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Impact of genetic variability in the *ABCG2* gene on ABCG2 expression, function, and interaction with AT1 receptor antagonist telmisartan

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ABSTRACT

The ATP-binding cassette transporter ABCG2 plays a prominent role in cardiovascular and cancer pathophysiology, is involved in the pathogenesis of gout, and affects pharmacokinetics of numerous drugs. Telmisartan, a widely used AT1 receptor antagonist, inhibits the transport capacity of ABCG2 and may cause drug–drug interactions, especially in individuals carrying polymorphism that facilitate the telmisartan–ABCG2 interaction. Thus, the aim of this study was to identify ABCG2 polymorphisms and somatic mutations with relevance for the telmisartan–ABCG2 interaction. For this purpose, a cellular system for the conditional expression of ABCG2 was established. ABCG2 variants were generated via site-directed mutagenesis. Interaction of telmisartan with these ABCG2 variants was investigated in HEK293-Tet-On cells using the pheophorbide A efflux assay. Moreover, expression of ABCG2 variants was studied in these cells. Importantly, protein levels of the Q141K and F489L variant were significantly reduced, a phenomenon that was partly reversed by pharmacological proteasome inhibition. Moreover, basal pheophorbide A efflux capacity of S248P, F431L, and F489L variants was significantly impaired. Interestingly, inhibition of ABCG2-mediated pheophorbide A transport by telmisartan was almost abolished in cells expressing the R482G variant, whereas it was largely increased in cells expressing the F489L variant. We conclude that the arginine residue at position 482 of the ABCG2 molecule is of major importance for the interaction of telmisartan with this ABC transporter. Furthermore, individuals carrying the F489L polymorphism may be at increased risk of developing adverse drug reactions in multi-drug regimens involving ABCG2 substrates and telmisartan.

1. Introduction

ATP-binding cassette (ABC)-transporters represent membrane-spanning ATP-dependent efflux transporters that comprise at least 48 different members in humans [1]. ABCG2, which belongs to the ABC transporter family, is thought to play a major role in the transport of various drugs and phase II metabolites [2] and is mainly localised at interfaces between the blood and important body compartments, e.g. placenta and central nervous system, where it reduces tissue-specific entry of drugs and xenobiotics [3,4]. Moreover, ABCG2 is considerably expressed at interfaces between the organism and its environment where drug excretion takes place, e.g. in the apical membrane of enterocytes and in the canalicular membrane of hepatocytes where it is involved in the intestinal and hepatobiliary excretion of various drugs and xenobiotics [2]. Thus, drugs which affect the activity of ABCG2 may cause drug–drug-interactions via reducing ABCG2-mediated elimination of drugs at above-mentioned sites. In addition, ABCG2 is highly expressed in various stem cell populations and has been postulated to play a role in the phenomenon of multidrug resistance of cancer and cancer stem cells [5,6]. Interestingly, recently a role of ABCG2 in uric acid transport and in the pathogenesis of gout has been demonstrated and has been linked to single nucleotide polymorphisms (SNPs) within the *ABCG2* gene, e.g. the Q141K (C421A) SNP [7]. Importantly, single nucleotide polymorphisms (SNPs) or acquired somatic mutations of ABCG2 may not only alter transport, disposition, and excretion of endogenous ABCG2 substrates but also the interaction of drugs with the transporter and hence, pharmacokinetics and tissue distribution of drugs, incidence of drug–drug interactions as well as drug response. Indeed, several SNPs and somatic mutations in the *ABCG2* gene have been described which may contribute to the considerable pharmacokinetic and pharmacodynamic variability of drugs which are transported by ABCG2 [8,9].

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AT1 receptor antagonists (ARBs) are a major group of first-line antihypertensive drugs which are also commonly prescribed in patients suffering from heart failure and diabetic nephropathy [10]. We have recently shown that the AT1 receptor antagonist telmisartan inhibits the activity of ABCG2 [11]. The inhibitory effect of telmisartan on ABCG2 function was unique within the class of AT1 receptor antagonists and may contribute to clinically relevant drug–drug interactions and altered drug deposition, e.g. in antihypertensive drug regimens involving telmisartan. However, the impact of genetic variability in the *ABCG2* gene on the interaction of telmisartan with the ABC transporter remains elusive, although it may affect the likelihood of drug–drug interactions in drug regimens involving telmisartan.

2. Materials and methods

A more detailed description of the materials and methods used in this study can be found in the Supplementary Materials and Methods Section.

