DMD Fast Forward. Published on September 28, 2018 as DOI: 10.1124/dmd.118.083030
This article has not been copyedited and formatted. The final version may differ from this version.

DMD # 83030

Polymorphisms of the multidrug pump ABCG2: a systematic review of their effect on protein expression, function and drug pharmacokinetics

Niall Heyes, Parth Kapoor and Ian D Kerr

School of Life Sciences
University of Nottingham
Queen's Medical Centre
Nottingham
NG7 2UH

DMD Fast Forward. Published on September 28, 2018 as DOI: 10.1124/dmd.118.083030
This article has not been copyedited and formatted. The final version may differ from this version.

Running Title: The impact of ABCG2 polymorphisms on drug pharmacokinetics

Correspondence should be addressed to:
Dr Ian Kerr, School of Life Sciences, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH
Ian.kerr@nottingham.ac.uk
Phone: 44 115 8230122
URL: http://www.nottingham.ac.uk/~mbzidk

Number of text pages: 46 (including references, tables and figure legends)
Number of Tables: 3
Number of Figures: 3
Number of References: approx. 175
Abstract word count: 233
Introduction word count: 1225
Discussion word count: 562

Abbreviations

ABC – ATP-binding cassette; AML – acute myeloid leukaemia; AUC – area under the curve, total drug exposure over time; CDK – cyclin-dependant kinase; CML – chronic myeloid leukaemia; DMARD – disease-modifying antirheumatic drug; DOAC – direct oral anti-coagulant; ERAD – endoplasmic-reticulum-associated protein degradation; GWAS – genome-wide association study; HDI – histone deacetylase inhibitor; HEK293 – human embryonic kidney 293 cell line; HNF4 – hepatocyte nuclear factor 4; HSC – haematopoietic stem cell; Jr – Junior blood group; Kt – concentration of substrate to reach half Vmax; MSD – membrane spanning domain; NBD – nucleotide binding domain; NSCLC – non-small-cell lung cancer; R-CHOP – rituximab plus cyclophosphamide–doxorubicin–vincristine–prednisone; SASP – sulfasalazine; SNP – single nucleotide polymorphism; SP1 – specificity protein 1; TKI – tyrosine kinase inhibitor

DMD Fast Forward. Published on September 28, 2018 as DOI: 10.1124/dmd.118.083030
This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT

The widespread expression and polyspecificity of the multidrug ABCG2 efflux transporter make it an important determinant of the pharmacokinetics of a variety of substrate drugs. Null ABCG2 expression has been linked to the Junior blood group. Polymorphisms affecting the expression or function of ABCG2 may have clinically important roles in drug disposition and efficacy. The most well studied SNP, Q141K (421C>A), is shown to decrease ABCG2 expression and activity, resulting in increased total drug exposure and decreased resistance to various substrates. The effect of Q141K can be rationalised by inspection of the ABCG2 structure and the effects of this SNP on protein processing may make it a target for pharmacological intervention. The V12M SNP (34G>A) appears to improve outcomes in cancer patients treated with tyrosine kinase inhibitors, but the reasons for this are yet to be established, and this residue's role in the mechanism of the protein is unexplored by current biochemical and structural approaches. Research into the less common polymorphisms is confined to *in vitro* studies, with several polymorphisms shown to decrease resistance to anti-cancer agents such as SN-38 and mitoxantrone. In this review we present a systematic analysis of the effects of ABCG2 polymorphisms on ABCG2 function and drug pharmacokinetics. Where possible, we use recent structural advances to present a molecular interpretation of the effects of SNPs, and indicate where we need further *in vitro* experiments to fully resolve how SNPs impact on ABCG2 function.

INTRODUCTION

The ATP-binding cassette (ABC) transporters are a family of membrane-bound protein pumps, which utilise the hydrolysis of ATP to transport compounds across membranes. They function in a variety of cellular processes such as nutrient uptake, osmotic homeostasis and protection from xenotoxins. Eukaryotic ABC transporters are possibly exclusively involved in the efflux of molecules from the cytosol; either out of the cell or into organelles within the cell. These molecules include endogenous compounds such as metabolic products and lipids, as well as xenobiotics and xenotoxins, which are of great clinical interest (Mo and Zhang, 2012). Humans possess 48 different ABC transporters, which are categorised into groups ABCA to ABCG (Dean et al., 2001). The three main ABC transporters under study in relation to drug uptake and elimination are ABCB1 (P-glycoprotein), ABCC1 (MRP1) and ABCG2 (breast cancer resistance protein). The ability of these 3 transporters to efflux multiple, structurally different drugs implicates them in multidrug resistance (MDR); the efflux of compounds from cells, lowering intracellular concentration and producing relative resistance to a broad variety of drugs (Wong et al., 2014).

The ATP-binding cassette sub-family G isoform 2 (ABCG2) protein is a MDR pump with a wide distribution in the human body, found in the small intestine, blood-brain barrier, blood-placenta barrier, liver canalicular membranes, proximal tubule cells of the kidney and the mammary gland (Horsey et al., 2016). The functions of ABCG2 at its widespread locations are summarised in Table 1, and to illustrate the diversity of its substrate repertoire a number of endogenous substrates or xenobiotic substrates is shown in Figure 1. This wide distribution and polyspecificity of ABCG2 make it an important determinant of the pharmacokinetics of various substrate drugs (Lee et al., 2015).

ABCG2 was originally identified in 1998 in placenta or in multidrug resistant (MDR) breast cancer cell lines; hence it was given the names breast cancer resistance protein/BCRP and placenta-specific ABC transporter (Allikmets et al., 1998; Doyle et al., 1998; Miyake et al., 1999). Both titles are misnomers: ABCG2 is certainly not restricted to the placenta (Table 1) and clinical correlations between ABCG2 and breast cancer staging, metastasis and outcome are equivocal (Faneyte et al., 2002; Xiang et al., 2011). On the other hand, the relationship between ABCG2 and the prognosis of haematological cancers is better developed. Poorer outcomes have been shown in large B-cell lymphoma (Kim et al., 2009b) and acute myeloid leukaemia (AML) in patients with higher ABCG2 levels (Benderra et al., 2004; Van den Heuvel-Eibrink et al., 2002). ABCG2 also has an established link to survival rates and therapy response in small cell and non-small cell lung cancer (see refs in (Horsey et al., 2016; Mo and Zhang, 2012)).

DMD Fast Forward. Published on September 28, 2018 as DOI: 10.1124/dmd.118.083030
This article has not been copyedited and formatted. The final version may differ from this version.

Along with cancer outcomes, there is a strong and well-researched link between ABCG2 and the development of gout (Woodward et al., 2013). Polymorphisms of *ABCG2* (throughout this manuscript we refer to the gene as *ABCG2* and the protein as ABCG2) can result in increased serum urate levels (hyperuricaemia); recent analysis factors correlated with renal urate clearance supports the hypothesis that ABCG2's role in gout is through decreased gut export of urea (Ichida et al., 2012; Kannangara et al., 2016). Interestingly, recent studies have also hypothesised a link between ABCG2 and the development of Parkinson's disease (PD) and Alzheimer's disease (AD). The common polymorphism 421 C>A (Q141K at the amino acid level, described in more detail below) was correlated to a delay in the onset of Parkinson's (Matsuo et al., 2015), due to an increased concentration of urate in the CNS, hypothesized to have a protective effect in the brain (Ascherio et al., 2009). ABCG2 has been found to be upregulated in the brains of Alzheimer's patients, and 421CC genotypes showed significantly increased susceptibility to Alzheimer's compared to CA and AA (Fehér et al., 2013; Xiong et al., 2009). At this time, it appears unclear as to the mechanism of the effects of ABCG2 on Alzheimer's development, although amyloid beta 1-40 peptide transport has been documented for ABCG2 (Do et al., 2012). Additional functions for ABCG2 in stem cell protection (both with respect to cancer stem cells and hematopoietic stem cells) and as a marker of autophagy have been described in the literature although full appreciation of the importance of ABCG2 in these scenarios remains to be revealed (Ding et al., 2016; Hirschmann-Jax et al., 2004; Krishnamurthy et al., 2004a; Zhou et al., 2001).

Many anti-cancer drugs have been discovered as ABCG2 substrates, such as camptothecin analogues (diflomotecan, irinotecan, topotecan), tyrosine kinase inhibitors (gefitinib, imatinib, erlotinib) and other agents (methotrexate, mitoxantrone). Cisplatin, paclitaxel and vinblastine are not substrates (Stacy et al., 2013) and the anthracyclines doxorubicin and daunorubicin are substrates only of drug selected ABCG2 variants (Robey et al., 2003). Other xenobiotics which have been discovered as substrates include statins, direct oral anticoagulants (DOACs), anti-HIV drugs, anti-rheumatics, immunosuppressants and antibiotics (see Figure 1 for some chemical structures of transported drugs and natural ABCG2 substrates which clearly demonstrates the chemical diversity of its substrates). Alongside traditional anti-cancer drugs, photodynamic therapy agents such as pheophorbide A and protoporphyrin A have been confirmed as substrates, possibly influencing the effects of photodynamic therapy in some individuals (Robey et al., 2005; Robey et al., 2004; Westover and Li, 2015). Polymorphisms reducing ABCG2 efflux activity may therefore be of potential importance in contributing to diet-induced phototoxicity, protoporphyria and other porphyrin-related disorders (Jonker et al., 2002). This exemplifies the importance of understanding how

polymorphisms affect ABCG2 function and for this we will need a detailed description of the structure of the resultant protein.

ABCG2 is coded for by the *ABCG2* gene at chromosome 4q22 (Bailey-Dell et al., 2001), and transcription of the gene is regulated by a TATA-less promoter region, containing several Specificity Protein 1 (SP1) transcription factor binding sites (Yang et al., 2013). Translation of the mRNA results in the production of a 655 amino acid protein (~65 kDa). Structurally, ABCG2 is known as a 'half transporter', meaning it contains only one membrane-spanning domain (MSD) and one nucleotide binding domain (NBD) in the polypeptide. The MSD of ABCG2 is found towards the C-terminus and is composed of six membrane-spanning α-helices, connected by alternating extracellular and intracellular loops (Figure 2). The 'half transporter' structure of ABCG2 means that it must either dimerise or oligomerise before it becomes functional (Horsey et al., 2016; Mo and Zhang, 2012), and although the basis of dimer formation is outside the scope of this review there has been much research in this field (Wong et al., 2016). The recent publication of structural models for ABCG2 (Khunweeraphong et al., 2017; Laszlo et al., 2016), and the emergence of cryo-electron microscopy data for the human protein (Jackson et al., 2018; Taylor et al., 2017) means that we are now close to being able to determine not only how a SNP in *ABCG2* might manifest at the level of protein function but also to relate this to its position in the 3D structure.

An important early finding in this regard was the original cloning of two *ABCG2* variants from drug resistant cell lines, with a substitution at amino acid 482 from arginine (R) to threonine (T) or glycine (G) (Doyle et al., 1998; Miyake et al., 1999). These R482G/T variants of ABCG2 were shown by the use of fluorescent probes and radioligand binding to have a broadened substrate specificity including anthacyclines (Clark et al., 2006; Honjo et al., 2001). Although it later transpired that the R482 variant sequences were drug-induced mutations (Honjo et al., 2001), it opened the question – could natural polymorphisms of *ABCG2* also affect its function?

Many single nucleotide polymorphisms have subsequently been identified in *ABCG2*, including promoter, intronic as well as protein coding changes (the latter being displayed in Figure 2). This review aims to provide a summary of the researched polymorphisms of the *ABCG2* gene, the impact of these on ABCG2 expression/function, and the resulting effects on pharmacokinetics.

The polymorphisms of ABCG2

Forty-five *ABCG2* polymorphisms were identified in the literature, 21 of which were not included due to lack of significant information. The excluded polymorphisms are (single amino acid notation) S13L, G51C, A149P, R160Q, R163K, Q166E, G268R, G354R, K360X, C369T, F373C, G406R, T434M, C474T, S476P, P480L, M515R, T542A, F571I and R575X. The majority of these have minor allele frequencies that are < 0.0001 and some even represent somatic mutations, and it is likely that most are of questionable clinically significance. Nevertheless, establishing the effects of ABCG2 polymorphisms at as many sites as possible would be beneficial in improving knowledge of the protein's mechanism. The remaining 24 polymorphisms (see Table 2 for frequency information and Figure 2, 3 for their location in the topology and 3D structure of ABCG2) will be described numerically from the N-terminus for convenience.

Polymorphisms in the NBD

The pivotal catalytic role played by the ABC transporter NBDs and our greater understanding of NBD structure and function should enhance our ability to rationalise the effects of NBD polymorphisms on ABCG2 function.

V12M - rs2231137

V12M (34 G>A; table 2) is one of the most common polymorphisms of *ABCG2*, with a highly variable frequency depending on ethnicity. The polymorphism was found at highest frequencies in Mexican Indians (90%), Pacific Islanders (64%) and South-Eastern Asians (45%), but more rarely in Caucasian (2-10.3%), African American (8.3%) and Middle Eastern populations (5%) (Kobayashi et al., 2005; Poonkuzhali et al., 2008; Zamber et al., 2003). Residue V12 is located intracellularly, close to the N-terminus of the polypeptide. This region has a substantial influence on the localisation of the pump into the plasma membrane, meaning polymorphisms may disrupt the insertion of ABCG2 into the membrane. Structural data currently do not include the N-terminal 30 amino acids and so the exact contribution this region makes to the fold of ABCG2 remains unclear (Taylor et al., 2017). *In vitro* examination of V12M shows expression-system dependent effects on ABCG2 localisation and expression level, which makes it important to verify the effects of polymorphisms in cell lines as close as possible to the *in vivo* setting (Imai et al., 2002; Kondo et al., 2004; Mizuarai et al., 2004; Morisaki et al., 2005). For V12M the closest to this comes in a study using Flip-In 293 cell line-transfectants where a reduced transport of SN-38 was observed (Tamura et al., 2007a). An *in vivo* study on liver tissues in a Hispanic population did find that *ABCG2* mRNA expression was lower in V12M, possibly as a result of alternative splicing (Poonkuzhali et al., 2008). However, lower ABCG2-V12M protein expression in tissues is yet to be found (Zamber et al., 2003).

If V12M does lower protein expression, this would impact on urate transport or cancer chemotherapy. There are conflicting reports as to the impact on V12M on the development of gout. Several studies have found no significant effect of V12M on urate transport and gout development (Higashino et al., 2017; Matsuo et al., 2009). Despite this, a recent meta-analysis of data concluded that V12M did exert a protective effect against gout. The study found an odds ratio of 0.76 (24% reduction in gout risk) when comparing Czech populations with New Zealand Polynesian and UK Biobank data (Stiburkova et al., 2017). In terms of cancer chemotherapy there are again conflicting reports. For tyrosine kinase inhibitor (TKI) therapy, overall survival in non-small-cell lung cancer (NSCLC) was found to be improved in AA/AG genotypes (Chen et al., 2015; Tamura et al., 2012). Other studies further supported the influence of the V12M SNP on TKI therapy in chronic myeloid leukaemia (CML) and renal cell metastatic cancer (Kim et al., 2009a; Tandia et al., 2017; van der Veldt et al., 2011); in the latter the presence of the 34A allele was linked to prolonged progression-free and overall survival in sunitinib therapy using Kaplan-Meier univariate analysis. Furthermore, 34AA/AG genotypes have been linked to improved outcomes in chronic myeloid leukaemia mediated by an increased toxicity of ctyarabine/anthracycline therapy, with an odds ratio of 8.41 (Hampras et al., 2010). In slight contrast, V12M has been found to show no effect on R-CHOP (rituximab plus cyclophosphamide–doxorubicin–vincristine–prednisone) therapy response or the side effects of R-CHOP treatment (Kim et al., 2008), although doxorubicin is not a good substrate of ABCG2 which at least partially explains this (Mao and Unadkat, 2015). Finally, in terms of cancer the 34 G>A allele was associated to lower survival rates in paediatric acute lymphoblastic leukaemia patients and diffuse large B-cell lymphoma (Hu et al., 2007; Zhai et al., 2012). At this time, it remains unclear as to the reason for poorer prognosis in V12M for certain cancers. Research into other ABCG2 substrates for the V12M SNP is limited with single reports indicating no effect on disposition for the anti-retroviral drugs lamivudine in healthy subjects (Kim et al., 2007) or in nelfinavir-receiving patients (Colombo et al., 2005)..

