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Functional Defect of Variants in the Adenosine Triphosphate–Binding Sites of ABCB4 and Their Rescue by the Cystic Fibrosis Transmembrane Conductance Regulator Potentiator, Ivacaftor (VX-770)

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ABCB4 (MDR3) is an adenosine triphosphate (ATP)-binding cassette (ABC) transporter expressed at the canalicular membrane of hepatocytes, where it mediates phosphatidylcholine (PC) secretion. Variations in the *ABCB4* gene are responsible for several biliary diseases, including progressive familial intrahepatic cholestasis type 3 (PFIC3), a rare disease that can be lethal in the absence of liver transplantation. In this study, we investigated the effect and potential rescue of *ABCB4* missense variations that reside in the highly conserved motifs of ABC transporters, involved in ATP binding. Five disease-causing variations in these motifs have been identified in *ABCB4* (G535D, G536R, S1076C, S1176L, and G1178S), three of which are homologous to the gating mutations of cystic fibrosis transmembrane conductance regulator (CFTR or ABCC7; i.e., G551D, S1251N, and G1349D), that were previously shown to be function defective and corrected by ivacaftor (VX-770; Kalydeco), a clinically approved CFTR potentiator. Three-dimensional structural modeling predicted that all five *ABCB4* variants would disrupt critical interactions in the binding of ATP and thereby impair ATP-induced nucleotide-binding domain dimerization and ABCB4 function. This prediction was confirmed by expression in cell models, which showed that the ABCB4 mutants were normally processed and targeted to the plasma membrane, whereas their PC secretion activity was dramatically decreased. As also hypothesized on the basis of molecular modeling, PC secretion activity of the mutants was rescued by the CFTR potentiator, ivacaftor (VX-770). *Conclusion*: Disease-causing variations in the ATP-binding sites of ABCB4 cause defects in PC secretion, which can be rescued by ivacaftor. These results provide the first experimental evidence that ivacaftor is a potential therapy for selected patients who harbor mutations in the ATP-binding sites of ABCB4. (HEPATOLOGY 2017;65:560-570)

ABCB4, also called MDR3 (multidrug resistance protein 3), is a phospholipid floppase almost exclusively expressed at the canalicular membrane of hepatocytes where its function is to translocate phosphatidylcholine (PC) into bile.[1,2] Variations in the *ABCB4* gene sequence cause several chronic and progressive liver diseases.[3] The most severe is progressive familial intrahepatic cholestasis type 3 (PFIC3), which develops early in childhood and most often requires liver transplantation (LT). Less-severe disorders are intrahepatic cholestasis of pregnancy (ICP) and low-phospholipid–associated

Abbreviations: 3D, three-dimensional; ABC, ATP-binding cassette; ATP, adenosine triphosphate; BSA, bovine serum albumin; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; HEK, human embryonic kidney; ICP, intrahepatic cholestasis of pregnancy; IgG, immunoglobulin G; LPAC, low-phospholipid–associated cholelithiasis; LT, liver transplantation; MDR3, multidrug resistance protein 3; MRP2, multidrug resistance-associated protein 2; MSD, membrane-spanning domain; NaTC, sodium taurocholate; NBD, nucleotide-binding domain; PC, phosphatidylcholine; PFIC3, progressive familial intrahepatic cholestasis type 3; UCDA, ursodeoxycholic acid; wt, wild type.

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cholelithiasis (LPAC) syndrome, which occur in young adults. Today, approximately 300 distinct disease-causing *ABCB4* variants have been reported, typically with homozygous status in PFIC3 and with heterozygous status in LPAC syndrome and ICP.[3-10] A major challenge is to find pharmacological treatments for the severe forms of these diseases. It has been reported that molecules designed to rescue trafficking defective CFTR (cystic fibrosis transmembrane conductance regulator) mutants can also be effective toward other adenosine triphosphate (ATP)-binding cassette (ABC) transporters.[11] For ABCB4, we have shown that variations, which impair ABCB4 folding in the endoplasmic reticulum, lead to premature degradation and could be rescued *in vitro* by treatments with cyclosporin A or C.[12,13] Two other chemical chaperones, 4-phenylbutyric acid and curcumin, have been recently proposed to rescue ABCB4 variants that impaired traffic.[14] However, the majority of variations affect PC secretion activity of ABCB4,[13] and no pharmacological treatment has been proposed for these mutations yet.

