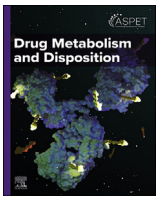
Drug Metabolism and Disposition 53 (2025) 100063

Drug Metabolism and Disposition

journal homepage: www.dmd.aspetjournals.org



ARTICLE

In vitro assessment of ATP-binding cassette transporters and their functional genetic polymorphisms on fluoroquinolone accumulation in human embryonic kidney 293 recombinant cell lines

Gwenaëlle Mahieu [1,2], Vincent Haufroid [3,4], Françoise Van Bambeke [1,*], Laure Elens [2,3]

[1] Pharmacologie Cellulaire et Moléculaire, Louvain Drug Research Institute (LDRI), Université Catholique de Louvain (UCLouvain), Brussels, Belgium
[2] Integrated PharmacoMetrics, PharmacoGenomics and Pharmacokinetics (PMGK) Research Group, Louvain Drug Research Institute (LDRI), Université Catholique de Louvain (UCLouvain), Brussels, Belgium
[3] Louvain Centre for Toxicology and Applied Pharmacology, Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain (UCLouvain), Brussels, Belgium
[4] Department of Clinical Chemistry, Cliniques Universitaires Saint-Luc, Brussels, Belgium

ARTICLE INFO

Article history:
Received 15 November 2024
Accepted 4 March 2025
Available online 12 March 2025

Key words:
anti-bacterial agents
Drug transporters
HEK293 cells

ABSTRACT

Fluoroquinolone tissue distribution and cellular accumulation are hindered by efflux transporters, including ATP-binding cassette subfamily B member 1 (ABCB1), ATP-binding cassette subfamily G member 2 (ABCG2), and ATP-binding cassette subfamily C member 4 (ABCC4). Genetic polymorphisms (single-nucleotide polymorphisms) can impact transporter activity, leading to interindividual variability in the systemic and cellular pharmacokinetics of their substrates. This study assesses the impact of these transporters on moxifloxacin and ciprofloxacin (CIP) cellular accumulation in vitro, and the effect of common single-nucleotide polymorphisms in *ABCB1* [c.1199G>A (rs2229109); common haplotype c.1236C>T (rs1128503), c.2677G>T/A (rs2032582), and c.3435C>T (rs1045642)] and *ABCG2* [c.421C>A (rs2231142)]. Recombinant human embryonic kidney (HEK) cell lines overexpressing wild-type or variant transporters were generated via stable plasmid transfection. The impact of transporter overexpression on fluoroquinolone cell disposition was assessed through accumulation experiments in the presence of specific inhibitors to establish the link between transporter expression and differential accumulation. Results indicated that ABCB1 overexpression reduced moxifloxacin cellular concentration by 30% but inconsistently with that of CIP and that zosuquidar or elacridar reversed these effects. ABCG2 had no impact. ABCC4 markedly reduced CIP accumulation by 25%, even at the basal level, an effect reversed by MK517. Contrarily to the wild-type and the c.1199A carriers, *ABCB1* CGT and TTT variants did not reduce antibiotic accumulation. In conclusion, moxifloxacin and CIP are substrates of the wild-type and 1199G>A ABCB1, while CGT and TTT haplotypes had a marginal impact on fluoroquinolone transport by ABCB1. CIP is a preferential ABCC4 substrate. Because of the large body distribution of these transporters, our findings may help rationalize their role and the impact of their polymorphisms in fluoroquinolone disposition in tissues and cells.

Significance Statement: This study demonstrates that moxifloxacin and ciprofloxacin are substrates of ABCB1, with ciprofloxacin also transported by ABCC4. Specific *ABCB1* polymorphisms (CGT and TTT haplotypes) reduce the ABCB1 transport capacity toward fluoroquinolones. These findings highlight the importance of considering ABCB1 and ABCC4 inducers or inhibitors, which may affect fluoroquinolone disposition in tissues and cells, as well as *ABCB1* polymorphisms that could explain interindividual variability in pharmacokinetic profiles.

* **Address correspondence to:** Prof. Françoise Van Bambeke, Pharmacologie Cellulaire et Moléculaire, Louvain Drug Research Institute (LDRI), Université Catholique de Louvain (UCLouvain), Avenue Emmanuel Mounier 73, B1.73.05 1200, Brussels, Belgium. E-mail: francoise.vanbambeke@uclouvain.be

This article has supplemental material available at dmd.aspetjournals.org.

F.V.B. and L.E. contributed equally to this work.

https://doi.org/10.1016/j.dmd.2025.100063

Introduction

The fluoroquinolones, moxifloxacin (MXF) and ciprofloxacin (CIP), are widely used in clinical practice because of their broad spectrum of activity, high bactericidal activity,[1-3] ability to accumulate in phagocytic and nonphagocytic cells,[4-6] and wide tissue distribution.[3] Because they can easily reach infection sites in various locations and accumulate therein, they exhibit useful activity against several intracellular bacteria thriving in different subcellular compartments.[7-10]

However, this accumulation is controlled and possibly limited by efflux transporters that affect their body disposition and intracellular accumulation in infected cells, impacting their activity against intracellular bacteria.[11-15] Transporter genes represent an important proportion of the human genome because transporters exert vital roles in the bioavailability and cellular trafficking of endogenous and exogenous compounds. The ATP-binding cassette (ABC) superfamily is composed of 48 efflux transporters classified into 7 subfamilies, designated A to G, based on their sequence homology.[16,17] Located in the plasma membrane, these ATP-dependent active transporters unidirectionally translocate a wide range of substances (lipids, bile acids, xenobiotics, and peptides) from the intracellular to the extracellular compartments.[16] By reducing the cellular concentration of potentially toxic compounds, they play a key role in body and tissue protection and detoxification.[18,19]

Several lines of evidence indicate that certain efflux transporters may impact fluoroquinolone cellular accumulation. A potential role of ATP-binding cassette subfamily B member 1 (ABCB1) (p-glycoprotein) for MXF[20,21] and CIP[22,23] and of ATP-binding cassette subfamily G member 2 (ABCG2) (breast cancer resistance protein)[24,25] and ATP-binding cassette subfamily C member 4 (ABCC4) (multidrug resistance protein (MRP) 4)[11-13,25] for CIP has been demonstrated. However, these data have been generated using different cell models (J774 macrophages, Calu-3 epithelial cells, Madin-Darby canine kidney II cells, and intestinal Caco-2 cells) or experimental procedures (transepithelial transport), rendering comparison difficult or impossible. Furthermore, these studies did not consider the potential influence of genetic polymorphisms.

In this study, we have evaluated the effect of these 3 ABC transporters on MXF and CIP transport activity using comparable in vitro models of recombinant HEK293 cell lines. We also assessed the potential functional impact of the most common coding single-nucleotide polymorphisms (SNPs) in ABCB1 and ABCG2 (see Supplemental Table 1, for a summary of the frequency of these SNPs in different ethnic groups). The *ABCB1* c.1199G>A coding SNP, located in exon 11 (rs2229109), has a reported minor allelic frequency of around 6% in Caucasians and is associated with a serine to asparagine substitution at position 400 (Ser400Asn) in the sixth cytoplasmic loop of the ABCB1 transporter, a domain involved in substrate recognition.[26] The 3 most common variants in the *ABCB1* coding region [rs1128503 (1236C>T, Gly412Gly), rs2032582 (2677G>T/A, Ala893Ser/Thr) and rs1045642 (3435C>T, Ile1145Ile)] are located in the sixth, 10th, and 12th cytoplasmic loops, respectively. Among these, rs1128503 and rs1045642 are synonymous, whereas rs2032582 is nonsynonymous. These variants are commonly found in strong linkage disequilibrium, with a minor allelic frequency of about 50% for the variant haplotype (TTT) in the Caucasian population.[15,27] Lastly, the *ABCG2* c.421C>A coding SNP located in exon 5 (rs2231142) has a reported minor allelic frequency of approximately 5%–10% in Caucasians[28] and is associated with a glutamine-to-lysine substitution at position 141 (Gln141Lys)[16] in the first cytoplasmic loop. Recent studies have associated this polymorphism with protein folding and trafficking default, partial degradation, and reduced overall and cell surface expression.[29]

2. Materials and methods

2.1. Drugs and chemicals

MXF-HCl and CIP-HCl were provided as microbiological standards by Bayer AG; rhodamine 123 (Rh123), LY335979 (zosuquidar HCl), GF120918 (elacridar), MK571 NaCl, and Ko143 hydrate were obtained from Sigma-Aldrich. Dulbecco's modified Eagle's medium, fetal bovine serum, penicillin/streptomycin (10,000 U mL^{-1}), and enzyme-free cell dissociation buffer were purchased from Gibco (Thermo Fisher Scientific); geneticin (G418) and hygromycin B were purchased from Roche, and pheophorbide A (PheA) was purchased from Sanbio. All flow cytometry antibodies [ABCB1 antibodies (fluorescein isothiocyanate mouse antihuman CD243, clone 17F9, reference 557002; and fluorescein isothiocyanate mouse IgG2b κ isotype control, clone 27−35, reference 555742); ABCG2 antibodies (Peridinin-Chlorophyll-Cyanine 5-5-a [PerCP-Cy5.5] mouse antihuman CD338, reference 561460; and PerCP-Cy5.5 mouse IgG2b κ isotype control, reference 558304)] were purchased from BD Biosciences. All chemicals used in drug quantification were of analytical grade.

2.2. Cell lines and cell culture

All HEK293 cells were grown in Dulbecco's modified Eagle's medium with 4.5 g L^{-1} glucose, L-glutamine, and sodium pyruvate supplemented with 10% (v/v) of fetal bovine serum and 1% (v/v) of penicillin/streptomycin solution at a temperature of 37 °C in a 5% CO_2 atmosphere. For subculture, cells were detached using an enzyme-free cell dissociation buffer. Cells were used between the second and the 13th passage, with verification of the transporter expression level before the first and after the last experiment.

HEK293 cells overexpressing the ABCC4 transporter were kindly supplied by Elaine M. Leslie from the University of Alberta (Canada) and cultured in the presence of G418 (600 μg mL^{-1}) to select stable recombinant cells.[30]

2.3. Generation of ABCB1 recombinant cells

The generation of stable recombinant cell lines has been described previously.[26,31] In short, HEK293 cells were transfected with a pcDNA3.1 vector with cDNA encoding either the ABCB1 wild-type (WT), a variant, or an empty vector using Lipofectamine (Thermo Fisher Scientific). Stable recombinant cells were selected by incubating the transfected cells with G418 (1 mg mL^{-1}) 48 hours after transfection. For $ABCB1_{1199G>A}$, 3 recombinant cell lines were generated: $HEK_{control}$ (pcDNA3.1 empty vector), HEK_{1199G} (WT), and HEK_{1199A} (variant), and designated as CTL, 1199G, and 1199A in this study. For $ABCB1_{1236C>T\ -\ 2677G>T\ -\ 3435C>T}$, 4 cell lines were used: $HEK_{control}$, $HEK_{1236C\text{-}2677G\text{-}3435C}$ (WT), and 2 different variant combinations, $HEK_{1236C\text{-}2677G\text{-}3435T}$ and $HEK_{1236T\text{-}2677T\text{-}3435T}$, referred to as CTL, CGC, CGT, and TTT, respectively.

2.4. Generation of ABCG2 recombinant cells

Details of the model development have been described previously.[32] Briefly, HEK293 cells were transfected by Lipofectamine (Thermo Fisher Scientific) with a pCMV3 vector containing cDNA encoding either the ABCG2 WT (421C), the variant (421A), or a pCMV3 empty vector. Stable recombinant cells were selected by incubating the transfected cells with hygromycin B (0.5 mg mL^{-1}) 48 hours after transfection. Three recombinant cell lines were generated, consisting of cell lines stably transfected with the pCMV3 empty vector ($HEK_{control}$), the WT vector (HEK_{421C}), or the

variant vector (HEK_{421A}). These will be further referred to as CTL, 421C, and 421A.