Q126X - rs72552713

The Q126X polymorphism (376 C>T) is a rare polymorphism found most commonly in Japanese populations, with a frequency between 0.9% and 2.4% (Honjo et al., 2002; Imai et al., 2002; Itoda et al., 2003; Kobayashi et al., 2005). Q126X has been investigated in other ethnicities such as Caucasians and African Americans, but in both of these populations the polymorphism was absent (Kobayashi et al., 2005; Mizuarai et al., 2004). The polymorphism results in a premature stop codon and likely results in nonsense mediated RNA decay and no protein expression in the membrane (Kobayashi et al., 2005). It is therefore expected that

the influence of this polymorphism on drug pharmacokinetics would be considerable, especially in homozygous carriers (Imai et al., 2002).

Many studies have found a strong connection between the Q126X polymorphism and an increased risk of developing gout (Li et al., 2015; Matsuo et al., 2009; Zhou et al., 2014), with Matsuo showing a significant odds ratio of 5.97. This study also found the Q126X haplotype was present in up to 13.5% of gout patients in a Japanese population (Matsuo et al., 2009). Studies focused on the effects of Q126X on drug pharmacokinetics are rare, most likely due to the low frequency and assumed outcome (i.e. absence of protein in homozygous individuals would clearly have a significant impact on intestinal drug uptake and biliary excretion). A small scale study has linked the Q126X polymorphism with sulfasalazine pharmacokinetics (Gotanda et al., 2015), and effects of Q126X on porphryin transport has also been shown (Tamura et al., 2012). Earlier studies had reported no interaction between Q126X and lamivudine/gefitinib therapy (Akasaka et al., 2010; Kim et al., 2007), suggesting that more studies are needed to fully understand the impact of this polymorphism on drug pharmacokinetics.

Q141K – rs2231142

Q141K (421 C>A) is probably the most widely studied, common variant of *ABCG2* which is found between the Walker A motif and the signature region of the NBD (Woodward et al., 2009). The polymorphism has a highly variable frequency depending on ethnicity. It is found commonly in Japanese (26.6-35%) and Chinese (34.2-35%) populations, but more rarely in Caucasian (8.7%-14%), Sub-Saharan (0.9%) and African American (0-5.3%) populations (De Jong et al., 2004; Imai et al., 2002). Multiple studies have looked at the influence of Q141K on the function of ABCG2, with differing results as to the impacts of the polymorphism. A reduction in overall ABCG2 protein expression has been identified in many studies, both *in vitro* and *in vivo* (Furukawa et al., 2009; Imai et al., 2002; Kobayashi et al., 2005; Kondo et al., 2004; Sarankó et al., 2013; Tamura et al., 2007b; Woodward et al., 2013). However, there are studies which have shown that the mutation also causes reduced ATPase activity, resulting in decreased transport activity (Mizuarai et al., 2004; Morisaki et al., 2005). A plausible structural explanation of this is that when the neutral Q141 is replaced by a positively charged lysine, an electrostatic repulsion occurs with TM helix 1a (see Figure 3B,C; potentially via an arginine residue in this helix, R383 (Polgar et al., 2009) or the adjacent lysine K382 (Laszlo et al., 2016)) leading to an ABCG2 folding defect (Taylor et al., 2017) and degradation by the endoplasmic reticulum associated protein degradation (ERAD) system (Furukawa et al., 2009)..

Reduced ABCG2 expression in the Q141K variant should result in increased serum drug concentrations through decreases in gut and urate clearance. The influence of Q141K on ABCG2 has been widely studied due to its connection with gout development, with a number of studies linking Q141K to reduced ATP-dependent urate transport (Woodward et al., 2009; Woodward et al., 2013) and an increased risk of gout (Cleophas et al., 2017; Higashino et al., 2017). In fact, Q141K is one of the most strongly predictive alleles for high serum urate levels and gout incidence. In addition to an increased gout incidence, Q141K was found to be associated with a reduced response to the gout treatment allopurinol in a genome-wide association study (GWAS) (Wen et al., 2015). Moreover, Q141K has been linked to poorer outcomes in prostate cancer, possibly due to reduced efflux of folate from the tumour (Sobek et al., 2017), resulting in high folate levels within the tumour, allowing cancer cells to proliferate more rapidly. However, the effects of Q141K are mixed, as it also reduces efflux of docetaxel in prostate tumours, resulting in an improved drug response (Sobek et al., 2017). A wide range of drugs have been investigated with respect to Q141K, as detailed in the following paragraphs.

Camptothecin analogues

Camptothecin analogues, anti-cancer agents which inhibit topoisomerase I, have shown mixed results in Q141K studies. Diflomotecan showed a 299% plasma level increase in heterozygous Q141K patients compared to wild-type (Sparreboom et al., 2004). Likewise, an *in vitro* study showed Q141K cells were more susceptible to diflomotecan, with IC_{50} values 1.2-2.3 fold lower compared to wild type (Morisaki et al., 2005). Another camptothecin analogue, 9-aminocamptothecin, showed significantly increased area under curve (AUC) in 421CA genotypes (Zamboni et al., 2006). This means Q141K most likely reduces transport of camptothecins compared to wild type. For other topoisomerase inhibitors, where one might also expect to see an increased oral bioavailability in Q141K individuals, the results are less consistent; increased topotecan oral bioavailability was shown by Sparreboom (Sparreboom et al., 2005) for 421CA genotypes, but was not found in a more recent study by Li (Li et al., 2013). Q141K was shown to have no significant influence on the disposition of irinotecan and its metabolite SN-38 in individuals in three studies (Han et al., 2007; Jada et al., 2007; Sai et al., 2010), in contrast to an earlier study suggesting a role for Q141K in irinotecan disposition (De Jong et al., 2004). A role for ABCG2 in irinotecan pharmacokinetics was however supported by *in vitro* studies of cellular resistance to SN-38 (Jandu et al., 2016; Tamura et al., 2007b). Therefore, more evidence on topotecan and irinotecan pharmacokinetics is needed before a conclusion on these can be made.

Tyrosine kinase inhibitors (TKIs)

Gefitinib pharmacokinetics were the first to be studied of the TKIs, with Li demonstrating that 421CA genotypes had 1.5-fold increased steady-state gefitinib accumulation compared to wild-type (Li et al., 2007). Q141K was also shown by the same group to increase the likelihood of diarrhoea after gefitinib treatment, but not skin toxicity (Cusatis et al., 2006). It was expected that erlotinib pharmacokinetics would show similar traits given its similarities to gefitinib. This was confirmed in studies showing a 24% reduction in the clearance of erlotinib in patients with at least 1 Q141K allele (Fukudo et al., 2013; Thomas et al., 2009). For sunitinib, both homozygous and heterozygous genotypes showed an increased dose-adjusted AUC compared to wild-type (Mizuno et al., 2012). Q141K showed an association with sunitinib therapy-induced thrombocytopenia, but not with early-onset haematoxicity (Kato et al., 2017; Low et al., 2016).

Studies examining imatinib response are more conflicting, although research appears to sway again towards an increased accumulation in Q141K carriers. The conflicting results are exemplified in Gardner's study which found increased Q141K accumulation *in vitro*, but not in 16 heterozygous patients *in vivo* (Gardner et al., 2006). Increased sensitivity to imatinib (and dasatinib and nilotinib) *in vitro* in Q141K expressing cells was also shown by Skoglund (Skoglund et al., 2014). Several studies have found Q141K showing no influence on imatinib concentration (Francis et al., 2015; Seong et al., 2013; Yamakawa et al., 2011), however other studies have found 1.4-fold dose-adjusted trough concentration to be higher in Q141K, along with reduced oral clearance and increased drug response (Au et al., 2014; Laszlo et al., 2016; Petain et al., 2008; Takahashi et al., 2010), and recent meta-analysis of 23 studies found that Q141K has a significant effect on improved imatinib treatment in CML in Asian populations (Jiang et al., 2017). Currently, no association has been found with *ABCG2* polymorphisms and telatinib/danusertib pharmacokinetics (Steeghs et al., 2011a; Steeghs et al., 2011b). Along with the changes in TKI disposition in ABCG2 polymorphisms, TKIs have been shown to inhibit ABCG2, with a potential role in drug-drug interactions, as discussed by Mao and Unadkat (Mao and Unadkat, 2015).

Other anti-cancer agents

It would be expected that loss-of-function polymorphisms such as Q141K would increase methotrexate toxicity, as inhibition of ABCG2 has consistently been shown to increase methotrexate AUC (Gervasini et al., 2017; Suthandiram et al., 2014; Vlaming et al., 2011. However, no patient studies have shown this link, with several studies showing no effect of Q141K on plasma concentrations and therapy response {El Mesallamy, 2014 #255). The anthracycline daunorubicin was one of the first ABCG2 substrates to be discovered, using MDR breast cancer cell lines. In Q141K expressing cell lines, daunorubicin and doxorubicin

were found to show an increased accumulation compared to wild-type (Tamura et al., 2007b), but worse outcomes were shown in Q141K AML patients treated with idarubicin (Tiribelli et al., 2013). In addition, the presence of the 421C allele was indicative of a weaker treatment response to anthracyclines in a Kurdish population (Ghafouri et al., 2016). The same study showed Q141K produced a weaker response to the taxane paclitaxel (Ghafouri et al., 2016), although Q141K patients were associated with longer progression free survival when treated with paclitaxel and platinum therapy in a study on ovarian cancer patients (Tian et al., 2012). Another taxane, docetaxel, was shown in a recent study to have reduced resistance in Q141K carriers compared to wild-type (Sobek et al., 2017), despite Q141K previously being shown to have no influence on docetaxel disposition (Baker et al., 2009; Chew et al., 2011). These conflicting findings, particularly for taxanes, reiterate the need for further investigation into the pharmacokinetics of anti-cancer agents in *ABCG2* variants.

Statins

The effects of Q141K on the family of statins has been extensively researched, with the majority of statins tested (notable exceptions being pitavastatin and pravastatin (Ieiri et al., 2007; Keskitalo et al., 2009b; Oh et al., 2013; Zhou et al., 2013c)) showing altered pharmacokinetics in individuals bearing the C421A allele. Rosuvastatin pharmacokinetics have been consistently shown to be influenced by Q141K, with many studies linking the polymorphism to an increase in plasma AUC. Both the 421CA and AA genotype show significant increases, with AA individuals showing 135% higher maximum plasma concentration and 140% higher AUC, thus creating drug efficacy at lower doses of rosuvastatin in individuals with the Q141K polymorphism (Birmingham et al., 2015; Keskitalo et al., 2009b; Lee et al., 2013; Liu et al., 2016; Tomlinson et al., 2010; Zhang et al., 2006; Zhou et al., 2013a). In addition, atorvastatin shows a 46% increased plasma AUC in the 421AA genotype, although there is limited evidence as to the effects of CA (Birmingham et al., 2015; Keskitalo et al., 2009b). Simvastatin acid has been shown to have a significantly increased concentration in Q141K patients in studies by Choi and Birmingham (Birmingham et al., 2015; Choi et al., 2015), but evidence for effects on the prodrug simvastatin lactone is more equivocal (Birmingham et al., 2015; Keskitalo et al., 2009a). Research into fluvastatin has also showed varying results. Zhou found that neither Q141K nor V12M influenced pharmacokinetics, however Keskitalo found a significant increase in AUC in 421AA individuals (Keskitalo et al., 2009a; Zhou et al., 2013b). Despite this apparently clear picture of ABCG2:statin interaction there are still ambiguities to resolve. Notably, whilst Q141K has been shown to result in an increased plasma concentration of atorvastatin and rosuvastatin, there was no effect on the elimination half-lives. This means altered pharmacokinetics are

due to tissue specific ABCG2:statin interactions, i.e. causing increased absorption into the blood, rather than changes in biliary excretion (Li and Barton, 2018).

Direct oral anti-coagulants (DOACs)

Recent research into anti-coagulants has found that dabigatran etexilate, rivaroxaban, edoxaban and apixaban are substrates of ABCG2 (Gong and Kim, 2013; Gong et al., 2013; Hodin et al., 2018; Mueck et al., 2013; Zhang et al., 2013). It was hypothesised that these drugs may be influenced by loss-of-function polymorphisms (e.g. Q141K) and drug-drug interactions, possibly risking patient safety and impacting on drug action. Apixaban was recently shown to have a 1.5-fold higher clearance rate in WT or heterozygous genotype compared to the 421AA genotype (Ueshima et al., 2017), supporting further studies to determine whether patients with C421A alleles are at greater risk of bleeding. This is an important field of potential study, especially due to the significant risks in under or over-dosing of anti-coagulants, and the lack of antidotes for some DOACs.

Anti-HIV medications

Several anti-HIV medications have been shown to act as ABCG2 substrates. Disposition of both lamivudine and nelfinavir seem to be unaffected by Q141K (Colombo et al., 2005; Kim et al., 2007). On the other hand, dolutegravir showed a significantly increased peak plasma concentration in the 421AA genotype compared to CC and CA. It was hypothesised this was due to reduced ABCG2 expression in the small intestine, which resulted in increased dolutegravir absorption (Tsuchiya et al., 2017). An increased drug concentration in Q141K patients has also been found for raltegravir (Tsuchiya et al., 2016).

Disease-modifying anti-rheumatic drugs (DMARDs)

Leflunomide, used in the treatment of rheumatoid arthritis, produces the active metabolite teriflunomide/A771726. A 70% increase in maximal serum concentration and 40% increase in AUC of teriflunomide has been shown in Q141K as a result of reduced ABCG2 activity (Kim et al., 2011; Wiese et al., 2012). The effects of Q141K on sulfasalazine (SASP) have been extensively researched but data remains conflicting. Studies by Urquhart, Ieiri and Gotanda all showed a significant increase in AUC of SASP for CA/AA genotypes, but this was not found in Adkison's study (Adkison et al., 2010; Gotanda et al., 2015; Ieiri et al., 2008; Urquhart et al., 2008). The increase in drug concentration may be caused by increased SASP absorption in the small intestine or decreased clearance in the liver (Ieiri et al., 2008).

Other drugs

A significant effect on the *in vitro* accumulation of diabetic medication glyburide/glibenclamide in cells expressing Q141K has been shown by Pollex (Pollex et al., 2010), with possible importance in the placenta during pregnancy. Increased glyburide accumulation in Q141K expressing cells was also found by Bircsak (173). Q141K has also recently been shown to influence the exposure of clozapine, an anti-psychotic medication (Bircsak et al., 2016). The disposition of the immunosuppressants tacrolimus and mizoribine have shown no change in Q141K (Fukao et al., 2011; Ogasawara et al., 2013), although mycophenolic acid showed more mixed results (Geng et al., 2012; Miura et al., 2008). There are also multiple drugs which have been studied with respect to Q141K which have so far shown no evidence of pharmacokinetic effects. No effects have been found so far for the angiotensin II inhibitors telmisartan and olmesartan (Chen et al., 2013; Kim et al., 2012; Yamada et al., 2011), nor for nitrofurantoin, an antibiotic used in bladder infections (Adkison et al., 2008; Huang et al., 2012). However, the wide variety of drugs potentially influenced by Q141K highlights the importance of testing new drugs for interactions with this specific polymorphism.