2.1. Preparation of the pTRE-Tight-BI-AcGFP1–ABCG2 expression vector

The pCMV6-XL5 vector containing a 2700 bp fragment of the human ABCG2 mRNA sequence (pCMV6-XL5-ABCG2; NM_004827.2) was purchased from OriGene Technologies Inc. (ID# SC117127, Rockville, USA) and was cloned non-directionally *Not*I/*Not*I into the pTRE-Tight-BI-AcGFP1 vector. The resulting pTRE-Tight-BI-AcGFP1–ABCG2 vector was finally sequenced to ensure lack of mutations in the ABCG2 sequence. The pTRE-Tight-BI-AcGFP1–ABCG2 vector enables the inducible expression of a reporter green fluorescent protein (AcGFP1) along with the gene of interest (ABCG2), e.g. in cells expressing the rtTA protein (Tet-On system; Supplemental Fig. 1A). For induction of gene expression the tetracycline derivative doxycycline was used.

2.2. Site-directed mutagenesis

Non-synonymous ABCG2 single nucleotide polymorphisms (SNPs) G34A (V12M), C421A (Q141K), T742C (S248P), T1291C (F431L), T1465C (F489L) as well as somatic mutation A1444G (R482G) were inserted into the ABCG2 cDNA sequence in the pTRE-Tight-BI-AcGFP1–ABCG2 plasmid using the QuickChange® Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, Waldbronn, Germany) with specific primers according to the manufacturer's instructions (Supplemental Fig. 1).

2.3. Cell culture

HEK293-Tet-On cells stably expressing the reverse tetracycline-controlled transactivator (rtTA2^S-M2) were purchased from Clontech (Cat-No. 630931; Heidelberg, Germany) and cultivated in Dulbecco's Modified Eagle Medium–High Glucose with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C with 5% CO_2.

2.4. Transient transfection of HEK293 Tet-On cells

HEK293-Tet-On cells were transiently transfected with the pTRE-Tight-BI-AcGFP1 vector additionally containing the ABCG2 wild-type sequence or the ABCG2 variant sequences, respectively, using the jetPRIME™ reagent according to the manufacturer's recommendations.

2.5. ABCG2 inhibition assay (PhA flow cytometry efflux assay)

The BCRP inhibition assay using PhA as fluorescent BCRP substrate was performed as described previously with minor modifications as specified in the extended Supplementary Methods Section [11,12].

2.6. Immunofluorescence staining and confocal laser scanning microscopy (CLSM)

Subcellular localization pattern of ABCG2 was studied in HEK293-Tet-On cells transiently transfected with pTRE-Tight-BI-AcGFP1–ABCG2 wild-type or the ABCG2 variants, respectively, as described previously [13]. ABCG2 as well as nuclei were visualised by an anti-ABCG2 antibody (BXP-21; 1:2000; abcam, Cambridge, UK) and phycoerythrin (PE)-labeled secondary anti-mouse antibody or Hoechst 33342, respectively. Cells were analysed with a Nikon A1 confocal microscope System (Nikon, Melville, USA)

2.7. Western blot analysis

Western blot analysis of cellular ABCG2 protein levels was performed as previously described [14,15]. Proteins were visualised using specific antibodies against ABCG2 (BXP-21, abcam, Cambridge, UK) at a dilution of 1:1000, AcGFP1 (Clontech; 1:5000) or β-Actin for normalization (Cell signaling Technology, Boston, USA; 1:2000).