2.5. Characterization of transfected cell lines

2.5.1. Characterization of transporter expression

2.5.1.1. Quantitative real-time polymerase chain reaction (qRT-PCR). qRT-PCR was performed to quantify the number of *ABCB1*, *ABCG2*, ATP-binding cassette subfamily C member 2 (*ABCC2*), and *ABCC4* gene copies in the different cell lines. Briefly, RNA was extracted from cells (5×10^6 cells) using the RNeasy Mini Kit from Qiagen and reverse transcribed into cDNA following the GoScript Reverse Transcription Mix, Oligo(dT) Protocol (Promega according to the manufacturer's instructions. qRT-PCR was carried out with SYBR-green (Bio-Rad) using the CFX96 Touch Real-Time PCR detection system (Bio-Rad). The *18S* expression was used to normalize the expression of *ABCB1*, *ABCG2, ABCC2,* and *ABCC4*. The primer sequences for *18S*, *ABCB1*, *ABCG2*, *ABCC2*, and *ABCC4* were as follows: (forward) 5'-CGG CTA CCA CAT CCA AGG AA -3' (reverse) 5'-ATA CGC TAT TGG AGC TGG AAT TAC C -3'; (forward) 5'-AGC AAA GGA GGC CAA CAT AC -3' (reverse) 5'-GGC TTC ATC CAA AAG CAA AA -3'; (forward) 5'-CAG GAC TCA ATG CAA CAG GA-3' (reverse) 5'-TTC AGG TAG GCA ATT GTG AGG-3'; (forward) 5'-CCT CAT TCA GAC CAT CC-3' (reverse) 5'-ATT CTC AAT GCC AGC TTC CT-3'; and (forward) 5'-AAA ATG TAC GCC TGG GAA AA-3' (reverse) 5'-GGT GAA GGT CAC AAA CAC GA-3'.

2.5.1.2. Western blot. The expression of transporter proteins was further characterized through western blot, according to a previously published method with certain modifications.[26,33] Cell pellets were harvested using an adapted Radio ImmunoPrecipitation Assay lysis buffer (sodium deoxycholate 1%, tetraethylammonium bromide 100 mM, NaCl 300 mM, IGEPAL (Sigma-Aldrich) 2%, SDS 0.2%, n-dodecyl-β-D-maltoside 0.4%, pH 7.2–7.5) supplemented with a complete protease inhibitor cocktail (Roche) and incubated for 15 minutes on ice. Following this incubation, the cell lysates were then vortexed for 30 seconds and further diluted in LDS Sample Buffer (Invitrogen, Thermo Fisher Scientific) containing 25% of sample reducing agent (Invitrogen, Thermo Fischer Scientific) and water to achieve total protein concentrations of 12, 6, and 3 μg for ABCB1; 10 μg for ABCG2 and ABCC4 in a final volume of 20 μL per sample. Each sample (20 μL) and 4 μL of protein ladder (PageRuler Prestained, Thermo Fischer Scientific) were loaded onto a 4%–12% Bis-Tris gel (NuPAGE, Thermo Fischer Scientific). The electrophoretic migration and the transfer of proteins onto a polyvinylidene difluoride transfer membrane (Thermo Fischer Scientific) were then achieved using a blotting pad (VWR) at an initial voltage of 175 V for 60 minutes, followed by a reduced voltage of 30 V for 60 minutes. Blots were blocked with bovine serum albumin 1% in Tris-buffered saline with 0.02% Tween (TBS-T) pH 8 for at least 60 minutes. After 3 washing steps with TBS-T during 5 minutes, membranes were incubated overnight at 4 °C with a monoclonal primary antibody depending on the targeting protein: anti-PgP (ab170904, rabbit, 1:10000 dilution, Abcam) for ABCB1; anti-MRP4 (ab233382, rabbit, 1:1000, Abcam) for ABCC4; anti-breast cancer resistance protein (ab207732, rabbit, 1:1000, Abcam) for ABCG2; and the monoclonal anti-β-Actin antibody (A1978, mouse, 1:2000 dilution, Sigma-Aldrich) for β-actin, diluted in TBS-T with milk powder (150 mg 5 mL^{-1} of TBS-T). The next day, after 3 steps of 5 minutes washing in TBS-T, membranes were incubated for 60 minutes with a secondary antibody diluted in TBS-T with milk powder (Anti-rabbit IgG horseradish peroxidase (A27036, 1:2500 dilution, Invitrogen) for ABCB1 and ABCG2; Anti-Rabbit IgG H&L (horseradish peroxidase) (ab205718, 1/20 000 dilution, Abcam) for ABCC4; and Anti-mouse IgG (H+L) (A27025, 1:2500 dilution, Invitrogen) for β-Actin). Subsequently, after 2 additional 5-minute washing steps in TBS-T and 1 washing step in Tris-buffered saline, protein detection was accomplished through chemiluminescence using horseradish peroxidase substrate (Immobilon Classico, Millipore).

2.5.1.3. Flow cytometry. ABCB1 and ABCG2 expressions were evaluated after 1 week of growth in the presence of the selection antibiotics (G418 and hygromycin B, respectively). The cell surface expression protein for ABCB1 and ABCG2 was evaluated by flow cytometry as previously described.[34] Briefly, for each cell line, 1×10^6 cells were collected by centrifugation and washed 3 times with ice-cold FACS buffer (PBS supplemented with 1% decomplemented FBS and 1 mM EDTA [Thermo Fisher Scientific]). Cells were then incubated for 45 minutes on ice in the dark with buffer containing the following antibodies (1×10^6 cells/condition): (1) anti-ABCB1 antibody (1:10 dilution in 100 μL of buffer) or anti-ABCG2 antibody (1:20 dilution in 100 μL of buffer); (2) with the corresponding isotype control (diluted 1:10 in 100 μL of buffer for ABCB1 or isotype control diluted 1:5 for ABCG2 in 100 μL of buffer); and (3) in 100 μL of buffer with no antibody. After incubation, the cells were washed with ice-cold buffer, centrifuged, and resuspended before being analyzed using a BD FACSVerse flow cytometer with BD FACSuite software. Additional data analysis and plotting were carried out in FlowJo (version 10.6.1).

2.5.2. Characterization of transporter functionality using fluorescent substrates

For ABCB1, the functionality of the overexpressed transporter was assessed using the reference substrate Rh123 and 2 different ABCB1 inhibitors, namely zosuquidar[26,35,36] and elacridar,[37] which differ by their mechanism of inhibition. Elacridar modulates ATPase activity, inhibiting ATP hydrolysis, essential for transporter activity.[37] By contrast, zosuquidar directly docks in the substrate pocket of the transporter, blocking the change of conformation required for substrate efflux.[36] The functionality of ABCG2 was assessed using PheA, a specific ABCG2 fluorescent substrate,[38] and the ABCG2 inhibitor Ko143.

For these functional assays, 350,000 cells were plated in 12-well plates in complete medium 1 day before the experiment was performed. The next day, cells were incubated either for 120 minutes at 37 °C in a 5% CO_2 atmosphere in the dark with 5 μM Rh123 alone or in combination with either 0.2 μM zosuquidar or 5 μM elacridar, or for 60 minutes with 5 μM PheA alone or in combination with 1 μM Ko143. At the end of the incubation, the supernatants were discarded, the cells were washed twice with PBS at 4 °C, and lysed by adding 200 μL of water per well followed by overnight agitation in the dark at 4 °C. After 10 minutes of centrifugation at 20,817 RCF, the fluorescence of Rh123 and PheA in the supernatant was measured, using a Spectramax M3 fluorimeter (Molecular Devices) with excitation and emission wavelengths set at 485/530 and 410/600 nm, respectively.

2.6. Fluoroquinolone accumulation experiments

One day before the experiment, 350,000 cells were seeded in 12-well plates in 2 mL of complete medium. The next day, the medium was removed and replaced with fresh complete medium containing either MXF or CIP at 4 μg mL^{-1} (9.13 and 10.36 μM, respectively), corresponding to the maximum plasma concentration (C_{max}; total drug concentration) observed in patients undergoing conventional therapy,[39,40] alone or combined with ABCB1 inhibitors (zosuquidar 0.2 μM or elacridar 5 μM), ABCG2 inhibitor (Ko143 1 μM), or a general MRP inhibitor (MK571 25 μM) to assess the role of ABCC4.[41] After 120 minutes of

incubation at 37 °C in a 5% CO_2 atmosphere, the medium was discarded, cells were washed twice with PBS at 4 °C, and lysed by adding 200 μL of glycine-HCl buffer (pH 3, exalting fluoroquinolone fluorescence for quantification[11,12]) followed by overnight agitation in the dark at 4 °C. The next day, both cells and supernatant were transferred to a 1.5 mL Eppendorf and centrifuged at 20,817 RCF for 10 minutes at 4 °C. Quantification of MXF and CIP was performed in the supernatant and proteins in the cell pellets for normalization.

2.7. Quantification of cell-associated fluoroquinolones

The cellular concentration of MXF and CIP was quantified by fluorometry using a Spectramax M3 fluorimeter with excitation and emission wavelengths set at 298/504 and 275/450 nm, respectively.[11,12] Calibration curves for both MXF and CIP were established within a concentration range of 10–300 ng mL^{-1} in glycine-HCl buffer (pH 3) and quantified in a similar manner. For each experiment, the absolute amount of MXF and CIP recovered from the cell extracts was normalized to the amount of proteins measured using a detergent-compatible Protein Assay from Bio-Rad according to the manufacturer's instructions.

2.8. Statistics and data analysis

All accumulation data are mean ± SEM expressed as a percentage of the CTL value (100%) of n experiments repeated at least 3 times ($N = 3$) in independent triplicates ($n = 3$ different wells), using GraphPad Prism version 8.0 (GraphPad software). Two-way ANOVA was performed under the null hypothesis that the means of the groups compared were equal. When the differences between the means were significant ($P < .05$), Tukey's multiple comparison tests were performed.

