines | ↑ | | | | [19] |
| TR | ABCB11 | - | Mouse liver | ↑↓ | | | Change in bile hydrophobicity and cholesterol absorption | [22] |
| VDR | ABCB1 | Calcitriol | Intestinal cell lines | ↑ | ↑ | ↑ | Lower substrate bioavailability | [23–25] |
| VDR | ABCB1 | Lithocholic acid | LS174T cells | ↑ | | | Lower substrate bioavailability | [24] |
| VDR | ABCB1 | Calcitriol | Rat brain endothelial cells | ↑ | ↑ | ↑ | Reduced penetration of substrates into CNS, reduced accumulation of amyloid beta protein | [26] |
| VDR | ABCB1 | Calcitriol | Mouse kidney | ↑ | ↑ | ↑ | Increased renal clearance | [27] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | and mouse brain | | | | of ABCB1 substrates | |
| VDR | ABCC2 | Calcitriol | Caco-2 cells | ↑ | ↑ | ↑ | Lower substrate bioavailability | [25] |
| VDR | ABCC2 | Calcitriol | Rat everted intestinal sacs | | | ↑ | Lower substrate bioavailability | [28] |
| ERβ | ABCB1 ABCC2 | 17α-ethinylestradiol genistein | Caco-2 cells | ↑ | ↑ | ↑ | Lower substrate bioavailability | [29] |
| ERβ | ABCB1 | 5α-androstane-3β-17β-diol | Human brain endothelial cells | | ↓ | | Increased penetration of ABCB1 substrates into the CNS | [31] |
| ERβ | ABCG2 | 17β-estradiol, 17α-ethinylestradiol | Rat brain endothelial cells | ↓ | ↓ | ↓ | Increased penetration of ABCG2 substrates into the CNS | [32,33],new 34Hartz |
| ER | ABCB1 | Zearalenone | Rat testes | ↓ | ↑↓ | | Changes in the contact of germinal cells with xenobiotics | [34] |
| ER | ABCC4 ABCC5 | Zearalenone | Rat testes | ↑ | ↓ | | Changes in the contact of germinal cells with xenobiotics | [34] |
| ER | ABCG2 | Zearalenone | Rat testes | ↓ | ↓ | | Changes in the contact of germinal cells with xenobiotics | [34] |
| ER | ABCB1 | Zearalenone | SerW3 cells | ↑ | ↓ | | Changes in the contact of germinal cells with xenobiotics | [34] |
| ER | ABCC4 | Zearalenone | SerW3 cells | ↑ | ↑ | | Changes in the contact of germinal cells with xenobiotics | [34] |
| ER | ABCC5 | Zearalenone | SerW3 cells | ↑ | ↑↓ | | Changes in the contact of germinal cells with xenobiotics | [34] |
| ERβ | ABCG2 | 17β-estradiol | Placental cell line (BeWo) Human term placenta | ↓ | ↓ | ↓ | Increased penetration of ABCG2 substrates into the fetal blood | [36,38] |
| ER and PR | ABCG2 | 17β-estradiol + progesterone | BeWo cells | ↑ | ↑ | ↑ | Reduced penetration of ABCG2 substrates into the fetal blood | [36] |
| ER | ABCG2 | para-nonylphenol, bisphenol A | Human term placenta | ↓ | ↓ | | Increased penetration of ABCG2 substrates into the fetal blood | [38] |
| ERα | ABCC3 | 17α-ethinylestradiol | Rat liver HepG2 cells | ↑ | ↑ | | Higher excretion of ABCC3 substrates into the sinusoidal blood | [39,40] |
| ERα | ABCB11 | 17β-estradiol, | Mouse liver | ↓ | ↓ | | Bile salt dependent-bile flow impairment | [42–43] |
| ERα | ABCB11 | 17α-ethinylestradiol | Mouse liver | | | ↓ | Bile salt dependent-bile flow impairment | [44] |
| ERα | ABCB1 | - | Breast cancer cell lines | ↑ | ↑ | ↑ | Increased resistance to ABCB1 substrates | [45] |
| ERα | ABCC11 | - | Breast cancer specimens | ↑ | | | Increased resistance to fluoropyrimidines and methotrexate | [46] |
| ERα | ABCG2 | 17β-estradiol | MCF-7 cells | ↑ | ↑ | ↑ | Increased resistance to ABCG2 substrates | [47] |
| ERβ | ABCG2 | 17β-estradiol | MDA-MB-453 cells | ↑ | ↑ | ↑ | Increased resistance to ABCG2 substrates | [50] |
| ER | ABCG2 | 17β-estradiol | MCF-7 cells | = | ↓ | ↓ | Decreased resistance to ABCG2 substrates | [51] |
| ERβ (and LXRα) | ABCA1 ABCG1 | 17β-estradiol | Vascular smooth muscle cells | ↑ | ↑ | ↑ | Increased efflux of cholesterol, inhibition of atherosclerotic plaque development | [55] |
| GR | ABCB1 | Dexamethasone | Human | ↑ (reporter | | | Reduced penetration of | [57] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | placental cell lines | gene) | | | ABCB1 substrates into the fetal blood | |
| GR | ABCB1 | Dexamethasone | HepG2 Caco-2 cells | ↑ | | | Lower substrate bioavailability | [58] |
| GR | ABCB1 ABCG2 | Dexamethasone | Rat brain endothelial cells | ↑ | ↑ | ↑ | Reduced penetration of ABCB1 substrates into the CNS | [59] |
| GR | ABCG2 | Dexamethasone | Breast cancer cell lines | ↓ | ↓ | ↓ | Reduced resistance to ABCG2 substrates | [60] |
| PR | ABCB1 | Progesterone | Human secretory and gestational endometrium | | ↑ | | Reduced intracellular accumulation / higher efflux of substrates | [62] |
| PR | ABCB1 | Progesterone | Porcine granulosa cells | ↑ | | | Reduced intracellular accumulation / higher efflux of ABCB1 substrates (e.g. steroid hormones) | [63] |
| PR | ABCG2 | Progesterone | BeWo cells | ↑ | ↑ | ↑ | Reduced penetration of ABCG2 substrates into the fetal blood | [64] |
| PR | ABCG2 | Progesterone | Breast cancer cell lines | ↓ | ↓ | ↓ | Reduced resistance to ABCG2 substrates | [66,67] |
| AR | ABCC3 ABCC4 | DHT | Mouse kidney | ↓ | ↓ | | Reduced transport of substrates in male individuals | [68] |
| AR | ABCC4 | DHT | Prostate cancer cell lines | ↑ | ↑ | ↑ | Increased resistance to ABCC4 substrates | [69] |

Species are only indicated for models different from human. ↑: increased expression/activity; ↓: decreased expression/activity; = : no changes; calcitriol: 1α,25-dihydroxyvitamin $D_3$; CNS: central nervous system; DHT: dihydrotestosterone.

## *4. Modulation of ABC transporters by xenobiotic receptors*

### *4.1. Pregnane X Receptor (PXR/NR1I2)*

PXR can be classified as orphan nuclear receptor due to the absence of a physiological ligand. Indeed, while physiological steroids have been reported to bind to PXR ligand binding pocket, this process exhibits lower affinity than xenobiotic binding and takes place usually at supra-physiological concentrations [8]. On the contrary, PXR displays a notably promiscuity concerning xenobiotic binding. For instance, a wide range of therapeutic agents and naturally occurring compounds were described as PXR agonists. Moreover, the huge spectrum of PXR target genes, including several members of the ABC transporter superfamily, confers this nuclear receptor a major role regarding protection against harmful xenobiotics, drug-drug and natural compound-drug interactions and multidrug resistance [72]. Noteworthy, PXR is characterized by a species-specific activation based on a high divergence in the sequence of the LBD. Consequently, strong agonists of human PXR (hPXR)

like rifampicin fail to activate murine PXR. On the contrary, strong agonists of murine PXR like pregnenolone-16α-carbonitrile (PCN) and cyproterone acetate trigger no activation of hPXR [8]. Below, we will summarize the most important cases concerning ABC transporter modulation by PXR and experimental strategies aiming at shortcutting PXR signalling, and thus PXR associated undesired effects. An extensive review of PXR agonists and their effects on different target genes can be found in [72] and [73]. The most important cases of ABC transporter regulation mediated by PXR, as well as by other xenobiotic receptors, are also presented in Table 2.

#### *4.1.1. Chemical barrier function*

ABC transporters play a major role in protecting the organism from harmful xenobiotics present in the environment or in the diet. Due to its extremely flexible ligand binding pocket allowing the binding of structurally different compounds, PXR functions as a xenosensor detecting increases in the exposition to xenobiotics and, consequently, triggering and increase in the expression of the transporters responsible for limiting the accumulation of these potentially toxic compounds. PXR expression mainly in cells from absorptive and secretory epithelia such as hepatocytes, enterocytes, renal tubular cells and brain endothelial cells further supports this protective role [8,74]. Moreover, PXR expression in the small intestine correlates positively with the expression of *ABCB1*, *ABCC1*, *ABCC2* and *ABCG2* also in absence of agonist, thus indicating not only a PXR role in the response to a chemical insult but also in the modulation of transporter basal expression [75].