ABCB4 belongs to the superfamily of ABC transporters, which are characterized by two membrane-spanning domains (MSDs) involved in substrate specificity and secretion, and two nucleotide-binding domains (NBDs) that bind and hydrolyze ATP to provide the energy required for the transport. The NBDs are well conserved throughout the family and contain specific motifs involved in ATP binding, such as the Walker A and Walker B motifs, the A-, D-, H-, and Q-loops, and the ABC signature, LSGGQ (Leu-Ser-Gly-Gly-Gln), which is unique to the family.[15] Five point variations located in the ATP-binding sites of ABCB4 (G535D, G536R, S1076C, S1176L, and G1178S) have been identified in patients with PFIC3 (S1076C),[16] LPAC syndrome (G536R, S1176L, and G1178S; this study), or ICP (G535D, G536R).[17,18] ATP-binding sites of ABCB4 have a strong homology with those of the chloride channel, CFTR (or ABCC7), another ABC transporter, which is deficient in cystic fibrosis (CF). The glycines, 536 and 1178, in the LSGGQ signature of ABCB4 and serine 1076 in the Walker A motif of ABCB4 are homologous to the glycines, 551 and 1349, and serine 1251 of CFTR, respectively. Variations of G551, G1349, and S1251 in CF patients do not affect CFTR expression at the plasma membrane, but lead to defective gating and a complete absence of chloride secretion.[19] G551D is the third-most common CF mutant and the first to be treated with a clinically approved CFTR potentiator, ivacaftor (also known as VX-770 or Kalydeco; Vertex pharmaceuticals, Boston, MA).[20-23] The approval of ivacaftor (VX-770) was extented to eight additional gating mutations of CFTR, including S1251N and G1349D.[19]

The aim of this work was to study whether the five disease-causing variations in the ATP-binding sites of

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ABCB4 also impaired ABCB4 function and whether they could be rescued by ivacaftor (VX-770).

Patients and Methods

PATIENTS' DATA ANALYSES

ABCB4 gene analysis was performed in patients referred to the Reference Center for Inflammatory Biliary Diseases (Hôpital Saint-Antoine, Paris, France) as reported.[24] Clinical phenotypes of patients were classified according to current spectrum of liver diseases related to *ABCB4* gene variations.

MOLECULAR MODELING

In order to get an accurate three-dimensional (3D) structure model of the ABCB4 NBD1/NBD2 assembly, we used as a template the experimental 3D structure of the MJ0796 NBD1/NBD2 heterodimer,[25] pdb 1l2t, as described for the modeling of the CFTR/ABCC7 NBD1/NBD2 assembly.[26] This experimental 3D structure of a hydrolytically inactive NBD dimer was solved at high resolution (1.9 Å) in the presence of ATP, thus allowing an accurate analysis of the ATP-binding sites. The model was constructed using Modeller (v9.14).[27]

ANTIBODIES AND REAGENTS

Mouse monoclonal anti-ABCB4 (P3-II-26) and anti-MRP2 (multidrug resistance-associated protein 2; M2-I-4) antibodies were purchased from Alexis Biochemicals (San Diego, CA), and the goat polyclonal anti-actin antibody was from Santa Cruz Biotechnology (Heidelberg, Germany). Goat anti-immunoglobulin G (IgG) 2b Alexa Fluor 488 and goat anti- IgG1 Alexa Fluor 594 secondary antibodies, peroxidase-conjugated secondary antibodies, and culture media were from Invitrogen-Life Technologies (Saint-Aubin, France). Ivacaftor (VX-770) was from Clinisciences (Nanterre, France). Sodium taurocholate (NaTC), ursodeoxycholic acid (UDCA), fatty-acid–free bovine serum albumin (BSA), and calf serum were from Sigma-Aldrich (Lyon, France).

DNA CONSTRUCTS AND MUTAGENESIS

The construction of the human wild-type (wt) *ABCB4*, isoform A (NM_000443.3), in the pcDNA3 vector was reported.[13] Site-directed mutagenesis was performed using the Quik-Change II XL mutagenesis kit from Agilent Technologies (Massy, France). DNA primers used for *ABCB4* mutagenesis were from Invitrogen-Life Technologies and are listed in Supporting Table S1. All constructs were verified by automated sequencing.

CELL CULTURE, TRANSFECTION, AND IMMUNOANALYSIS

ABCB4-wt, ABCB4-G535D, ABCB4-G536R, ABCB4-S1076C, ABCB4-S1176L, and ABCB4-G1178S encoding vectors were transiently transfected in HepG2 and human embryonic kidney (HEK) 293 cells as published.[24,28] Of note is that, in these conditions, HepG2 cells form canaliculi and display virtually no endogenous ABCB4 expression. Immunofluorescence and immunoblotting analyses were performed as described.[28]

IVACAFTOR (VX-770) TREATMENT AND PC SECRETION ANALYSIS

HEK293 cells were seeded on polylysine-precoated six-well plates at a density of 1.3×10^6 cells/well. Six hours after seeding, cells were transiently transfected with 1 μg of ABCB4-encoding plasmids using Turbofect (Thermo-Fisher Scientific, Villebon-sur-Yvette, France), following the manufacturer's instructions. Twenty-four hours posttransfection, cells were washed twice with Hanks' balance salt solution, and then the medium was replaced by phenol red-free Dulbecco's modified Eagle's medium containing 0.5 mmol/L of NaTC and 0.02% fatty-acid–free BSA in the presence or absence of 10 μmol/L of ivacaftor, 50 μM/L of UDCA, and 10 μmol/L of ivacaftor plus 50 μM/L of UDCA. Media were collected after 24 hours. Measurement of PC content in collected media was performed as described.[24]

STATISTICAL ANALYSIS

Data are shown as means ± SD. Statistical significance among means was determined using the Student *t* test. A P value < 0.05 was considered significant.