3. Results

3.1. Characterization and validation of the recombinant models

3.1.1. Transporter expression

The expression of the different transporters of interest was quantified by qRT-PCR across all models, revealing marked increases in recombinant transporter transcripts compared with control cells (100-fold for *ABCB1* [Fig. 1A], 1000-fold for *ABCG2* [Fig. 1B], and 36-fold for *ABCC4* [Fig. 1C]). At the protein level, western blot analysis showed stronger signals in recombinant

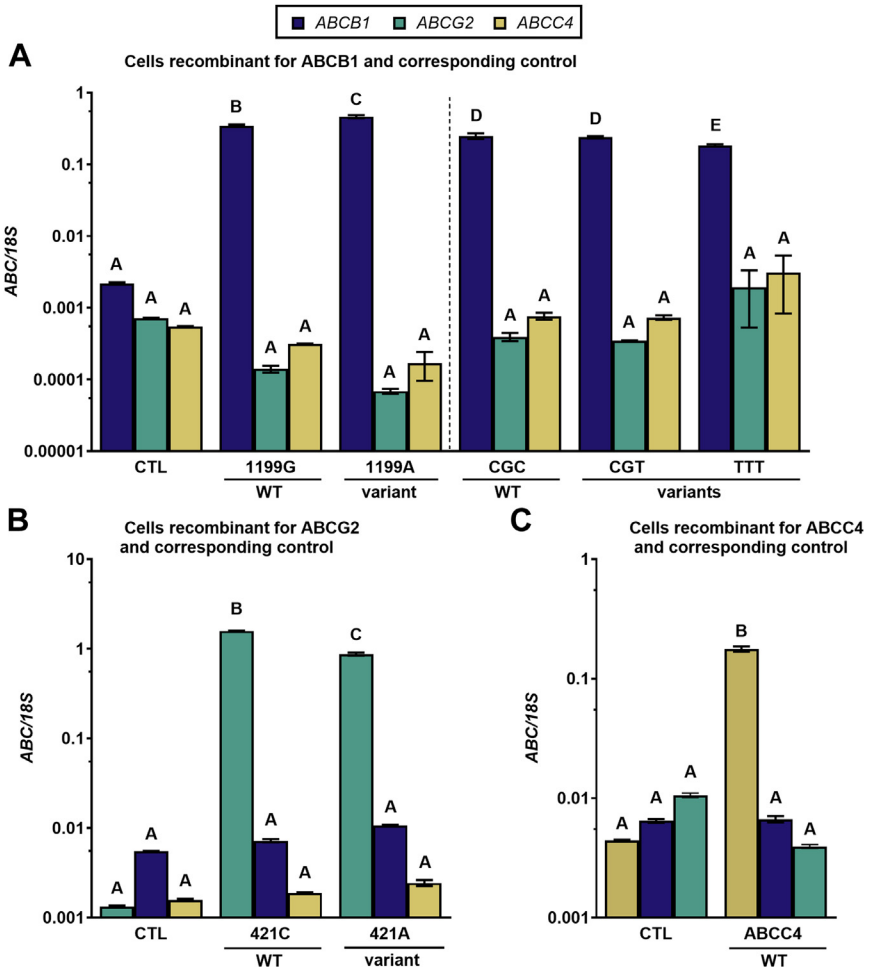


Fig. 1. ABC transporter RNA transcripts. ABC transporter RNA expression levels normalized to the *18S* expression level (housekeeping gene) in (A) $HEK_{control}$ (CTL), $HEK_{1199G>A}$ (1199G, 1199A), and $HEK_{1236C>T\text{-}2677G>T\text{-}3435C>T}$ (CGC, CGT, TTT) cell lines transfected to overexpress ABCB1 WT or its variants; (B) $HEK_{control}$ (CTL) and $HEK_{421C>A}$ (421C, 421A) cell lines transfected to overexpress ABCG2 WT or its variants; (C) $HEK_{control}$ (CTL) and HEK_{ABCC4} (ABCC4) cell lines transfected to overexpress ABCC4 WT. The $HEK_{control}$ (CTL) in each panel corresponds to the clone used to overexpress the corresponding transporter and transfected by the corresponding empty vector. Results are reported as mean ± SD ($n = 3$). Statistical analysis: 2-way ANOVA with Tukey post hoc test, comparison between all transporters in all cell lines. Values with different letters are statistically different from each other ($P < .05$). ABC, ATP-binding cassette; ABCB1, ATP-binding cassette subfamily B member 1; ABCC4, ATP-binding cassette subfamily C member 4; ABCG2, ATP-binding cassette subfamily G member; HEK, human embryonic kidney; WT, wild-type.

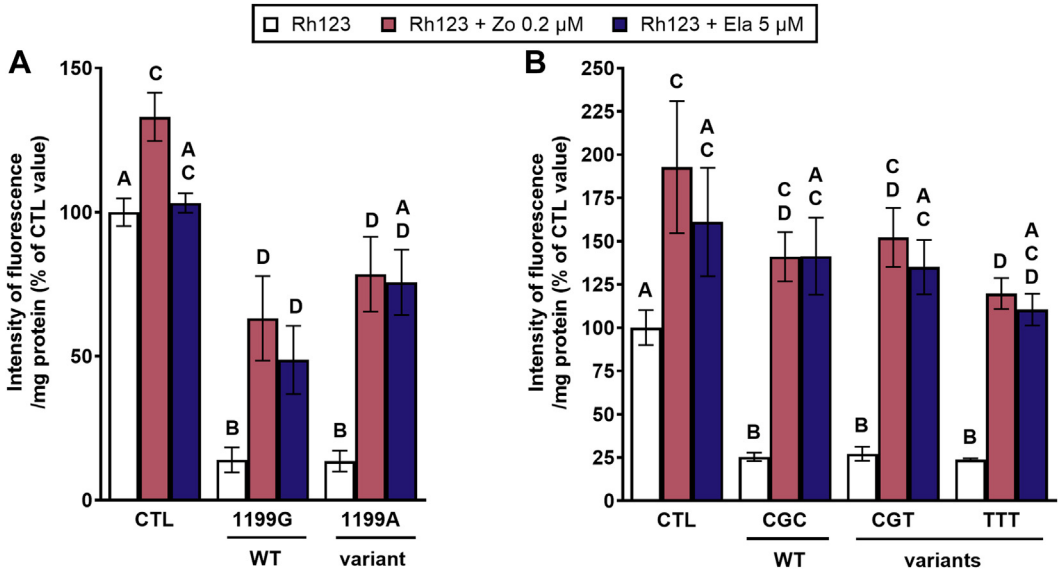


Fig. 2. Impact of ABCB1 transporter on the cellular accumulation of rhodamine 123 (Rh123). Cell-associated fluorescence of Rh123 (5 μM) after 2 hours of incubation in control conditions or in the presence of the ABCB1 inhibitors zosuquidar (Zo; 0.2 μM) or elacridar (Ela; 5 μM) in (A) $HEK_{control}$ (CTL), $HEK_{wild\ type}$ (1199G), $HEK_{variant}$ (1199A), and (B) $HEK_{control}$ (CTL), $HEK_{wild\ type}$ (CGC), and $HEK_{variant}$ (CGT, TTT) cell lines. The $HEK_{control}$ (CTL) in each panel corresponds to the clone used to overexpress the corresponding transporter and transfected by the corresponding empty vector. Intensity of fluorescence is reported in arbitrary units. Results are reported as mean ± SEM ($n = 9$) expressed as a percentage of the CTL value (100%). Statistical analysis: 2-way ANOVA with Tukey post hoc test, comparison between all conditions in all cell lines. Values with different letters are statistically different from each other ($P < .05$). ABCB1, ATP-binding cassette subfamily B member 1; HEK, human embryonic kidney.

models than in controls (Supplemental Figs. 1–3). Flow cytometry analysis of membrane transporter expression indicated that over 92% of the recombinant cell populations expressed the transporter, whereas control cells were negative for transporter expression (Supplemental Fig. 4, for flow cytometry profiles of ABCB1 and ABCG2; no data for ABCC4). The expression of ABCC2, also described as a multidrug transporter, was similar and negligible (<0.1%) in all cell lines (Supplemental Fig. 5).

3.1.2. Transporter functionality

The functionality of ABCB1 and ABCG2 was evaluated by measuring their capacity to export specific fluorescent substrates, Rh123 and PheA, respectively. The results (Fig. 2, A and B) show significantly lower intracellular Rh123 fluorescence in cells overexpressing ABCB1 compared with control (CTL) cells, with a reduction of approximately 4-fold ($P < .0009$) and no differences in Rh123 accumulation between WT (1199G, CGC) and variant (1199A, CGT, TTT) cell lines, indicating no impact of the studied polymorphisms on Rh123 accumulation. The addition of the specific ABCB1 inhibitor zosuquidar (Zo; Ki = 59 nM)[35] slightly increased the accumulation of Rh123 in control cells, which express a low but detectable basal level of *ABCB1* RNA transcripts (Fig. 1A). Most evidently, both zosuquidar and the nonspecific ABCB1 inhibitor elacridar (Ela; Ki = 1.16 nM)[42] restored Rh123 cellular accumulation in ABCB1-transfected cells, bringing its concentration back to levels closer to those measured in the corresponding CTL cells.

Similar findings were obtained for the ABCG2 transporter and the PheA substrate. As shown in Fig. 3, PheA fluorescence levels were lower in recombinant ABCG2 cells (421C, 421A) compared with control cells (CTL), with a reduction of approximately 50% ($P < .0026$). The addition of the ABCG2-specific inhibitor Ko143 (Ki = 25.9 nM)[43,44] restored PheA cell-associated fluorescence in all recombinant cells. No effect of the 421C>A *ABCG2* polymorphism was observed.

3.2. Effect of ABCB1 overexpression and polymorphisms on cellular fluoroquinolone concentrations

To evaluate the effect of transporters and inhibitors under clinically relevant conditions, experiments were conducted using fluoroquinolone concentrations corresponding to their C_{max}.

3.2.1. 1199G>A polymorphism

A reduction of approximately 30% in the cellular concentration of MXF was observed in cell lines expressing either WT (1199G) or mutant (1199A) ABCB1 compared with the control (CTL) cells (Fig. 4A1) ($P < .0040$). No significant difference in MXF concentration was observed between cells expressing the WT or the variant protein. The addition of zosuquidar or elacridar abolished the effect of the overexpressed transporter, resulting in cellular concentrations similar to those observed in the CTL cell line, while Ko143 and the nonspecific inhibitor MK571[42] were ineffective. In contrast, CIP showed the same level of cellular concentration in all cell lines, unaffected by Ko143, but significantly increased by MK571 in the CTL cell line ($P < .0001$) or zosuquidar and elacridar in both transfected cell lines (Fig. 4A2) ($P < .0470$).

3.2.2. 1236C>T–2677G>T–3435C>T polymorphisms

Here, in addition, the cellular concentration of MXF was significantly lower in the cell line overexpressing WT ABCB1 (CGC) compared with control cells (CTL) (Fig. 4B1), showing a reduction of approximately 30% ($P < .0003$). Additionally, MXF concentration tended to be higher in CGT and TTT cells when compared with the WT haplotype (CGC) and no more different from that measured in CTL cells, suggesting that these 2 SNP haplotypes have a reduced effect on MXF transport. Regarding inhibitors, zosuquidar and, to a larger extent, elacridar increased MXF concentration in cells expressing the WT haplotype, but not significantly in cells expressing *ABCB1* polymorphisms. As observed in the experiments conducted with the 1199G>A polymorphism, Ko143 and MK571

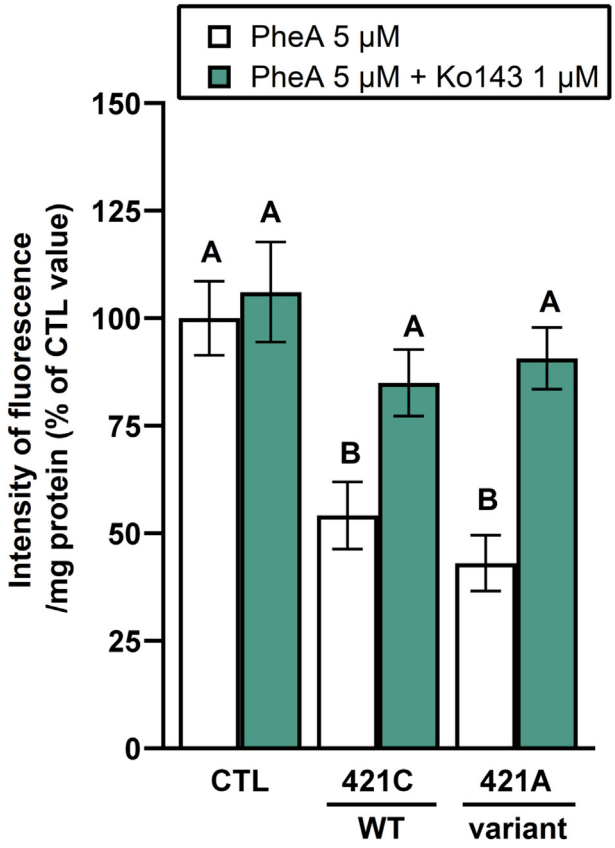


Fig. 3. Impact of ABCG2 transporter on the cellular accumulation of pheophorbide A (PheA). Cell-associated fluorescence of PheA (5 μM) after 1 hour of incubation in control conditions or the presence of the ABCG2 inhibitor Ko143 (1 μM) in $HEK_{control}$ (CTL), $HEK_{wild\ type}$ (421C), and $HEK_{variant}$ (421A). The $HEK_{control}$ (CTL) in each panel corresponds to the clone used to overexpress the corresponding transporter and transfected by the corresponding empty vector. Intensity of fluorescence is reported in arbitrary units. Results are reported as mean ± SEM ($n = 9$) expressed as a percentage of the CTL value (100%). Statistical analysis: 2-way ANOVA with Tukey post hoc test, comparison between all conditions in all cell lines. Values with different letters are statistically different from each other ($P < .05$). ABCG2, ATP-binding cassette subfamily G member 2; HEK, human embryonic kidney.

had no significant effect on MXF cellular disposition in any cell line. Regarding CIP, a small reduction in its cellular concentration was observed in the WT versus CTL cells, contrary to what was reported in panel A2. It is attributed to the fact these are 2 different clones of WT cells, with possibly variable basal expression levels for this transporter. Notably, CIP cellular concentration tended to be higher in cells overexpressing CGT or TTT variants than the WT transporter, reaching values similar to those measured in control cells. Elacridar, and to a lower extent zosuquidar, increased its accumulation in cells overexpressing the ABCB1 WT transporter or the CGT variant, but not the TTT variant (Fig. 4B2). Here, in addition, MK571 significantly increased CIP cellular disposition in CTL cells by approximately 3-fold ($P < .0001$), whereas Ko143 had no effect on CIP disposition in any cell line.