Concerning the cytoprotective function of PXR activation, a mitigation of hepatotoxicity associated with cholic acid induced cholestasis has been described in mice cotreated with a PXR agonist. This effect relied on the up-regulation of sinusoidal ABCC3 by activated PXR, thus providing an alternative excretion pathway for bile salts under conditions of bile flow impairment [76]. Similarly, an increase in the expression of ABCB1 [77], ABCC2 [78] and ABCG2 [74] has been reported in brain endothelial cells from different species by treatment with PXR agonists like PCN, rifampicin or hyperforin. Like any transporter induction at the blood-brain barrier, it may be beneficial in preventing the brain penetration of toxic compounds but it may also limit the uptake of drugs targeting the central nervous system. In this regard, a decrease in the antinociceptive effect of the

ABCB1 substrate methadone has been reported in hPXR-expressing transgenic mice cotreated with rifampicin [77].

### *4.1.2. Drug-drug interactions*

Rifampicin is an antibiotic used in the treatment of several bacterial infections such as tuberculosis and leprosy. In addition, rifampicin was characterized as a strong hPXR agonist and, moreover, as an ABCB1 and ABCC2 inducer through a mechanism relying on binding of the activated nuclear receptor to PXR response elements in the promoters of the mentioned ABC transporters [58,79,80]. Furthermore, in the case of ABCB1, the transporter induction was associated with a lower bioavailability of the well-known ABCB1 substrate digoxin [81,82]. Likewise, treatment with rifampicin resulted in hepatic and intestinal ABCC2 up-regulation [58,83]. Moreover, healthy volunteers pretreated with rifampicin exhibited a decreased exposure to morphine-6-glucuronide and a reduced antinociceptive effect [84], agreeing well with the well-known transport of morphine glucuronides by ABCC2 [85]. Similarly, pretreatment with rifampicin decreased the AUC of the immunosuppressant agent mycophenolic acid and increased the urinary excretion of glucuronide conjugates, substrates of ABCC2, in renal allograft recipients [86].

St John´s wort is a frequent component of natural formulations used in the treatment of depression. Hyperforin constitutes one of the main active principles found in available extracts. Interestingly, hyperforin was also one of the first PXR agonists described [87]. In line with this finding, intake of St John´s wort was associated with intestinal ABCB1 up-regulation and a lower oral bioavailability of its substrates fexofenadine, cyclosporine and talinolol in healthy volunteers [88–90]. Moreover, decreased cyclosporine bioavailability due to the intake St John´s wort was also observed in transplanted patients and even associated with a case of acute transplant rejection [Bauer et al., Br J Clin Pharmacol, 2003->now 93; Ruschitzka et al., Lancet, 2000->now 94]. Furthermore, up-regulation of hepatic ABCC2 was described in rats exposed to St John´s wort, suggesting that the inducing effects and consequently the interaction potential are not restricted to ABCB1 and its substrates [91].

Antiviral drugs also exhibit a high interaction potential, partially due to their function as PXR agonists. For instance, amprenavir, atazanavir, lopinavir, nelfinavir, ritonavir and saquinavir were

reported to up-regulate ABCB1 expression in the intestinal cell line LS180, frequently used as a model to screen drug-drug interactions. Concomitant activation of a PXR reporter gene assay and attenuation of the induction in PXR knock-down cells points to a mediation of this nuclear receptor in the transporter induction [92]. Similarly, the non-nucleoside reverse transcriptase inhibitor rilpivirine, more recently approved for HIV therapy, also exhibited a PXR-dependent ABCB1 up-regulation in the same cell line [93]. Considering that HIV/AIDS patients may be polymedicated, a decrease in the oral bioavailability of coadministered drugs is likely to occur. In addition, PXR-dependent up-regulation of ABCB1 was described in human brain microvessel endothelial cells exposed to amprenavir, darunavir, lopinavir and efavirenz, the latter resulting from a concomitant PXR and CAR (constitutive androstane receptor) activation (see also section 4.2.2) [94]. Considering that several inducing antiviral agents are also ABCB1 substrates, this transporter up-regulation may limit their uptake into the brain and thus contribute to the constitution of the central nervous system as a pharmacological sanctuary site for HIV.

Carbamazepine is an antiepileptic drug activating PXR *in vitro* [95]. In line with this finding, healthy volunteers treated with carbamazepine exhibited an increase in intestinal *ABCB1* and *ABCC2* mRNA expression as well as an increase in ABCC2 at the protein level. In addition, treatment with carbamazepine resulted in a lower exposition and a higher renal clearance of the ABCB1 and ABCC2 substrate talinolol. These results indicate a probable induction of ABC transporters also at the renal level [96]. Noteworthy, while the alterations in the pharmacokinetics of talinolol were still within the accepted therapeutic window, a similar induction in patients cotreated with carbamazepine and ABCB1 and ABCC2 substrates with a narrow therapeutic range may result in a lower therapeutic efficacy.

Spironolactone is an antagonist of the mineralocorticoid receptor used in the clinical practice as diuretic and also demonstrated to activate PXR [8]. Spironolactone up-regulated ABCB1 in the human hepatic cell line HepG2. Prevention of the effect by PXR knock-down suggests a participation of this nuclear receptor in the transporter induction [97]. Furthermore, a similar ABCB1 induction was observed in the intestine and liver of rats treated with spironolactone. Interestingly, exposure to the diuretic led to a reduced bioavailability of the ABCB1 substrate digoxin resulting from a diminished

intestinal absorption rather than from an enhanced biliary excretion, thus suggesting potential drug-drug interactions especially with orally administered ABCB1 substrates [98].

### *4.1.3. Nutrient-drug interactions*

Exposure to different spectra of natural compounds present in the environment and in the diet appears as a possible driving force that led to the divergent evolution of the PXR ligand binding domain across species [8]. In this regard, natural compounds were also reported to modulate ABC transporters via PXR activation. For instance, purified tangeritin, a flavonoid present in the peel of citrus fruits and ginkgolide A and B, present in *Ginkgo biloba* extracts used in traditional medicine were described to up-regulate PXR transcriptional activity and ABCB1 protein expression in LS180 cells. Also, a reduced intracellular accumulation of the ABCB1 model substrate rhodamine-123 was observed [99]. Moreover, clementine juice, mandarin juice and grapefruit juice increased PXR activity as well as AhR activity in the same intestinal cell line. This effect was associated with an up-regulation of *ABCC2* mRNA by the three treatments. In addition, grapefruit juice up-regulated *ABCB1*, *ABCC3* and *ABCG2* mRNA expression while treatment with mandarin juice resulted also in *ABCC3* up-regulation. These observations indicate an inducing effect of citrus fruits also in a form available for human consumption and highly suggest potential interactions with coadministered medications [100]. Similarly, β-carotene, abundant in plants and fruits and also used as food colorant up-regulated PXR activity and *ABCB1* and *ABCC2* expression in HepG2 cells [101]. Also, members of the vitamin E group were reported to induce PXR. In fact, γ-tocotrienol and the vitamin E metabolite α-tocopherol-13´-COOH up-regulated PXR activity and ABCB1 expression and reduced rhodamine-123 accumulation in LS180 cells [102]. In a similar way, piperine, a compound present in pepper, was reported to increase PXR activity and ABCB1 expression in rat small intestine. Moreover, this transporter induction correlated with a decrease exposure to the ABCB1 substrate diltiazem and its active metabolite desacetyldiltiazem after oral administration [103]. These findings highlight the importance of considering dietary habits and intake of nutritional supplements during the establishment of a medication plan, especially for those therapeutic agents exhibiting narrow therapeutic windows.

#### *4.1.4. Multidrug resistance*

PXR-mediated induction of ABC transporters was also associated to acquired multidrug resistance in cancer. For example, a PXR-dependent up-regulation of ABCB1 by vincristine, vinblastine, flutamide, docetaxel and paclitaxel has been reported in a colon adenocarcinoma cell line. A concomitant decrease in rhodamine-123 accumulation indicates an increase in the transporter efflux activity [104]. Similarly, treatment with the tyrosine kinase inhibitors erlotinib, gefitinib, nilotinib, sorafenib and vandetanib resulted in PXR-dependent ABCB1 induction [105]. Also, ABCC3 basal expression was modulated by PXR in colon carcinoma [106]. In addition, other cancer types also exhibit PXR-mediated multidrug resistance. For instance, an induction in ABCB1 expression and an increase in the resistance to its substrates paclitaxel and tamoxifen have been reported in the breast cancer cell lines MDA-MB-231 and MCF-7, respectively. Moreover, experimental inhibition of ABCB1 expression using a short hairpin RNA resulted in higher sensitivity to paclitaxel and vinblastine [107]. In a similar way, exposure to a PXR agonist up-regulated ABCB1 expression in non-small-cell lung cancer and prostate cancer cell lines and, consequently, increased resistance to paclitaxel [108,109]. This evidence clearly indicates an important role of PXR in the autoinduction of chemoresistance as well as promoting a decreased therapeutic response upon cotreatment with other PXR agonists.