TABLE 1. Characteristics of Patients With *ABCB4* Variations

Patients (No./Sex)	Onset	Nucleotide Variant	Amino-Acid Variant	Domain	Homologous *CFTR* Variant	*ABCB4* Allelic Status	Diagnosis	Reference
1/F	Adolescent	c. 1605G>A	G535D	S	—	HTZ	Cholelithiasis; ICP Biliary cirrhosis	(17)
2/F	Young adult	c. 1606 G>A	G536R	S	G551D	HTZ	ICP	(18)
3/F	Young adult	c. 1606 G>A	G536R	S	G551D	HTZ	LPAC	This study
4/M	Child	c.3228 A>T	S1076C	WA	S1251N	HMZ	PFIC3	(16)
5/M	Young adult	c.3527 C>T	S1176L	S	—	HTZ	LPAC	This study
6/F	Young adult	c.3532 G>A	G1178S	S	G1349D	HTZ	LPAC	This study

Nucleotide variant corresponds to the complementary DNA of the NM_000443.3 (*ABCB4*, transcript variant A, messenger RNA).
Abbreviations: F, female; M, male; S, LSGGQ signature; WA, Walker A; HTZ, heterozygous; HMZ, homozygous.

Results

ABCB4 VARIATIONS AND PATIENTS

The main characteristics of the patients are shown in Table 1. The S1076C variation was identified with a homozygous status in a PFIC3 patient.[16] The G535D was described with a heterozygous status in a patient who developed cholelithiasis, ICP, and biliary cirrhosis.[17] The G536R was described with a heterozygous status in a patient with ICP.[18] G536R was also identified, as well as S1176L and G1178S, all with heterozygous status in patients with LPAC syndrome (this study).

HOMOLOGY OF DISEASE-CAUSING VARIATIONS IN THE ATP-BINDING SITES OF ABCB4 AND CFTR/ABCC7

Distribution of the five disease-causing variations of interest in this study is shown in Fig. 1 (left panel).

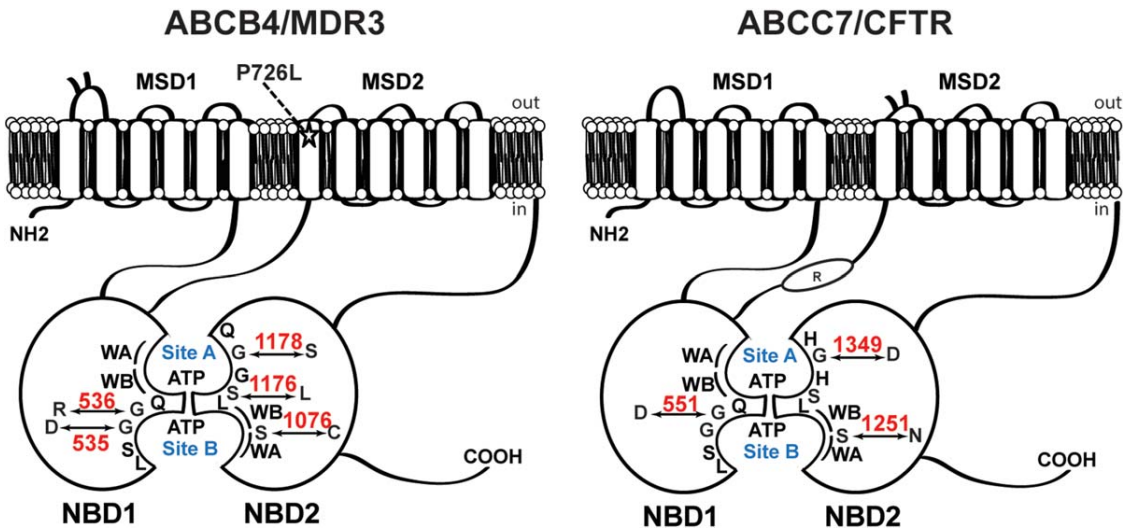

FIG. 1. Organization of ATP-binding sites in ABCB4/MDR3 and ABCC7/CFTR. Both proteins are composed of two MSDs (MSD1 and MSD2) and two NBDs (NBD1 and NBD2). The two ATP-binding pockets (sites A and B) formed by the ABC signature (LSGGQ) and Walker A (WA) and Walker B (WB) motifs are represented. In ABCC7/CFTR, ABC signature of site A is degenerated (LSHGH). The five disease-causing variations of ABCB4 investigated here are indicated in red. G536, in the signature of the NBD1 of ABCB4, is homologous to G551 (in red) in the signature of the NBD1 of CFTR; S1076 in the Walker A of NBD2 of ABCB4 is homologous to S1251 (in red) in the Walker A of NBD2 of CFTR; and G1178 in the signature of the NBD2 of ABCB4 is homologous to G1349 (in red) in the signature of the NBD2 of CFTR. The P726L, located in the transmembrane domain of ABCB4 is used as a control variant in this study.