3.3. Effect of ABCG2 overexpression and 421C>A polymorphism on cellular fluoroquinolone concentrations

Given that ABCG2 is known to facilitate the transport of CIP, we investigated the effect of ABCG2 overexpression and the impact of the 421C>A polymorphism on the cellular accumulation of MXF and CIP (Fig. 5, A and B). Both WT (421C) and variant (421A) ABCG2 cell lines showed similar cellular concentrations of MXF or CIP compared with control cell lines that express the transporter to a very low basal level (Fig. 1B). Consistently, the addition of Ko143 had no effect on the MXF or CIP accumulation in any ABCG2 overexpressing cell lines. Furthermore, the addition of elacridar and MK571 increased the accumulation of MXF and CIP, respectively, in CTL cell lines ($P < .0001$).

3.4. Effect of ABCC4 overexpression on cellular fluoroquinolone concentrations

To elucidate our observation that MK571 increases CIP accumulation in both CTL cell lines, we evaluated the impact of the ABCC4 transporter on MXF and CIP accumulation in a HEK293 recombinant model overexpressing ABCC4. ABCC4 was specifically considered rather than other ABCC multidrug transporters, based on previous data indicating that neither ABCC1 nor ABCC2 was involved in CIP transport.[12,45] The results presented in Fig. 6, A and B indicate that the ABCC4 transporter had no impact on MXF accumulation but significantly reduced that of CIP, with a reduction of approximately 25% ($P < .0222$). Furthermore, MK571 enhanced CIP accumulation in recombinant cells expressing ABCC4 and CTL cells ($P < .0001$), consistent with our previous experiments. This effect of MK571 on CIP accumulation was concentration-dependent in recombinant cells but not in CTL cells.

4. Discussion

In this study, we assessed the functional relevance of ABCB1, ABCG2, and ABCC4 transporters and key genetic polymorphisms in *ABCB1* and *ABCG2* (1199G>A; 1236C>T-2677G>T-3435C>T and 421C>A) for MXF and CIP cellular disposition. Stable HEK293 cell lines overexpressing WT (1199G and CGC for *ABCB1*; 421C for *ABCG2*) or variant alleles (1199A, CGT, and TTT for *ABCB1*; 421A for *ABCG2*) were generated.

Although membrane localization of the transporters was previously demonstrated in our models,[26,31,32,46] we tested the functionality of the overexpressed proteins by analyzing the accumulation of the ABCB1 and ABCG2 substrates[26,38] and assessing the effects of specific transporter inhibitors. We observed that Rh123 accumulation was 4-fold lower in all ABCB1 transfected cell lines compared with CTL cells. These findings align with previous studies using the same cell model and further validate our experimental setting.[26,31] Furthermore, we demonstrate that Rh123 accumulation in CTL cells was slightly affected by the presence of the ABCB1 inhibitor zosuquidar (consistent with low ABCB1 basal expression in this model) and sensitive to both zosuquidar and elacridar in transfected cell lines. The concomitant use of the specific, competitive ABCB1 inhibitor zosuquidar[35] and the nonspecific, inhibiting ATP-hydrolysis, inhibitor elacridar enables more comprehensive ABCB1 inhibition by targeting different mechanisms.[36,37] It allows the comparison of their efficacy and assessment of interactions with *ABCB1* polymorphisms and prevents compensatory drug efflux via other transporters such as ABCG2, also inhibited by elacridar (Ki = 12.3 nM).[43]

For the ABCG2 transporter, a similar conclusion can be drawn as PheA accumulation was 2-fold higher in CTL cells compared with those overexpressing ABCG2. Moreover, the ABCG2-specific inhibitor Ko143, which has no effect on PheA accumulation in CTL cells that express *ABCG2* to very low levels, restores it to CTL values in transfected cell lines.

Collectively, these experiments validate that both recombinant proteins (ABCB1 and ABCG2) and their variants are overexpressed, functional, and could be effectively inhibited by their respective inhibitors in this model.

The results obtained with CIP and MOX on these functional models are summarized in a table for clarity (see Supplemental

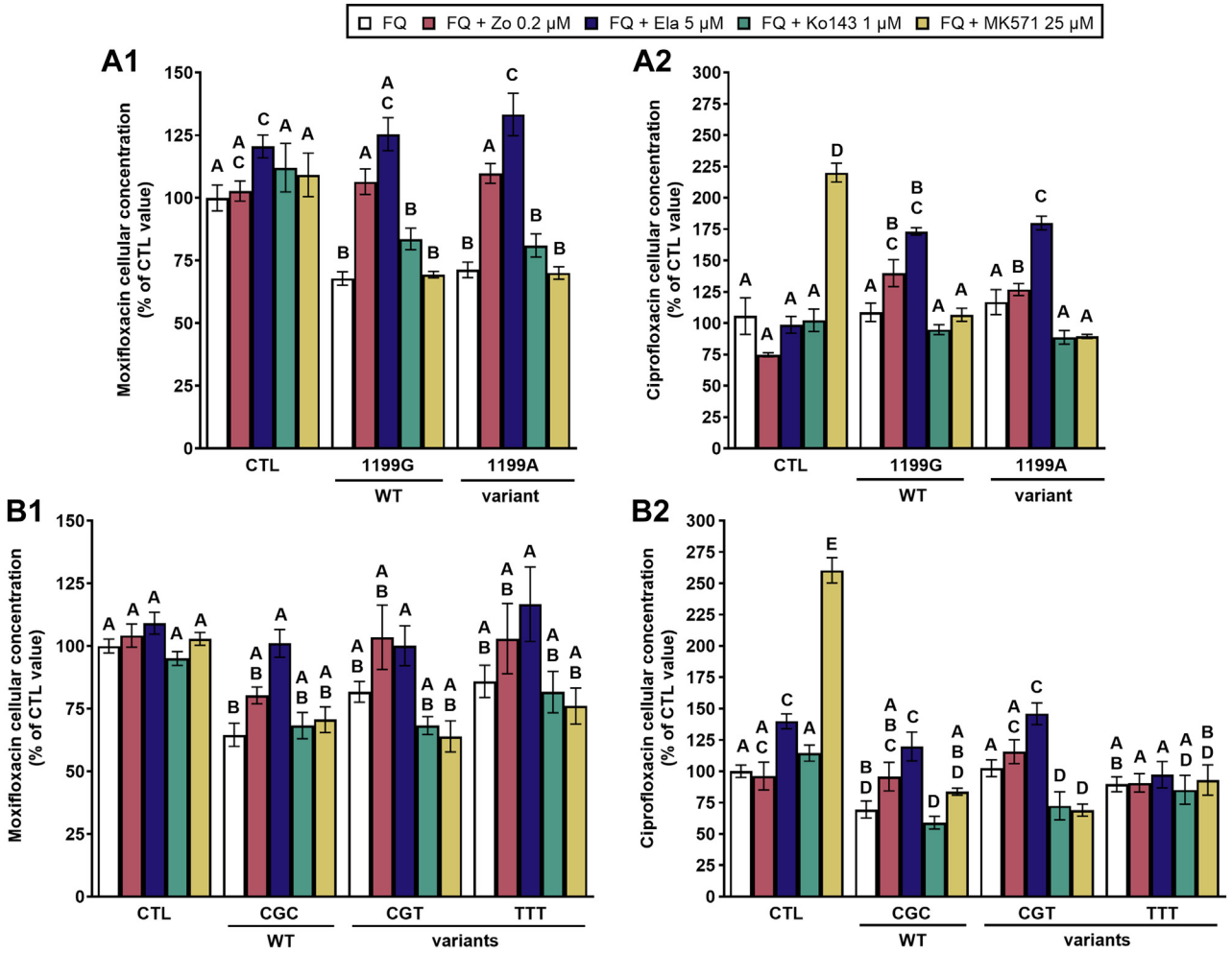


Fig. 4. Impact of ABCB1 and its polymorphisms on MXF and CIP cellular accumulation. Cellular accumulation of the fluoroquinolones (FQ) MXF and CIP (4 μg mL^{-1}) after 2 hours of incubation in control conditions or the presence of zosuquidar (Zo; 0.2 μM); elacridar (Ela; 5 μM); Ko 143 (1 μM), or MK571 (25 μM) in (A1 and A2) HEK$_{control}$ (CTL), HEK$_{wild\ type}$ (1199G), HEK$_{variant}$ (1199A), and (B1 and B2) HEK$_{control}$ (CTL), HEK$_{wild\ type}$ (CGC), HEK$_{variant}$ (CGT, TTT). The HEK$_{control}$ (CTL) in each panel corresponds to the clone used to overexpress the corresponding transporter and transfected by the corresponding empty vector. Results are reported as mean ± SEM ($n = 9$) expressed as a percentage of the CTL value (100%). Statistical analysis: 2-way ANOVA with Tukey post hoc test, comparison between all conditions in all cell lines. Values with different letters are statistically different from each other ($P < .05$). ABCB1, ATP-binding cassette subfamily B member 1; CIP, ciprofloxacin; HEK, human embryonic kidney; MXF, moxifloxacin.

Table 2). Consistent with other in vitro studies,[20,21] our findings (reduced accumulation in recombinant cell lines, reversed by specific inhibitors) confirm the ABCB1 role in the active transport of MXF. Likewise, the specific effects of ABCB1 inhibitors on CIP accumulation confirm that this transporter also plays a role in CIP cellular disposition, though to a lesser extent than for MXF.[22,23] ABCB1 transports various substrates that do not necessarily share structural similarities,[47] making it difficult to develop theories based on substrate structure only, even for drugs within the same pharmacological class. In this respect, it is worth mentioning that MXF is more lipophilic than CIP and essentially present in zwitterionic form at neutral pH.[12] This might explain why MXF is better transported because ABCB1 substrates are mainly hydrophobic and amphipathic compounds.[47]

Strikingly, we observe that the nonspecific MRP transporter inhibitor MK571 (Ki < 2 μM)[43] increases by approximately 3-fold in CIP accumulation in all CTL clones. This effect may be due to ABCC4 basal expression in these cells. Confirming this hypothesis and in accordance with previous observations in J774 macrophages overexpressing ABCC4,[13,48] CIP accumulation is reduced by approximately 25% in HEK cells overexpressing ABCC4, and higher MK571 concentrations (50 μM) are needed to restore CIP accumulation in these cells. Consequently, our hypothesis is that ABCC4 basal expression in all HEK293 cell lines reduces CIP accumulation and, hence, the expected role of ABCB1 and ABCG2[24] in transfected cells. These results highlight the importance of carefully considering the basal expression of transporters in a given cell line because they can have a nonnegligible impact on drug substrate accumulation, leading to erroneous interpretation of the overexpressed transporter's real impact in the same cell line. In the present case, the effects of nonspecific and, to a lesser extent, specific ABCB1 inhibitors remain observable in cells overexpressing ABCB1 or its variants, but it might have been different in cells that did not express ABCC4 and accumulated more CIP.