#### *4.1.5. PXR inhibition as therapeutic approach*

Antagonism of PXR may represent a possible strategy to counteract PXR-mediated drug-drug or nutrient-drug interactions as well as to prevent acquired resistance to anticancer therapy. Although several natural and synthetic compounds were tested for PXR inhibition *in vitro*, supporting clinical evidence is still lacking. For instance, A-792611, developed as HIV protease inhibitor, prevented the activation of a PXR response element by the well-known agonists rifampicin and ritonavir in primary human hepatocytes. Moreover, A-792611 reduced *ABCB1* expression, even in the presence of ritonavir [110]. To date, no studies have confirmed the clinical suitability of A-792611 as an antagonist. Also the antifungal drug ketoconazole was reported to antagonize PXR activation *in vitro*

[111]. However, a clinical study involving healthy volunteers treated with hyperforin and ketoconazole failed to corroborate this antagonistic effect *in vivo* [112].

An extensive study on the effect of natural extracts on PXR transcriptional activity in LS180 cells pointed out an antagonistic effect of milk thistle, observed in terms of prevention of the induction of a reporter gene under the control of a PXR response element by rifampicin and also by the chemotherapeutic agent paclitaxel. A similar effect was observed after exposure to commercially available milk thistle based products. Analysis of individual extract components indicated silybin and isosilybin as the main compounds responsible for the observed effect [113]. Similarly, sesamin and resveratrol, compounds abundant in sesame seeds and red wine, respectively, prevented PXR activation in HepG2 and LS174T cells [114,115]. Considering the low toxicity of these compounds, they constitute promising alternatives to antagonize PXR-mediated induction of ABC transporters by drugs and natural compounds and thus the associated pharmacological interactions and multidrug resistance. Clinical studies should be performed to confirm this antagonistic potential *in vivo*.

In addition, bitter melon extracts reduced PXR-mediated induction of *ABCB1*, *ABCC2* and *ABCG2* and attenuated resistance to doxorubicin in human colon cancer cells. However, the effect was due to a decrease in PXR expression by the natural extract instead of an antagonistic effect as described above [116]. A similar mechanism of action relying on PXR down-regulation was found for the mycotoxin ochratoxin A, ingested as food contaminant. In this case, ochratoxin A prevented the induction of a reporter gene under the control of a PXR response element in the human embryonic cell line HEK293T as well as the induction of the typical PXR target gene *CYP3A4* by rifampicin in human hepatocytes [117]. Although ochratoxin A toxicity limits its therapeutic applications to avoid PXR-mediated transporter induction, its intake bears high toxicological relevance since it might lead to an increased bioavailability and potential toxicity of transporter substrates.

### *4.2. Constitutive Androstane Receptor (CAR/NR1I3)*

Like PXR, CAR was originally classified as an orphan receptor and functions as a xenosensor in the regulation of ABC transporters in connection with chemical barrier functions and the clearance of endobiotics and xenobiotics. CAR structure, activators, regulation and mechanism of action were

extensively reviewed by di Masi et al. [72]. Following, we will summarize the main biological processes where a transporter modulation by CAR was described.

### *4.2.1. Systemic clearance of xenobiotics*

Similar to PXR, under physiological conditions CAR is mainly expressed in liver, intestine and kidney and modulates the expression of several ABC transporters with pharmacological and toxicological relevance. CAR over-expression was sufficient to increase ABCB1 expression in the human intestinal cell lines LS174T [118] and Caco-2 [119]. Moreover, CAR was reported to bind as a heterodimer with the retinoid X receptor α (RXRα/NR2B1) to several response elements in the *ABCB1* promoter. Furthermore, exposure of Caco-2 cells to the CAR activator phenobarbital resulted in the induction of *ABCB1, ABCC1* and *ABCC2* mRNA and protein expression [58]. On the contrary, exposure of cells to CAR inverse agonists such as clotrimazole and meclizine resulted in an inhibition of *ABCB1* promoter activity, indicating also the possibility of a transcriptional down-regulation by this nuclear receptor [118]. Concerning positive transporter regulation, an activation of CAR by the antimalarial drug artemisin concomitantly with an induction of *ABCB1* expression in LS174T cells was reported. Nevertheless, the inducing effect on the transporter expression appears to be more dependent on the simultaneous PXR activation [120]. In addition, natural compounds were also described to modulate ABCB1 expression through a CAR-dependent mechanism. This was, for instance, the case of baicalein, a compound present in several oriental medicine formulations. Baicalein increased CAR transcriptional activity and CAR binding to DNA response elements and consequently *ABCB1* expression in the same intestinal cell line. This up-regulatory effect bears a high potential for micronutrient-drug interactions considering that herbal medicines, as well as diet associated compounds, do not require a medical prescription and thus might remained unconsidered during the medication process [121].

Similarly, CAR was reported to modulate ABC transporters and thus xenobiotic clearance at the hepatic level. For instance, phenobarbital increased ABCB1, ABCC1 and ABCG2 expression in human hepatocytes [58,122]. Additionally, an induction of *Abcc3*, *Abcc6* (multidrug resistance associated protein 6, *Mrp6)*, *Abcc10* (multidrug resistance associated protein 7, *Mrp7*) and *Abcc11* was

reported in mouse liver [123]. *Abcc2*, *Abcc3* and *Abcc4* modulation by the prototypical CAR activator 1,4-Bis(3,5-dichloro-2-pyridyloxy)benzene (TCPOBOP) was further evaluated in liver of wild type and *CAR*$^{-/-}$ male and female mice. The induction was observed only in wild type mice and the effect was gender-independent [124]. Induction of ABCC2 and ABCC3 by phenobarbital was also reported in rat liver, concomitantly with an activation of CAR, also pointing out a causal association between the nuclear receptor and the drug transporter [80,125]. These observations highlight the role of CAR in the regulation of ABC transporters in pharmacologically relevant tissues like intestine and liver, thus suggesting a potential risk of drug-drug interactions upon administration of CAR modulating compounds and ABC transporter substrates. This could be the case, for example, during administration of valproic acid, used in the clinical practice as antiepileptic agent, for which pharmacologically relevant concentrations resulted in an induction of the transcriptional activity of *ABCB1* promoter in the hepatic cell line HepG2 as well as in an induction of *ABCB1* mRNA levels in the intestinal cell line LS174T [126]. Moreover, the induction was reversed by the cotreatment with the CAR inverse agonist androstenol, thus indicating further possible pharmacological interactions depending on the presence of other receptor modulators.

#### *4.2.2. Blood-brain barrier*

Similarly to CAR-mediated modulation of ABC transporters in liver and intestine, this nuclear receptor plays an important role in the modulation of the blood-brain barrier permeability. For instance, phenobarbital up-regulated ABCB1, ABCC2 and ABCG2 expression and activity in mouse and rat brain capillaries. Protein phosphatase 2A (PP2A) catalyzes a key step in the CAR activation cascade. Hereby, pretreatment with the PP2A inhibitor okadaic acid prevented the transporter up-regulation. Moreover, no inducing effect was observed in capillaries isolated from *CAR*$^{-/-}$ mice, thus clearly indicating a participation of this nuclear receptor in the up-regulation of the above-mentioned transporters [127]. Acetaminophen is a widely consumed analgesic and, also, a well-known CAR activator [128]. Indeed, acetaminophen increased ABCB1 expression in rat brain microvessels through a CAR-dependent mechanism. The effect was associated with a reduced uptake of morphine into the brain and a reduced antinociceptive effect of the opioid [129]. ABCB1 modulation by CAR was also

confirmed in porcine brain capillary endothelial cells where the treatment with the CAR agonist 6-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO) leads to an ABCB1 and also ABCG2 induction at the mRNA and protein level. Prevention of the effect by the CAR inverse agonist meclizine further indicates a regulatory role of this nuclear receptor [130]. Finally, a similar effect was described in a human brain microvessel endothelial cell line, where the antiretrovirals abacavir, efavirenz and nevirapine up-regulated ABCB1 expression concomitantly with a CAR activation [94]. This inducing effect may be associated with a reduced penetration of coadministered antiretroviral drugs substrates of this transporter.

#### *4.2.3. Clearance of endobiotics*

Bile flow impairment as well as a higher production of bile acids from dietary sources may lead to an increase in bile acids in serum. ABCC4 locates to the basolateral membrane of the hepatocyte and mediates the efflux of bile acids into the sinusoidal blood. CAR was demonstrated as a molecular mediator of this process. For instance, treatment of mice with the prototypical agonist TCPOBOP resulted in an enhanced hepatic ABCC4 expression both at mRNA and protein level, being this effect absent in *CAR*$^{-/-}$ mice [131]. Mediation by CAR was further confirmed in an *in vivo* reporter assay where phenobarbital treatment resulted in the induction of a reporter gene under the control of *Abcc4* promoter. Similarly, patients suffering from obstructive cholestasis exhibited higher CAR protein levels, higher CAR nuclear translocation, which indicates higher nuclear receptor activation, and also an increase in ABCC4 expression. Although the compounds specifically mediating CAR activation in cholestatic disease are not completely known, concomitant induction of the bile acid sulfotransferase 2A1 (*Sult2a1*) by CAR in mice suggests a common mechanism aiming at conjugating and extruding (i.e. detoxifying) highly toxic unconjugated bile acids [131,132].