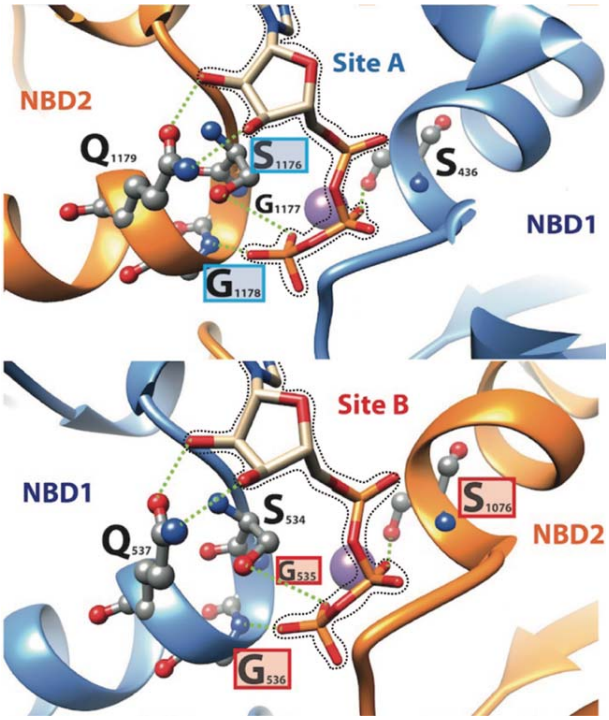

FIG. 2. 3D structure modeling of the two ATP-binding sites (sites A [up] and B [bottom]) of human ABCB4, in which the mutations studied here are boxed on a colored background. Interaction between the ATP molecules (dashed black line) and the different residues are indicated by hatched green lines.

They affect positions that all belong to the two ATP-binding sites of ABCB4 (site A and site B), formed at the interface of the head-to-tail NBD1/NBD2 heterodimer. Site A is formed by the NBD1 Walker A and Walker B motifs and the NBD2 LSGGQ signature, whereas site B is formed by the NBD2 Walker A and Walker B motifs and the NBD1 signature. Three of these ABCB4 variations (G536R, S1076C, and G1178S; Fig. 1, left panel) are homologous to the gating mutations of CFTR/ABCC7 (G551D, S1251N, and G1349D), respectively (Fig. 1 [right panel] and Supporting Fig. S1).

MOLECULAR MODELING OF ABCB4 MUTANTS

3D structure modeling showed that G536 and G1178 of ABCB4 were located at equivalent positions of the ABC signature sequences of NBD1 and NBD2, respectively (LSGGQ motif, in which the mutated glycine is underlined). In this position, the nitrogen atom is hydrogen bonded to one of the oxygen atoms of the ATP γ-phosphate (Fig. 2 and Supporting Fig. S2). The lack of a side chain exposes the protein backbone so that the ATP γ-phosphate can form this hydrogen bond with the backbone amide. At this position, there is not enough room to accommodate a long side chain, such as that of arginine. Missense mutations would thus lead to steric hindrance. Steric hindrance is also predicted for mutation of G535 in site B, for which the backbone nitrogen atom is in Van der Waals contact with the ATP γ-phosphate (Fig. 2 and Supporting Fig. S2). In this case also, there is no room to accommodate a larger side chain. In site B, the oxygen atom of the S1076 side chain (Walker A motif) of NBD2 is hydrogen bonded to the ATP β-phosphate (Fig. 2 and Supporting Fig. S2). Furthermore, in site A, the oxygen atom of the S1176 side chain in the NBD2 ABC signature is also hydrogen bonded to the ATP γ-phosphate. Substitution of both these serine residues would lead to disruption of these critical contacts with ATP (Fig. 2 and Supporting Fig. S2). In summary, structure modeling indicated that the five mutants tested in this study were likely to disrupt critical interactions for ATP binding and thereby to interrupt ATP-induced NBD dimerization from which one would expect a defect in ABCB4 function.