Lastly, we did not find any significant effect of ABCG2 or its genetic polymorphisms on CIP and MXF cellular accumulation. To our knowledge, MXF transport by ABCG2 has not been reported so far, and its role in CIP transport does not seem predominant in the presence of other efflux transporters.[24,25]

We did not detect any significant effect of certain SNPs investigated. Specifically, MXF or CIP cellular accumulation in cells overexpressing *ABCB1* 1199A or *ABCG2* 421A variant alleles did not

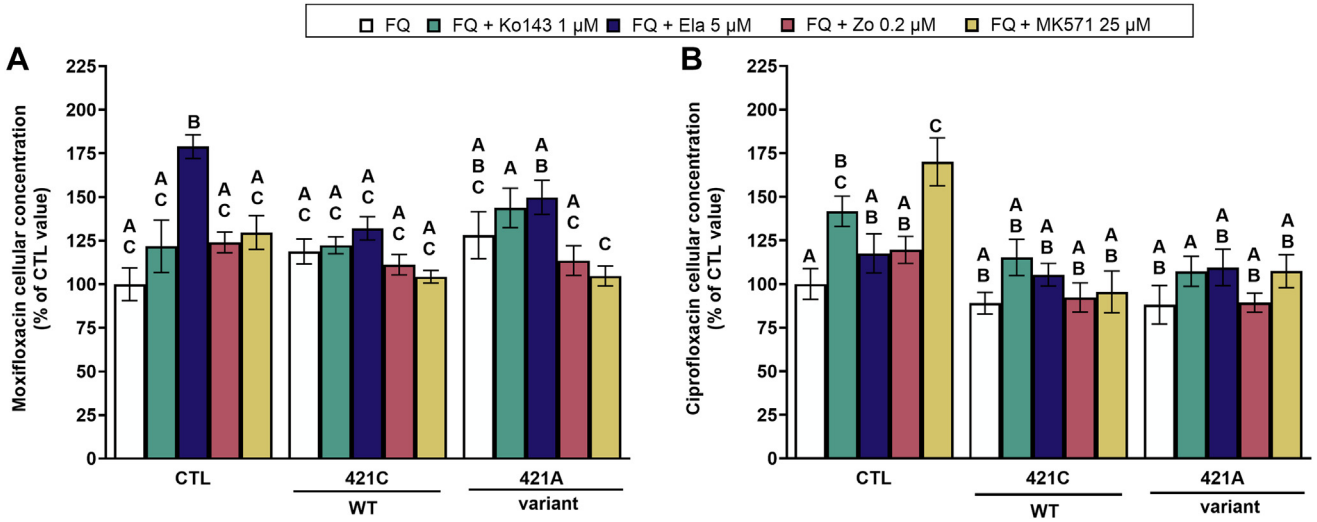


Fig. 5. Impact of ABCG2 and polymorphisms on MXF (A) and CIP (B) cellular accumulation. Cellular accumulation of the fluoroquinolones (FQ) MXF (A) and CIP (B) (4 μg mL^{-1}) after 1 hour of incubation in control conditions or the presence of Ko 143 (1 μM); elacridar (Ela; 5 μM); zosuquidar (Zo; 0.2 μM) or MK571 (25 μM) in HEK$_{control}$ (CTL), HEK$_{wild\ type}$ (421C), or HEK$_{variant}$ (421A). The HEK$_{control}$ (CTL) in each panel corresponds to the clone used to overexpress the corresponding transporter and transfected by the corresponding empty vector. Results are reported as mean ± SEM (n = 9) expressed as a percentage of the CTL value (100%). Statistical analysis: 2-way ANOVA with Tukey post hoc test, comparison between all conditions in all cell lines. Values with different letters are statistically different from each other (P < .05). ABCG2, ATP-binding cassette subfamily G member 2; CIP, ciprofloxacin; HEK, human embryonic kidney; MXF, moxifloxacin.

differ from that measured in cells overexpressing the corresponding WT transporter. Previous studies have demonstrated that the 1199G>A SNP influences the transporter activity in a substrate-dependent manner. For instance, the 1199A variant exhibited reduced ABCB1 activity toward tacrolimus in recombinant HEK293 and K512 cell lines, while it enhanced ABCB1 activity toward vinblastine in K562 recombinant cell lines.[26] However, this variant did not significantly affect cyclosporin A, Rh123 (HEK293 and K562), doxorubicin (K562), or rivaroxaban (HEK293),[49] darunavir (HEK293)[34] or bictegravir (HEK293) transport.[50] Similarly, the in vitro effect of the 1199G>A SNP was less pronounced for nilotinib compared with imatinib (HEK293), a better ABCB1 substrate.[51] Given its localization in the sixth cytoplasmic loop involved in substrate recognition, the 1199G>A polymorphism may selectively

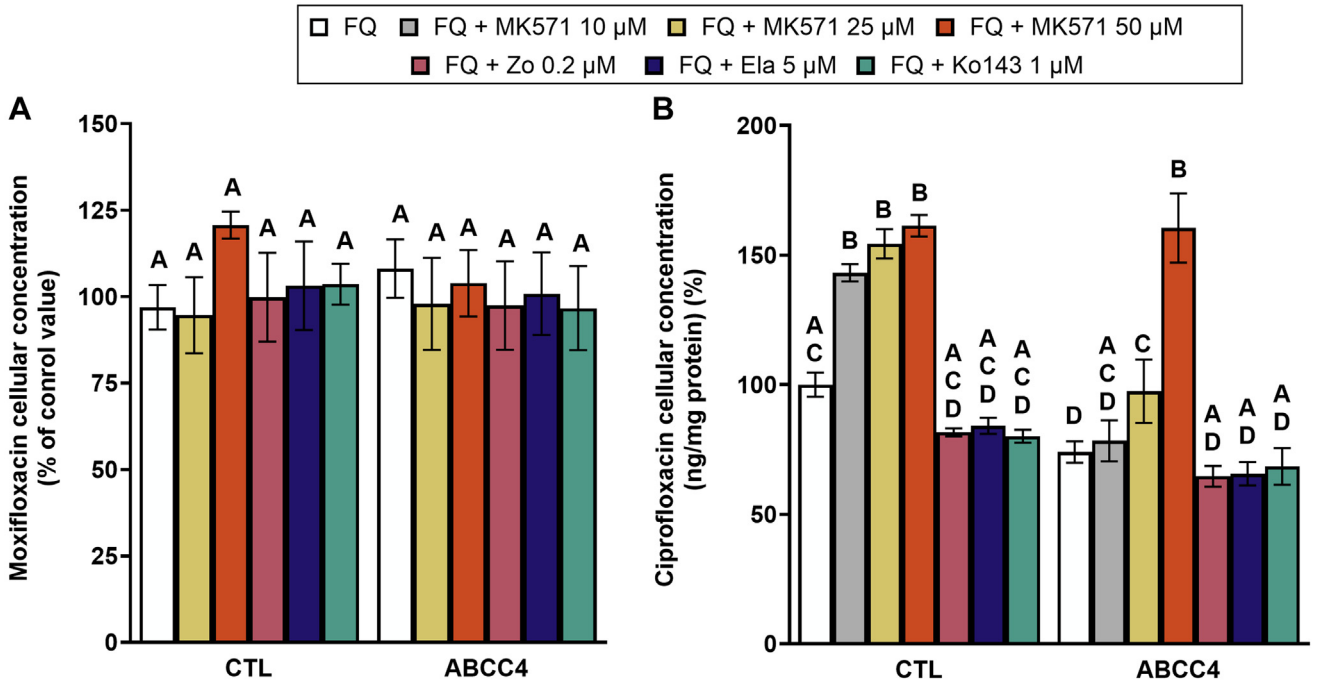


Fig. 6. Impact of ABCC4 on MXF and CIP cellular accumulation. Cellular accumulation of the fluoroquinolones (FQ) MXF (A) and CIP (B) (4 μg mL^{-1}) after 1 hour of incubation in control conditions or the presence of MK571 (10, 25, and 50 μM), zosuquidar (Zo; 0.2 μM); elacridar (Ela; 5 μM); Ko 143 (1 μM) in HEK$_{control}$ (CTL) and HEK$_{ABCC4}$ (ABCC4). Results are reported as mean ± SEM (n = 9) expressed as a percentage of the CTL value (100%). The HEK$_{control}$ (CTL) in each panel corresponds to the clone used to overexpress the corresponding transporter and transfected by the corresponding empty vector. Results are reported as mean ± SEM (n = 9) expressed as a percentage of the CTL value (100%). Statistical analysis: 2-way ANOVA with Tukey post hoc test, comparison between all conditions in all cell lines. Values with different letters are statistically different from each other (P < .05). ABCC4, ATP-binding cassette subfamily C member 4; CIP, ciprofloxacin; HEK, human embryonic kidney; MXF, moxifloxacin.

influence the binding of certain substrates while leaving others unaffected. Two distinct binding sites have been identified in ABCB1, namely H- and R-sites. H-site is involved in highly hydrophobic substrate recognition, whereas R-site recognizes smaller substrates and facilitates ATP hydrolysis, both essential for ABCB1 transport.[52] At the present time, however, it is unknown if 1199G>A *ABCB1* polymorphism might impact substrate interaction with these sites and in which site MXF or other substrates bind in this pocket, making it difficult to rationalize how it may affect various drugs.[53]

In contrast to the WT ABCB1 protein, the TTT and CGT haplotypes either do not or only minimally reduce the cellular accumulation of MXF and CIP. Both haplotypes share the variant at the third nucleotide (c.3435C>T), suggesting its possible causative role. To our knowledge, this is the first in vitro study that has investigated the role of these polymorphisms on fluoroquinolone disposition. Limited in vivo studies have shown a specific impact on fluoroquinolone pharmacokinetics. For example, the *ABCB1* 3435C>T (CGT) polymorphism is associated with a decrease in the sitafloxacin C_{max}.[54] It introduces a rare codon that causes a ribosome translational pause, which affects protein folding kinetics by chaperones, thereby altering ABCB1 function.[55] Additionally, the *ABCB1* 2677T>G/A (rs2032582) polymorphism correlates with a 40% reduction in MXF prehepatic bioavailability in humans, leading to a decrease in the area under the curve.[56] These data suggest that the reduced function of this variant increases fluoroquinolone cellular and tissue distribution, supported by our in vitro data, which further identifies the c.3435C>T SNP within the haplotype as the causative variant.

As for the TTT variant haplotype, although there is an ongoing debate about the functional implications of this haplotype, the literature indicates a trend toward a lower impact of this SNP on drug transport compared with the WT. For example, the *ABCB1* TTT variant haplotype is linked to a lower decrease in the accumulation of imatinib compared with *ABCB1* CGC,[31] also in line with our own findings with MXF and CIP.

In summary, ABCB1, which is predominantly expressed in epithelial cells, plays a pivotal role in the absorption, distribution, and excretion of various substrates, including antibiotics. This study has demonstrated that this transporter significantly influences fluoroquinolone cellular concentration in vitro, with a more pronounced effect on MXF than on CIP, and highlights the impact of specific polymorphisms. These findings may help explain the limited in vivo data suggesting that ABCB1 genetic polymorphisms can affect fluoroquinolone disposition. Noteworthy, the 3435TT genotype is more common in White populations, whereas the CC genotype is more frequent in Africans,[57] indicating the potential value of comparing fluoroquinolone pharmacokinetics in these populations.