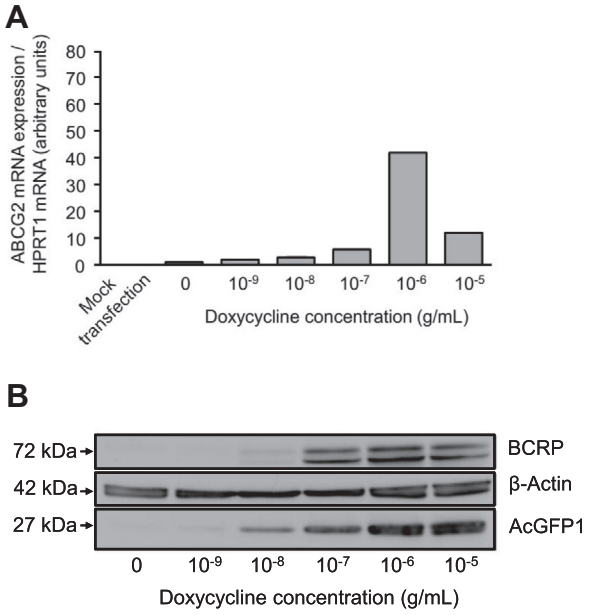

Fig. 1. (A) Doxycycline-dependent induction of ABCG2 mRNA expression in HEK293-Tet-On cells transiently transfected with pTRE-Tight-Bl-AcGFP1–ABCG2 wild-type vector in the absence or presence of increasing concentrations of doxycycline. Data are shown as mean of two independent experiments. (B) Representative Western blot analysis (from a series of three independent analyses) of doxycycline-dependent concomitant expression of ABCG2- and AcGFP1 protein in HEK293-Tet-On cells transiently transfected with the pTRE-Tight-Bl-AcGFP1–ABCG2 wild-type vector. (C) Representative flow cytometric analyses of doxycycline-induced AcGFP1 expression and ABCG2-mediated PhA efflux in HEK293-Tet-On cells transiently transfected with pTRE-Tight-Bl-AcGFP1–ABCG2 wild-type vector (transfection efficiency of approximately 50%). (D and E) Gating of AcGFP1-positive HEK293-Tet-On cells reveals concomitant expression of functional ABCG2 as evidenced by increased PhA efflux and decreased PhA-associated fluorescence as compared with AcGFP1-negative HEK293-Tet-On cells. Black dotted lines indicate the gate used to distinguish between AcGFP1-negative and -positive cells. Red dotted lines indicate the representative maximal basal PhA efflux niveau accomplished in non-induced AcGFP1-negative cells transiently transfected with ABCG2 wild-type.

2.8. Real time RT-PCR

Real time RT-PCR analysis of mRNA expression was performed as described previously [13,15] using pre-made probes for ABCG2 (Hs01053790_m1) and Hypoxanthine–guanine phosphoribosyltransferase 1 (HPRT1; Hs02800695_m1). We performed relative quantification of gene expression using the delta–delta CT method.

2.9. Statistical analyses

Data were analysed using GraphPad Prism Version 5.02 (GraphPad Software, San Diego, CA, USA). Statistical analyses were performed using one-way analysis of variance followed by the Fisher's protected least significant difference test or the Student's *t*-test as appropriate. Data are given as mean ± standard deviation (SD) or if indicated as mean ± standard error of the mean (SEM). Probability values were considered significant at a $P < 0.05$.

3. Results

3.1. Conditional expression of AcGFP1 and ABCG2 in HEK293-Tet-On cells

In a first step, we validated our experimental system for the conditional concomitant expression of ABCG2 and AcGFP1 in HEK293-Tet-On cells. For this purpose, HEK293-Tet-On cells were transiently transfected with the pTRE-Tight-Bl-AcGFP1–ABCG2 wild-type vector and induced with increasing concentrations of doxycycline (10^{-9} to 10^{-5} g/mL) for 24 h. As shown in Fig. 1A, we observed a dose-dependent increase of ABCG2 mRNA expression