MATURATION AND CANALICULAR LOCALIZATION OF ABCB4 MUTANTS

To determine their functional impact, the five point mutations were introduced into the *ABCB4* complementary DNA by site-directed mutagenesis. Expression of the mutants was compared to that of ABCB4-wt after transient transfection in polarized HepG2 hepatoma cells. These cells form neo-bile canaliculi in culture and allow localization studies. Forty-eight hours after transfection, cells were fixed, stained, and analyzed by confocal microscopy to determine whether the mutants reached the canalicular membrane. Figure 3A shows that ABCB4-wt was exclusively detected at the canalicular membrane of HepG2 cells as shown[13,24,28] and colocalized with endogenously expressed MRP2, used as a canalicular marker.[29] Like ABCB4-wt, all the mutants displayed canalicular localization and colocalized with MRP2 (Fig. 3A). The electrophoretic patterns obtained by western blotting analysis of the

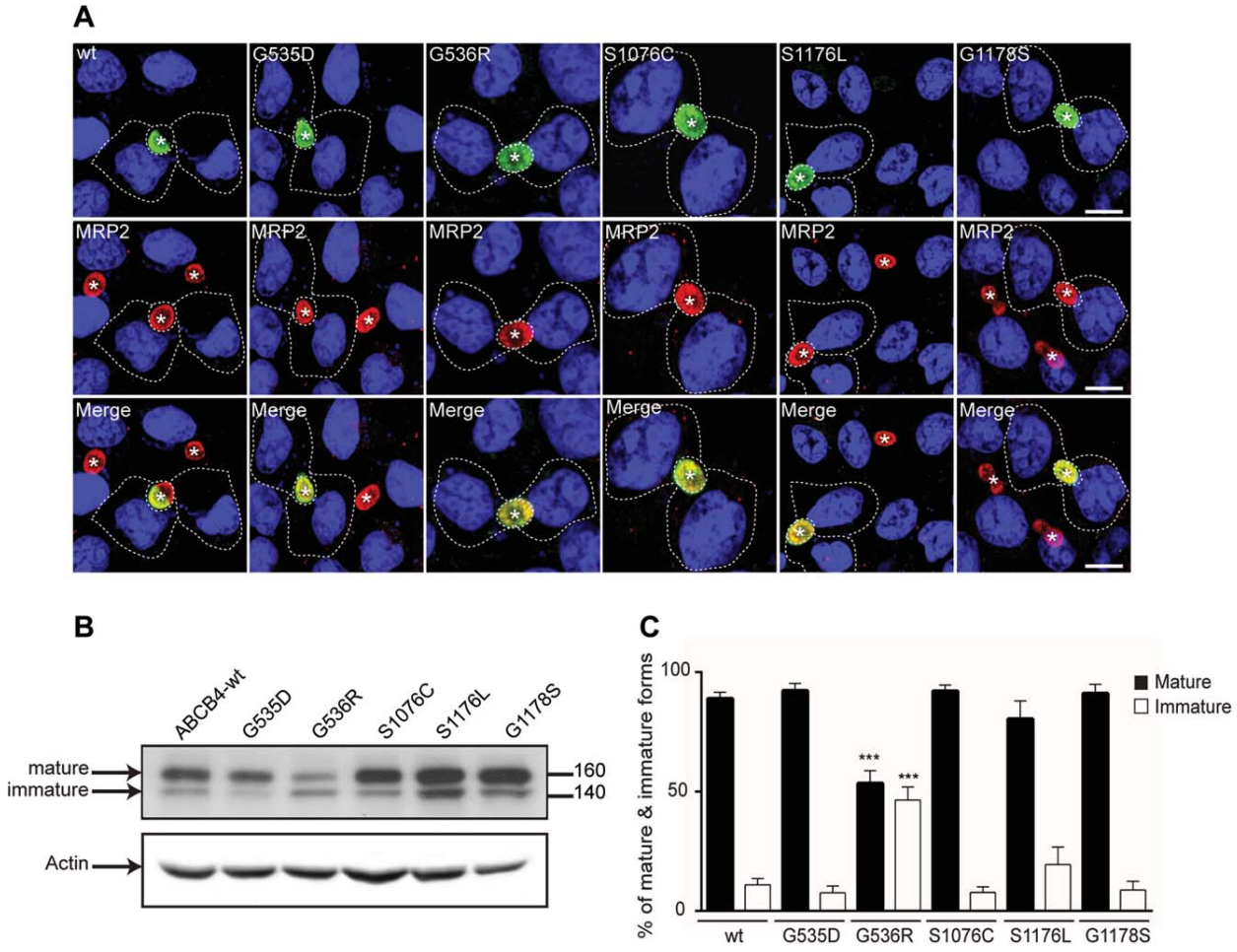

FIG. 3. Expression and processing of ABCB4 mutants in HepG2 cells. (A) Localization of ABCB4-wt and mutants by immunofluorescence and confocal microscopy. HepG2 cells transiently expressing ABCB4-wt or the mutants were fixed, permeabilized, and costained with anti-ABCB4 (P3-II-26) and anti-MRP2 (M2-I-4) monoclonal antibodies, followed by goat anti-IgG2b Alexa Fluor 488– and goat anti-IgG1 594–conjugated secondary antibodies, and visualized by confocal microscopy. In transfected cells, ABCB4-wt and the mutants are expressed at the canalicular membrane and colocalized with endogenously expressed MRP2. Nuclei are stained with Draq5 (in blue). Transfected cells are indicated by dashed lines. *Bile canaliculus. Bar = 10 μm. (B) HepG2 cells transiently expressing ABCB4-wt or the mutants were lysed and analyzed by immunoblotting using P3II-26 antiboby. Molecular masses are indicated on the right (in kDa). Actin was used as a loading control. (C) Quantification of experiments shown in (B). The mature and immature bands were separately quantified on gels, and their relative amounts were calculated. Results are means ($\pm$ SD) of at least three experiments. ***$P < 0.001$, compared to the wt.

ABCB4 mutants compared to the wt protein are shown in Fig. 3B. Quantification of the results showed that all mutants were similar to the wt protein and migrated predominantly as a mature form, with the exception of G536R for which the mature and immature forms were equally abundant (Fig. 3C). From these results, we concluded that none of the five point mutations impaired the intracellular processing of ABCB4 to the canalicular membrane, although the mature form of G536R may be less stable than normal.

PC SECRETION ACTIVITY OF ABCB4 MUTANTS AND EFFECT OF IVACAFTOR (VX-770)

Structure modeling of ABCB4-G535D, ABCB4-G536R, ABCB4-S1076C, ABCB4-S1176L, and ABCB4-G1178S predicted that their transport function would be altered, a possibility further supported by their normal canalicular localization. PC secretion activity of ABCB4 mutants was examined in the nonpolarized