Conversely, ABCC4, expressed in phagocytic cells,[13] reduces by approximately 25% the intracellular CIP concentration, potentially impacting its efficacy against intracellular pathogens.[58] The consequences of these modulations need to be addressed in terms of efficacy, risks of resistance selection, or toxicity in well designed in vivo or clinical studies.

Despite these important findings, some limitations of this work should be acknowledged. Indeed, using a cell model overexpressing a specific transporter may not accurately reflect its role in the body where its expression could be lower, and multiple transporters may interact to modulate drug disposition. However, these models remain valuable in pharmacology for understanding substrate specificity and comparing the impact of genetic polymorphisms. A model incorporating all relevant transporters may nevertheless usefully complement this work to assess the individual contribution and relative importance of each transporter in MXF and CIP disposition. Additionally, although we confirm the overexpression of ABCC4 in the recombinant cell lines, we could not sort exclusively recombinant cells that express ABCC4 for technical reasons, which could introduce variability in our results. Second, only key polymorphisms of *ABCB1* and *ABCG2* were explored, but the study would need to be extended to others in the future. *ABCC4* polymorphisms could also be investigated, although they are rare in the population and not associated so far with clinically significant changes in drug pharmacokinetics.[59] Lastly, the in vitro nature of the study does not capture the complexity of pharmacokinetic processes such as tissue distribution or hepatic or renal elimination which involve transporters in vivo. Nevertheless, our work may help in understanding interindividual variations in fluoroquinolone pharmacokinetics among patients.

To conclude, in HEK293 recombinant cell models, our study indicates that ABCB1 plays a significant role in MXF transport, whereas ABCC4 is more crucial for CIP transport. Additionally, we show that *ABCB1* CGT and TTT haplotypes seem to reduce ABCB1 efflux ability toward these compounds. Importantly, our work also highlights the need to consider the interfering role of the basal expression of transporters when studying drug transport in recombinant cell lines to correctly interpret the resulting data.

Abbreviations

ABC, ATP-binding cassette; ABCB1, ATP-binding cassette subfamily B member 1; ABCC2, ATP-binding cassette subfamily C member 2; ABCC4, ATP-binding cassette subfamily C member 4; ABCG2, ATP-binding cassette subfamily G member 2; CIP, ciprofloxacin; HEK, human embryonic kidney; MRP, multidrug resistance protein; MXF, moxifloxacin; PheA, pheophorbide A; qRT-PCR, quantitative real-time polymerase chain reaction; Rh123, Rhodamine 123; SNP(s), single-nucleotide polymorphism(s); TBS-T, Tris-buffered saline with 0.02% Tween; WT, wild-type.

Acknowledgments

The authors thank Nadtha Panin, Maxime Lingursky, and Virginie Mohymont for technical support for cell culture and specific experiments. The authors also thank Elaine M. Leslie for kindly providing HEK293 cells overexpressing the ABCC4 transporter.

Financial support

This work was supported by the Belgian Fonds de la Recherche Scientifique [GRANT T.0205.22] to F.V.B.

Conflict of interest

Gwenaëlle Mahieu is a research fellow (FC43775), and Françoise Van Bambeke is a research director from the Belgian Fonds de la Recherche Scientifique (FRS-FNRS). All other authors declare no conflicts of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authorship contribution

Participated in research design: Mahieu, Bambeke, Elens.
Conducted experiments: Mahieu.
Contributed new reagents or analytic tools: Haufroi.
Performed data analysis: Mahieu, Bambeke.

Wrote and contributed to the writing of the manuscript: Mahieu, V.H., Bambeke, Elens.

Supplemental material

This article has supplemental material available at dmd.aspetjournals.org.

References

1. Hooper DC. *Fluoroquinolones. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 2000:404–423.
2. King DE, Malone R, Lilley SH. New classification and update on the quinolone antibiotics. *Am Fam Physician*. 2000;61(9):2741–2748.
3. Van Bambeke F, Michot JM, Van Eldere J, Tulkens PM. Quinolones in 2005: an update. *Clin Microbiol Infect*. 2005;11(4):256–280. http://dx.doi.org/10.1111/j.1469-0691.2005.01131.x
4. Easmon CS, Crane JP. Uptake of ciprofloxacin by macrophages. *J Clin Pathol*. 1985;38(4):442–444. http://dx.doi.org/10.1136/jcp.38.4.442
5. Nix DE, Goodwin SD, Peloquin CA, Rotella DL, Schentag JJ. Antibiotic tissue penetration and its relevance: models of tissue penetration and their meaning. *Antimicrob Agents Chemother*. 1991;35(10):1947–1952. http://dx.doi.org/10.1128/AAC.35.10.1947
6. Tulkens PM. Intracellular distribution and activity of antibiotics. *Eur J Clin Microbiol Infect Dis*. 1991;10(2):100–106. http://dx.doi.org/10.1007/BF01964420
7. Carlier MB, Scorneaux B, Zenebergh A, Desnottes JF, Tulkens PM. Cellular uptake, localization and activity of fluoroquinolones in uninfected and infected macrophages. *J Antimicrob Chemother*. 1990;26(Suppl B):27–39. http://dx.doi.org/10.1093/jac/26.suppl_b.27
8. Carryn S, Van Bambeke F, Mingeot-Leclercq MP, Tulkens PM. Comparative intracellular (THP-1 macrophage) and extracellular activities of β-lactams, azithromycin, gentamicin, and fluoroquinolones against *Listeria monocytogenes* at clinically relevant concentrations. *Antimicrob Agents Chemother*. 2002;46(7):2095–2103. http://dx.doi.org/10.1128/AAC.46.7.2095-2103.2002
9. Veziris N, Truffot-Pernot C, Aubry A, Jarlier V, Lounis N. Fluoroquinolone-containing third-line regimen against Mycobacterium tuberculosis in vivo. *Antimicrob Agents Chemother*. 2003;47(10):3117–3122. http://dx.doi.org/10.1128/AAC.47.10.3117-3122.2003
10. Barcia-Macay M, Seral C, Mingeot-Leclercq MP, Tulkens PM, Van Bambeke F. Pharmacodynamic evaluation of the intracellular activities of antibiotics against *Staphylococcus aureus* in a model of THP-1 macrophages. *Antimicrob Agents Chemother*. 2006;50(3):841–851. http://dx.doi.org/10.1128/AAC.50.3.841-851.2006
11. Michot JM, Van Bambeke F, Mingeot-Leclercq MP, Tulkens PM. Active efflux of ciprofloxacin from J774 macrophages through an MRP-like transporter. *Antimicrob Agents Chemother*. 2004;48(7):2673–2682. http://dx.doi.org/10.1128/AAC.48.7.2673-2682.2004
12. Michot J-M, Seral C, Van Bambeke F, Mingeot-Leclercq M-P, Tulkens PM. Influence of efflux transporters on the accumulation and efflux of four quinolones (ciprofloxacin, levofloxacin, garenoxacin, and moxifloxacin) in J774 macrophages. *Antimicrob Agents Chemother*. 2005;49(6):2429–2437. http://dx.doi.org/10.1128/AAC.49.6.2429-2437.2005
13. Marquez B, Caceres NE, Mingeot-Leclercq M-P, Tulkens PM, Van Bambeke F. Identification of the efflux transporter of the fluoroquinolone antibiotic ciprofloxacin in murine macrophages: studies with ciprofloxacin-resistant cells. *Antimicrob Agents Chemother*. 2009;53(6):2410–2416. http://dx.doi.org/10.1128/AAC.01428-08
14. Vallet CM, Marquez B, Nhiri N, et al. Modulation of the expression of ABC transporters in murine (J774) macrophages exposed to large concentrations of the fluoroquinolone antibiotic moxifloxacin. *Toxicology*. 2011;290(2-3):178–186. http://dx.doi.org/10.1016/j.tox.2011.09.003
15. Van Bambeke F. Antibiotic efflux pumps in eukaryotic cells: occurrence and impact on antibiotic cellular pharmacokinetics, pharmacodynamics and toxicodynamics. *J Antimicrob Chemother*. 2003;51(5):1067–1077. http://dx.doi.org/10.1093/jac/dkg225
16. Choudhuri S, Klaassen CD. Structure, function, expression, genomic organization, and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP), and ABCG2 (BCRP) efflux transporters. *Int J Toxicol*. 2006;25(4):231–259. http://dx.doi.org/10.1080/10915810600746023
17. Vasiliou V, Vasiliou K, Nebert DW. Human ATP-binding cassette (ABC) transporter family. *Hum Genomics*. 2009;3(3):281–290. http://dx.doi.org/10.1186/1479-7364-3-3-281
18. Fardel O, Lecureur V, Guillouzo A. The P-glycoprotein multidrug transporter. *Gen Pharmacol*. 1996;27(8):1283–1291. http://dx.doi.org/10.1016/S0306-3623(96)00081-X
19. Schinkel AH, Jonker JW. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. *Adv Drug Deliv Rev*. 2012;64:138–153. http://dx.doi.org/10.1016/j.addr.2012.09.027
20. Brillault J, De Castro WV, Harnois T, Kitzis A, Olivier J-C, Couet W. P-glycoprotein-mediated transport of moxifloxacin in a calu-3 lung epithelial cell model. *Antimicrob Agents Chemother*. 2009;53(4):1457–1462. http://dx.doi.org/10.1128/AAC.01253-08
21. Brillault J, De Castro WV, Couet W. Relative contributions of active mediated transport and passive diffusion of fluoroquinolones with various lipophilicities in a calu-3 lung epithelial cell model. *Antimicrob Agents Chemother*. 2010;54(1):543–545. http://dx.doi.org/10.1128/AAC.00733-09
22. Griffiths NM, Hirst BH, Simmons NL. Active secretion of the fluoroquinolone ciprofloxacin by human intestinal epithelial Caco-2 cell layers. *Br J Pharmacol*. 1993;108(3):575–576. http://dx.doi.org/10.1111/j.1476-5381.1993.tb12844.x
23. Rodríguez-Ibáñez M, Nalda-Molina R, Montalar-Montero M, Bermejo MV, Merino V, Garrigues TM. Transintestinal secretion of ciprofloxacin, grepafloxacin and sparfloxacin: *in vitro* and *in situ* inhibition studies. *Eur J Pharm Biopharm*. 2003;55(2):241–246. http://dx.doi.org/10.1016/S0939-6411(02)00185-6
24. Ando T, Kusuhara H, Merino G, Alvarez AI, Schinkel AH, Sugiyama Y. Involvement of breast cancer resistance protein (*ABCG2*) in the biliary excretion mechanism of fluoroquinolones. *Drug Metab Dispos*. 2007;35(10):1873–1879. http://dx.doi.org/10.1124/dmd.107.014969
25. Haslam IS, Wright JA, O'Reilly DA, Sherlock DJ, Coleman T, Simmons NL. Intestinal ciprofloxacin efflux: the role of breast cancer resistance protein (ABCG2). *Drug Metab Dispos*. 2011;39(12):2321–2328. http://dx.doi.org/10.1124/dmd.111.038323
26. Dessilly G, Elens L, Panin N, et al. ABCB1 1199G>A genetic polymorphism (Rs2229109) influences the intracellular accumulation of tacrolimus in HEK293 and K562 recombinant cell lines. *PLoS One*. 2014;9(3):e91555. http://dx.doi.org/10.1371/journal.pone.0091555
27. Wolking S, Schaeffeler E, Lerche H, Schwab M, Nies AT. Impact of genetic polymorphisms of ABCB1 (MDR1, P-Glycoprotein) on drug disposition and potential clinical implications: update of the literature. *Clin Pharmacokinet*. 2015;54(7):709–735. http://dx.doi.org/10.1007/s40262-015-0267-1
28. Hira D, Terada T. BCRP/ABCG2 and high-alert medications: biochemical, pharmacokinetic, pharmacogenetic, and clinical implications. *Biochem Pharmacol*. 2018;147:201–210. http://dx.doi.org/10.1016/j.bcp.2017.10.004
29. Homolya L. Medically important alterations in transport function and trafficking of ABCG2. *Int J Mol Sci*. 2021;22(6):2786. http://dx.doi.org/10.3390/ijms22062786
30. Li J, Bauer M, Moe B, Leslie EM, Li X-F. Multidrug resistance protein 4 (MRP4/ABCC4) protects cells from the toxic effects of halobenzoquinones. *Chem Res Toxicol*. 2017;30(10):1815–1822. http://dx.doi.org/10.1021/acs.chemrestox.7b00156
31. Dessilly G, Panin N, Elens L, Haufroid V, Demoulin J-B. Impact of ABCB1 1236C > T-2677G > T-3435C > T polymorphisms on the anti-proliferative activity of imatinib, nilotinib, dasatinib and ponatinib. *Sci Rep*. 2016b;6:29559. http://dx.doi.org/10.1038/srep29559
32. Hoste E, Deldicque L, Muccioli GG, et al. Silencing drug transporters in human primary muscle cells modulates atorvastatin pharmacokinetics: a pilot study. *Br J Pharmacol*. Published online Feb 5, 2025. http://dx.doi.org/10.1111/bph.17449
33. Mahieu G, Sennesael A-L, Pochet L, et al. *In vitro* assessment of the risk of ABCB1-mediated drug-drug interaction between rivaroxaban and tacrolimus in human embryonic kidney 293 recombinant cell lines. *Res Pract Thromb Haemost*. 2024;8(5):102521. http://dx.doi.org/10.1016/j.rpth.2024.102521
34. Stillemans G, Djokoto HP, Delongie K-A, et al. Effect of four ABCB1 genetic polymorphisms on the accumulation of darunavir in HEK293 recombinant cell lines. *Sci Rep*. 2021;11(1):9000. http://dx.doi.org/10.1038/s41598-021-88365-7
35. Dantzig A, Law K, Cao J, Starling J. Reversal of multidrug resistance by the P-Glycoprotein modulator, LY335979, from the bench to the clinic. *Curr Med Chem*. 2001;8(1):39–50. http://dx.doi.org/10.2174/0929867013373903
36. Alam A, Kowal J, Broude E, Roninson I, Locher KP. Structural insight into substrate and inhibitor discrimination by human P-glycoprotein. *Science*. 2019;363(6428):753–756. http://dx.doi.org/10.1126/science.aav7102
37. Dash RP, Jayachandra Babu R, Srinivas NR. Therapeutic potential and utility of elacridar with respect to P-glycoprotein inhibition: an insight from the published *in vitro*, preclinical and clinical studies. *Eur J Drug Metab Pharmacokinet*. 2017;42(6):915–933. http://dx.doi.org/10.1007/s13318-017-0411-4
38. Robey RW, Steadman K, Polgar O, et al. Pheophorbide a is a specific probe for ABCG2 function and inhibition. *Cancer Res*. 2004;64(4):1242–1246. http://dx.doi.org/10.1158/0008-5472.CAN-03-3298
39. Lettieri JT, Rogge MC, Kaiser L, Echols RM, Heller AH. Pharmacokinetic profiles of ciprofloxacin after single intravenous and oral doses. *Antimicrob Agents Chemother*. 1992;36(5):993–996. http://dx.doi.org/10.1128/AAC.36.5.993
40. Stass H, Kubitza D. Pharmacokinetics and elimination of moxifloxacin after oral and intravenous administration in man. *J Antimicrob Chemother*. 1999;43(suppl_2):83–90. http://dx.doi.org/10.1093/jac/43.suppl_2.83
41. Tivnan A, Zakaria Z, O'Leary C, et al. Inhibition of multidrug resistance protein 1 (MRP1) improves chemotherapy drug response in primary and recurrent glioblastoma multiforme. *Front Neurosci*. 2015;9:218. http://dx.doi.org/10.3389/fnins.2015.00218
42. Kallem R, Kulkarni CP, Patel D, et al. A simplified protocol employing elacridar in rodents: A screening model in drug discovery to assess P-gp mediated efflux at the blood brain barrier. *Drug Metab Lett*. 2012;6(2):134–144.
43. Badagnani I. Abstracts from the 10th European regional ISSX meeting. *Drug Metab Rev*. 2008;40(sup1):1–172. http://dx.doi.org/10.1080/03602530801949332