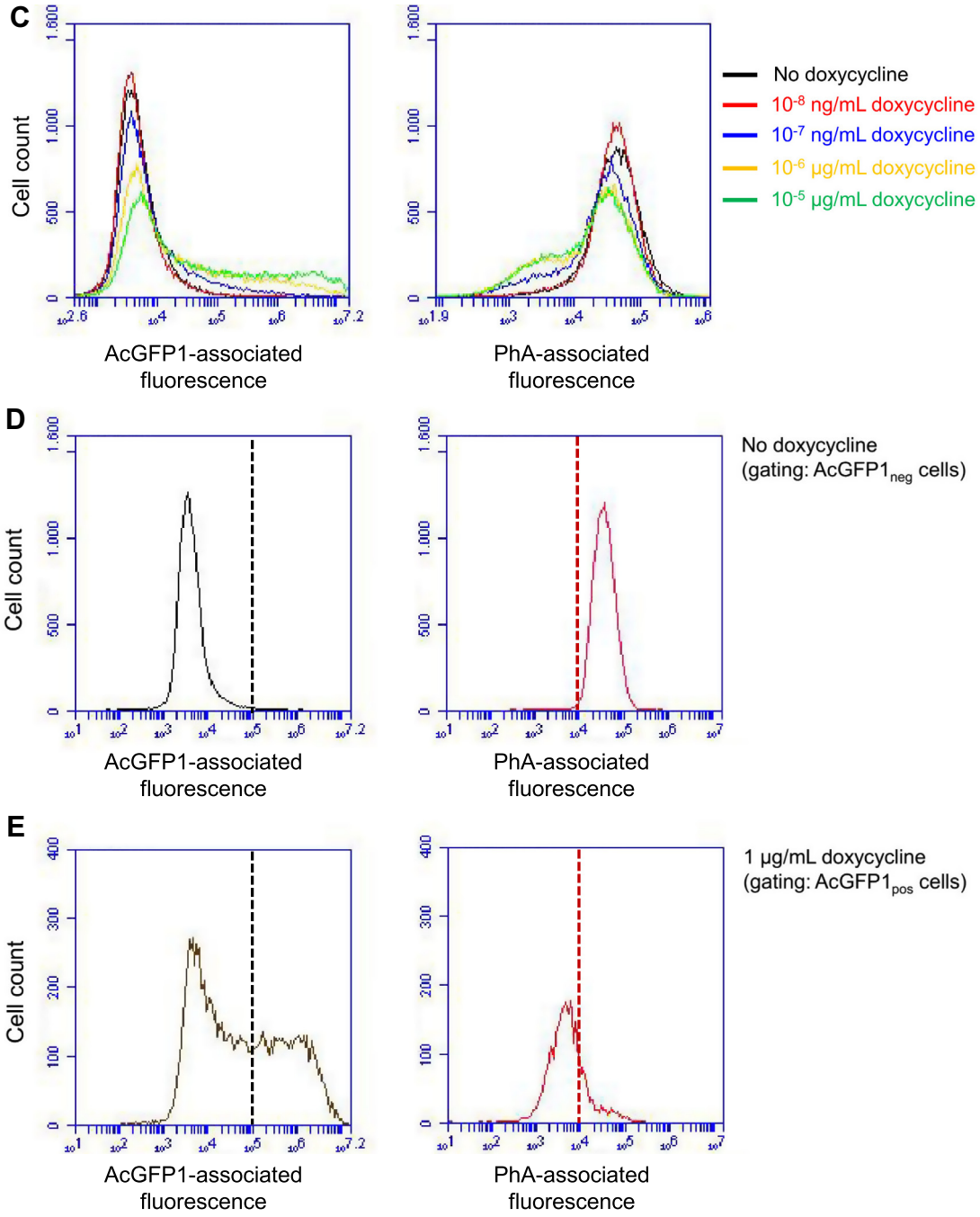

Fig. 1 (*continued*)

with a maximal induction of ABCG2 mRNA expression at doxycycline concentrations of 10^{-6} g/mL. Moreover, a very similar doxycycline response with respect to ABCG2 and AcGFP1 protein expression was detected by Western blot analysis (Fig. 1B). Additionally, we carried out flow cytometric analysis of ABCG2 function using the PhA efflux assay. Again, HEK293-Tet-On cells transiently transfected with the pTRE-Tight-Bl-AcGFP1–ABCG2 wild-type vector were induced with increasing concentrations of doxycycline for 24 h. Subsequently, AcGFP1 expression and ABCG2-mediated PhA efflux capacity of transiently transfected cells was analysed flow cytometrically. Increasing concentrations of doxycycline gradually increased the fraction of AcGFP1-positive HEK293-Tet-On cells (Fig. 1C). Moreover, a concomitant concentration-dependent decrease in PhA-associated fluorescence was observed in these cells reaching a maximum at a concentration of 10^{-6} g/mL doxycycline, thereby demonstrating doxycycline-dependent concomitant expression of functional ABCG2 and AcGFP1 protein in transiently transfected HEK293-Tet-On cells (Fig. 1C). Specific gating of AcGFP1-negative and AcGFP1-positive HEK293-Tet-On cells in this experimental setting revealed a reduction of PhA-associated fluorescence in AcGFP1-positive cells of approximately 90% as compared with AcGFP1-negative non-induced cells (Fig. 1D and E).

3.2. Effect of ABCG2 mutations on ABCG2 mRNA and protein expression as well as subcellular localization of the efflux transporter

To elucidate the impact of ABCG2 mutations on ABCG2 mRNA and protein expression, mRNA and proteins were extracted from doxycycline-treated (1 μg/mL; 24 h) HEK293-Tet-On cells transiently transfected with pTRE-Tight-Bl-AcGFP1–ABCG2 wild-type/variants. To ensure comparability, all genotypes were analysed simultaneously within the same set of experiments (transfection and induction procedure, reverse transcription and real-time PCR analysis). mRNA expression of the respective ABCG2 variants was not significantly different as compared with ABCG2 wild-type (Fig. 2A). In contrast, ABCG2 protein levels of cells transfected with the Q141K and the F489L mutants, respectively, were lower than those transfected with ABCG2 wild-type (Fig. 2B and C). Concomitant treatment of transfected HEK293-Tet-On cells with increasing concentrations of the proteasome inhibitor PS-341 (bortezomib) for 24 h slightly increased protein levels of variants Q141K and F489L but not that of ABCG2 wild-type (Fig. 2D). These data suggest that increased proteasomal degradation of both variants may play a role in the observed reduction of protein levels. Nevertheless, all mutants were primarily located at the plasma membrane as evidenced by confocal laser scanning microscopy (Supplemental Fig. 2).

3.3. Impact of ABCG2 mutations on ABCG2-mediated basal PhA transport and telmisartan-induced inhibition of ABCG2-mediated PhA efflux

Again, all genotypes were analysed simultaneously within the same set of experiments (transfection and induction procedure as well as PhA efflux assay). Non-induced (AcGFP1-and ABCG2-negative) transfected HEK293-Tet-On cells served as a control for potential non-specific effects of telmisartan and FTC, respectively, and as indicator of baseline cellular PhA accumulation in the absence of relevant ABCG2 expression. For analysis of basal PhA efflux, the averaged median PhA-associated fluorescence of non-induced HEK293-Tet-On cells transiently transfected with the pTRE-Tight-Bl-AcGFP1–ABCG2 wild-type vector was set to 100%. The average PhA-associated fluorescence in non-induced HEK293-Tet-On cells transiently transfected with the various ABCG2 variants was not significantly different as compared with that observed in HEK293-Tet-On cells transfected with ABCG2 wild-type (wild-type (100 ± 12.1%), V12M (106.7 ± 2.0%), Q141K (97.1 ± 9.3%), S248P (99.1 ± 9.8%), F431L (104.7% ± 10.9%), R482G