In addition, a role of CAR in the modulation of cholesterol homeostasis has been demonstrated in mice fed with a high fat diet. Indeed, CAR activation by TCPOBOP leads to an induction in hepatic bile salt synthesis from cholesterol and an increased bile salt excretion due to an ABCB11 up-regulation. Simultaneously, CAR activation was associated with a down-regulation in hepatic and intestinal ABCG5 and ABCG8 expression, which mediate cholesterol efflux into the bile and the

intestinal lumen, respectively. The final result was a decrease in cholesterol accumulation in the liver [133].

Bilirubin is a product of heme catabolism. Its detoxification relies on its uptake by hepatocytes followed by conjugation and excretion by ABCC2. In this regard, CAR activation by TCPOBOP was reported to induce bilirubin clearance in mice, consistent with the well-known induction of *Abcc2* by CAR. Moreover, a direct CAR-mediated regulation of *Abcc2* by bilirubin and *CAR*$^{-/-}$ mice exhibiting higher plasma bilirubin levels than wild type ones after a hemolytic stimulus have been reported. Additionally, the authors demonstrated a lower CAR expression in neonate compared to adult liver, which may probably account for the higher bilirubin levels observed in newborn children [134].

### *4.3. Aryl hydrocarbon receptor (AhR)*

Although not belonging to the nuclear receptor superfamily, AhR exhibits a mechanism of action and regulatory properties on ABC transporters similar to those of classical nuclear receptors. Indeed, AhR is a member of the basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) family of transcription factors. In the inactive state (i.e. in absence of ligand), AhR is retained in the cytoplasm. Binding of ligands (e.g. aromatic hydrocarbons, drugs) triggers conformational changes within the receptor leading to the exposure of the nuclear localization signal and thus to the nuclear translocation and binding to dioxin-response elements within the promoters of target genes [2,135].

Among ABC transporters, ABCG2 was reported to be modulated through AhR after exposure to different agonists of this transcription factor in different cell types or tissues. For instance, TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) induced ABCG2 expression in human cell lines of intestinal, hepatic and mammary gland origin. A similar induction was reported in primary human colonocytes and hepatocytes. Moreover, the inducing effect of TCDD was prevented by coincubation with the AhR antagonist 3′,4′-dimethoxyflavone, thus indicating a causal relationship between the activation of AhR and ABCG2 induction [122,136,137]. A similar induction was observed in the intestinal cell line LS174T after exposure to the AhR agonist 3-methylcholanthrene [138]. Moreover, an inducing effect was also reported in normal bovine mammary epithelial cells. In this case, the environmental pollutant TCDD and the fungicide prochloraz up-regulated ABCG2 expression. This finding may suggest an

increase in the transport of ABCG2 substrates into the milk during exposure to AhR agonists [139]. Similarly, *Abcg2* induction by 3-methylcholanthrene was reported in normal mouse liver [140]. In addition to environmental pollutants, certain drugs can also activate AhR thereby inducing ABC transporters. For instance, ABCG2 induction by oltipraz [137] and obatoclax [141] were reported in the intestinal cell lines Caco-2 and LS180, respectively. Likewise, the histone deacetylase inhibitor romidepsin, used in cancer therapy, was able to induce ABCG2 in glioblastoma, colon and breast cancer cell lines, while the effect was prevented by AhR knock-down [142].

In addition, other ABC transporters different from ABCG2 can be induced by AhR agonists in epithelia of pharmacotoxicological relevance. For example, oltipraz induced *ABCB1*, *ABBC2*, *ABCC3* and *ABCC4* mRNA in primary human hepatocytes, probably in an AhR-dependent way. ABCC4 induction was further confirmed at the protein level [122,143]. Similarly, a gender-specific induction of *Abcc2*, *Abcc3* and *Abcc4* in mouse liver by TCDD was reported, where the absence of the effect in AhR knock-out animals clearly indicates mediation by this nuclear receptor [124]. In line with these observations, 3,3',4,4',5-pentachlorobiphenyl (PCB126), also an environmental toxicant, induced murine hepatic *Abcc2*, *Abcc3*, *Abcc5*, *Abcc6* and *Abcc10* at the mRNA level [123]. Additionally, ABCB1, ABCC2 and ABCG2 induction by TCDD was reported at the blood-brain barrier [144]. These results suggest a reduced penetration of transporter substrates into the brain during exposure to AhR agonists like environmental toxicants, also resulting in an enhanced protection against potentially harmful compounds but concomitantly in a reduced delivery of therapeutic agents to targets within the central nervous system.

AhR also mediates ABC transporter modulation in disease states. For example, liver fibrosis was associated to hepatic ABCC2 down-regulation resulting from a concomitant down-regulation in AhR expression, probably as a consequence of the increase in proinflammatory cytokines [145]. Moreover, an AhR role was described in tumor diseases such as head and neck squamous cell carcinoma (HNSCC). Here, a constitutive AhR activation has been reported in patient samples. This effect was associated with a more aggressive phenotype. Moreover, exposure of HNSCC cell lines to an AhR agonist resulted in a clear ABCG2 induction, which may result in an enhanced resistance to chemotherapeutic agents. Transporter induction was prevented by treatment with the AhR antagonist

GNF351, suggesting AhR inhibition as a potential strategy to overcome induction of drug transporters and thus acquired multidrug resistance [146]. Indeed, this approach has already been proven in esophageal carcinoma cell lines, where treatment with the AhR antagonists kaempferol and salicylamide partially reverted acquired resistance to the ABCG2 substrates 5-fluorouracil and irinotecan [147].