"Rescuing" the mis-trafficked Q141K Isoform

The pharmacological modulation of misfolded ABC transporters has been intensively studied in the case of ABCC7/CFTR (cystic fibrosis transmembrane conductance regulator) (De Boeck et al., 2014; Vauthier et al., 2017). The F508del variant of CFTR, which is found in approximately 70% of cystic fibrosis patients, is located in a structurally equivalent position to Q141K which has prompted significant research into the use of pharmacological chaperones, to increase Q141K protein expression at the cell membrane. A number of small molecules were discovered by Woodward to increase the expression of Q141K ABCG2 within the membrane including as 4-phenybutyrate (a histone deacetylase inhibitor), and the CFTR corrector VRT-325 (Woodward et al., 2013). In the case of VRT-325, it was found to restore function (uric acid transport) of Q141K expressing cells, showing its potential benefits in gout treatment. Other histone deacetylase inhibitors (HDIs), such as romidepsin, have been shown to rescue Q141K ABCG2, causing restored ABCG2 expression in the membrane and increased ABCG2 function (Basseville et al., 2012). The potential for ABCG2 modulation in gout therapy is in its infancy but there is promise in this area.

Less well studied SNPs in the NBD

The T153M (458 C>T; rs753759474) polymorphism was shown to cause decreased ABCG2 expression, resulting in decreased resistance to SN-38 (irinotecan), due to reduced efflux activity (Mizuarai et al., 2004; Stiburkova et al., 2017; Yoshioka et al., 2007). A recent study linked T153M to gout induction, along with the nearby polymorphism R147W. It was

hypothesised that these effects may be due to a disruption in ATP-binding (Stiburkova et al., 2017), although no *in vitro* examination of the effect of this SNP on ATP binding has been performed. Indeed, examination of the structure of ABCG2 indicates that both T153M and R147W are positioned close to the NBD:TMD interface (Figure 3B). Similar to Q141K, these residues may either disturb the folding of the protein and/or the allosteric communication of ATP hydrolysis with drug binding and transport. Further *in vitro* examination of these SNPs is certainly warranted as it may shed light on the mechanism of the transporter.

Close to the Walker B catalytic glutamate residue (E211 in human ABCG2) there are two SNPs, I206L (rs12721643) and F208S (rs1061018) (Zamber et al., 2003). Their location in the Walker B region meant that impacts on ATP-binding are expected, particularly for the drastic change from Phe to Ser in the F208S variant. *In vitro* experiments on cells expressing the I206L variant indicated that there was significantly lower ABCG2 expression compared to wild-type (Vethanayagam et al., 2005), which is paralleled by other mutations in neighbouring residues (Cui et al., 2001)). F208S (623 T>C) expression is also reduced and furthermore the mutated protein is not localised to the cell surface membrane. Concomitantly, F208S was found to show no resistance to SN-38 (irinotecan) and mitoxantrone in studies by Yoshioka and Tamura, and is defective in porphyrin transport (Yoshioka et al., 2007). This makes it likely to be a loss-of-function polymorphism, a view reinforced by transport studies of TKI in cells expressing this variant (Skoglund et al., 2014). For both I206L and F208S there are no studies regarding the effects of this SNP in patients to date.

Polymorphism rs3116448, which results in S248P (742 T>C) shows an impaired efflux function and defective porphyrin transport compared to wild-type protein (Deppe et al., 2014; Tamura et al., 2006), despite showing similar expression at the plasma membrane. S248P has been shown to reduce drug resistance for SN-38 and mitoxantrone compared to wild-type (Tamura et al., 2007b). This is a result of impaired transport which will result in an increased concentration within target cells. Structurally S248 is located immediately C-terminal to a conserved NBD motif – the Histidine switch region (Mao and Unadkat, 2015) – which is known to be critical in ATP hydrolysis and so a dramatic effect on protein function is expected here.

Finally, in a loop towards the end of the NBD, the SNP P269S (805 C>T) is located close to the linker region; it is found in Korean and Vietnamese populations at a frequency of 0.2% (Sang et al., 2007). The activity of P269S ABCG2 was decreased modestly compared to wild-type, despite similar protein expression levels, for a range of drugs including prazosin,

estrone-3-sulphate and mitoxantrone (Higashino et al., 2017; Kondo et al., 2004; Sang et al., 2007). Similarly, there is no effect of this SNP on urate transport suggesting P269S is not clinically relevant in gout, and the clinical significance of this SNP on pharmacokinetics remains to be determined.

Polymorphisms in the linker region

The recent structural advances for ABCG2 now mean that interpretations of linker region SNPs will be more informed (Taylor et al., 2017). Only one SNPs in the linker region of ABCG2 has been described in any detail. D296H (886 G>C) was originally identified by Polgar (Polgar et al., 2008), and was shown to reduce ABCG2 expression and increased the toxicity of several TKIs (Skoglund et al., 2014). This can be rationalised by inspection of the structure of ABCG2, which shows D296 to be located at an NBD:NBD interaction site. It is feasible that disruption of NBD:NBD interface by this SNP could have dramatic effects on the transporter's ability to hydrolyse ATP, although this remains to be seen.

Polymorphisms in the MSD

The membrane spanning region of ABCG2, like other ABC transporters, is the site at which transported substrates interact (see Figure 3D for the proposed drug binding site in ABCG2). The structural basis for multidrug transport by ABCG2 is not fully understood but is believed to be underpinned by multiple pharmacologically distinct and potentially spatially distinct drug interaction sites (Clark et al., 2006; Cox et al., 2018). A loop between two of the helices which comprise the MSD is also the location of the single N-linked glycosylation site in the transporter, full maturation of which is necessary for ABCG2 stability (Nakagawa et al., 2009). It might therefore be expected that mutations in the MSD would impact on drug specificity as well as protein stability.

F431L

F431L (1291 T>C) is found within the TM2 segment of the MSD. Studies found that ABCG2 expression at the cell surface membrane was not affected by the polymorphism, although there was a loss-of-function (Deppe et al., 2014; Sjöstedt et al., 2017; Yoshioka et al., 2007). This loss-of-function results in an impaired transport of porphyrins (Tamura et al., 2007b), a decreased resistance to SN-38 and mitoxantrone compared to wild-type (Tamura et al., 2007a) and differences in response to TKIs and ABCG2-specific inhibitors (Kawahara et al., 2010; Yoshioka et al., 2007). This may result in an increased risk of hypersensitivity to certain anti-cancer agents, especially methotrexate, where transport is completely abolished. Yoshioka hypothesised the F431 residue may be important in substrate recognition (184), and inspection of the structure of ABCG2 (Figure 3D) shows the location of this residue

close to a proposed drug binding pocket (Taylor et al., 2017), and the recent determination of the structure of ABCG2 with a bound inhibitor confirms the importance of this residue in transporter pharmacology (Jackson et al., 2018).

S441N – rs758900849

The S441N polymorphism (1322 G>A), also in TM2, has been shown to cause instability in the protein, presumably by disrupting the TM helical fold, despite the relatively conservative nature of the substitution. This results in enhance proteasomal degradation of S441N variant protein, and a lower overall protein expression level within the plasma membrane (Nakagawa et al., 2008). The lower protein expression in S441N appears to completely eliminate the transport function of ABCG2 in multiple studies (Sjöstedt et al., 2017; Tamura et al., 2007a; Tamura et al., 2007b; Tamura et al., 2006), in various different cell based systems; these describe reductions in/abolition of transport of urate, porphyrins, SN-38 and mitoxantrone. How this translates to effects on ABCG2 in individuals with either GA or AA haplotypes is unclear. Notably the polymorphism S441N is close to numerous residues identified in structural and functional work on ABCG2 as being important for drug binding and transport. It lies adjacent to S440, shown to be critical in the transport of mitoxantrone and pheophorbide A (Cox et al., 2018), and is located at a site where multiple structural and molecular docking studies predict drugs may bind (Cox et al., 2018; Laszlo et al., 2016; Taylor et al., 2017).

F489L – rs192169063

F489L (1465 T>C) is found in the TM3 segment of the MSD, producing ABCG2 with impaired functioning, but with similar expression levels in the membrane (Sjöstedt et al., 2017). This impaired transport has been found to be around 10% of wild-type, with F489L ABCG2 shown to be unable to transport methotrexate (Tamura et al., 2006). Although not the exact residue change seen in the SNP, it is interesting that Kerr and colleagues have also expressed an F489A variant, which showed a contribution to a drug binding site in molecular docking studies, a reduced drug export function (reduced pheophorbide A transport), but with no effect on protein expression (Cox et al., 2018). In contrast, a study by Deppe found that F498L reduced levels of ABCG2, which was reversed by proteasome inhibition (Deppe et al., 2014), suggesting a protein processing defect.

N590Y – rs34264773

N590Y (1768 A>T) is located in the extracellular loop between TM5c and TM6a and was found at a frequency of 1% in a Caucasian population (Zamber et al., 2003). Results on this polymorphism are limited to *in vitro* studies which do not show strong agreement;

Vethanayagam found that N590Y increased the expression of ABCG2 within the membrane, but reduced drug transport and resistance to mitoxantrone and topotecan to just 30% of wild-type (Vethanayagam et al., 2005). This was not supported by Yoshioka, who showed comparable expression to wild-type, and no change in SN-38 pharmacokinetics (Yoshioka et al., 2007). Research on the pharmacokinetics of F489L and N590Y is limited and conflicting, so no reliable judgement can be made on their effects. It is interesting to note that the sidechain of N590 is in very close proximity to N596, which is the site of glycosylation in ABCG2, so an effect of this SNP on processing and maturation of the transporter would not be unexpected.

D620N – rs34783571

The D620N polymorphism (1858 G>A) is also located at the very extracellular end of the TM6a helix and its sidechain interacts with the polar headgroup region of the lipid bilayer (Taylor et al., 2017). The impact of the polymorphism on protein expression again shows contradictory results. Vethanayagam and colleagues showed a 240% increased ABCG2 expression compared to wild-type, similar to N590Y. This study also showed that the mutated pump had 50% of the efflux activity of the wild-type pump, with a concomitant 50% decrease in methotrexate resistance, although there was no effect on mitoxantrone or topotecan resistance (Vethanayagam et al., 2005). On the other hand, another study found a decrease in ABCG2 expression (Yoshioka et al., 2007) and reduced resistance to SN-38 (Yoshioka et al., 2007), whilst a further report indicated no effect on efflux activity (Morisaki et al., 2005). Reduced excretion of urate in the kidney may result in a possibly damaging effect of D620N in gout (Stiburkova et al., 2017), although samples sizes are not currently high enough to provide firm evidence of this.

Polymorphisms in non-coding regions

Mutations in the introns may affect ABCG2 expression and function via effects on mRNA splicing or RNA stability, although the exact mechanisms of many such polymorphisms remain unclear. Both 16702 C>T and 12283 T>C have been shown to increase ABCG2 expression in liver samples (Poonkuzhali et al., 2008), with 16702 C>T showing some association with skin toxicity and rash in erlotinib therapy. These effects of 16702 C>T are likely caused by the gain of a GATA4 transcription factor binding site (Rudin et al., 2008). Conversely, 1143 C>T was shown to lower ABCG2 expression in the same study with 1143CT/TT carriers showing an increased concentration response to erlotinib (Poonkuzhali et al., 2008; Rudin et al., 2008), and CT carriers have shown a minor link to sorafenib response (Tandia et al., 2017). Another study looked at the effects of intronic polymorphisms on methotrexate therapy. Two polymorphisms were identified with an enhanced response,

5566 C>T in intron 2 and 3531 G>C in intron 9. The 18271 G>A (rs1564481) polymorphism was shown to correlate with the pharmacokinetics of, and improved response to, therapy with flavopiridol, a CDK9 inhibitor used in the treatment of acute myeloid leukaemia (Ni et al., 2010). No effect on flavopiridol pharmacokinetics was found for other intron polymorphisms 152 G>A, 10130 A>G, 14952 G>T and 18271 G>A.

Promoter mutations may affect expression via effects on the transcription of the *ABCG2* gene. The -15622 C>T polymorphism has been shown to lower ABCG2 protein expression in tissue (Poonkuzhali et al., 2008). Both heterozygous and homozygous haplotypes result in a 40-50% increases in maximum concentration in patients treated with erlotinib, along with increased incidence of side effects such as diarrhoea (Rudin et al., 2008). An increased risk of side effects was also shown in gefitinib therapy, although Lemos did not find any link between the TT genotype and therapy outcomes (Lemos et al., 2011). Conversely, the -15994 C>T (rs7699188) polymorphism significantly increases ABCG2 expression. These effects are possibly due to the gain of an HNF4 (hepatocyte nuclear factor 4) binding site, a transcription factor which may be involved in ABCG2 expression. The increased expression as a result of this polymorphism has been shown to increase the clearance of oral imatinib *in vivo* (Poonkuzhali et al., 2008).

Junior Blood Group

As well as its influence on pharmacokinetics, ABCG2 has recently been explained as having a key role in the Junior (Jr) blood group system, which is especially common in Asian populations. Several millions of people are predicted to not express *ABCG2*, producing the Jr(a-) blood group, because the Jr antigen is within the ABCG2 protein (Saison et al., 2012; Zelinski et al., 2012). Antibodies to the Jr(a-) blood group may result in blood transfusion reactions, but do not commonly cause severe haemolytic disease of the new-born, despite some reports of this (Zelinski et al., 2012). The Jr(a-) blood group is induced by *ABCG2* null alleles, which lead to an insertion of a premature stop codon, such as the Q126X (376 C>T) polymorphism (Honjo et al., 2002; Zelinski et al., 2012). These individuals have no ABCG2 expression of the surface of their erythrocytes. Homozygous Q126X was detected in 80% of Jr(a-) blood samples in a recent study in a Japanese population (Tanaka et al., 2017). Saison also suggested that the R236X polymorphism (706 C>T) may be the basis of Jr(a-) blood type in Romani Gypsy diaspora communities of Southwestern Europe (Saison et al., 2012), and a homozygous R246X (736 C>T) variant has been found, resulting in Jr(a-) erythrocytes in two Caucasian individuals. Heterozygous R246X was shown to lower ABCG2 expression approximately 50% in vivo during flow cytometry analysis of erythrocytes (Kasza et al., 2012). Along with these, it is predicted that the E334X variant also produces Jr(a-)

DMD Fast Forward. Published on September 28, 2018 as DOI: 10.1124/dmd.118.083030
This article has not been copyedited and formatted. The final version may differ from this version.

blood group (Zelinski et al., 2012). Recently, an increasing catalogue of alleles has been linked to the Jr blood group, with studies by Hue-Roye reporting R113X, G262X, Q531X and S340del as mutations causing Jr(a-) (Zelinski et al., 2012). It is not known yet whether individuals with the Jr(a-) blood group have altered pharmacokinetics of drugs, although they would be expected to have similar pharmacokinetics to those detailed in the Q126X section above. An interesting line of research would be the differences in drug disposition and treatment outcomes in populations with/without the Jr(a-) group.

Perspectives for future research

The effects of the Q141K polymorphism are well-researched, with a variety of substrate drugs being shown to have altered pharmacokinetics. These effects are likely caused by the decreased ABCG2 expression in the membrane in the various ABCG2 sites, a result of increased instability and degradation of this isoform. The influence of Q141K on pharmacokinetics may be a result of increased oral availability (e.g. due to reduced excretion in the small intestine), decreased efflux of drugs from the target cells or decreased transport across barriers (e.g. BBB).