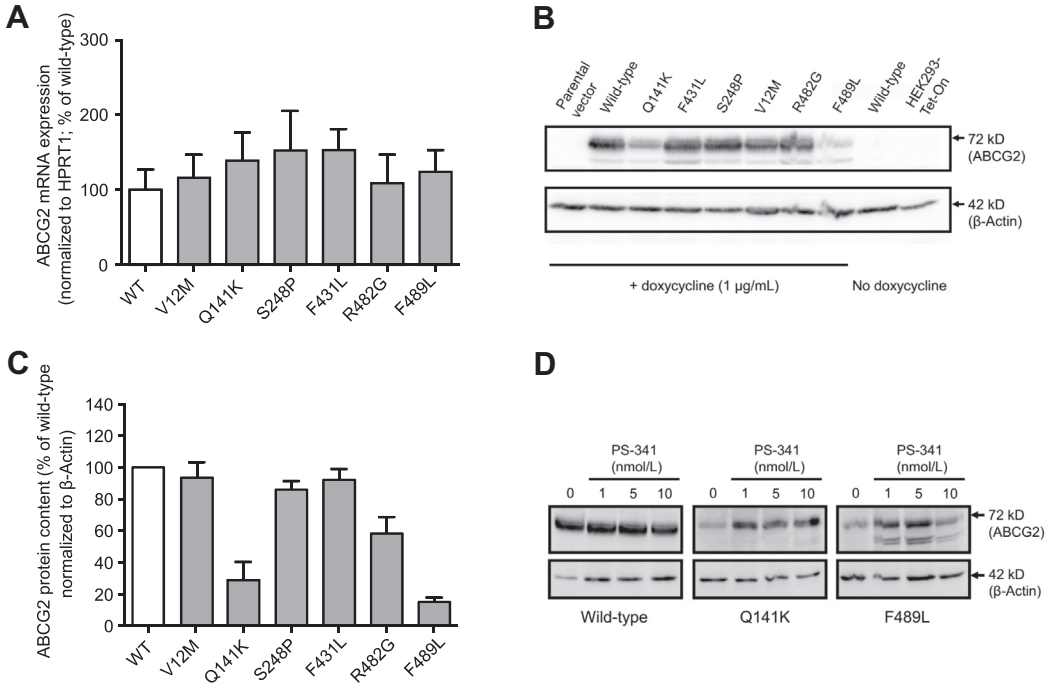

Fig. 2. (A) Analysis of ABCG2 mRNA expression in doxycycline-treated (1 μg/mL; 24 h) HEK293-Tet-On cells transiently transfected with ABCG2 wild-type or ABCG2 variants, respectively. Data are shown as mean ± SEM (n = 3–7). (B) Representative Western blot and (C) densitometric analysis of ABCG2 protein levels of HEK293-Tet-On cells transiently transfected with ABCG2 wild-type or ABCG2 variants in the absence or presence of 1 μg/mL doxycycline. (C) Data are shown as mean ± SEM (n = 3). (D) Impact of increasing concentrations of the proteasome inhibitor PS-341 (bortezomib) on ABCG2 protein levels in doxycycline-treated (1 μg/mL; 24 h) HEK293-Tet-On cells transiently transfected with ABCG2 wild-type or the ABCG2 variants Q141K and F489L, respectively.

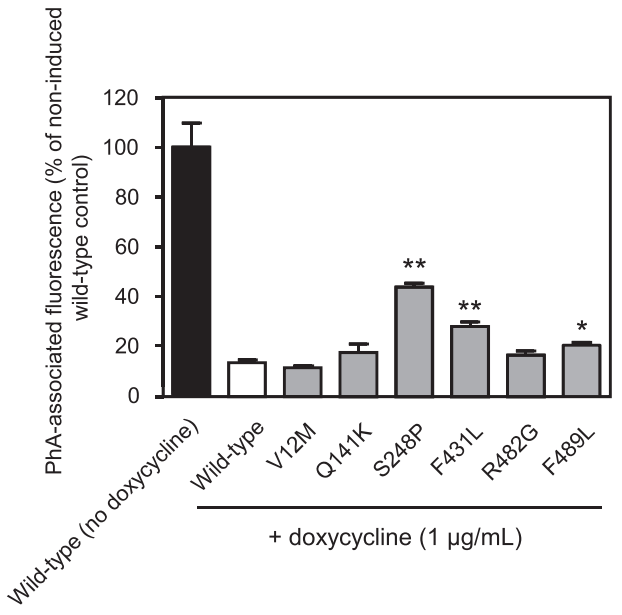

Fig. 3. PhA efflux assay: basal PhA efflux of either untreated (AcGFP1-negative) or doxycycline-treated (1 µg/mL; 24 h; AcGFP1-positive) HEK293-Tet-On cells transiently transfected with ABCG2 wild-type or ABCG2 variants, respectively. Data are shown as mean ± SEM (n = 3–4); $^{*}P < 0.05/^{**}P < 0.01$ vs. doxycycline-induced AcGFP1-positive HEK293-Tet-On cells transiently transfected with ABCG2 wild-type.