Table **2.** ABC transporter modulation by xenobiotic receptors

| Receptor | Transporter | Modulatory compounds | Model or tissue | Effect reported | | | Physiological process or biological effect | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | mRNA | Protein | Activity | | |
| PXR | ABCB1<br>ABCC1<br>ABCC2<br>ABCG2 | - | Small intestine | ↑ | | | Lower substrate bioavailability | [75] |
| PXR | ABCC3 | PCN | Liver of mice under cholic acid induced cholestasis | ↑ | | | Higher sinusoidal excretion and reduced hepatotoxicity by bile salts | [76] |
| PXR | ABCB1 | PCN | Mice brain endothelial cells | | ↑ | ↑ | Reduced penetration of transporter substrates into the central nervous system | [77] |
| PXR | ABCB1<br>ABCC2 | Rifampicin | Brain endothelial cells of hPXR-expressing mice | | ↑ | ↑ | Reduced penetration of transporter substrates into the central nervous system | [77] |
| PXR | ABCC2 | PCN | Rat brain endothelial cells | | ↑ | ↑ | Reduced penetration of transporter substrates into the central nervous system | [78] |
| PXR | ABCB1<br>ABCG2 | Hyperforin<br>Rifampicin | Porcine brain endothelial cells | ↑ | ↑ | ↑ | Reduced penetration of transporter substrates into the central nervous system | [74] |
| PXR | ABCB1<br>ABCC2 | Rifampicin | Hepatic and intestinal cell lines | ↑ | ↑ | | Lower substrate bioavailability | [58,79,80] |
| PXR | ABCB1 | Rifampicin | Human liver and small intestine | | ↑ | ↑ | Lower substrate bioavailability | [81,82] |
| PXR | ABCC2 | Rifampicin | Human small intestine | ↑ | ↑ | ↑ | Lower substrate bioavailability | [83,84,86] |
| PXR | ABCB1 | Hyperforin | Human small intestine | ↑ | ↑ | ↑ | Lower substrate bioavailability | [88–90] |
| PXR | ABCC2 | Hyperforin | Rat liver | | ↑ | | Lower substrate bioavailability | [91] |
| PXR | ABCB1 | Amprenavir<br>Atazanavir<br>Lopinavir<br>Nelfinavir<br>Ritonavir<br>Saquinavir | LS180 cells | ↑ | | | Lower substrate bioavailability | [92] |
| PXR | ABCB1 | Rilpivirine | LS180 | ↑ | ↑ | | Lower substrate bioavailability | [93] |
| PXR | ABCB1 | Amprenavir<br>Darunavir<br>Lopinavir<br>Efavirenz | Human brain endothelial cells | | ↑ | ↑ | Lower penetration of ABCB1 substrates into the CNS | [94] |
| PXR | ABCB1 | Carbamazepine | Small intestine | ↑ | = | ↑ | Lower substrate bioavailability | [96] |
| PXR | ABCC2 | Carbamazepine | Small intestine | ↑ | ↑ | | Lower substrate bioavailability | [96] |
| PXR | ABCB1 | Spironolactone | Rat liver and small intestine, HepG2 cells | ↑ | ↑ | ↑ | Lower substrate bioavailability | [97,98] |
| PXR | ABCB1 | Tangeritin<br>Gingkolide A and B | LS180 cells | ↑ | ↑ | ↑ | Lower substrate bioavailability | [99] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PXR and AhR | ABCC2 | Clementine juice | LS180 cells | ↑ | ↑ | | Lower substrate bioavailability | [100] |
| PXR and AhR | ABCB1 | Grapefruit juice | LS180 cells | ↑ | ↑ | | Lower substrate bioavailability | [100] |
| PXR and AhR | ABCC2<br>ABCC3<br>ABCG2 | Grapefruit juice | LS180 cells | ↑ | | | Lower substrate bioavailability | [100] |
| PXR and AhR | ABCC2<br>ABCC3 | Mandarin juice | LS180 cells | ↑ | | | Lower substrate bioavailability | [100] |
| PXR | ABCB1<br>ABCC2 | β-carotene | HepG2 cells | ↑ | | | Lower substrate bioavailability | [101] |
| PXR | ABCB1 | α-tocopherol-13´-COOH,<br>γ-tocotrienol | LS180 cells | | ↑ | ↑ | Lower substrate bioavailability | [102] |
| PXR | ABCB1 | Piperine | Rat small intestine | | ↑ | ↑ | Lower substrate bioavailability | [103] |
| PXR | ABCB1 | Docetaxel<br>Flutamide<br>Paclitaxel<br>Vinblastine<br>Vincristine | LS180 cells | ↑ (reporter gene) | ↑ | ↑ | Multidrug resistance, lower substrate bioavailability | [104] |
| PXR | ABCB1 | Erlotinib<br>Gefitinib<br>Nilotinib<br>Sorafenib<br>Vandetanib | LS180 cells | | ↑ | ↑ | Multidrug resistance, lower substrate bioavailability | [105] |
| PXR | ABCC3 | Rifampicin | Colon carcinoma specimens | ↑ | ↑ | | Multidrug resistance | [106] |
| PXR | ABCB1 | SR12813 | Breast cancer cell lines | ↑ | | ↑ | Multidrug resistance | [107] |
| PXR | ABCB1 | SR12813 | Prostate cancer cell lines | ↑ | | ↑ | Multidrug resistance | [108] |
| PXR | ABCB1 | SR12813 | Non-small cell lung cancer cell lines | ↑ | ↑ | ↑ | Multidrug resistance | [109] |
| PXR | ABCB1 | A-792611 | Primary human hepatocytes | ↓ | | | Prevention of PXR activation by well-known agonists | [110] |
| PXR and CAR | ABCB1 | Artemisin | LS174T cells | ↑ | | ↓ | Lower substrate bioavailability | [120] |
| CAR | ABCB1 | - | Intestinal cell lines | ↑ | | | Lower substrate bioavailability | [118,119] |
| CAR | ABCB1<br>ABCC1<br>ABCC2 | Phenobarbital | Caco-2 cells | ↑ | ↑ | | Lower substrate bioavailability | [58] |
| CAR | ABCB1<br>ABCC1<br>ABCG2 | Phenobarbital | Human hepatocytes | ↑ | ↑ | | Lower substrate bioavailability | [58,122] |
| CAR | ABCC3<br>ABCC6<br>ABCC10<br>ABCC11 | Phenobarbital | Mouse liver | ↑ | | | Lower substrate bioavailability | [123] |
| CAR | ABCC2 | Phenobarbital | Rat liver | ↑ | | | Lower substrate bioavailability | [80] |
| CAR | ABCC3 | Phenobarbital | Rat liver | ↑ | ↑ | | Lower substrate bioavailability | [125] |
| CAR | ABCB1<br>ABCC2<br>ABCG2 | Phenobarbital | Mouse and rat brain capillaries | | ↑ | ↑ | Lower penetration of transporter substrates into the CNS | [127] |
| CAR | ABCB1 | Clotrimazole<br>Meclizine | LS174T cells | ↓ (reporter gene) | | | Higher substrate bioavailability | [118] |
| CAR | ABCB1 | Baicalein | LS174T cells | ↑ | ↑ | | Lower substrate bioavailability | [121] |
| CAR | ABCC2<br>ABCC3<br>ABCC4 | TCPOBOP | Mouse liver | ↑ | | | Lower substrate bioavailability | [124] |
| CAR | ABCB1 | Valproic acid | HepG2 and LS174T cells | ↑ | | | Lower substrate bioavailability | [126] |
| CAR | ABCB1 | Acetaminophen | Rat brain endothelial cells | | ↑ | ↑ | Lower penetration of ABCB1 substrates into the CNS | [129] |
| CAR | ABCB1<br>ABCG2 | CITCO | Porcine brain endothelial cells | ↑ | ↑ | ↑ | Lower penetration of transporter substrates into the CNS | [130] |
| CAR | ABCC4 | TCPOBOP | Mouse liver | ↑ | ↑ | | Higher excretion of ABCC4 substrates into the sinusoidal | [131] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | blood | |
| CAR | ABCC4 | - | Liver of mice with cholestatic disease | ↑ | ↑ | | Higher excretion of ABCC4 substrates into the sinusoidal blood | [132] |
| CAR | ABCB11 | TCPOBOP | Mouse liver | ↑ | | ↑ | Higher canalicular secretion of bile salts, lower cholesterol accumulation in the liver | [133] |
| CAR | ABCC2 | Bilirubin | Mouse liver | ↑ | | | Higher biliary excretion of ABCC2 substrates including bilirubin | [134] |
| AhR | ABCG2 | TCDD | Human primary hepatocytes | ↑ | | | Lower substrate bioavailability | [122] |
| AhR | ABCG2 | TCDD | Intestinal and breast cancer cell lines | ↑ | ↑ | ↑ | Lower substrate bioavailability, multidrug resistance | [136,137] |
| AhR | ABCG2 | 3-MC | LS174T cells | ↑ | ↑ | | Lower substrate bioavailability | [138] |
| AhR | ABCG2 | 3-MC | Mouse liver | ↑ | | | Lower substrate bioavailability | [140] |
| AhR | ABCG2 | TCDD Prochloraz | Normal bovine mammary gland | ↑ | ↑ | | Increase secretion of ABCG2 substrates into the milk | [139] |
| AhR | ABCG2 | Oltipraz | Caco-2 cells | ↑ | ↑ | | Lower substrate bioavailability | [137] |
| AhR | ABCB1 ABCC2 ABCC3 | Oltipraz | Primary human hepatocytes | ↑ | | | Lower substrate bioavailability | [122] |
| AhR | ABCC4 | Oltipraz | Primary human hepatocytes | ↑ | ↑ | | Lower substrate bioavailability | [143] |
| AhR | ABCG2 | Obatoclax | LS180 cells | ↑ | | | Lower bioavailability of ABCG2 substrates | [141] |
| AhR | ABCG2 | Romidepsin | Glioblastoma, colon and breast cancer cell lines | ↑ | | ↑ | Multidrug resistance | [142] |
| AhR | ABCC2 ABCC3 ABCC4 | TCDD | Mouse liver | ↑ | | | Lower substrate bioavailability | [124] |
| AhR | ABCB1 ABCC2 ABCG2 | TCDD | Rat brain endothelial capillaries | ↑ | ↑ | ↑ | Lower penetration of transporter substrates into the CNS | [144] |
| AhR | ABCC2 ABCC3 ABCC5 ABCC6 ABCC10 | PCB126 | Mouse liver | ↑ | | | Lower substrate bioavailability | [123] |
| AhR | ABCC2 | - | Fibrotic liver biopsies | ↓ | | | Higher substrate bioavailability | [145] |
| AhR | ABCG2 | BaP | Head and neck squamous cell carcinoma specimens | ↑ | ↑ | | Multidrug resistance | [146] |

Species are only indicated for models different from human. ↑: increased expression/activity; ↓: decreased expression/activity; 3-MC: 3-methylcholantrene; Bap: Benzo[a]pyrene; CITCO: 6-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime; CNS: central nervous system; PCB126: 3,3',4,4',5-pentachlorobiphenyl; PCN: pregnenolone-16α-carbonitrile; TCDD: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; TCPOBOP: 1,4-Bis(3,5-dichloro-2-pyridyloxy)benzene.

## *5. Modulation of ABC transporters by bile acid and lipid receptors*

### *5.1. Peroxisome proliferator-activated receptors (PPARα/NR1C1, PPARβ/δ/NR1C2 and PPARγ/NR1C3)*

The PPAR subfamily can be divided into PPARα, PPARβ/δ and PPARγ, being all nuclear receptors functioning as heterodimers with RXRα and associated with cellular development, metabolism and differentiation. Moreover, pharmacological modulation of PPARs is an already established strategy for the treatment of several metabolic diseases [148]. Among PPARs target genes, several ABC transporters can be found. This results in the modulation of hepatic trafficking of endobiotics in order to respond to alterations in the cell metabolism. For instance PPARα, activated under physiological conditions by fatty acids and their derivatives and pharmacologically by fibrates, has been described to mediate the induction of the canalicular phospholipid transporter ABCB4 (Mdr2) in the liver of fasting mice. Although the physiological relevance of this up-regulation is not clear, one hypothesis proposes that a higher secretion of phospholipids into the bile may protect the biliary tree cells from the harmful potential of the increased bile acid secretion that usually takes place during fasting [149]. In agreement with this protective function of PPARα, PPARβ/δ activation resulted in an enhanced cholesterol uptake from the bile into the cholangiocytes followed by an ABCA1 up-regulation at the basolateral membrane. This process was potentiated during simultaneous activation of PPARβ/δ and LXRβ (NR1H2) (also discussed in section 5.2) [150]. This mechanism may be involved in the modulation of bile composition and thus may be associated with the formation of gallstones.