44. Zattoni IF, Delabio LC, Dutra JP, et al. Targeting breast cancer resistance protein (BCRP/ABCG2): functional inhibitors and expression modulators. *Eur J Med Chem*. 2022;237:114346. http://dx.doi.org/10.1016/j.ejmech.2022.114346
45. Lowes S, Simmons NL. Multiple pathways for fluoroquinolone secretion by human intestinal epithelial (Caco-2) cells. *Br J Pharmacol*. 2002;135(5):1263−1275. http://dx.doi.org/10.1038/sj.bjp.0704560
46. Banerjee M, Carew MW, Roggenbeck BA, et al. A novel pathway for arsenic elimination: human multidrug resistance protein 4 (MRP4/ABCC4) mediates cellular export of dimethylarsinic acid (DMAV) and the diglutathione conjugate of monomethylarsonous acid (MMAIII). *Mol Pharmacol*. 2014;86(2):168−179. http://dx.doi.org/10.1124/mol.113.091314
47. Chufan EE, Sim H-M, Ambudkar SV. Molecular basis of the polyspecificity of P-glycoprotein (ABCB1): recent biochemical and structural studies. *Adv Cancer Res*. 2015;125:71−96. http://dx.doi.org/10.1016/bs.acr.2014.10.003
48. Michot J-M, Heremans MF, Caceres NE, Mingeot-Leclercq M-P, Tulkens PM, Van Bambeke F. Cellular accumulation and activity of quinolones in ciprofloxacin-resistant J774 macrophages. *Antimicrob Agents Chemother*. 2006;50(5):1689−1695. http://dx.doi.org/10.1128/AAC.50.5.1689-1695.2006
49. Sennesael A-L, Panin N, Vancraeynest C, et al. Effect of ABCB1 genetic polymorphisms on the transport of rivaroxaban in HEK293 recombinant cell lines. *Sci Rep*. 2018;8(1):10514. http://dx.doi.org/10.1038/s41598-018-28622-4
50. De Greef J, Akue M, Panin N, et al. Effect of ABCB1 most frequent polymorphisms on the accumulation of bictegravir in recombinant HEK293 cell lines. *Sci Rep*. 2024;14(1):16290. http://dx.doi.org/10.1038/s41598-024-66809-0
51. Dessilly G, Elens L, Panin N, Karmani L, Demoulin J-B, Haufroid V. *ABCB1* 1199G>A polymorphism (rs2229109) affects the transport of imatinib, nilotinib and dasatinib. *Pharmacogenomics*. 2016a;17(8):883−890. http://dx.doi.org/10.2217/pgs-2016-0012
52. Ferreira RJ, Ferreira M-JU, Dos Santos DJVA. Molecular docking characterizes substrate-binding sites and efflux modulation mechanisms within P-glycoprotein. *J Chem Inf Model*. 2013;53(7):1747−1760. http://dx.doi.org/10.1021/ci400195v
53. Chufan EE, Kapoor K, Sim H-M, et al. Multiple transport-active binding sites are available for a single substrate on human P-glycoprotein (ABCB1). *PLoS One*. 2013;8(12):e82463. http://dx.doi.org/10.1371/journal.pone.0082463
54. Sun L-N, Sun G-X, Yang Y-Q, et al. Effects of *ABCB1*, *UGT1A1*, and *UGT1A9* genetic polymorphisms on the pharmacokinetics of sitafloxacin granules in healthy subjects. *Clin Pharmacol Drug Dev*. 2021;10(1):57−67. http://dx.doi.org/10.1002/cpdd.848
55. Kimchi-Sarfaty C, Oh JM, Kim I-W, et al. A “silent” polymorphism in the MDR1 gene changes substrate specificity. *Science*. 2007;315(5811):525−528. http://dx.doi.org/10.1126/science.1135308
56. Naidoo A, Ramsuran V, Chirehwa M, et al. Effect of genetic variation in UGT1A and ABCB1 on moxifloxacin pharmacokinetics in South African patients with tuberculosis. *Pharmacogenomics*. 2018;19(1):17−29. http://dx.doi.org/10.2217/pgs-2017-0144
57. Schaeffeler E, Eichelbaum M, Brinkmann U, et al. Frequency of C3435T polymorphism of MDR1 gene in African people. *Lancet*. 2001;358(9279):383−384. http://dx.doi.org/10.1016/S0140-6736(01)05579-9
58. Lismond A, Tulkens PM, Mingeot-Leclercq M-P, Courvalin P, Van Bambeke F. Cooperation between prokaryotic (Lde) and eukaryotic (MRP) efflux transporters in J774 macrophages infected with Listeria monocytogenes: studies with ciprofloxacin and moxifloxacin. *Antimicrob Agents Chemother*. 2008;52(9):3040−3046. http://dx.doi.org/10.1128/AAC.00105-08
59. PharmGKB. The Pharmacogenomics Knowledgebase. n.d.. Accessed January 3, 2025. https://www.pharmgkb.org/

Supplemental Data

Table S1: Frequency of the SNPs investigated in different ethnic groups.

		Frequency (%)*			
		European	South Asian	African	East Asian
ABCB1	1199G>A	3.28	0.92	0.30	0.00
	1236C>T	41.55	58.69	13.62	62.70
	2677G>T/A	40.95/1.79	59.20/5.01	1.97/0.08	39.78/13.39
	3435C>T	51.79	57.46	14.98	39.78
ABCG2	421C>A	9.44	9.71	1.29	29.07

*Allelic frequencies are from the 1000 Genomes projects found in http://www.pharmgkb.org/search/.

Table S2: Summary of the main inhibitor effects on ciprofloxacin and moxifloxacin cellular concentration.