(92.7 ± 10.4%), and F489L (94.1 ± 9.1%)). To analyse the impact of doxycycline-induced ABCG2 expression on cellular PhA accumulation, median PhA-associated fluorescence was selectively analysed in AcGFP1-positive HEK293-Tet-On cells (Fig. 1E). Induction of ABCG2 wild-type expression reduced PhA-associated fluorescence to 13.8 ± 1.8% of non-induced ABCG2 wild-type-transfected cells, demonstrating expression of functional ABCG2 (Fig. 3). PhA-associated fluorescence was similar in doxycycline-induced AcGFP1-positive HEK293-Tet-On cells transfected with the ABCG2 variants V12M (11.8 ± 0.9%), Q141K (17.9 ± 5.8%), and R482G (17.0 ± 2.8%). However, basal PhA efflux was significantly lower in HEK293-Tet-On cells transfected with the ABCG2 variants S248P (44.2 ± 2.8%; $P < 0.01$ vs. doxycycline-induced ABCG2 wild-type), F431L (28.4 ± 3.1%; $P < 0.01$ vs. doxycycline-induced ABCG2 wild-type), and F489L (20.9 ± 2.0%; $P < 0.05$ vs. doxycycline-induced ABCG2 wild-type), demonstrating that these mutations significantly reduce ABCG2-mediated PhA transport in HEK293-Tet-On cells.

Next, we analysed the impact of ABCG2 mutations on telmisartan-induced inhibition of PhA efflux in transiently transfected HEK293-Tet-On cells. As expected, telmisartan concentration-dependently inhibited ABCG2-dependent PhA efflux (Fig. 4). However, telmisartan-induced inhibition of PhA efflux did not reach a plateau in HEK293-Tet-On cells transiently transfected with ABCG2 wild-type or the denoted ABCG2 variants so that comparative IC_{50} calculations were not possible. Inhibitory efficacy of telmisartan was not altered by the ABCG2 polymorphisms V12M and Q141K but tended to be lower in the ABCG2 variants S248P and F431L (Fig. 4A and C). Interestingly, the inhibitory effect of telmisartan on ABCG2-mediated PhA efflux was almost abolished by the R482G mutation (Fig. 4B and C), thereby indicating that the arginine residue at position 482 of the ABCG2 molecule may be of major importance for the interaction of telmisartan with the ABC transporter. In contrast, the inhibitory efficacy of telmisartan was significantly increased by the F489L mutation (Fig. 4B and C), indicating that the F489L mutation may facilitate the inhibitory interaction of telmisartan with ABCG2.

4. Discussion

Within the class of ARBs, telmisartan is a highly selective non-peptide-substituted benzimidazole AT1 receptor antagonist, which is characterised by a unique pharmacokinetic and pharmacodynamic profile. For instance, telmisartan activates peroxisome proliferator-activated receptor (PPAR)-γ and PPAR-α at therapeutically relevant concentrations and may thus exert beneficial metabolic effects in patients with hypertension [16,17]. In addition, we have demonstrated that telmisartan also possesses an exceptional pharmacological profile within the class of angiotensin receptor blockers (ARB) in that it inhibits the activity of ABC-transporters, such as ABCB1, ABCC2, and ABCG2 [10,11]. As telmisartan often is used in multi-drug regimens for blood pressure and cardiovascular risk reduction [10,18], an in-depth analysis of its ABC transporter interaction potential is needed to identify individuals at risk of developing telmisartan-induced drug–drug interactions. Hence, the aim of this study was to explore genetic aspects in the ABCG2–telmisartan interaction, with ABCG2 being one of the most relevant ABC transporters for drug pharmacokinetics.