PPARγ regulates fatty acid and glucose metabolism. Moreover, PPARγ and the associated coactivator PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) mediated up-regulation of hepatic ABCC2, ABCG2, ABCG5 and ABCG8 in a model of streptozotocin-induced diabetes in mice. This effect was not observed in pregnant mice with streptozotocin-induced diabetes. This finding may implicate a differential disposition of transporter substrates like therapeutic drugs and also cholesterol under hyperglycemia and pregnancy [151].

In addition PPARs can mediate ABC transporter modulation by drugs. For instance, clofibrate up-regulated ABCB1, ABCC2, ABCC3, ABCC4 and ABCG2 in liver of wild type mice, while the effect was absent in PPARα knock-out animals [124,152]. Additionally, gemfibrozil, another clinically relevant PPARα ligand, was also reported to induce ABCC2 in the hepatic cell line HepG2 [153].

These results suggest potential interactions with drugs substrate of the up-regulated transporters during treatment with fibrates.

Apart from the regulation of hepatic transporters, PPARα activation by linoleic acid or clofibrate resulted in an increase in the expression and activity of ABCB1, ABCC2 and ABCG2 in isolated rat brain capillaries. Moreover, fasting mice also exhibited an increased ABCB1 activity at the blood-brain barrier, whereas this effect was not observed in PPARα knock-out mice [154]. Similarly, PPARα activated by clofibrate up-regulated ABCG2 in human cerebral microvessel endothelial cells [155]. These findings suggest a reduced penetration of substrates of the up-regulated transporters into the central nervous system during treatment with fibrates or exposure to other PPARα agonists. Likewise, the PPARγ agonist rosiglitazone up-regulated ABCG2 expression and activity in a placental cell line. This indicates a potential of PPARγ activation in the strengthening of the placental barrier [156].

### *5.2. Liver X Receptors (LXRα/NR1H3 and LXRβ/NR1H2)*

LXRs comprise LXRα and LXRβ and function as heterodimers with RXRα to regulate the expression of their target genes. Both LXRs are activated by the same ligands, mainly oxysterols resulting from cholesterol oxidation. However, whereas LXRα is expressed in liver, intestine, adrenal glands, adipose tissue and macrophages, LXRβ is ubiquitously expressed. LXR target genes are mainly associated with cholesterol metabolism and homeostasis, thus conferring these nuclear receptors an important role in the pathogenesis of dyslipidemia and atherosclerosis [157]. For instance, ABCA1 and ABCG1 are key mediators of reverse cholesterol transport. They are expressed in macrophages and mediate the efflux of cholesterol, which is then loaded in high density lipoprotein (HDL) particles and transported to the liver. In this context, LXR activation by 22-(R)-hydroxycholesterol resulted in ABCA1 up-regulation in the human macrophagic cell line THP-1. In addition, the inducing effect was modulated by protein kinase C (PKC), c-Jun N-terminal kinase (JNK) and phosphatidylinositide 3-kinase (PI3K) [158,159]. A similar role of LXR in the modulation of cholesterol efflux was reported in vascular smooth muscle cells, also involved in the formation of the atherogenic plaque. As described in section 3.3.4., treatment with 17β-estradiol leads to an ERβ-dependent up-regulation of LXRα and, consequently, to an induction of ABCA1 and ABCG1 [55]. In

relation, exposure of the intestinal cell line Caco-2 to cholesterol, the combination of its metabolite 22-(R)-hydroxycholesterol and 9-cis retinoic acid or the LXR synthetic agonist T0901317 resulted in ABCA1 and ABCG1 up-regulation and an increase in the basolateral transport of cholesterol. Thus, upon an increase in cholesterol intake with the diet, this mechanism may lead to an increase in nascent HDL particles which may serve as acceptors to transport cholesterol from peripheral tissues to the liver leading finally to the biliary excretion and maintenance of the homeostatic levels [160]. Finally, treatment with the LXR agonist T0901317 was also described to induce *ABCA1* mRNA at the blood-brain barrier. This may contribute to cholesterol homeostasis in the central nervous system [161].

In addition to transporters related to cholesterol homeostasis, T0901317 was reported to up-regulate ABCC2 in rat liver and HepG2 cells through an LXRα-dependent mechanism [162]. This suggests an altered excretion of ABCC2 substrates upon changes in LXR activity, for instance by variable cholesterol levels.

### *5.3. Farnesoid X Receptor α (FXRα/NR1H4)*

After its isolation from a rat liver cDNA library and with no physiological ligand known, FXR was indexed as orphan receptor. After some time, it left this category when bile acids were identified as endogenous ligands [163]. Nowadays, FXR is considered the master regulator of bile acid homeostasis, tightly modulating their levels in the liver and in the whole organism. In addition to this main function, FXR was demonstrated to play an important role in lipoprotein, glucose and lipid metabolism. FXR is mainly expressed in liver, intestine, adrenal gland and kidney [164]. To regulate its target genes, FXR binds to the DNA response elements associated with its partner RXRα. Two FXR genes were discovered: *FXRα (NR1H4)* and *FXRβ (NR1H5)*. While *FXRβ* is a pseudogene in humans and, consequently, is not associated with ABC transporter modulation, *FXRα* presents four isoforms (FXRα1-4) which have a tissue-dependent expression.

The role of FXR as a regulator of ABCB11 was first demonstrated *in vivo* using FXR knock-out mice. These animals showed a decrease in basal *Abcb11* mRNA expression when compared to wild-type mice and a loss of *Abcb11* induction by cholic acid rich feeding [165]. In another study, bile acids up-regulated ABCB11 in human hepatocytes. The analysis of their relative potency pointed out

chenodeoxycholic acid (CDCA) as the most potent ABCB11 activator followed by deoxycholic acid (DCA) and lithocholic acid (LCA) [166]. This ranking is identical to the rank order of potency of these compounds to activate FXR. Shortly afterwards, another group shed some light on the molecular mechanism behind this regulation, demonstrating that bile acids activate human *ABCB11* gene transcription after the binding of FXR/RXRα to the inverted repeat element (IR-1) in its promoter [167]. One year later, using a siRNA-mediated FXR knock-down in HepG2 cells, the participation of this nuclear receptor in bile acid-induced human *ABCB11* transcriptional up-regulation was demonstrated [168]. Finally, individuals with neonatal cholestasis due to homozygous mutation of *FXR* exhibited undetectable ABCB11 expression in their livers as a direct consequence of the loss of FXR function, further confirming the association between this nuclear receptor and the main bile salt efflux transporter [169]. In addition to changes in its constitutive expression, ABCB11 can be down-regulated in cholestasis through different mechanisms. For example, pro-inflammatory cytokines such as tumor necrosis factor α (TNFα) and interleukin 1β (IL-1β) were shown to decrease ABCB11 expression at both mRNA and protein levels in mice by affecting FXR/RXRα binding to IR-1 [170]. Moreover, FXR modulation was also suggested as a mechanism underlying intrahepatic cholestasis of pregnancy. In fact, sulfated metabolites of progesterone antagonized FXR activation by bile salts and thereby ABCB11 induction by this nuclear receptor [171]. In addition, post-translational modifications of FXR can also play a role in modulation of ABCB11 expression. In this regard, bile duct ligated mice showed an increased SUMOylation of FXR leading to a decreased binding of the nuclear receptor to *Abcb11* promoter [172].

Disturbance of bile acid homeostasis is also a common feature in hepatocellular carcinoma (HCC). ABCB11 expression was drastically decreased in HCC and adjacent liver tissue [173]. This down-regulation was associated with an increased FXRα1/FXRα2 ratio, showing that ABCB11 is under isoform-specific control by FXR. Since TNFα and interleukin 6 (IL-6) were shown to alter FXR isoform expression in Huh-7 cells, the authors proposed these proinflammatory cytokines as perpetrators of ABCB11 dysregulation in HCC.

In addition to bile acids, other endogenous metabolites such as intermediates in their synthesis from cholesterol can regulate ABCB11. For example, 22(R)-hydroxycholesterol induced ABCB11

expression in human hepatocytes and Huh-7 cells through activation of FXR [174]. Using site-directed mutagenesis in an *ABCB11* promoter reporter, it was demonstrated that the IR-1 element is necessary for the maximal inducing potency. Also, polyunsaturated fatty acids were reported to be among the list of endogenous ligands of FXR. Indeed, arachidonic acid, docosahexanoic acid and linolenic acid induced *ABCB11* mRNA expression in a dose-dependent fashion in HepG2 cells [175].