Summarising the effects of the less common polymorphisms in the NBD and polymorphisms in the MSD (see table 3) shows varied conclusions, with polymorphisms found to increase, decrease or have no change on levels of ABCG2 expression *in vitro*, but there is limited research into their effects on drug therapy. More research into the *in vivo* effects of these polymorphisms on ABCG2 expression and drug efficacy is required before any definitive conclusions can be drawn. An ideal outcome of research into the *ABCG2* polymorphisms would be to establish a grouping system for polymorphisms, based on their characteristics of protein expression, efflux activity and substrate-specific effects. One grouping method was presented previously, based upon protein expression, transport activity, drug resistance and prazosin-stimulated ATPase activity (Tamura et al., 2007a). Based on the accumulation of limited research into rarer polymorphisms, we propose an updated six-group system based on ABCG2 expression and effects on activity or resistance (Table 4). To substantiate and validate this though requires much more work on the rarer SNPs.

Where will such information come from? One novel method involves the use of antibody-based flow cytometry analysis to quantify the levels of ABCG2 in erythrocytes via a blood sample, coupled with genomic analysis of the DNA in the same sample. The use of blood samples could be a well-tolerated method of analysing ABCG2 levels as it is non-invasive and quantifiable with good anti-ABCG2 antibodies. This method has been used to describe significant decreases in protein expression in heterozygous Q141K and R236X carriers

(Kasza et al., 2012). This proved the method could be successful in showing the varying tissue levels of ABCG2 for differing genotypes. An expansion of this method to discover the *in vivo* expression levels of the many other natural polymorphisms detailed in this review would be an ideal next step in research, as it would remove the more subjective interpretation of cell line studies. Complementary functional studies in cell lines do have an important role to play as long as changes in transport are correlated with the ABCG2 expression level to ensure that an accurate picture of a SNP's effect on drug transport is obtained. One additional question that is not often discussed in the literature is the effect of heterozygous allele in the final assembled ABCG2 dimer. Specifically, in a heterozygous individual there is a possibility that following translation and trafficking ABCG2 dimers are present in three forms: WT-SNP, SNP-SNP, or WT-WT. Given that translation occurs through the action of polyribosomes on the same mRNA is seems feasible that SNP-SNP ABCG2 dimers and WT-WT dimers would occur rather than "mixed-dimers", but this remains to be determined. Finally, better structural data now sets the scene for molecular docking approaches that should be able to rationalise effects in patients and enable better predictions regarding possible drug interactions with this important MDR pump.

Acknowledgements

The authors thank other members of the Kerr lab for informative discussions, particularly Megan Cox and Aaron Horsey.

References

Adkison KK, Vaidya SS, Lee DY, Koo SH, Li L, Mehta AA, Gross AS, Polli JW, Humphreys JE, Lou Y and Lee EJD (2010) Oral sulfasalazine as a clinical BCRP probe substrate: Pharmacokinetic effects of genetic variation (C421A) and pantoprazole coadministration. *Journal of Pharmaceutical Sciences* **99**:1046-1062.

Adkison KK, Vaidya SS, Lee DY, Koo SH, Li L, Mehta AA, Gross AS, Polli JW, Lou Y and Lee EJ (2008) The ABCG2 C421A polymorphism does not affect oral nitrofurantoin pharmacokinetics in healthy Chinese male subjects. *Br J Clin Pharmacol* **66**:233-239.

Akasaka K, Kaburagi T, Yasuda Si, Ohmori K, Abe K, Sagara H, Ueda Y, Nagao K, Imura J and Imai Y (2010) Impact of functional ABCG2 polymorphisms on the adverse effects

of gefitinib in Japanese patients with non-small-cell lung cancer. *Cancer Chemotherapy and Pharmacology* **66**:691-698.

Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V and Dean M (1998) A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. *Cancer Research* **58**:5337-5339.

Ascherio A, LeWitt PA, Xu K, Eberly S, Watts A, Matson WR, Marras C, Kieburtz K, Rudolph A, Bogdanov MB, Schwid SR, Tennis M, Tanner CM, Beal MF, Lang AE, Oakes D, Fahn S, Shoulson I, Schwarzschild MA and Parkinson Study Group DI (2009) Urate as a predictor of the rate of clinical decline in Parkinson disease. *Arch Neurol* **66**:1460-1468.

Au A, Aziz Baba A, Goh AS, Wahid Fadilah SA, Teh A, Rosline H and Ankathil R (2014) Association of genotypes and haplotypes of multi-drug transporter genes ABCB1 and ABCG2 with clinical response to imatinib mesylate in chronic myeloid leukemia patients. *Biomedicine and Pharmacotherapy* **68**:343-349.

Bailey-Dell KJ, Hassel B, Doyle La and Ross DD (2001) Promoter characterization and genomic organization of the human breast cancer resistance protein (ATP-binding cassette transporter G2) gene. *Biochimica et biophysica acta* **1520**:234-241.

Baker SD, Verweij J, Cusatis Ga, van Schaik RH, Marsh S, Orwick SJ, Franke RM, Hu S, Schuetz EG, Lamba V, Messersmith Wa, Wolff aC, Carducci Ma and Sparreboom a (2009) Pharmacogenetic pathway analysis of docetaxel elimination. *Clinical pharmacology and therapeutics* **85**:155-163.

Basseville A, Tamaki A, Ierano C, Trostel S, Ward Y, Robey RW, Hegde RS and Bates SE (2012) Histone deacetylase inhibitors influence chemotherapy transport by modulating expression and trafficking of a common polymorphic variant of the ABCG2 efflux transporter. *Cancer Research* **72**:3642-3651.

Benderra Z, Faussat AM, Sayada L, Perrot JY, Chaoui D, Marie JP and Legrand O (2004) Breast cancer resistance protein and P-glycoprotein in 149 adult acute myeloid leukemias. *Clin Cancer Res* **10**:7896-7902.

Bircsak KM, Gupta V, Yuen PYS, Gorczyca L, Weinberger BI, Vetrano AM and Aleksunes LM (2016) Genetic and Dietary Regulation of Glyburide Efflux by the Human Placental Breast Cancer Resistance Protein Transporter. *The Journal of pharmacology and experimental therapeutics* **357**:103-113.

Birmingham BK, Bujac SR, Elsby R, Azumaya CT, Wei C, Chen Y, Mosqueda-Garcia R and Ambrose HJ (2015) Impact of ABCG2 and SLCO1B1 polymorphisms on pharmacokinetics of rosuvastatin, atorvastatin and simvastatin acid in Caucasian and Asian subjects: A class effect? *European Journal of Clinical Pharmacology* **71**:341-355.

Chen WQ, Shu Y, Li Q, Xu LY, Roederer MW, Fan L, Wu LX, He FZ, Luo JQ, Tan ZR, He YJ, Zhou HH, Chen X and Zhang W (2013) Polymorphism of ORM1 is associated with the pharmacokinetics of telmisartan. *Plos One* **8**:e70341.

Chen X, Chen D, Yang S, Ma R, Pan Y, Li X and Ma S (2015) Impact of ABCG2 polymorphisms on the clinical outcome of TKIs therapy in Chinese advanced non-small-cell lung cancer patients. *Cancer Cell International* **15**:43.

Chew SC, Singh O, Chen X, Ramasamy RD, Kulkarni T, Lee EJD, Tan EH, Lim WT and Chowbay B (2011) The effects of CYP3A4, CYP3A5, ABCB1, ABCC2, ABCG2 and SLCO1B3 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of docetaxel in nasopharyngeal carcinoma patients. *Cancer Chemotherapy and Pharmacology* **67**:1471-1478.

Choi HY, Bae K-S, Cho S-H, Ghim J-L, Choe S, Jung JA, Jin S-J, Kim H-S and Lim H-S (2015) Impact of CYP2D6, CYP3A5, CYP2C19, CYP2A6, SLCO1B1, ABCB1, and ABCG2 gene polymorphisms on the pharmacokinetics of simvastatin and simvastatin acid. *Pharmacogenetics and Genomics* **25**:595-608.

Clark R, Kerr ID and Callaghan R (2006) Multiple drugbinding sites on the R482G isoform of the ABCG2 transporter. *British journal of pharmacology* **149**:506-515.

Cleophas MC, Joosten LA, Stamp LK, Dalbeth N, Woodward OM and Merriman TR (2017) ABCG2 polymorphisms in gout: Insights into disease susceptibility and treatment approaches. *Pharmacogenomics and Personalized Medicine* **10**:129-142.

Colombo S, Soranzo N, Rotger M, Sprenger R, Bleiber G, Furrer H, Buclin T, Goldstein D, Decosterd L and Telenti A (2005) Influence of ABCB1, ABCC1, ABCC2, and ABCG2 haplotypes on the cellular exposure of nelfinavir in vivo. *Pharmacogenetics and genomics* **15**:599-608.

Cooray HC, Blackmore CG, Maskell L and Barrand MA (2002) Localisation of breast cancer resistance protein in microvessel endothelium of human brain. *Neuroreport* **13**:2059-2063.

Cox MH, Kapoor P, Briggs DA and Kerr ID (2018) *Biochemical Journal* **in press**.

Cui L, Hou YX, Riordan JR and Chang XB (2001) Mutations of the Walker B motif in the first nucleotide binding domain of multidrug resistance protein MRP1 prevent conformational maturation. *Archives of biochemistry and biophysics* **392**:153-161.

Cusatis G, Gregorc V, Li J, Spreafico A, Ingersoll RG, Verweij J, Ludovini V, Villa E, Hidalgo M, Sparreboom A and Baker SD (2006) Pharmacogenetics of ABCG2 and adverse reactions to gefitinib. *Journal of the National Cancer Institute* **98**:1739-1742.

De Boeck K, Zolin A, Cuppens H, Olesen HV and Viviani L (2014) The relative frequency of CFTR mutation classes in European patients with cystic fibrosis. *Journal of Cystic Fibrosis* **13**:403-409.

De Jong FA, Marsh S, Mathijssen RHJ, King C, Verweij J, Sparreboom A and McLeod HL (2004) ABCG2 pharmacogenetics: Ethnic differences in allele frequency and assessment of influence on irinotecan disposition. *Clinical Cancer Research* **10**:5889-5894.

Dean M, Rzhetsky A and Allikmets R (2001) The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res* **11**:1156-1166.

Deppe S, Ripperger A, Weiss J, Ergün S and Benndorf RA (2014) Impact of genetic variability in the ABCG2 gene on ABCG2 expression, function, and interaction with

AT1 receptor antagonist telmisartan. *Biochemical and Biophysical Research Communications* **443**:1211-1217.

Ding R, Jin S, Pabon K and Scotto KW (2016) A role for ABCG2 beyond drug transport: Regulation of autophagy. *Autophagy* **12**:737-751.

Do TM, Noel-Hudson MS, Ribes S, Besengez C, Smirnova M, Cisternino S, Buyse M, Calon F, Chimini G, Chacun H, Scherrmann JM, Farinotti R and Bourasset F (2012) ABCG2- and ABCG4-mediated efflux of amyloid-beta peptide 1-40 at the mouse blood-brain barrier. *J Alzheimers Dis* **30**:155-166.

Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK and Ross DD (1998) A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proceedings of the National Academy of Sciences of the United States of America* **95**:15665-15670.

Faneyte IF, Kristel PMP, Maliepaard M, Scheffer GL, Scheper RJ, Schellens JHM and van de Vijver MJ (2002) Expression of the breast cancer resistance protein in breast cancer. *Clinical cancer research* **8**:1068-1074.

Fehér Á, Juhász A, László A, Pákáski M, Kálmán J and Janka Z (2013) Association between the ABCG2 C421A polymorphism and Alzheimer's disease. *Neuroscience Letters* **550**:51-54.

Francis J, Dubashi B, Sundaram R, Pradhan SC and Chandrasekaran A (2015) Influence of Sokal, Hasford, EUTOS scores and pharmacogenetic factors on the complete cytogenetic response at 1??year in chronic myeloid leukemia patients treated with imatinib. *Medical Oncology* **32**:213.

Fukao M, Ishida K, Sakamoto T, Taguchi M, Matsukura H, Miyawaki T and Hashimoto Y (2011) Effect of genetic polymorphisms of SLC28A1, ABCG2, and ABCC4 on bioavailability of mizoribine in healthy Japanese males. *Drug metabolism and pharmacokinetics* **26**:538-543.

Fukudo M, Ikemi Y, Togashi Y, Masago K, Kim YH, Mio T, Terada T, Teramukai S, Mishima M, Inui K-I and Katsura T (2013) Population pharmacokinetics/pharmacodynamics of

erlotinib and pharmacogenomic analysis of plasma and cerebrospinal fluid drug concentrations in Japanese patients with non-small cell lung cancer. *Clinical Pharmacokinetics* **52**:593-609.

Furukawa T, Wakabayashi K, Tamura A, Nakagawa H, Morishima Y, Osawa Y and Ishikawa T (2009) Major SNP (Q141K) variant of human ABC transporter ABCG2 undergoes lysosomal and proteasomal degradations. *Pharmaceutical Research* **26**:469-479.

Gardner ER, Burger H, van Schaik RH, van Oosterom AT, de Bruijn EA, Guetens G, Prenen H, de Jong FA, Baker SD, Bates SE, Figg WD, Verweij J, Sparreboom A and Nooter K (2006) Association of enzyme and transporter genotypes with the pharmacokinetics of imatinib. *Clinical Pharmacology and Therapeutics* **80**:192-201.

Geng F, Jiao Z, Dao YJ, Qiu XY, Ding JJ, Shi XJ, Li ZD and Zhong MK (2012) The association of the UGT1A8, SLCO1B3 and ABCC2/ABCG2 genetic polymorphisms with the pharmacokinetics of mycophenolic acid and its phenolic glucuronide metabolite in Chinese individuals. *Clinica Chimica Acta* **413**:683-690.

Gervasini G, de Murillo SG, Jiménez M, de la Maya MD and Vagace JM (2017) Effect of polymorphisms in transporter genes on dosing, efficacy and toxicity of maintenance therapy in children with acute lymphoblastic leukemia. *Gene* **628**:72-77.

Ghafouri H, Ghaderi B, Amini S, Nikkhoo B, Abdi M and Hoseini A (2016) Association of ABCB1 and ABCG2 single nucleotide polymorphisms with clinical findings and response to chemotherapy treatments in Kurdish patients with breast cancer. *Tumor Biology* **37**:7901-7906.

Gong IY and Kim RB (2013) Importance of pharmacokinetic profile and variability as determinants of dose and response to dabigatran, rivaroxaban, and apixaban. *Canadian Journal of Cardiology* **29**:S24-S33.

Gong IY, Mansell SE and Kim RB (2013) Absence of both MDR1 (ABCB1) and Breast Cancer Resistance Protein (ABCG2) Transporters Significantly Alters Rivaroxaban Disposition and Central Nervous System Entry. *Basic and Clinical Pharmacology and Toxicology* **112**:164-170.

Gotanda K, Tokumoto T, Hirota T, Fukae M and Ieiri I (2015) Sulfasalazine disposition in a subject with 376C>T (nonsense mutation) and 421C>A variants in the ABCG2 gene. *British Journal of Clinical Pharmacology* **80**:1236-1237.

Hampras SS, Sucheston L, Weiss J, Baer MR, Zirpoli G, Singh PK, Wetzler M, Chennamaneni R, Blanco JG, Ford L and Moysich KB (2010) Genetic polymorphisms of ATP-binding cassette (ABC) proteins, overall survival and drug toxicity in patients with Acute Myeloid Leukemia. *International journal of molecular epidemiology and genetics* **1**:201-207.

Han JY, Lim HS, Yoo YK, Eun SS, Yong HP, Sung YL, Lee JE, Dea HL, Heung TK and Jin SL (2007) Associations of ABCB1, ABCC2, and ABCG2 polymorphisms with irinotecan-pharmacokinetics and clinical outcome in patients with advanced non-small cell lung cancer. *Cancer* **110**:138-147.