Indeed, genetic variations, e.g. various single nucleotide polymorphisms (SNPs), in the *ABCG2* gene have been described which may affect its ability to interact with and/or extrude xenobiotic or endogenous substrates, such as urate, from the intracellular milieu to the extracellular space [7,19]. Moreover, genetic variations in the *ABCG2* gene may reduce the protein expression level and alter subcellular ABCG2 distribution in relevant tissues, e.g. intestinal epithelial cells, thereby increasing the exposure to drugs which are transported by ABCG2. Therefore, we studied several ABCG2 genetic variants which have previously been linked to altered drug pharmacokinetics in vivo or to an altered ABCG2 substrate spectrum in vitro [2,19]. For this purpose, we established and validated a conditional expression system for ABCG2, generated the above-mentioned variants via site-directed mutagenesis, and expressed them in HEK293-Tet-On cells. Although the mRNA expression levels of these ABCG2 variants were similar to ABCG2 wild-type, protein levels of the Q141K and the F489L variants were significantly reduced in our experimental system. These findings are in accordance with experimental data from other groups [19], although the reduction of the F489L variant was more pronounced in our cellular expression model. Moreover, a reduced protein level of the Q141K variant has been described in vitro and interestingly, the Q141K variant has been linked to an impaired uric acid efflux in vitro as well as gout disease in vivo [7,20,21]. Hence, further investigations of a potential role of the F489L mutation in the pathogenesis of gout could not only be of scientific interest but also of clinical importance. Moreover, reduced ABCG2 protein levels in individuals carrying the F489L mutation could also affect the pharmacokinetic profile of ABCG2 substrates, e.g. small-molecule receptor tyrosine kinase inhibitors, and increase the likelihood of adverse drug reactions. The reason for the reduced protein levels of the Q141K and F489L variant are currently unclear, but enhanced proteasomal degradation has been proposed to participate in reduction of cellular Q141K protein content [22]. Indeed, inhibition of proteasomal activity using the proteasome inhibitor bortezomib slightly increased the protein levels of both the Q141K and the F489L variant in HEK293-Tet-On cells, thereby suggesting a role of the proteasome in decreasing the protein content of both ABCG2 variants. Surprisingly, the Q141K variant did not exhibit a reduced pheophorbide A (PhA) efflux in HEK293-Tet-On cells as evidenced by flow cytometric analyses. In addition, PhA efflux of the F489L variant was only marginally impaired in our experimental system. These data suggest that (1) the observed reduction in ABCG2 protein content caused by the Q141K or F489L polymorphisms may be compensated by a more efficient PhA transport