Besides its well-known role in the maintenance of the bile salt homeostasis, FXR also plays a role in the modulation of ABC transporters in other physiological and pathological processes. For instance, hepatic ABCB1, ABCC1 and ABCC2 can be up-regulated by FXR ligands. In this regard it has been demonstrated that CDCA increased both mRNA and protein levels of these transporters together with FXR expression in HepG2 cells [58]. This report is in agreement with another work demonstrating an increase in *ABCC2* mRNA levels in HepG2 cells and human, mouse and rat hepatocytes after treatment with either CDCA or the synthetic FXR agonist GW4064 [80]. These results suggest an increase in the efflux of ABCC2 substrates into the bile upon increase in the intrahepatic bile salt concentration.

Additionally, FXR expression was described in several tumor cells. In breast cancer, for instance, an association between FXR and the modulation of ABCC2 has been observed. Indeed, exposure of MCF-7 cells to GW4064 resulted in an induction of this transporter. However, the effect did not correlate with an increased resistance to the ABCC2 substrate paclitaxel [176]. On the contrary, in a colon adenocarcinoma cell line, FXR activation resulted in ABCG2 up-regulation, whereas this higher transporter expression was associated with an increased resistance to the ABCG2 substrate mitoxantrone. Moreover, FXR was proposed as a mediator of acquired resistance to cisplatin in the same cells [177]. All cases of ABC transporter modulation mediated by FXR or by other lipid receptors are presented in Table 3.

Table **3.** ABC transporter modulation by bile acid and lipid receptors

| Receptor | Transporter | Modulatory compounds | Model or tissue | Effect reported | | | Physiological process or biological effect | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | mRNA | Protein | Activity | | |
| PPARα | ABCB4 | - | Liver of fasting mice | ↑ | ↑ | | Higher secretion of phospholipids into the bile | [149] |
| PPARβ/δ and | ABCA1 | T0901317 | Mouse | ↑ | ↑ | ↑ | Higher cholesterol uptake from | [150] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| LXRβ | | GW501516 | cholangiocytes | | | | the bile and basolateral secretion | |
| PPARγ | ABCC2<br>ABCG2<br>ABCG5<br>ABCG8 | - | Diabetic mice liver | ↑ | ↑ | ↑ | Lower substrate bioavailability, pregnancy associated bioavailability differences | [151] |
| PPARα | ABCB1<br>ABCC3<br>ABCC4<br>ABCG2 | Clofibrate | Mouse liver | ↑ | ↑ | | Lower substrate bioavailability | [152] |
| PPARα | ABCC2 | Clofibrate | Mouse liver | ↑ | | | Lower substrate bioavailability | [124] |
| PPARα | ABCG2 | Clofibrate | Human brain endothelial cells | ↑ | ↑ | ↑ | Lower penetration of transporter substrates into the CNS | [155] |
| PPARα | ABCC2 | Fenofibrate<br>Gemfibrozil | Human hepatocytes and HepG2 cells | ↑ | | | Lower substrate bioavailability | [153] |
| PPARα | ABCB1<br>ABCC2<br>ABCG2 | Linoleic acid<br>Clofibrate | Rat brain endothelial capillaries | | ↑ | ↑ | Lower penetration of transporter substrates into the CNS | [154] |
| PPARα | ABCB1 | - | Brain endothelial capillaries of fasting mice | | | ↑ | Lower penetration of ABCB1 substrates into the CNS | [154] |
| PPARγ | ABCG2 | Rosiglitazone | BeWo cells | ↑ | ↑ | ↑ | Lower penetration of ABCG2 substrates into the fetal blood | [156] |
| LXR | ABCA1 | 22-(R)-OHC | Human macrophagic cell line THP-1 | ↑ | ↑ | | Increased efflux of cholesterol, inhibition of atherosclerotic plaque development | [158,159] |
| LXRα (and ERβ) | ABCA1<br>ABCG1 | 17β-estradiol | Vascular smooth muscle cells | ↑ | ↑ | ↑ | Increased efflux of cholesterol, inhibition of atherosclerotic plaque development | [55] |
| LXR | ABCA1<br>ABCG1 | Cholesterol<br>22-(R)-OHC + 9-cis-retinoic acid<br>T0901317 | Caco-2 cells | ↑ | ↑ | ↑ | Increased cholesterol transport from the intestine to the liver | [160] |
| LXR | ABCA1 | T0901317 | Rat brain endothelial cells | ↑ | | | Reduced cholesterol accumulation in the CNS | [161] |
| LXR | ABCC2 | T0901317<br>CDCA | Rat liver and HepG2 cells | ↑ | ↑ | | Lower substrate bioavailability | [162] |
| FXR | ABCB11 | CDCA<br>DCA<br>LCA | Primary human hepatocytes and mouse liver | ↑ | ↑ | | Higher bile salt dependent-bile flow | [166] |
| FXR | ABCB11 | - | Liver of individuals with mutated FXR | | ↓ | | Neonatal cholestasis | [169] |
| FXR | ABCB11 | - | Liver of pregnant women | | ↓ | | Intrahepatic cholestasis of pregnancy | [171] |
| FXR | ABCB11 | - | Hepatocellular carcinoma liver specimens | ↓ | ↓ | | Disturbance of bile acid homeostasis | [173] |
| FXR | ABCB11 | 22-(R)-OHC | Primary human hepatocytes and Huh7 cells | ↑ | | | Higher bile salt dependent-bile flow, maintenance of cholesterol homeostasis | [174] |
| FXR | ABCB11 | Arachidonic acid<br>Docosahexanoic acid<br>Linolenic acid | HepG2 cells | ↑ | | | Higher bile salt dependent-bile flow | [175] |
| FXR | ABCB1<br>ABCC1<br>ABCC2 | CDCA | HepG2 cells | ↑ | ↑ | | Lower substrate bioavailability | [58] |
| FXR | ABCC2 | GW4064<br>CDCA | HepG2 cells<br>Human, mouse and rat hepatocytes | ↑ | | | Lower substrate bioavailability | [80] |
| FXR | ABCG2 | GW4064 | LS174T cells | ↑ | | | Multidrug resistance | [177] |

Species are only indicated for models different from human. ↑: increased expression/activity, ↓: decreased expression/activity; 22-(R)-OHC: 22-(R)-hydroxycholesterol; CDCA chenodeoxycholic acid; CNS: central nervous system; DCA: deoxycholic acid; LCA: lithocholic acid.

### *6. Modulation of ABC transporters by retinoid receptors*

### *6.1. Retinoic acid receptors (RARα/NR1B1, RARβ/NR1B2, RARγ/NR1B3)*

Retinoic acid receptors (RARs) exist as three different isoforms RARα, RARβ and RARγ and function as heterodimers with RXRα [178]. All-*trans*-retinoic acid (ATRA) is used for the treatment of acute promyelocytic leukemia and constitutes one of the major RAR ligands. ATRA in combination with verapamil was described to decrease ABCB1 expression in the leukemia cell line L1210. The effect, however, appears not to be directly mediated by RAR binding to *ABCB1* promoter, but as result of RXRα sequestering. Consequently, less RXRα may be available to mediate transcriptional activation by classical ABCB1 inducing receptors like PXR [179]. On the contrary, ATRA was described to induce ABCB1 expression in hepatocarcinoma and melanoma cell lines with the effect being enhanced by RARα over-expression. However, only hepatocarcinoma cells additionally exhibited an increase in ABCB1 transport activity, thus indicating cell specific mechanisms of ABCB1 regulation by retinoids [180].

Obstructive cholestasis is characterized by ABCC2 down-regulation. In this regard, bile duct ligation was associated with an IL-1β increase which led to a RARα down-regulation. Moreover, nuclear translocation of RARα and binding of RARα/RXR heterodimers to rat *Abcc2* promoter were also impaired. This suggests a role for RARα signalling in ABCC2 down-regulation [181].

In addition, RAR activated by ATRA can modulate ABC transporters under physiological conditions. For instance, ATRA up-regulates ABCG2 expression and activity in the intestinal cell line Caco-2. A similar effect was observed with Am580 and CD2608, specific agonists for RARα and RXR, respectively. Moreover, combination of both agonists resulted in a synergistic effect, further suggesting a role of the RARα/RXR heterodimer in ABCG2 regulation [182]. This effect may be associated with a more efficient intestinal excretion as well as a reduced absorption of diet toxicants substrate of ABCG2. Similarly, the absorption of orally administered drugs may be diminished upon exposure to ATRA.

Cholesterol excretion from macrophages was also reported to be modulated by RAR. Indeed, ATRA up-regulated ABCA1 in murine peritoneal and human macrophages without exhibiting a major effect on other typical LXR target genes. Moreover, a similar effect was observed after exposure of macrophages to the synthetic RAR pan-agonist 4-[(*E*)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-

naphthalenyl)-1-propenyl]benzoic acid (TTNPB), further suggesting a RAR-mediated effect. Over-expression experiments attributed RARγ, an isoform not expressed in liver, a major role in ABCA1 induction by ATRA in macrophages. Although RARγ activation may not be associated with the same side effects observed for LXR activation, RAR may be related to a different spectrum of side effects not allowing their utilization in atherosclerosis treatment [183].