Transporter involved	Polymorphisms	Substrate (Concentration)	Inhibitors (Concentration)	Key observations (vs. CTL)
ABCB1	1199G (WT)	Moxifloxacin (9.13 µM)	Zosuquidar (0.2 µM)	Increase cellular concentration (Significant effect)
			Elacridar (5 µM)	Increase cellular concentration (Significant effect)
			Ko143 (1 µM)	No significant effect
			MK571 (25 µM)	No significant effect
		Ciprofloxacin (10.36 µM)	Zosuquidar (0.2 µM)	Increase cellular concentration (Significant effect)
			Elacridar (5 µM)	Increase cellular concentration (Significant effect)
			Ko143 (1 µM)	No significant effect
			MK571 (25 µM)	No significant effect
	1199A (Variant)	Moxifloxacin (9.13 µM)	Zosuquidar (0.2 µM)	Increase cellular concentration (Significant effect)
			Elacridar (5 µM)	Increase cellular concentration (Significant effect)
			Ko143 (1 µM)	No significant effect
			MK571 (25 µM)	No significant effect
		Ciprofloxacin (10.36 µM)	Zosuquidar (0.2 µM)	Increase cellular concentration (Significant effect)
			Elacridar (5 µM)	Increase cellular concentration (Significant effect)
			Ko143 (1 µM)	No significant effect
			MK571 (25 µM)	No significant effect
	Haplotype CGC 1236C ; 2677G ; 3435C (WT)	Moxifloxacin (9.13 µM)	Zosuquidar (0.2 µM)	Increase cellular concentration (No significant effect)
			Elacridar (5 µM)	Increase cellular concentration (Significant effect)
			Ko143 (1 µM)	No significant effect
			MK571 (25 µM)	No significant effect
		Ciprofloxacin (10.36 µM)	Zosuquidar (0.2 µM)	Increase cellular concentration (No significant effect)
			Elacridar (5 µM)	Increase cellular concentration (Significant effect)
			Ko143 (1 µM)	No significant effect

			MK571 (25 µM)	No significant effect
	Haplotype CGT 1236C ; 2677G 3435T (Variant)	Moxifloxacin (9.13 µM)	Zosuquidar (0.2 µM) Elacridar (5 µM) Ko143 (1 µM) MK571 (25 µM)	No significant effect No significant effect No significant effect No significant effect
		Ciprofloxacin (10.36 µM)	Zosuquidar (0.2 µM) Elacridar (5 µM) Ko143 (1 µM) MK571 (25 µM)	Increase cellular concentration (No significant effect) Increase cellular concentration (Significant effect) No significant effect No significant effect
	Haplotype TTT 1236T ; 2677T 3435T (Variant)	Moxifloxacin (9.13 µM)	Zosuquidar (0.2 µM) Elacridar (5 µM) Ko143 (1 µM) MK571 (25 µM)	No significant effect No significant effect No significant effect No significant effect
		Ciprofloxacin (10.36 µM)	Zosuquidar (0.2 µM) Elacridar (5 µM) Ko143 (1 µM) MK571 (25 µM)	No significant effect No significant effect No significant effect No significant effect
ABCG2	421C (WT)	Moxifloxacin (9.13 µM)	Zosuquidar (0.2 µM) Elacridar (5 µM) Ko143 (1 µM) MK571 (25 µM)	No significant effect No significant effect No significant effect No significant effect
		Ciprofloxacin (10.36 µM)	Zosuquidar (0.2 µM) Elacridar (5 µM) Ko143 (1 µM) MK571 (25 µM)	No significant effect No significant effect No significant effect No significant effect
	421A (Variant)	Moxifloxacin (9.13 µM)	Zosuquidar (0.2 µM) Elacridar (5 µM) Ko143 (1 µM) MK571 (25 µM)	No significant effect No significant effect No significant effect No significant effect
		Ciprofloxacin (10.36 µM)	Zosuquidar (0.2 µM) Elacridar (5 µM) Ko143 (1 µM) MK571 (25 µM)	No significant effect No significant effect No significant effect No significant effect
ABCC4	WT	Moxifloxacin (9.13 µM)	Zosuquidar (0.2 µM) Elacridar (5 µM) Ko143 (1 µM) MK571 (25 µM) MK571 (50 µM)	No significant effect No significant effect No significant effect No significant effect No significant effect
		Ciprofloxacin (10.36 µM)	Zosuquidar (0.2 µM) Elacridar (5 µM) Ko143 (1 µM) MK571 (10 µM) MK571 (25 µM) MK571 (50 µM)	No significant effect No significant effect No significant effect No significant effect Increase cellular concentration (Significant effect) Increase cellular concentration (Significant effect)

Figure S1. ABCB1 expression analysis by western blot. Cells transfected with empty plasmid ($HEK_{control}$ [CTL]) and cells transfected with vector cDNA encoding the ABCB1 transporter ($HEK_{wild\ type}$ [1199G], $HEK_{variant}$ [1199A]). Dilutions (from the left to the right): 12, 6, 3, and 1.5 µg of proteins

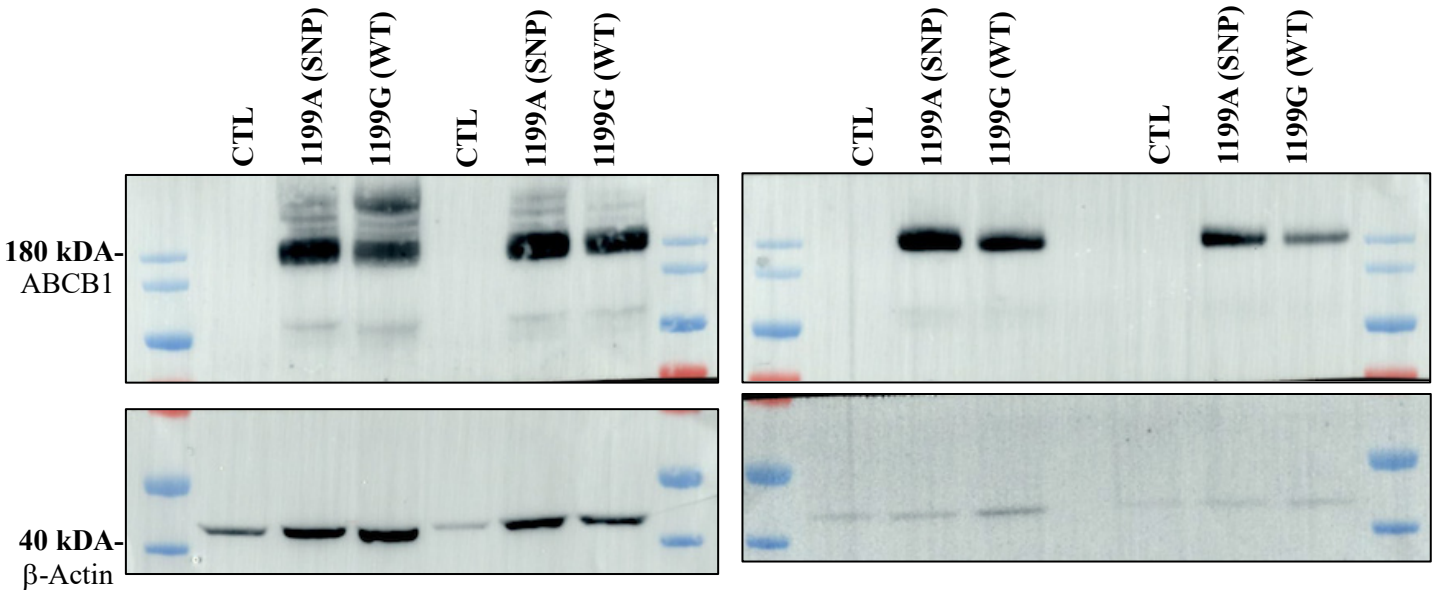


Figure S2. ABCC4 expression analysis by western blot. Cells transfected with empty plasmid ($HEK_{control}$ [CTL]) and cells transfected with vector cDNA encoding the ABCC4 transporter (HEK_{ABCC4} [ABCC4]).

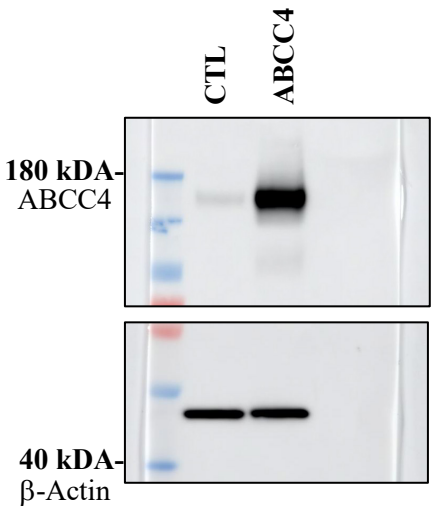


Figure S3. ABCG2 expression analysis by western blot. Cells transfected with empty plasmid ($HEK_{control}$ [CTL]) and cells transfected with vector cDNA encoding the ABCG2 transporter ($HEK_{variant}$ [421A], $HEK_{wild\ type}$ [421C]).

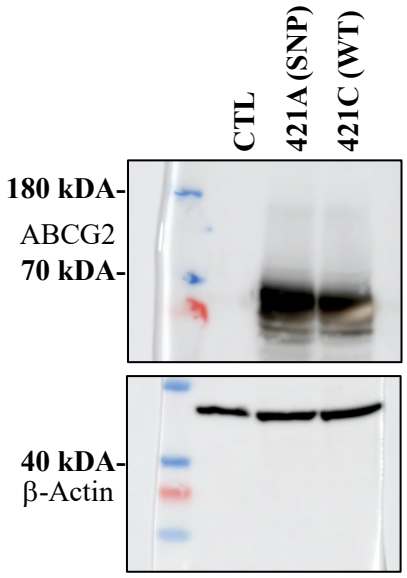
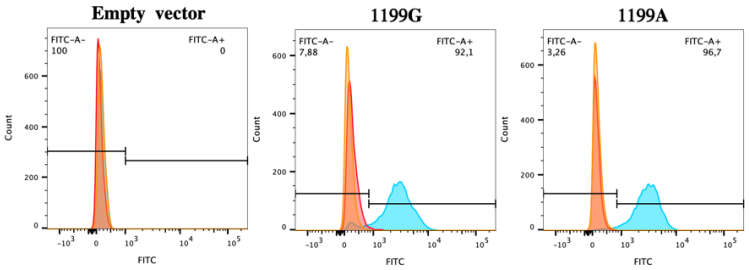
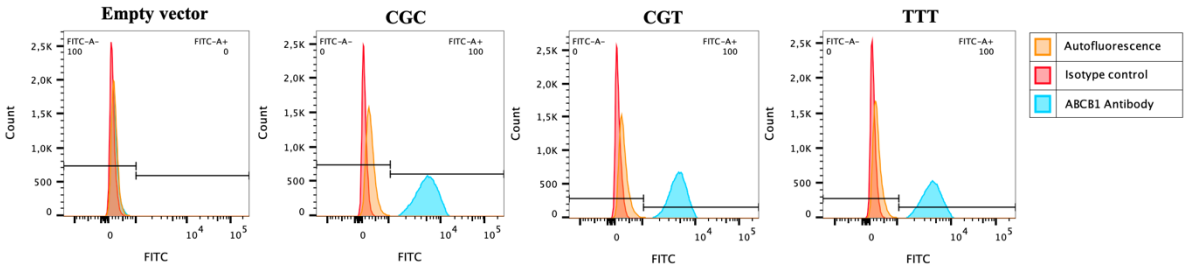


Figure S4. (A, B, C) ABCB1 and ABCG2 cell surface expression assessed by flow cytometry of HEK293 transfected cells stained with ABCB1 antibody, isotype control and no staining (autofluorescence). (A) Empty pcDNA3.1 vector, $ABCB1_{1199G}$, $ABCB1_{1199A}$. **(B)** Empty pcDNA3.1 vector, $ABCB1_{CGC}$, $ABCB1_{CGT}$, $ABCB1_{TTT}$. FITC, Fluorescein isothiocyanate. **(C)** Empty pcMV3 vector, $ABCG2_{421C}$, $ABCG2_{421A}$, PerCP (Peridinin chlorophyll)-Cy5-5-a (cyanine).

(A)



(B)



(C)

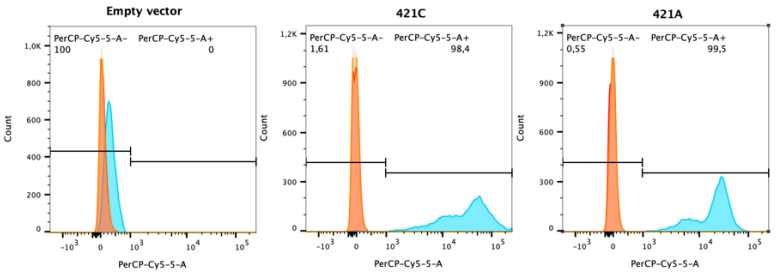


Figure S5. ABCC2 transporter RNA transcripts. ABCC2 transporter RNA expression levels normalized to the *18S* expression level (housekeeping gene) in HEKcontrol (CTL), HEK1199G>A (1199G, 1199A) and HEK1236C>T-2677G>T-3435C>T (CGC, CGT, TTT) cell lines; HEKcontrol (CTL) and HEK421C>A (421C, 421A) cell lines; HEKcontrol (CTL) and HEKABCC4 (ABCC4) cell lines.