Haslam IS, El-Chami C, Faruqi H, Shahmalak A, O'Neill CA and Paus R (2015) Differential expression and functionality of ATP-binding cassette transporters in the human hair follicle. *Br J Dermatol* **172**:1562-1572.

Higashino T, Takada T, Nakaoka H, Toyoda Y, Stiburkova B, Miyata H, Ikebuchi Y, Nakashima H, Shimizu S, Kawaguchi M, Sakiyama M, Nakayama A, Akashi A, Tanahashi Y, Kawamura Y, Nakamura T, Wakai K, Okada R, Yamamoto K, Hosomichi K, Hosoya T, Ichida K, Ooyama H, Suzuki H, Inoue I, Merriman TR, Shinomiya N and Matsuo H (2017) Multiple common and rare variants of ABCG2 cause gout. *RMD Open* **3**:e000464.

Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, Goodell MA and Brenner MK (2004) A distinct "side population" of cells with high drug efflux capacity in human tumor cells. *Proc Natl Acad Sci U S A* **101**:14228-14233.

Hodin S, Basset T, Jacqueroux E, Delezay O, Clotagatide A, Perek N, Mismetti P and Delavenne X (2018) In Vitro Comparison of the Role of P-Glycoprotein and Breast Cancer Resistance Protein on Direct Oral Anticoagulants Disposition. *European Journal of Drug Metabolism and Pharmacokinetics* **43**:183-191.

Honjo Y, Hrycyna CA, Yan QW, Medina-Pérez WY, Robey RW, Van de Laar A, Litman T, Dean M and Bates SE (2001) Acquired mutations in the MXR/BCRP/ABCP gene alter substrate specificity in MXR/BCRP/ABCP-overexpressing cells. *Cancer Research* **61**:6635-6639.

Honjo Y, Morisaki K, Mickley Huff L, Robey RW, Hung J, Dean M and Bates SE (2002) Single-Nucleotide Polymorphism (SNP) analysis in the ABC half-transporter ABCG2 (MXR/BCRP/ABCP1). *Cancer Biology and Therapy* **1**:696-702.

Horsey AJ, Cox MH, Sarwat S and Kerr ID (2016) The multidrug transporter ABCG2: still more questions than answers. *Biochemical Society Transactions* **44**:824-830.

Hu L-L, Wang X-X, Chen X, Chang J, Li C, Zhang Y, Yang J, Jiang W and Zhuang S-M (2007) BCRP gene polymorphisms are associated with susceptibility and survival of diffuse large B-cell lymphoma. *Carcinogenesis* **28**:1740-1744.

Huang L, Be X, Tchaparian EH, Colletti AE, Roberts J, Langley M, Ling Y, Wong BK and Jin L (2012) Deletion of Abcg2 Has Differential Effects on Excretion and Pharmacokinetics of Probe Substrates in Rats. *Journal of Pharmacology and Experimental Therapeutics* **343**:316-324.

Huss WJ, Gray DR, Greenberg NM, Mohler JL and Smith GJ (2005) Breast cancer resistance protein-mediated efflux of androgen in putative benign and malignant prostate stem cells. *Cancer Research* **65**:6640-6650.

Ichida K, Matsuo H, Takada T, Nakayama A, Murakami K, Shimizu T, Yamanashi Y, Kasuga H, Nakashima H, Nakamura T, Takada Y, Kawamura Y, Inoue H, Okada C, Utsumi Y, Ikebuchi Y, Ito K, Nakamura M, Shinohara Y, Hosoyamada M, Sakurai Y, Shinomiya N, Hosoya T and Suzuki H (2012) Decreased extra-renal urate excretion is a common cause of hyperuricemia. *Nat Commun* **3**:764.

Ieiri I, Suwannakul S, Maeda K, Uchimaru H, Hashimoto K, Kimura M, Fujino H, Hirano M, Kusuhara H, Irie S, Higuchi S and Sugiyama Y (2007) SLCO1B1 (OATP1B1, an Uptake Transporter) and ABCG2 (BCRP, an Efflux Transporter) Variant Alleles and

Pharmacokinetics of Pitavastatin in Healthy Volunteers. *Clinical Pharmacology & Therapeutics* **82**:541-547.

Ieiri I, Yamasaki Y, Sasaki T, Hirota T, Higuchi S, Kimura M, Irie S, Kusuhara H and Sugiyama Y (2008) Pharmacogenetic characterization of sulfasalazine disposition based on NAT2 and ABCG2 (BCRP) gene polymorphisms in humans. *Japanese Pharmacology and Therapeutics* **36**:131-134.

Imai Y, Nakane M, Kage K, Tsukahara S, Ishikawa E, Tsuruo T, Miki Y and Sugimoto Y (2002) C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. *Molecular cancer therapeutics* **1**:611-616.

Itoda M, Saito Y, Shirao K, Minami H, Ohtsu A, Yoshida T, Saijo N, Suzuki H, Sugiyama Y, Ozawa S and Sawada J-i (2003) Eight novel single nucleotide polymorphisms in ABCG2/BCRP in Japanese cancer patients administered irinotacan. *Drug metabolism and pharmacokinetics* **18**:212-217.

Jackson SM, Manolaridis I, Kowal J, Zechner M, Taylor NMI, Bause M, Bauer S, Bartholomaeus R, Bernhardt G, Koenig B, Buschauer A, Stahlberg H, Altmann KH and Locher KP (2018) Structural basis of small-molecule inhibition of human multidrug transporter ABCG2. *Nat Struct Mol Biol* **25**:333-340.

Jada SR, Lim R, Wong CI, Shu X, Lee SC, Zhou Q, Goh BC and Chowbay B (2007) Role of UGT1A1*6, UGT1A1*28 and ABCG2 c.421C>A polymorphisms in irinotecan-induced neutropenia in Asian cancer patients. *Cancer Science* **98**:1461-1467.

Jandu H, Aluzaite K, Fogh L, Thrane SW, Noer JB, Proszek J, Do KN, Hansen SN, Damsgaard B, Nielsen SL, Stougaard M, Knudsen BR, Moreira J, Hamerlik P, Gajjar M, Smid M, Martens J, Foekens J, Pommier Y, Brünner N, Schrohl A-S and Stenvang J (2016) Molecular characterization of irinotecan (SN-38) resistant human breast cancer cell lines. *BMC Cancer* **16**:34.

Jiang Z-P, Zhao X-L, Takahashi N, Angelini S, Dubashi B, Sun L and Xu P (2017) Trough concentration and ABCG2 polymorphism are better to predict imatinib response in chronic myeloid leukemia: a meta-analysis. *Pharmacogenomics* **18**:35-56.

Jonker JW, Buitelaar M, Wagenaar E, Van Der Valk Ma, Scheffer GL, Scheper RJ, Plosch T, Kuipers F, Elferink RPJO, Rosing H, Beijnen JH and Schinkel AH (2002) The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. *Proceedings of the National Academy of Sciences of the United States of America* **99**:15649-15654.

Kannangara DR, Phipps-Green AJ, Dalbeth N, Stamp LK, Williams KM, Graham GG, Day RO and Merriman TR (2016) Hyperuricaemia: contributions of urate transporter ABCG2 and the fractional renal clearance of urate. *Ann Rheum Dis* **75**:1363-1366.

Kasza I, Várady G, Andrikovics H, Koszarska M, Tordai A, Scheffer GL, Németh A, Szakács G and Sarkadi B (2012) Expression Levels of the ABCG2 Multidrug Transporter in Human Erythrocytes Correspond to Pharmacologically Relevant Genetic Variations. *Plos One* **7**.

Kato R, Kato Y, Matsuura T, Kanehira M, Takata R and Obara W (2017) Characteristics of early-onset hematotoxicity of sunitinib in Japanese patients with renal cell carcinoma. *BMC Cancer* **17**:214.

Kawahara H, Noguchi K, Katayama K, Mitsuhashi J and Sugimoto Y (2010) Pharmacological interaction with sunitinib is abolished by a germ-line mutation (1291T>C) of BCRP/ABCG2 gene. *Cancer Science* **101**:1493-1500.

Keskitalo JE, Pasanen MK, Neuvonen PJ and Niemi M (2009a) Different effects of the ABCG2 c.421C>A SNP on the pharmacokinetics of fluvastatin, pravastatin and simvastatin. *Pharmacogenomics* **10**:1617-1624.

Keskitalo JE, Zolk O, Fromm MF, Kurkinen KJ, Neuvonen PJ and Niemi M (2009b) ABCG2 Polymorphism Markedly Affects the Pharmacokinetics of Atorvastatin and Rosuvastatin. *Clinical Pharmacology & Therapeutics* **86**:197-203.

Khunweeraphong N, Stockner T and Kuchler K (2017) The structure of the human ABC transporter ABCG2 reveals a novel mechanism for drug extrusion. *Sci Rep* **7**:13767.

Kim CO, Cho SK, Oh ES, Park MS and Chung J-Y (2012) Influence of ABCC2, SLCO1B1, and ABCG2 polymorphisms on the pharmacokinetics of olmesartan. *Journal of cardiovascular pharmacology* **60**:49-54.

Kim DH, Sriharsha L, Xu W, Kamel-Reid S, Liu X, Siminovitch K, Messner HA and Lipton JH (2009a) Clinical Relevance of a Pharmacogenetic Approach Using Multiple Candidate Genes to Predict Response and Resistance to Imatinib Therapy in Chronic Myeloid Leukemia. *Clinical Cancer Research* **15**:4750-4758.

Kim HS, Sunwoo YE, Ryu JY, Kang HJ, Jung HE, Song IS, Kim EY, Shim JC, Shon JH and Shin JG (2007) The effect of ABCG2 V12M, Q141K and Q126X, known functional variants in vitro, on the disposition of lamivudine. *British Journal of Clinical Pharmacology* **64**:645-654.

Kim IS, Kim HG, Kim DC, Eom HS, Kong SY, Shin HJ, Hwang SH, Lee EY and Lee GW (2008) ABCG2 Q141K polymorphism is associated with chemotherapy-induced diarrhea in patients with diffuse large B-cell lymphoma who received frontline rituximab plus cyclophosphamide/doxorubicin/vincristine/ prednisone chemotherapy. *Cancer Science* **99**:2496-2501.

Kim JE, Singh RR, Cho-Vega JH, Drakos E, Davuluri Y, Khokhar FA, Fayad L, Medeiros LJ and Vega F (2009b) Sonic hedgehog signaling proteins and ATP-binding cassette G2 are aberrantly expressed in diffuse large B-cell lymphoma. *Mod Pathol* **22**:1312-1320.

Kim KA, Joo HJ and Park JY (2011) Effect of ABCG2 genotypes on the pharmacokinetics of A771726, an active metabolite of prodrug leflunomide, and association of A771726 exposure with serum uric acid level. *European Journal of Clinical Pharmacology* **67**:129-134.

Kobayashi D, Ieiri I, Hirota T, Takane H, Maegawa S, Kigawa J, Suzuki H, Nanba E, Oshimura M, Terakawa N, Otsubo K, Mine K and Sugiyama Y (2005) Functional

assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. *Drug metabolism and disposition* **33**:94-101.

Kondo C, Suzuki H, Itoda M, Ozawa S, Sawada JI, Kobayashi D, Ieiri I, Mine K, Ohtsubo K and Sugiyama Y (2004) Functional analysis of SNPs variants of BCRP/ABCG2. *Pharmaceutical Research* **21**:1895-1903.

Krishnamurthy P, Ross DD, Nakanishi T, Bailey-Dell K, Zhou S, Mercer KE, Sarkadi B, Sorrentino BP and Schuetz JD (2004a) The stem cell marker Bcrp/ABCG2 enhances hypoxic cell survival through interactions with heme. *J Biol Chem* **279**:24218-24225.

Krishnamurthy P, Ross DD, Nakanishi T, Bailey-Dell K, Zhou S, Mercer KE, Sarkadi B, Sorrentino BP and Schuetz JD (2004b) The stem cell marker Bcrp/ABCG2 enhances hypoxic cell survival through interactions with heme. *Journal of Biological Chemistry* **279**:24218-24225.

Laszlo L, Sarkadi B and Hegedus T (2016) Jump into a New Fold-A Homology Based Model for the ABCG2/BCRP Multidrug Transporter. *Plos One* **11**:e0164426.

Lee CA, O'Connor MA, Ritchie TK, Galetin A, Cook JA, Ragueneau-Majlessi I, Ellens H, Feng B, Taub ME, Paine MF, Polli JW, Ware JA and Zamek-Gliszczynski MJ (2015) Breast cancer resistance protein (ABCG2) in clinical pharmacokinetics and drug interactions: practical recommendations for clinical victim and perpetrator drug-drug interaction study design. *Drug Metab Dispos* **43**:490-509.

Lee H-K, Hu M, Lui SS, Ho C-S, Wong C-K and Tomlinson B (2013) Effects of polymorphisms in ABCG2 , SLCO1B1 , SLC10A1 and CYP2C9 / 19 on plasma concentrations of rosuvastatin and lipid response in Chinese patients. *Pharmacogenomics* **14**:1283-1294.

Lemos C, Giovannetti E, Zucali Pa, Assaraf YG, Scheffer GL, van der Straaten T, D'Incecco A, Falcone A, Guchelaar H-J, Danesi R, Santoro A, Giaccone G, Tibaldi C and Peters GJ (2011) Impact of ABCG2 polymorphisms on the clinical outcome and toxicity of gefitinib in non-small-cell lung cancer patients. *Pharmacogenomics* **12**:159-170.

Li J, Cusatis G, Brahmer J, Sparreboom A, Robey RW, Bates SE, Hidalgo M and Baker SD (2007) Association of variant ABCG2 and the pharmacokinetics of epidermal growth factor receptor tyrosine kinase inhibitors in cancer patients. *Cancer Biol Ther* **6**:432-438.

Li N, Song Y, Du P, Shen Y, Yang J, Gui L, Wang S, Wang J, Sun Y, Han X and Shi Y (2013) Oral topotecan: Bioavailability, pharmacokinetics and impact of ABCG2 genotyping in Chinese patients with advanced cancers. *Biomedicine and Pharmacotherapy* **67**:801-806.

Li R and Barton HA (2018) Explaining Ethnic Variability of Transporter Substrate Pharmacokinetics in Healthy Asian and Caucasian Subjects with Allele Frequencies of OATP1B1 and BCRP: A Mechanistic Modeling Analysis. *Clinical Pharmacokinetics* **57**:491-503.

Li R, Miao L, Qin L, Xiang Y, Zhang X, Peng H, Mailamuguli, Sun Y and Yao H (2015) A meta-analysis of the associations between the Q141K and Q126X ABCG2 gene variants and gout risk. *International Journal of Clinical and Experimental Pathology* **8**:9812-9823.

Liu M, Wu X-J, Zhao G-L, Zhang T, Xu S-S, Sun Y-X, Qiu F and Zhao L-M (2016) Effects of Polymorphisms in NR1H4, NR1I2, SLCO1B1, and ABCG2 on the Pharmacokinetics of Rosuvastatin in Healthy Chinese Volunteers. *Journal of cardiovascular pharmacology* **68**:383-390.

Low SK, Fukunaga K, Takahashi A, Matsuda K, Hongo F, Nakanishi H, Kitamura H, Inoue T, Kato Y, Tomita Y, Fukasawa S, Tanaka T, Nishimura K, Uemura H, Hara I, Fujisawa M, Matsuyama H, Hashine K, Tatsugami K, Enokida H, Kubo M, Miki T and Mushiroda T (2016) Association study of a functional variant on ABCG2 gene with sunitinib-induced severe adverse drug reaction. *Plos One* **11**:e0148177.