### *6.2. Retinoid X receptors (RXRα/NR2B1, RXRβ/NR2B2, RXRγ/NR2B3)*

Retinoid X receptors exist as three different isoforms: RXRα, mainly expressed in the liver, kidney, intestine and skin; RXRβ, which exhibits ubiquitous expression and RXRγ, expressed in brain and muscle. Although RXRs play an essential role as heterodimerization partners with other nuclear receptors, mice bearing deletions in RXR AF-1 or AF-2 function exhibit development abnormalities similar to those exhibited by RXR knock-out mice. This suggests a role of RXR own activation functions and thus of the receptor independent of its well-known role as heterodimerization partner. In this regard, the formation of RXR/RXR homodimers has already been described [184].

ABC transporter modulation by RXR was described, for example, in the central nervous system, where exposure of rat astrocytes to 9-cis retinoic acid resulted in ABCA1 and ABCG1 up-regulation and enhanced cholesterol efflux. Although 9-cis retinoic was described to activate both RXR and RAR, treatment with a pure RAR agonist resulted in a down-regulation of both ABCA1 and ABCG1, thus suggesting 9-cis retinoic acid effect to take place through a mediator different than RAR, probably RXR. Taking into consideration that intracellular cholesterol levels were associated with the pathogenesis of Alzheimer disease, RXR signaling appears as a potential candidate in the development of future prevention or therapeutic strategies [185]. In this regard, treatment with the RXRα agonist methyl-2-amino-6-(tert-butyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (TBTC) ameliorated behavioral deficit in a model of Alzheimer´s disease in mice [186].

A role of RXRα was also described in the modulation of the response to the FXR agonist CDCA. Here, an attenuation of ABCB11 up-regulation under cotreatment with CDCA and the 9-cis retinoic acid in HepG2 cells respect to cells treated only with CDCA has been described. Moreover, RXRα activation by 9-cis retinoic acid promoted a decrease in the binding of the FXR/RXRα heterodimer to

ABCB11 promoter. Similarly, an *in vivo* study demonstrated a higher ABCB11 up-regulation by cholic acid in mice fed a vitamin A deficient diet compared with animals fed a normal diet. Preventing ABCB11 induction may be a useful strategy, for instance, in cases of extrahepatic obstructive cholestasis [187]. All cases of ABC transporter modulation by RXR and RAR are listed in Table 4.

Table **4.** ABC transporter modulation by retinoid receptors

| **Receptor** | **Transporter** | **Modulatory compounds** | **Model or tissue** | **Effect reported** | | | **Physiological process or biological effect** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| | | | | **mRNA** | **Protein** | **Activity** | | |
| RXRα | ABCB1 | ATRA + verapamil | Leukemia cell line L1210 | ↓ | ↓ | ↓ | Chemosensitization | [179] |
| RARα | ABCB1 | ATRA | Hepatocarcinoma and melanoma cell lines | ↑ | | ↑ | Multidrug resistance | [180] |
| RARα | ABCC2 | Proinflammatory cytokines | Liver of rats with bile duct ligation | ↓ | ↓ | | Lower biliary excretion of ABCC2 substrates during obstructive cholestasis | [181] |
| RARα and RXRα | ABCG2 | ATRA | Caco-2 cells | ↑ | ↑ | ↑ | Lower substrate bioavailability | [182] |
| RARγ | ABCA1 | ATRA<br>TTPNB | Mouse and human macrophages | ↑ | ↑ | ↑ | Inhibition of atherosclerotic plaque development | [183] |
| RXR | ABCA1<br>ABCG1 | 9-cis retinoic acid<br>TBTC | Rat astrocytes | ↑ | ↑ | ↑ | Maintenance of cholesterol homeostasis in the brain and prevention of Alzheimer´s disease | [185,186] |
| RXR | ABCB11 | 9-cis retinoic acid | Mouse liver | ↓ | | | Prevention of FXR- mediated induction by CDCA | [187] |

Species are only indicated for models different from human. ↑: increased expression/activity; ↓: decreased expression/activity; ATRA: all-*trans*-retinoid acid, CDCA chenodeoxycholic acid; TBTC: methyl-2-amino-6-(tert-butyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate; TTPNB: 4-[(*E*)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid.

### *7. Clinical applications: FXR and LXR*

Alterations in ABC transporter expression and activity by nuclear receptors underlie several physiological and pathological processes. Thus, the pharmacological modulation of these signaling pathways may provide new therapeutic strategies to manage long-standing diseases. For instance, modulation of FXR has been suggested as a possible approach to counteract cholestatic disorders. In particular, treatment with natural FXR agonists like boldine, isolated from *Peumus boldus* tree or with synthetic agonists, appears as a possible strategy to manage intrahepatic cholestasis, as demonstrated in rats [Cermanova et al., Toxicol Appl Pharmacol, 2015; Liu Y et al., 2003]. Transcriptional activation of transporters like ABCB4 and ABCB11 has been proposed as the underlying mechanism [Liu Y et al., 2003; Cermanova et al., Toxicol Appl Pharmacol, 2015]. In this regard, a phase IV clinical trial evaluating the use of the FXR agonist obeticholic acid in the treatment of primary biliary cholangitis is being performed [www.clinicaltrials.gov/ct2/show/NCT02308111]. On the contrary, in particular cases of obstructive cholestasis, a decrease in the biliary secretion can be beneficial. Here, oleanolic acid has been demonstrated to antagonize FXR activation and, this way, to decrease ABCB11 expression, thus reducing bile flow and preventing further liver injury [Chen P et al., Eur J Pharmacol, 2015].

LXR was proposed as a potential therapeutic target to treat hypercholesterolemia. To date, several synthetic modulators of LXR exhibited several side effects that made them unsuitable for their application in the clinical practice [157]. In contrast, plant sterols appear as a promising pharmacologic tool to inhibit intestinal cholesterol uptake and to promote its transport back into the lumen. ABCA1 induction by phytosterols via LXR has been demonstrated in Caco-2 cells and proposed as a mechanism underlying the decrease in cholesterol absorption by phytosterols [Plat and Mensik, FASEB J, 2002; De Smet et al., Mol Nutr Food Res, 2012]. However, the role of LXR remains controversial. In fact, a study using LXR deficient mice pointed out also a LXR independent mechanism as responsible for the effect of phytosterols on cholesterol absorption [Cedó et al., Mol Nutr Food Res, 2017]. Further studies should be performed to assess the real relevance of LXR in the well-known beneficial effect of phytosterols on cholesterol absorption.

## *8. Conclusions*

The axis nuclear receptor-ABC transporter plays a crucial role in several physiological processes, whereas its malfunction frequently constitutes the molecular basis of different diseases. Modulation of this regulatory axis represents an important strategy aiming, for instance, at protecting the organism from external and internal chemical insults. This challenging function takes place at systemic level but exhibits also an important tissue-specific component. For instance, nuclear receptors contribute notably to the maintenance of the blood-brain barrier integrity. On the contrary, nuclear receptor modulation can also represent a hindrance to be overcome to reach an appropriate bioavailability and delivery of therapeutic agents. Furthermore, modulation of nuclear receptors constitutes a promising approach for the treatment of life-threatening diseases like atherosclerosis, Alzheimer as well as metabolic disorders. There is abundant information linking the receptor activation (or inhibition) by several compounds with the transporter modulation and, this way, with a desired pharmacological effect. Moreover, the structural aspects governing the modulator-receptor and the receptor-gene promoter interaction are, in most of the cases, partially identified. Nevertheless, modulatory compounds exhibiting a great potential on the bench usually fail to prove themselves for clinical application. Further research in the field of identification and design of tolerable and bioavailable nuclear receptor modulators will allow using the already acquired knowledge in the design of better therapeutic strategies.

## *9. List of abbreviations*

AF-1 = activation function 1

AF-2 = activation function 2

AhR = aryl hydrocarbon receptor

AR = androgen receptor

ATRA = all-*trans*-retinoic acid

AUC = area under the curve

CAR = constitutive androstane receptor

CITCO = 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime

CPA = cyproterone acetate

DBD = DNA binding domain

DHT = dihydrotestosterone

ER = estrogen receptor

FXR = farnesoid X receptor

FXR = farnesoid X receptor

GR = glucocorticoid receptor

IL = interleukin

IR = inverted repeat

LBD = ligand binding domain

LXR = liver X receptor

miRNA = microRNA

NCoR = nuclear receptor corepressor

PCN = pregnenolone-16α-carbonitrile

PGC1α = peroxisome proliferator-activated receptor γ - coactivator 1α

PP2A = protein phosphatase 2A

PPAR = peroxisome proliferator activated receptor

PR = progesterone receptor

PXR = pregnane X receptor

RAR = retinoid acid receptor

RXR = retinoid X receptor

TBTC = methyl-2-amino-6-(tert-butyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate

TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

TCPOBOP = 1,4-bis(3,5-dichloro-2-pyridyloxy)benzene

TNFα = tumor necrosis factor α

TR = thyroid hormone receptor

TTNPB = 4-[(*E*)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid

VDR = vitamin D receptor

VSMC = vascular smooth muscle cells

## *10. Conflict of interest*

The authors declare that they have no conflict of interest.

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