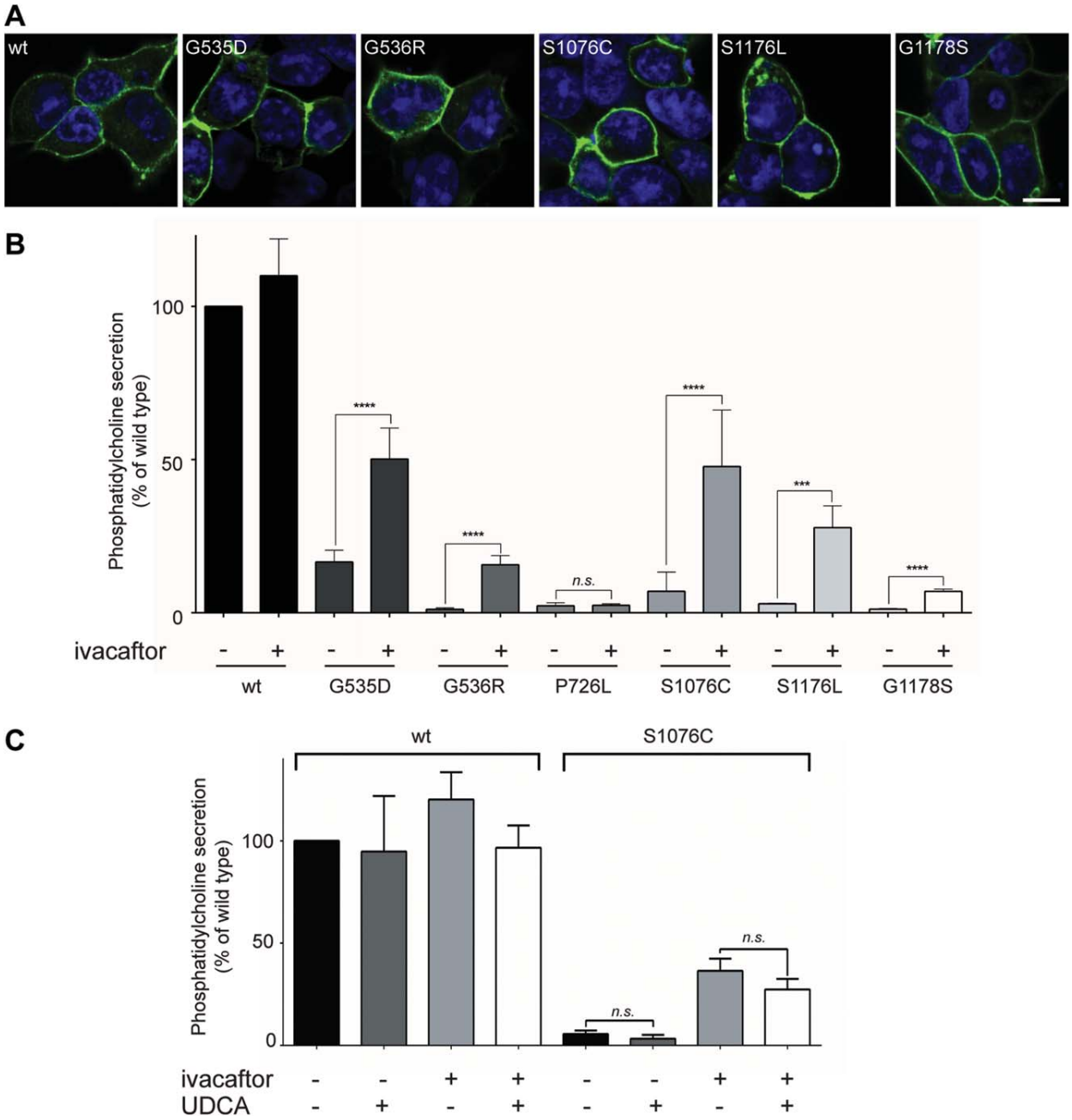

FIG. 4. PC secretion by ABCB4 mutants and effect of ivacaftor (VX-770). (A) Localization of ABCB4-wt and mutants in HEK293 cells. HEK293 cells transiently expressing ABCB4-wt or the mutants were fixed, permeabilized, and processed for immunofluorescence using the P3II-26 monoclonal antibody against ABCB4, followed by Alexa Fluor 488–conjugated secondary antibody. ABCB4-wt and the mutants are expressed at the plasma membrane. Nuclei are stained with Draq5 (in blue). Bar = 10 μm. (B) HEK293 cells were transfected with plasmids encoding ABCB4-wt or the mutants, and PC secretion was measured after 24 hours in the absence (–) or presence (+) of 10 µmol/L of ivacaftor (VX-770). PC secretion was normalized to amounts of ABCB4-wt or mutant mature forms and expressed as a percentage of the secretion activity of ABCB4-wt. Results are means (± SD) of at least six independent experiments performed in triplicate. ***$P < 0.001$; ****$P <$ 0.0001. (C) PC secretion activity was measured as in (B) after treatment of HEK293 cells 24 hours with 10 µmol/L of ivacaftor (VX-770) alone or in combination with 50 µmol/L of UDCA. Abbreviation: n.s., not significant.

epithelial HEK293 cells, which provide the best-suited model for such studies. PC secretion was measured in the culture medium after transient transfection, as described.[24] As in HepG2 cells, all mutants were localized predominantly at the plasma membrane (Fig. 4A). However, cells expressing the ABCB4 mutants released almost no PC compared to the cells expressing the wt protein (Fig. 4B). These results confirmed that, as predicted by structural modeling, disease-causing mutations in the ATP-binding sites of ABCB4 impaired the PC secretion activity of this transporter.