Maliepaard M, Scheffer G, Faneyte I, van Gastelen M, Pijnenborg A, Schinkel A, van De Vijver M, Scheper R and Schellens J (2001) Subcellular localization and distribution

of the breast cancer resistance protein transporter in normal human tissues. *Cancer research* **61**:3458-3464.

Mao Q and Unadkat JD (2015) Role of the Breast Cancer Resistance Protein (BCRP/ABCG2) in Drug Transport—an Update. *The AAPS Journal* **17**:65-82.

Matsuo H, Takada T, Ichida K, Nakamura T, Nakayama A, Ikebuchi Y, Ito K, Kusanagi Y, Chiba T, Tadokoro S, Takada Y, Oikawa Y, Inoue H, Suzuki K, Okada R, Nishiyama J, Domoto H, Watanabe S, Fujita M, Morimoto Y, Naito M, Nishio K, Hishida A, Wakai K, Asai Y, Niwa K, Kamakura K, Nonoyama S, Sakurai Y, Hosoya T, Kanai Y, Suzuki H, Hamajima N and Shinomiya N (2009) Common defects of ABCG2, a high-capacity urate exporter, cause gout: a function-based genetic analysis in a Japanese population. *Sci Transl Med* **1**:5ra11.

Matsuo H, Tomiyama H, Satake W, Chiba T, Onoue H, Kawamura Y, Nakayama A, Shimizu S, Sakiyama M, Funayama M, Nishioka K, Shimizu T, Kaida K, Kamakura K, Toda T, Hattori N and Shinomiya N (2015) ABCG2 variant has opposing effects on onset ages of Parkinson's disease and gout. *Annals of Clinical and Translational Neurology* **2**:302-306.

Memon N, Bircsak KM, Archer F, Gibson CJ, Ohman-Strickland P, Weinberger BI, Parast MM, Vetrano AM and Aleksunes LM (2014) Regional expression of the BCRP/ABCG2 transporter in term human placentas. *Reproductive Toxicology* **43**:72-77.

Miura M, Kagaya H, Satoh S, Inoue K, Saito M, Habuchi T and Suzuki T (2008) Influence of drug transporters and UGT polymorphisms on pharmacokinetics of phenolic glucuronide metabolite of mycophenolic acid in Japanese renal transplant recipients. *Therapeutic drug monitoring* **30**:559-564.

Miyake K, Mickley L, Litman T, Zhan Z, Robey R, Cristensen B, Brangi M, Greenberger L, Dean M, Fojo T and Bates SE (1999) Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: Demonstration of homology to ABC transport genes. *Cancer Research* **59**:8-13.

Mizuarai S, Aozasa N and Kotani H (2004) Single nucleotide polymorphisms result in impaired membrane localization and reduced atpase activity in multidrug transporter ABCG2. *International Journal of Cancer* **109**:238-246.

Mizuno T, Fukudo M, Terada T, Kamba T, Nakamura E, Ogawa O, Inui K-i and Katsura T (2012) Impact of Genetic Variation in Breast Cancer Resistance Protein (BCRP/ABCG2) on Sunitinib Pharmacokinetics. *Drug Metabolism and Pharmacokinetics* **27**:631-639.

Mo W and Zhang JT (2012) Human ABCG2: Structure, function, and its role in multidrug resistance. *International Journal of Biochemistry and Molecular Biology* **3**:1-27.

Morisaki K, Robey RW, Özvegy-Laczka C, Honjo Y, Polgar O, Steadman K, Sarkadi B and Bates SE (2005) Single nucleotide polymorphisms modify the transporter activity of ABCG2. *Cancer Chemotherapy and Pharmacology* **56**:161-172.

Mueck W, Kubitza D and Becka M (2013) Co-administration of rivaroxaban with drugs that share its elimination pathways: Pharmacokinetic effects in healthy subjects. *British Journal of Clinical Pharmacology* **76**:455-466.

Nakagawa H, Tamura A, Wakabayashi K, Hoshijima K, Komada M, Yoshida T, Kometani S, Matsubara T, Mikuriya K and Ishikawa T (2008) Ubiquitin-mediated proteasomal degradation of non-synonymous SNP variants of human ABC transporter ABCG2. *The Biochemical journal* **411**:623-631.

Nakagawa H, Wakabayashi-Nakao K, Tamura A, Toyoda Y, Koshiba S and Ishikawa T (2009) Disruption of N-linked glycosylation enhances ubiquitin-mediated proteasomal degradation of the human ATP-binding cassette transporter ABCG2. *FEBS Journal* **276**:7237-7252.

Ni W, Ji J, Dai Z, Papp A, Johnson AJ, Ahn S, Farley KL, Lin TS, Dalton JT, Li X, Jarjoura D, Byrd JC, Sadee W, Grever MR and Phelps MA (2010) Flavopiridol Pharmacogenetics: Clinical and Functional Evidence for the Role of SLCO1B1/OATP1B1 in Flavopiridol Disposition. *Plos One* **5**:e13792.

Ogasawara K, Chitnis SD, Gohh RY, Christians U and Akhlaghi F (2013) Multidrug resistance-associated protein 2 (MRP2/ABCC2) haplotypes significantly affect the pharmacokinetics of tacrolimus in kidney transplant recipients. *Clinical Pharmacokinetics* **52**:751-762.

Oh ES, Kim CO, Cho SK, Park MS and Chung J-Y (2013) Impact of ABCC2, ABCG2 and SLCO1B1 Polymorphisms on the Pharmacokinetics of Pitavastatin in Humans. *Drug Metabolism and Pharmacokinetics* **28**:196-202.

Pascal LE, Oudes AJ, Petersen TW, Goo YA, Walashek LS, True LD and Liu AY (2007) Molecular and cellular characterization of ABCG2 in the prostate. *BMC urology* **7**:6.

Petain A, Kattygnarath D, Azard J, Chatelut E, Delbaldo C, Geoerger B, Barrois M, Séronie-Vivien S, LeCesne A and Vassal G (2008) Population pharmacokinetics and pharmacogenetics of imatinib in children and adults. *Clinical Cancer Research* **14**:7102-7109.

Polgar O, Ediriwickrema LS, Robey RW, Sharma A, Hegde RS, Li Y, Xia D, Ward Y, Dean M, Ozvegy-Laczka C, Sarkadi B and Bates SE (2009) Arginine 383 is a crucial residue in ABCG2 biogenesis. *Biochimica et Biophysica Acta - Biomembranes* **1788**:1434-1443.

Polgar O, Robey RW and Bates SE (2008) ABCG2: Structure, Function and Role in Drug Response. *Expert opinion on drug metabolism & toxicology* **4**:1-15.

Pollex EK, Anger G, Hutson J, Koren G and Piquette-Miller M (2010) Breast cancer resistance protein (BCRP)-mediated glyburide transport: Effect of the C421A/Q141K BCRP single-nucleotide polymorphism. *Drug Metabolism and Disposition* **38**:740-744.

Poonkuzhali B, Lamba J, Strom S, Sparreboom A, Thummel K, Watkins P and Schuetz E (2008) Association of breast cancer resistance protein/ABCG2 phenotypes and novel promoter and intron 1 single nucleotide polymorphisms. *Drug metabolism and disposition: the biological fate of chemicals* **36**:780-795.

Robey RW, Honjo Y, Morisaki K, Nadjem TA, Runge S, Risbood M, Poruchynsky MS and Bates SE (2003) Mutations at amino-acid 482 in the ABCG2 gene affect substrate and antagonist specificity. *British journal of cancer* **89**:1971-1978.

Robey RW, Steadman K, Polgar O and Bates SE (2005) ABCG2-mediated transport of photosensitizers: Potential impact on photodynamic therapy. *Cancer Biology and Therapy* **4**:187-194.

Robey RW, Steadman K, Polgar O, Morisaki K, Blayney M, Mistry P and Bates SE (2004) Pheophorbide a Is a Specific Probe for ABCG2 Function and Inhibition. *Cancer Research* **64**:1242-1246.

Robillard KR, Hoque MT and Bendayan R (2012) Expression of ATP-Binding Cassette Membrane Transporters in Rodent and Human Sertoli Cells: Relevance to the Permeability of Antiretroviral Therapy at the Blood-Testis Barrier. *Journal of Pharmacology and Experimental Therapeutics* **340**:96-108.

Rudin CM, Liu W, Desai A, Karrison T, Jiang X, Janisch L, Das S, Ramirez J, Poonkuzhali B, Schuetz E, Fackenthal DL, Chen P, Armstrong DK, Brahmer JR, Fleming GF, Vokes EE, Carducci MA and Ratain MJ (2008) Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity. *Journal of Clinical Oncology* **26**:1119-1127.

Sai K, Saito Y, Maekawa K, Kim SR, Kaniwa N, Nishimaki-Mogami T, Sawada JI, Shirao K, Hamaguchi T, Yamamoto N, Kunitoh H, Ohe Y, Yamada Y, Tamura T, Yoshida T, Matsumura Y, Ohtsu A, Saijo N and Minami H (2010) Additive Effects of drug transporter genetic polymorphisms on irinotecan pharmacokinetics/pharmacodynamics in Japanese cancer patients. *Cancer Chemotherapy and Pharmacology* **66**:95-105.

Saison C, Helias V, Ballif BA, Peyrard T, Puy H, Miyazaki T, Perrot S, Vayssier-Taussat M, Waldner M, Le Pennec P-Y, Cartron J-P and Arnaud L (2012) Null alleles of ABCG2 encoding the breast cancer resistance protein define the new blood group system Junior. *Nature Genetics* **44**:174-177.

Sang SL, Jeong HE, Yi JM, Jung HJ, Jang JE, Kim EY, Lee SJ and Shin JG (2007) Identification and functional assessment of BCRP polymorphisms in a Korean population. *Drug Metabolism and Disposition* **35**:623-632.

Sarankó H, Tordai H, Telbisz Á, Özvegy-Laczka C, Erdos G, Sarkadi B and Hegedus T (2013) Effects of the gout-causing Q141K polymorphism and a CFTR ??F508 mimicking mutation on the processing and stability of the ABCG2 protein. *Biochemical and Biophysical Research Communications* **437**:140-145.

Scharenberg CW, Harkey MA and Torok-Storb B (2002) The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors. *Blood* **99**:507-512.

Seong SJ, Lim M, Sohn SK, Moon JH, Oh SJ, Kim BS, Ryoo HM, Chung JS, Joo YD, Bang SM, Jung CW, Kim DH, Park SY, Yoon SS, Kim I, Lee HG, Won JH, Min YH, Cheong JW, Park JS, Eom KS, Hyun MS, Kim MK, Kim H, Park MR, Park J, Kim CS, Kim HJ, Kim YK, Park EK, Zang DY, Jo DY, Lee HW and Yoon YR (2013) Influence of enzyme and transporter polymorphisms on trough imatinib concentration and clinical response in chronic myeloid leukemia patients. *Annals of Oncology* **24**:756-760.

Sjöstedt N, van den Heuvel JJMW, Koenderink JB and Kidron H (2017) Transmembrane Domain Single-Nucleotide Polymorphisms Impair Expression and Transport Activity of ABC Transporter ABCG2. *Pharmaceutical Research* **34**:1626-1636.

Skoglund K, Boiso Moreno S, Jönsson J-I, Vikingsson S, Carlsson B and Gréen H (2014) Single-nucleotide polymorphisms of ABCG2 increase the efficacy of tyrosine kinase inhibitors in the K562 chronic myeloid leukemia cell line. *Pharmacogenetics and Genomics* **24**:52-61.

Sobek KM, Cummings JL, Bacich DJ and O'Keefe DS (2017) Contrasting roles of the ABCG2 Q141K variant in prostate cancer. *Experimental Cell Research* **354**:40-47.

Sparreboom A, Gelderblom H, Marsh S, Ahluwalia R, Obach R, Principe P, Twelves C, Verweij J and McLeod HL (2004) Diflomotecan pharmacokinetics in relation to ABCG2 421C>A genotype. *Clinical Pharmacology and Therapeutics* **76**:38-44.

Sparreboom A, Loos WJ, Burger H, Sissung TM, Verweij J, Figg WD, Nooter K and Gelderblom H (2005) Effect of ABCG2 genotype on the oral bioavailability of topotecan. *Cancer biology & therapy* **4**:650-658.

Stacy AE, Jansson PJ and Richardson DR (2013) Molecular Pharmacology of ABCG2 and Its Role in Chemoresistance. *Mol Pharmacol* **84**:655-669.

Steeghs N, Gelderblom H, Wessels J, Eskens FALM, De Bont N, Nortier JWR and Guchelaar HJ (2011a) Pharmacogenetics of telatinib, a VEGFR-2 and VEGFR-3 tyrosine kinase inhibitor, used in patients with solid tumors. *Investigational New Drugs* **29**:137-143.

Steeghs N, Mathijssen RHJ, Wessels JAM, De Graan AJ, Van Der Straaten T, Mariani M, Laffranchi B, Comis S, De Jonge MJA, Gelderblom H and Guchelaar HJ (2011b) Influence of pharmacogenetic variability on the pharmacokinetics and toxicity of the aurora kinase inhibitor danusertib. *Investigational New Drugs* **29**:953-962.

Stiburkova B, Pavelcova K, Zavada J, Petru L, Simek P, Cepek P, Pavlikova M, Matsuo H, Merriman TR and Pavelka K (2017) Functional non-synonymous variants of ABCG2 and gout risk. *Rheumatology* **56**:1982-1992.

Suthandiram S, Gan G-G, Zain SM, Bee P-C, Lian L-H, Chang K-M, Ong T-C and Mohamed Z (2014) Effect of polymorphisms within methotrexate pathway genes on methotrexate toxicity and plasma levels in adults with hematological malignancies. *Pharmacogenomics* **15**:1479-1494.

Taipalensuu J, Tornblom H, Lindberg G, Einarsson C, Sjoqvist F, Melhus H, Garberg P, Sjostrom B, Lundgren B and Artursson P (2001) Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers. *J Pharmacol Exp Ther* **299**:164-170.

Takahashi N, Miura M, Scott SA, Kagaya H, Kameoka Y, Tagawa H, Saitoh H, Fujishima N, Yoshioka T, Hirokawa M and Sawada K (2010) Influence of CYP3A5 and drug transporter polymorphisms on imatinib trough concentration and clinical response

among patients with chronic phase chronic myeloid leukemia. *Journal of Human Genetics* **55**:731-737.

Tamura A, Onishii Y, An R, Koshiba S, Wakabayashi K, Hoshijima K, Priebe W, Yoshida T, Kometani S, Matsubara T, Mikuriya K and Ishikawa T (2007a) In Vitro Evaluation of Photosensitivity Risk Related to Genetic Polymorphisms of Human ABC Transporter ABCG2 and Inhibition by Drugs. *Drug Metabolism and Pharmacokinetics* **22**:428-440.

Tamura A, Wakabayashi K, Onishi Y, Takeda M, Ikegami Y, Sawada S, Tsuji M, Matsuda Y and Ishikawa T (2007b) Re-evaluation and functional classification of non-synonymous single nucleotide polymorphisms of the human ATP-binding cassette transporter ABCG2. *Cancer Science* **98**:231-239.

Tamura A, Watanabe M, Saito H, Nakagawa H, Kamachi T, Okura I and Ishikawa T (2006) Functional validation of the genetic polymorphisms of human ATP-binding cassette (ABC) transporter ABCG2: identification of alleles that are defective in porphyrin transport. *Molecular pharmacology* **70**:287-296.

Tamura M, Kondo M, Horio M, Ando M, Saito H, Yamamoto M, Horio Y and Hasegawa Y (2012) Genetic polymorphisms of the adenosine triphosphate-binding cassette transporters (ABCG2, ABCB1) and gefitinib toxicity. *Nagoya journal of medical science* **74**:133-140.