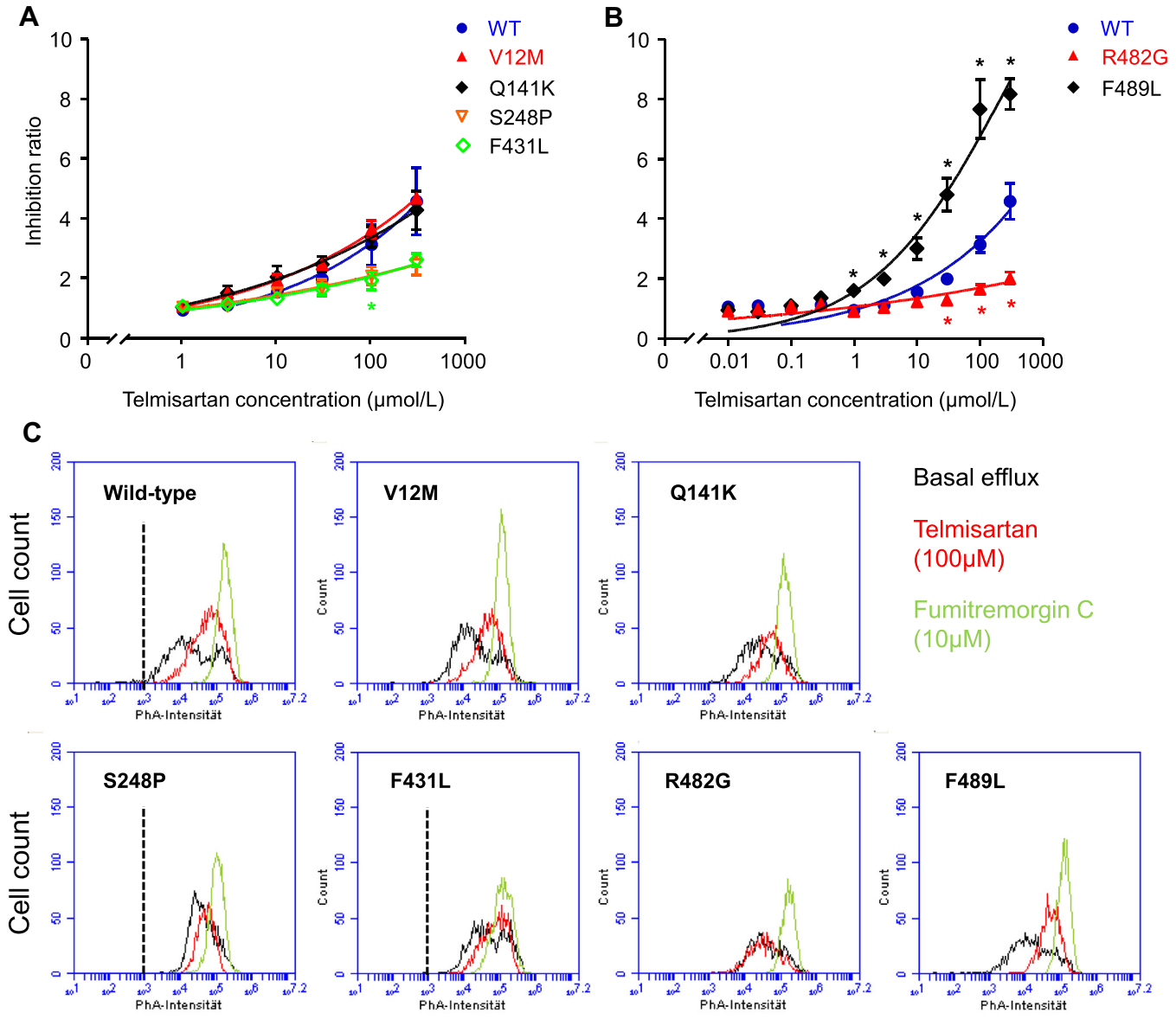

Fig. 4. (A and B) PhA efflux assay: concentration-dependent inhibition of ABCG2-mediated PhA efflux by telmisartan in HEK293-Tet-On cells transiently transfected with ABCG2 wild-type or ABCG2 variants, respectively. The inhibition ratio (a measure of drug-induced ABCG2 inhibition) for each genotype was calculated as a ratio between the median PhA-associated fluorescence (MF) with and without inhibitor during the efflux period in doxycycline-induced (1 µg/mL; 24 h) HEK293-Tet-On cells normalised to the effect of the inhibitor on MF in non-induced cells. Data are shown as mean ± SEM (n = 4–8); $^{*}P < 0.05$ vs. wild-type. (C) Representative flow cytometric plots of genotype-specific ABCG2-mediated basal PhA efflux (black lines) as well as genotype-specific telmisartan- and fumitremorgin C-induced inhibition of ABCG2-mediated PhA efflux. Dotted lines delineate the representative maximal basal PhA efflux niveau accomplished in ABCG2 wild-type-transfected HEK293-Tet-On cells.

by the aberrant protein or that (2) in the overexpression system used protein content is not linearly linked to ABCG2-dependent substrate transport. Nevertheless, data from clinical investigations indicate that the Q141K variant may be associated with a reduced ABCG2-mediated substrate clearance in affected individuals [8,9,21] and that thus, clinical investigations are needed to further explore this issue with respect to the F489L variant. Moreover, ABCG2 variants S248P and F431L displayed a significantly reduced PhA transport, although protein expression was similar to that of ABCG2 wild-type. These findings are partly in concordance with data from further groups demonstrating an impaired PhA transport in Sf9 membrane vesicles containing the S248P variant [19]. Moreover, SN-38 and methotrexate transport activity has been shown to be reduced in PA317 cells as well as in Sf9 membrane vesicles expressing the F431L variant [19,23], thereby indicating that substrate transport may be generally reduced by this polymorphism.

Interestingly, the somatic mutation R482G, which frequently occurs in drug-resistant cancer cell lines and has been associated with an altered ABCG2 substrate spectrum with regard to cytotoxic drugs [1], almost completely abolished telmisartan-induced inhibition of PhA transport in our experimental system. These data suggest that the arginine residue at position 482 of the ABCG2 molecule is of major relevance for the interaction of telmisartan with ABCG2 and that removal of the positive charge at this position in ABCG2 may largely reduce the ability of the ABC transporter to interact with telmisartan. Indeed, experimental evidence points to a prominent role of Arg-482 in ABCG2-drug interaction. For instance, the R482G mutation abolished ABCG2-mediated transport of the dihydrofolate reductase inhibitor methotrexate, whereas it acted as a gain-of-function mutation with respect to doxorubicin or rhodamine 123 [1,24]. Hence, our findings add to the available evidence on the important role of the R482G mutation for

ABCG2-drug interaction. Moreover, our data demonstrate that the germ-line mutation F489L is associated with a more efficient telmisartan-induced inhibition of ABCG2-mediated PhA transport. The reason for this phenomenon remains unclear, but it is tempting to speculate that the F489L mutation may induce structural changes within the third transmembrane domain of the ABCG2 molecule to facilitate ABCG2-associated recognition and/or binding of telmisartan. This finding may also be of clinical importance, as individuals carrying the F489L mutation could be prone to telmisartan-induced ABCG2-dependent drug–drug interactions, e.g. in concomitant use with ABCG2 substrates. In this context, future clinical investigations will help to elucidate the impact of ABCG2 and ABCG2 polymorphisms on the effectiveness and safety of antihypertensive drug regimens involving telmisartan.

In summary, our work provides evidence that the arginine residue at position 482 of the ABCG2 molecule may be of major importance for the interaction of telmisartan with the ABC transporter. Moreover, our data suggest that clinical investigations are needed to clarify whether individuals carrying the F489L mutation may be prone to ABCG2-dependent drug–drug interactions in drug regimens involving telmisartan.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.12.119.

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