Considering the conservation of motifs between ABCB4 and CFTR/ABCC7 (Fig. 1 and Supporting Fig. S1), we hypothesized that ivacaftor, a potentiator of CFTR gating mutations, could also correct homologous mutations in ABCB4. Therefore, we tested the effect of ivacaftor on the PC secretion activity of ABCB4 mutants. HEK293 cells transiently expressing ABCB4-wt or the mutants were treated with 10 μmol/L of ivacaftor (VX-770), for 24 hours. This concentration was previously validated for the rescue of CFTR G551D variant,[20] and we verified that it was not cytotoxic toward HEK293 cells (data not shown). Treatment with ivacaftor increased the PC secretion activity by 3-fold for ABCB4-G535D, 13.7-fold for ABCB4-G536R, 6.7-fold for ABCB4-S1076C, 9.4-fold for ABCB4-S1176L, and 5.7-fold for ABCB4-G1178S (Fig. 4B). These effects on activity are in line with those reported for CFTR/ABCC7[20] and indicate that the functional defect of ABCB4 mutants can be corrected by the CFTR potentiator, ivacaftor (VX-770). The effect of ivacaftor was also tested on another function-defective ABCB4 mutant located in the transmembrane domain of ABCB4, P726L[13] (Fig. 1, left panel). In contrast to the studied mutants of the ATP-binding sites, ivacaftor was ineffective on the P726L mutant (Fig. 4B), suggesting selectivity of ivacaftor toward mutants located in the ATP-binding-sites. In addition, given that UDCA represents the only treatment in patients with ABCB4-related diseases, we tested whether the combination of treatments with ivacaftor and UDCA may increase the PC secretion activity of ABCB4. We performed this experiment on ABCB4-wt and ABCB4-S1076C mutant, which was the most responsive to ivacaftor. In our cell model, we found that treatment with UDCA had no effect on PC secretion activity of ABCB4 (Fig. 4C).

Discussion

In the present study, we show that five disease-causing variations in *ABCB4* (ABCB4-G535D, ABCB4-G536R, ABCB4-S1076C, ABCB4-S1176L, and ABCB4-G1178S), all found in highly conserved amino-acid sequences of the LSGGQ signature and Walker A motif involved in ATP binding and hydrolysis, lead to a loss of ABCB4 function, which can be rescued by the clinically approved CFTR potentiator, ivacaftor (VX-770).