Tanaka M, Kamada I, Takahashi J, Kimura T and Tani Y (2017) Genotyping of the ABCG2 gene using Matrix-Associated Laser Desorption/Ionisation, Time-of-Flight Mass Spectrometry. *Transfusion Medicine* **28**:255-260.

Tandia M, Mhiri A, Paule B, Saffroy R, Cailliez V, Noé G, Farinotti R and Bonhomme-Faivre L (2017) Correlation between clinical response to sorafenib in hepatocellular carcinoma treatment and polymorphisms of P-glycoprotein (ABCB1) and of breast cancer resistance protein (ABCG2): monocentric study. *Cancer Chemotherapy and Pharmacology* **79**:759-766.

Taylor NMI, Manolaridis I, Jackson SM, Kowal J, Stahlberg H and Locher KP (2017) Structure of the human multidrug transporter ABCG2. *Nature* **546**:504-509.

Thomas F, Rochaix P, White-Koning M, Hennebelle I, Sarini J, Benlyazid A, Malard L, Lefebvre JL, Chatelut E and Delord JP (2009) Population pharmacokinetics of erlotinib and its pharmacokinetic/pharmacodynamic relationships in head and neck squamous cell carcinoma. *European Journal of Cancer* **45**:2316-2323.

Tian C, Ambrosone CB, Darcy KM, Krivak TC, Armstrong DK, Bookman MA, Davis W, Zhao H, Moysich K, Gallion H and Deloia JA (2012) Common variants in ABCB1, ABCC2 and ABCG2 genes and clinical outcomes among women with advanced stage ovarian cancer treated with platinum and taxane-based chemotherapy: A Gynecologic Oncology Group study. *Gynecologic Oncology* **124**:575-581.

Tiribelli M, Fabbro D, Franzon A, Fanin R, Damante G and Damiani D (2013) Q141K polymorphism of ABCG2 protein is associated with poor prognosis in adult acute myeloid leukemia treated with idarubicin-based chemotherapy. *Haematologica* **98**.

Tomlinson B, Hu M, Lee VWY, Lui SSH, Chu TTW, Poon EWM, Ko GTC, Baum L, Tam LS and Li EK (2010) ABCG2 Polymorphism is associated with the low-density lipoprotein cholesterol response to rosuvastatin. *Clinical Pharmacology and Therapeutics* **87**:558-562.

Tsuchiya K, Hayashida T, Hamada A, Oka S and Gatanaga H (2016) Brief Report: High Peak Level of Plasma Raltegravir Concentration in Patients With ABCB1 and ABCG2 Genetic Variants. *Journal of acquired immune deficiency syndromes (1999)* **72**:11-14.

Tsuchiya K, Hayashida T, Hamada A, Oki S, Oka S and Gatanaga H (2017) High plasma concentrations of dolutegravir in patients with ABCG2 genetic variants. *Pharmacogenetics and Genomics* **27**:416-419.

Ueshima S, Hira D, Fujii R, Kimura Y, Tomitsuka C, Yamane T, Tabuchi Y, Ozawa T, Itoh H, Horie M, Terada T and Katsura T (2017) Impact of ABCB1, ABCG2, and CYP3A5 polymorphisms on plasma trough concentrations of apixaban in Japanese patients with atrial fibrillation. *Pharmacogenetics and Genomics* **27**:329-336.

Urquhart BL, Ware Ja, Tirona RG, Ho RH, Leake BF, Schwarz UI, Zaher H, Palandra J, Gregor JC, Dresser GK and Kim RB (2008) Breast cancer resistance protein (ABCG2) and drug disposition: intestinal expression, polymorphisms and sulfasalazine as an in vivo probe. *Pharmacogenetics and genomics* **18**:439-448.

Van den Heuvel-Eibrink M, Wiemer E, Prins A, Meijerink J, Vossebeld P, Van Der Holt B, Pieters R and Sonneveld P (2002) Increased expression of the breast cancer resistance protein (BCRP) in relapsed or refractory acute myeloid leukemia (AML). *Leukemia* **16**:833-839.

van der Veldt AA, Eechoute K, Gelderblom H, Gietema J, Guchelaar HJ, van Erp NP, van den Eertwegh AJ, Haanen JB, Mathijssen RH and Wessels JA (2011) Genetic polymorphisms associated with a prolonged progression-free survival in patients with metastatic renal cell cancer treated with sunitinib. *Clin Cancer Res* **17**:620-629.

Vauthier V, Housset C and Falguières T (2017) Targeted pharmacotherapies for defective ABC transporters. *Biochemical Pharmacology* **136**:1-11.

Vethanayagam RR, Wang H, Gupta A, Zhang Y, Lewis F, Unadkat JD and Mao Q (2005) Functional analysis of the human variants of breast cancer resistance protein: I206L, N590Y, and D620N. *Drug Metabolism and Disposition* **33**:697-705.

Vlaming ML, van Esch A, van de Steeg E, Pala Z, Wagenaar E, van Tellingen O and Schinkel AH (2011) Impact of abcc2 [multidrug resistance-associated protein (MRP) 2], abcc3 (MRP3), and abcg2 (breast cancer resistance protein) on the oral pharmacokinetics of methotrexate and its main metabolite 7-hydroxymethotrexate. *Drug Metab Dispos* **39**:1338-1344.

Vlaming MLH, Lagas JS and Schinkel AH (2009) Physiological and pharmacological roles of ABCG2 (BCRP): Recent findings in Abcg2 knockout mice. *Advanced Drug Delivery Reviews* **61**:14-25.

Wen CC, Yee SW, Liang X, Hoffmann TJ, Kvale MN, Banda Y, Jorgenson E, Schaefer C, Risch N and Giacomini KM (2015) Genome-wide association study identifies ABCG2

(BCRP) as an allopurinol transporter and a determinant of drug response. *Clinical pharmacology and therapeutics* **97**:518-525.

Westover D and Li F (2015) New trends for overcoming ABCG2/BCRP-mediated resistance to cancer therapies. *Journal of experimental & clinical cancer research : CR* **34**:159.

Wiese MD, Schnabl M, O'Doherty C, Spargo LD, Sorich MJ, Cleland LG and Proudman SM (2012) Polymorphisms in cytochrome P450 2C19 enzyme and cessation of leflunomide in patients with rheumatoid arthritis. *Arthritis Research & Therapy* **14**:R163.

Wong K, Briddon SJ, Holliday ND and Kerr ID (2016) Plasma membrane dynamics and tetrameric organisation of ABCG2 transporters in mammalian cells revealed by single particle imaging techniques. *Biochimica et Biophysica Acta - Molecular Cell Research* **1863**:19-29.

Wong K, Ma J, Rothnie A, Biggin PC and Kerr ID (2014) Towards understanding promiscuity in multidrug efflux pumps. *Trends in Biochemical Sciences* **39**:8-16.

Woodward OM, Kottgen A, Coresh J, Boerwinkle E, Guggino WB and Kottgen M (2009) Identification of a urate transporter, ABCG2, with a common functional polymorphism causing gout. *Proceedings of the National Academy of Sciences* **106**:10338-10342.

Woodward OM, Tukaye DN, Cui J, Greenwell P, Constantoulakis LM, Parker BS, Rao A, Kottgen M, Maloney PC and Guggino WB (2013) Gout-causing Q141K mutation in ABCG2 leads to instability of the nucleotide-binding domain and can be corrected with small molecules. *Proceedings of the National Academy of Sciences* **110**:5223-5228.

Xiang L, Su P, Xia S, Liu Z, Wang Y, Gao P and Zhou G (2011) ABCG2 is associated with HER-2 Expression, lymph node metastasis and clinical stage in breast invasive ductal carcinoma. *Diagnostic Pathology* **6**:90.

Xiong H, Callaghan D, Jones A, Bai J, Rasquinha I, Smith C, Pei K, Walker D, Lue L-F, Stanimirovic D and Zhang W (2009) ABCG2 is up-regulated in Alzheimer's brain with

cerebral amyloid angiopathy and may act as a gatekeeper at the blood-brain barrier for Aβ1-40 peptides. *J Neurosci* **17**:5463-5475.

Yamada A, Maeda K, Ishiguro N, Tsuda Y, Igarashi T, Ebner T, Roth W, Ikushiro S and Sugiyama Y (2011) The impact of pharmacogenetics of metabolic enzymes and transporters on the pharmacokinetics of telmisartan in healthy volunteers. *Pharmacogenetics and genomics* **21**:523-530.

Yamakawa Y, Hamada A, Nakashima R, Yuki M, Hirayama C, Kawaguchi T and Saito H (2011) Association of genetic polymorphisms in the influx transporter SLCO1B3 and the efflux transporter ABCB1 with imatinib pharmacokinetics in patients with chronic myeloid leukemia. *Therapeutic drug monitoring* **33**:244-250.

Yang WJ, Song MJ, Park EY, Lee JJ, Park JH, Park K, Park JH and Kim HP (2013) Transcription factors Sp1 and Sp3 regulate expression of human ABCG2 gene and chemoresistance phenotype. *Molecules and Cells* **36**:368-375.

Yoshioka S, Katayama K, Okawa C, Takahashi S, Tsukahara S, Mitsuhashi J and Sugimoto Y (2007) The identification of two germ-line mutations in the human breast cancer resistance protein gene that result in the expression of a low/non-functional protein. *Pharmaceutical Research* **24**:1108-1117.

Zamber CP, Lamba JK, Yasuda K, Farnum J, Thummel K, Schuetz JD and Schuetz EG (2003) Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine. *Pharmacogenetics* **13**:19-28.

Zamboni WC, Ramanathan RK, McLeod HL, Mani S, Potter DM, Strychor S, Maruca LJ, King CR, Jung LL, Parise RA, Egorin MJ, Davis TA and Marsh S (2006) Disposition of 9-nitrocamptothecin and its 9-aminocamptothecin metabolite in relation to ABC transporter genotypes. *Investigational New Drugs* **24**:393-401.

Zelinski T, Coghlan G, Liu X-Q and Reid ME (2012) ABCG2 null alleles define the Jr(a−) blood group phenotype. *Nature Genetics* **44**:131-132.

Zhai X, Wang H, Zhu X, Miao H, Qian X, Li J, Gao Y, Lu F and Wu Y (2012) Gene polymorphisms of ABC transporters are associated with clinical outcomes in children with acute lymphoblastic leukemia. *Archives of medical science : AMS* **8**:659-671.

Zhang D, He K, Herbst JJ, Kolb J, Shou W, Wang L, Balimane PV, Han YH, Gan J, Frost CE and Humphreys WG (2013) Characterization of efflux transporters involved in distribution and disposition of apixaban. *Drug Metab Dispos* **41**:827-835.

Zhang W, Yu BN, He YJ, Fan L, Li Q, Liu ZQ, Wang A, Liu YL, Tan ZR, Fen-Jiang, Huang YF and Zhou HH (2006) Role of BCRP 421C>A polymorphism on rosuvastatin pharmacokinetics in healthy Chinese males. *Clinica Chimica Acta* **373**:99-103.

Zhang Z, Uchida Y, Hirano S, Ando D, Kubo Y, Auriola S, Akanuma S-i, Hosoya K-i, Urtti A, Terasaki T and Tachikawa M (2017) Inner blood-retinal barrier dominantly expresses breast cancer resistance protein: Comparative quantitative targeted absolute proteomics (QTAP) study of CNS barriers in pig. *Molecular Pharmaceutics* **14**:acs.molpharmaceut.7b00493.

Zhou D, Liu Y, Zhang X, Gu X, Wang H, Luo X, Zhang J, Zou H and Guan M (2014) Functional polymorphisms of the ABCG2 gene are associated with gout disease in the Chinese Han male population. *International Journal of Molecular Sciences* **15**:9149-9159.

Zhou Q, Chen QX, Ruan ZR, Yuan H, Xu HM and Zeng S (2013a) CYP2C9*3(1075A > C), ABCB1 and SLCO1B1 genetic polymorphisms and gender are determinants of inter-subject variability in pitavastatin pharmacokinetics. *Pharmazie* **68**:187-194.

Zhou Q, Ruan ZR, Jiang B, Yuan H and Zeng S (2013b) Simvastatin pharmacokinetics in healthy Chinese subjects and its relations with CYP2C9, CYP3A5, ABCB1, ABCG2 and SLCO1B1 polymorphisms. *Pharmazie* **68**:124-128.

Zhou Q, Ruan ZR, Yuan H, Xu DH and Zeng S (2013c) ABCB1 Gene polymorphisms, ABCB1 haplotypes and ABCG2 c.421C > A are determinants of inter-subject variability in rosuvastatin pharmacokinetics. *Pharmazie* **68**:129-134.

Zhou S, Schuetz JD, Bunting KD, Colapietro AM, Sampath J, Morris JJ, Lagutina I, Grosveld GC, Osawa M, Nakauchi H and Sorrentino BP (2001) The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med* **7**:1028-1034.

Zhou S, Zong Y, Ney PA, Nair G, Stewart CF and Sorrentino BP (2005) Increased expression of the Abcg2 transporter during erythroid maturation plays a role in decreasing cellular protoporphyrin IX levels. *Blood* **105**:2571-2576.

Authorship Contribution

All authors participated in collection and analysis of data, and contributed to the writing of the manuscript.

Footnotes

Requests for reprints should be sent to Ian Kerr, School of Life Sciences, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, U.K.

DMD Fast Forward. Published on September 28, 2018 as DOI: 10.1124/dmd.118.083030
This article has not been copyedited and formatted. The final version may differ from this version.

Figure Legends

Figure 1. Chemical structures of ABCG2 physiological substrates and pharmacological drug substrates. Structures obtained from PubChem database.

Figure 2. Topology map of ABCG2. The membrane topology of ABCG2 is shown with TM helices represented as cylinders and the NBD as an oval. The location of the SNPs is indicated using the single letter amino acid code, as is the location of the N-glycosylation site (N596) and intra- and inter-molecular disulphide bonds (C592-C608 and C603-C603).

Figure 3. Structural localisation of SNPs within ABCG2. **A.** The structure of ABCG2 (Taylor et al., 2017) is shown in ribbon format with the location of SNPs indicate by the blue colouring of residues. **B, C.** An expanded view of the NBD:MSD interface is shown to highlight the interaction between Q141 and K382/R383 in wild type ABCG2 (**B**) that is destabilised by the introduction of the Q141K SNP (**C**). **D.** The ABCG2 drug binding cavity ("cavity 1" in the terminology of (Taylor et al., 2017)) is shown with residues in pink proposed to interact with substrates. Of the known SNPs (cyan) only F431 shown in stick format localises to this cavity.

Table 1 — An overview of the expression and functions of ABCG2

Location of ABCG2	Action/function at site	Reference
Small intestine enterocytes	Efflux of compounds into lumen of GI tract; reduced absorption of drugs into bloodstream; protection of body from absorption of toxins & xenobiotics; extra-renal clearance of uric acid	(Maliepaard et al., 2001; Taipalensuu et al., 2001)
Liver canalicular membrane	Transport of compounds from bloodstream into hepatocytes and liver canaliculi; increased excretion of compounds via bile	(Maliepaard et al., 2001)
Proximal tubule cells in kidney	Transport from blood into proximal renal tubule; increased excretion of compounds-via urine; role in excretion of uric acid	(Maliepaard et al., 2001)
Blood-brain-barrier	Efflux of compounds from endothelial cells into microvessels; protection of brain from toxic compounds; role in tolerance to CNS drugs e.g. opioids; potential role in CNS diseases e.g. Alzheimer's & PD	(Cooray et al., 2002)
Placental syncytiotrophoblasts	Transports compounds across placenta from foetus into maternal circulation; protection of foetus from toxins & xenobiotics	(Maliepaard et al., 2001; Memon et al., 2014)
Mammary gland	Transports compounds into breast milk; modulation of vitamin levels; potential harmful value to infant if mother is receiving drug treatment whilst breast feeding	(Vlaming et al., 2009)
Erythrocytes	Efflux of porphyrins from cell; upregulated in erythroid maturation; modulation of protoporphyrin IX - determinant in protoporphyria	(Zhou et al., 2005)

Haematopoietic stem cells (HSCs)	Efflux of porphyrins from cell; protection from hypoxia and accumulation of haem; role in stem cell phenotype; role in haematological cancer prognosis	(Krishnamurthy et al., 2004b; Scharenberg et al., 2002)
Hair follicle	Protection of the root sheath	(Haslam et al., 2015)
Basal epithelial cells of prostate	Efflux of differentiation-inducing molecules; role in self-renewal & development of prostate stem cells; role in regulation of testosterone	(Huss et al., 2005; Pascal et al., 2007)
Blood-testis-barrier	Efflux of compounds from Sertoli cells into interstitium; protection of developing spermatozoa from xenobiotics & toxins	(Robillard et al., 2012)
Harderian gland	Transport into tubulo-alveolar lumen of gland secretions of lipids & porphyrins	(Vlaming et al., 2009)
Inner blood-retinal barrier	Efflux of toxins out of retina; protection from phototoxicity	(Vlaming et al., 2009; Zhang et al., 2017)
In addition to these locations, ABCG2 has been found in the adrenal gland, cervix and uterus, bladder and lungs		

DMD Fast Forward. Published on September 28, 2018 as DOI: 10.1124/dmd.118.083030
This article has not been copyedited and formatted. The final version may differ from this version.

Table 2. Allelic variants of ABCG2

Allelic variant	SNP reference	global MAF	prevalent populations
V12M	rs2231137	0.158	native Mexicans, SE Asia and Pacific
Q126X	rs72552713	0.001	Japan
Q141K	rs2231142	0.119	Asia
R147W	rs372192400	0.0001	
T153M	rs199753603	0.0002	
I206L	rs12721643	0.0003	
F208S	rs1061018	<0.0001	
S248P	rs3116448	< 0.0001	
P269S	rs3116448	< 0.0001	Korea, Vietnam
D296H	rs41282401	0.0002	
F431L	rs750568956	< 0.0001	
S441N	rs758900849	< 0.0001	
F489L	rs192169063	0.001	
N590Y	rs34264773	0.0004	
D620N	rs34783571	0.003	

Abbreviation: MAF: minor allele frequency obtained from dnSNP (https://www.ncbi.nlm.nih.gov/projects/SNP)

Table 3. Summary of the effects of less-well studied *ABCG2* SNPs on expression and function

Base change	Amino acid change	Location	Test systems-cell lines	Effects on ABCG2	Effects on drug transport	Reference
458 C>T	T153M	NBD	Cancer cell lines HCT116, blood cells from patients, murine fibroblast PA317 cells	Decreased expression, impaired efflux activity, possible disruption to ATP binding	Lower SN-38 resistance, defective urate transport	(Mizuarai et al., 2004; Stiburkova et al., 2017; Yoshioka et al., 2007)
616 A>C	I206L	NBD	HEK293 cells, murine fibroblast PA317 cells	Decreased expression, no effect on efflux	No effects shown	(Vethanayagam et al., 2005; Yoshioka et al., 2007)
623 T>C	F208S	NBD	Blood leukocytes, chronic myeloid leukaemia K562 cells	Decreased expression, impaired efflux activity, possible disruption to ATP binding	Sensitivity to SN-38 & mitoxantrone, defective porphyrin and TKI transport	(Itoda et al., 2003; Skoglund et al., 2014)
742 T>C	S248P	Linker	HEK293-Tet-On-cells, Flp-In293 cells, Sf9 cells	Similar expression, impaired efflux activity	Lower SN-38 & mitoxantrone resistance; defective porphyrin transport	(Deppe et al., 2014; Tamura et al., 2007b; Tamura et al., 2006)
805 C>T	P269S	Linker	HEK cells, cellular localisation monitored in LLC-PK1 cells, Sf9	Similar expression, impaired efflux activity	No effect on urate transport	(Higashino et al., 2017; Kondo et al., 2004; Sang et al., 2007)

			cells			
886 G>C	D296H	Linker	Chronic myeloid leukaemia K562 cells	Decreased expression	Increased sensitivity to TKIs	(Skoglund et al., 2014)
1291 T>C	F431L	TMH2	HEK293-Tet-On-cells, chronic myeloid leukaemia K562 cells, Flp-In293 cells, Sf9 cells, murine fibroblast PA317 cells	Similar expression, impaired efflux activity or drug substrate binding	Increase sensitivity to SN-38, mitoxantrone & methotrexate; defective porphyrin transport	(Deppe et al., 2014; Kawahara et al., 2010; Tamura et al., 2007b; Tamura et al., 2006; Yoshioka et al., 2007)
1322 G>A	S441N	TMH2	Cellular localisation monitored in LLC-PK1 cells, Flp-In293 cells, Sf9 cells, HEK283 cells	Decreased expression; impaired or abrogated efflux activity; change in substrate specificity	Sensitivity to SN-38 & mitoxantrone; defective porphyrin transport; defective urate transport	(Kondo et al., 2004; Nakagawa et al., 2008; Sjöstedt et al., 2017; Tamura et al., 2007b; Tamura et al., 2006)
1465 T>C	F489L	TMH3	HEK293 Tet-On-cells, HEK293 cells, Sf9 cells, Flp-In293 cells	Similar or reduced expression level; impaired efflux	Sensitivity to SN-38, mitoxantrone & methotrexate	(Deppe et al., 2014; Sjöstedt et al., 2017; Tamura et al., 2007b; Tamura et al., 2006)
1574 T>G	L525R	TMH4	Sf9 cells, HEK283 cells, chronic myeloid leukaemia K562	Decreased expression level	Sensitivity to TKIs; proximal residue M523 is involved in drug	(Sjöstedt et al., 2017; Skoglund et al., 2014)

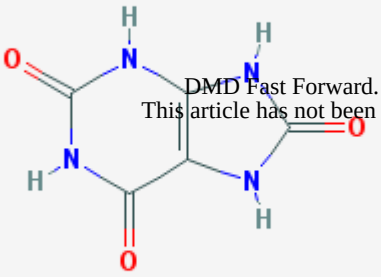
			cells		recognition	
1582 G>A	A528T	TMH4	Chronic myeloid leukaemia K562 cells	Similar expression level to WT; increased efflux activity	No effects to date	(Skoglund et al., 2014)
1768 A>T	N590Y	TMH5-6 loop	HEK293 cells, murine fibroblast PA317 cells, DNA isolation from liver and intestinal donors	Similar or increased expression	Increased sensitivity to mitoxantrone & topotecan; no change in SN-38 pharmacokinetics	(Vethanayagam et al., 2005; Yoshioka et al., 2007; Zamber et al., 2003)
1858 G>A	D620N	TMH5-6 loop	HEK293 cells, chronic myeloid leukaemia K562 cells, blood cells, Sf9 cells	Ambiguous; documented increases and decreases in both expression and activity	Ambiguous; documented increased sensitivity or no change in sensitivity; defective urate transport?	(Morisaki et al., 2005; Skoglund et al., 2014; Stiburkova et al., 2017; Tamura et al., 2006; Vethanayagam et al., 2005)

DMD Fast Forward. Published on September 28, 2018 as DOI: 10.1124/dmd.118.083030
This article has not been copyedited and formatted. The final version may differ from this version.

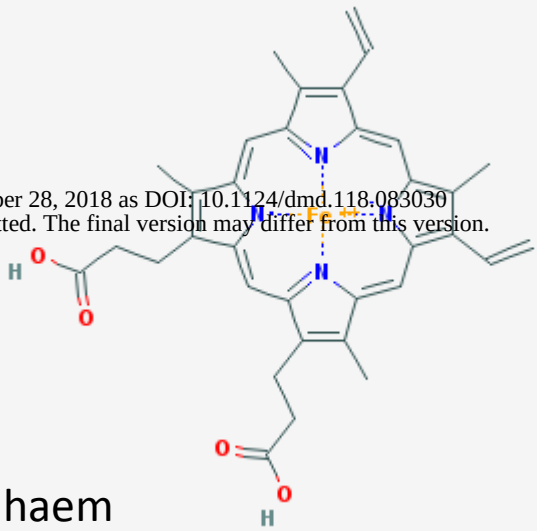
Table 4. A potential categorisation of *ABCG2* SNPs.

Group	Example SNPs	Cell surface expression	Drug transport
1a	Wild-type	As wild-type	As wild-type
1b	S248P, P269S, F431L	As wild-type	Impaired
2a	V12M, Q126X, Q141K, T153M, F208S, S441N, F489L, -15622 C>T, 1143 C>T	Reduced	Impaired
2b	I206L	Reduced	Elevated
3a	N590Y, D620N	Increased	Impaired
3b	-15994 C>T	Increased	Elevated

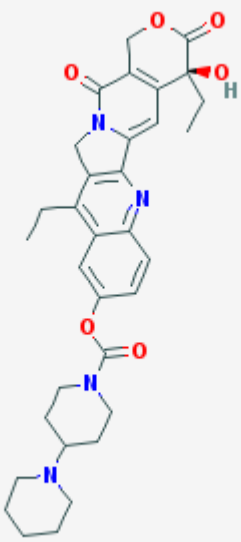
Figure 1



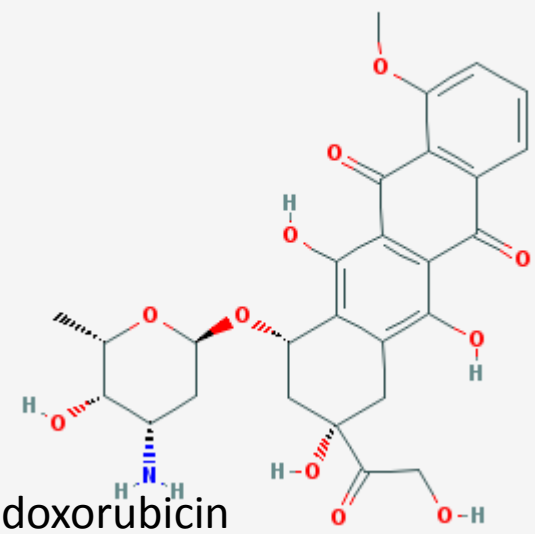
urate



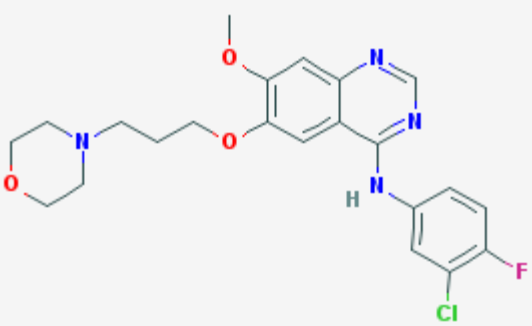
haem



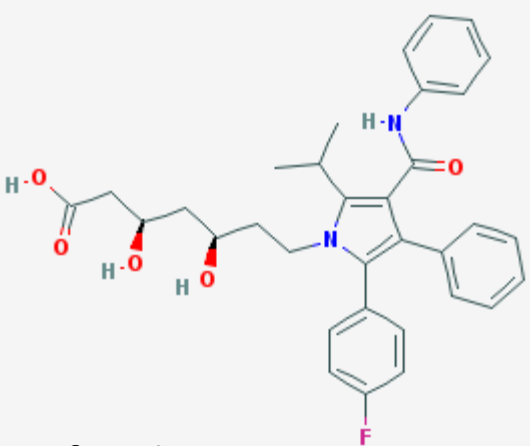
irinotecan



doxorubicin

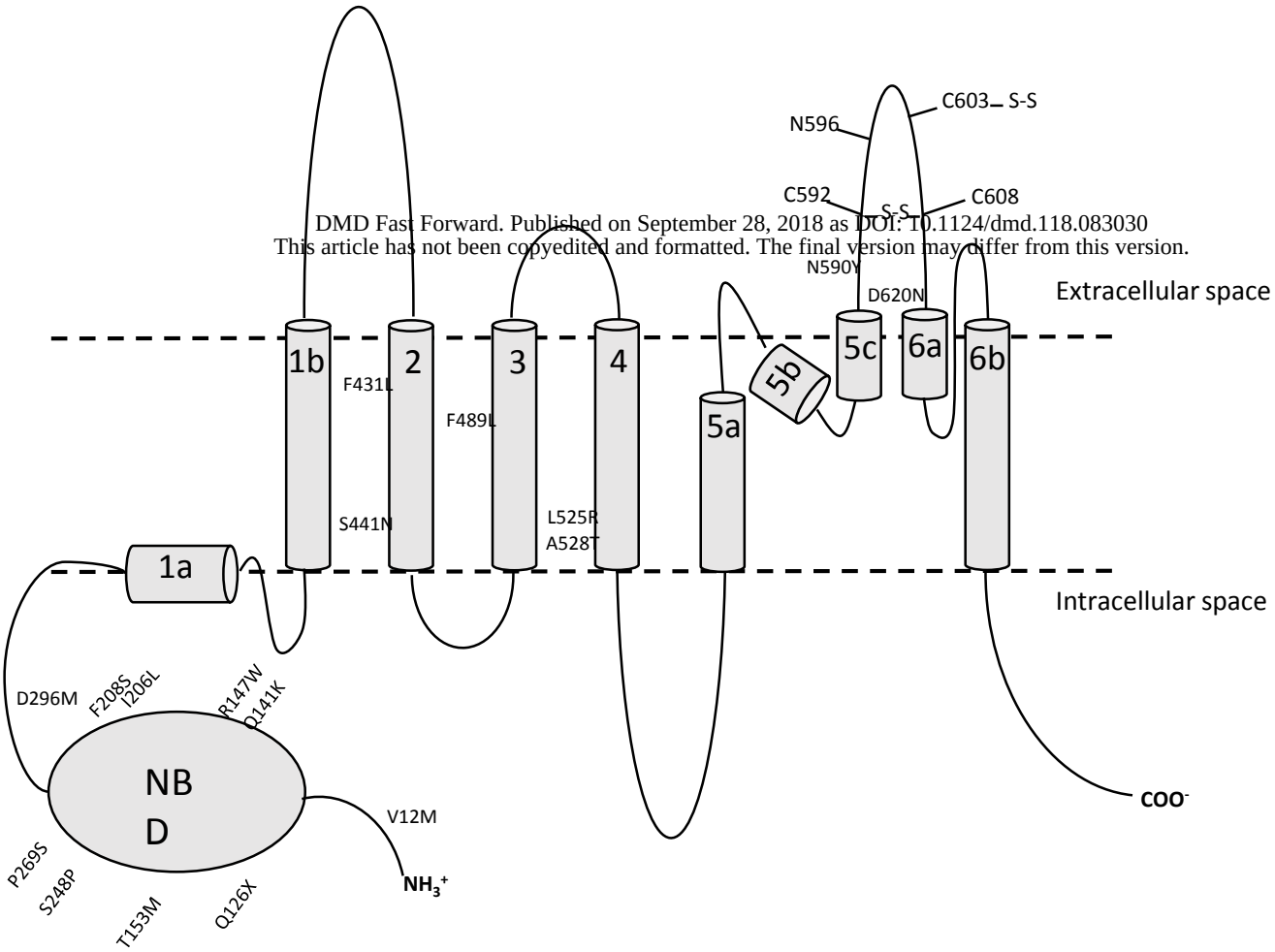


atorvastatin



gefitinib

Figure 2



DMD Fast Forward. Published on September 28, 2018 as DOI: 10.1124/dmd.118.083030
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 3