The G535D variation was identified in a heterozygous patient who developed cholelithiasis in adolescence, followed by ICP and biliary cirrhosis in adulthood (no. 1, Table 1), whose case was previously reported.[17] Immunohistochemical staining of ABCB4 in liver biopsies from this patient previously showed the presence of ABCB4 at the canalicular membrane, which had suggested that ABCB4-G535D did not affect the targeting of the protein to the plasma membrane.[17] In agreement with this observation, we found that ABCB4-G535D was detected at the canalicular membrane of HepG2 cells. The G536R variation was identified in 2 heterozygous patients, 1 with ICP (no. 2, Table 1)[18] and the other with LPAC (no. 3, Table 1; this study); the S1076C variation was identified in a homozygous patient with PFIC3 who is on waiting-list for LT (no. 4, Table 1),[16] and the S1176L and G1178S variants were identified in heterozygous patients with LPAC (nos. 5 and 6, Table 1; this study). Although efficiently targeted to the canalicular membrane of HepG2 cells, all these mutants displayed major activity defects. All were located in the two ATP-binding sites and therefore could affect ABCB4 transport function by interfering with ATP binding and/or hydrolysis.

Based on the analysis of sequence alignment (Supporting Fig. S1) and structure-function relationships between ABCB4 and CFTR/ABCC7, we found that among the five mutants, three (ABCB4-G536R, ABCB4-S1076C, and ABCB4-G1178S) had counterparts in CFTR (CFTR-G551D, CFTR-S1251N, and CFTR-G1349D, respectively; Fig. 1 and Supporting Fig. S1). In CFTR, these mutations have been classified as gating mutations that inactivate the opening of the channel and prevent transport of chloride anions.[19] Interestingly, these CFTR mutants can be rescued by the CFTR potentiator, ivacaftor (VX-770).[19] Ivacaftor (VX-770) is a clinically approved small molecule that was identified by high-throughput screening and that targets gating defects in disease-causing CFTR mutations, including G551D, S1251N, and G1349D. It was safe when used in CF patients harboring gating mutations. Treatment with ivacaftor (VX-770) leads to significant improvements in respiratory function in these patients.[30]

So far, no specific therapeutic tool has been proposed for mutations that affect ABCB4 function. The fact that the five function-defective mutants of ABCB4 can be overcome by ivacaftor suggests that the specificity of this molecule is not restricted to CFTR/ABCC7, but rather to a common binding site involving the most conserved regions within ATP-binding sites or at the NBD1/NBD2 interface. The selectivity of ivacaftor (VX-770) toward mutants located in the ATP-binding sites is strengthened by the observation that this molecule did not rescue the P726L function-defective ABCB4 mutant, which is not involved in ATP binding or hydrolysis.

On the basis of 3D structure modeling, the mutations would cause steric hindrance or disrupt critical interactions for ATP binding (Fig. 2 and Supporting Fig. S2). This might interrupt ATP-induced NBD dimerization, as suggested for CFTR in several studies.[31,32] Several theories have been proposed to explain the mechanism of CFTR-G551D defect and rescue by ivacaftor. It was shown that CFTR-G551D exhibited a thermal instability at 37 °C, and that it was counteracted by ivacaftor.[33] Conversely, other studies have suggested an enhanced stability of the G551D protein, which would be diminished by ivacaftor.[34] Evidence was also provided to indicate that G551D converted ATP-binding site B into an inhibitory site,[35,36] probably through electrostatic repulsion between the negative charges of the acidic residue and the ATP γ-phosphate. Irrespective of the consequence of G551D defect, ivacaftor could regulate the mutant stability through direct binding at the dimer interface[37] or through an allosteric mechanism.[36,38,39]

Our findings provide the first experimental evidence making ivacaftor a potential therapeutic drug in patients with ABCB4 function deficiency attributed to mutations in the ATP-binding sites. So far, UDCA has been the only treatment in patients with ABCB4-related diseases and provided benefit essentially in patients with ICP or LPAC syndrome.[40] Although the mechanisms of UDCA actions are complex,[41] its beneficial effect in patients with ABCB4-related diseases is mostly attributed to a decrease in the toxicity of bile acids in the absence of sufficient levels of PC in bile.[40] In most PFIC3 patients, such as patient no. 4 in this study, UDCA likely slows down disease progression, but does not avoid LT. Therefore, treatment with ivacaftor could provide a benefit in such patients. Whether the level of correction provided by ivacaftor is sufficient to be clinically relevant requires confirmation by clinical trials. One can expect that it will be the case given that a small percentage of ABCB4 PC secretion activity might suffice to improve PFIC3 symptoms.[16]

Of all ABCB4 disease-causing variations, those in the ATP-binding domains that were herein investigated are restricted to one (private variations) or very few subjects from few families. However, beyond this limitation, the current findings pave the way for personalized medicine in genetic diseases; they also provide further evidence that the same drug could be effective toward several ABC transporters with homologous variations.

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