ABCG2 Pharmacogenetics: Ethnic Differences in Allele Frequency and Assessment of Influence on Irinotecan Disposition

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ABSTRACT

Purpose: The ATP-binding cassette transporter ABCG2 (breast cancer resistance protein) is an efflux protein that plays a role in the detoxification of various xenobiotic substrates, including the irinotecan metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). The ABCG2 421C>A polymorphism has been associated with reduced protein expression and altered function in vitro. The aim of this study was to evaluate the ethnic distribution and potential functional consequence of the ABCG2 421C>A genotype in cancer patients treated with irinotecan.

Experimental Design: ABCG2 genotyping was performed using Pyrosequencing on DNA from 88 American Caucasians, 94 African Americans, 938 Africans, and 95 Han Chinese, as well as in 84 European Caucasian patients treated with irinotecan undergoing additional blood sampling for pharmacokinetic studies.

Results: Significant differences in allele frequencies were observed between the given world populations (P < 0.001), the variant allele being most common in the Han Chinese population with a frequency as high as 34%. The mean area under the curve of irinotecan and SN-38 were 19,851 and 639 ng h/mL, respectively. The frequency of the variant allele (10.7%) was in line with results in American Caucasians. No significant changes in irinotecan pharmacokinetics were observed in relation to the ABCG2 421C>A genotype, although one of two homozygous variant allele carriers showed extensive accumulation of SN-38 and SN-38 glucuronide.

Conclusions: The ABCG2 421C>A polymorphism appears to play a limited role in the disposition of irinotecan in European Caucasians. It is likely that the contribution of this genetic variant is obscured by a functional role of other polymorphic proteins.

INTRODUCTION

The ATP-binding cassette (ABC) transporters represent the largest family of transmembrane proteins that bind ATP and use the energy to drive the transport of various molecules across cell membranes (1). On the basis of the arrangement of molecular structural components, i.e., the nucleotide binding domain and the topology of transmembrane domains, human proteins are classified into seven distinct families (ABCA to ABCG; ref. 1). The ABCG subfamily consists of several half transporters that are generally thought to form homo- or heterodimers to create the active transporter (2, 3). Very recently, it was reported that human ABCG2 probably exists as a homotetramer with a possibility of a higher form of oligomerization (3). The human ABCG2 gene is located on chromosome 4q22 and encodes a 655 amino acid polypeptide (4, 5). In vitro studies have indicated that, apart from topotecan (6, 7), the irinotecan metabolites 7-ethyl-10-hydroxycamptothecin (SN-38) and its glucuronide conjugate SN-38G (8, 9) are very good substrates for ABCG2. Furthermore, overexpression of ABCG2 reportedly confers cancer cell resistance to various anticancer drugs, including topotecan and SN-38 (10, 11). ABCG2 is endogenously expressed at high levels in human placenta, the small intestine and colon, and the bile canalicular membrane (12). This localization suggests that the physiologic role of ABCG2 may be to regulate intestinal absorption and biliary secretion of potentially toxic xenobiotics by active transport mechanisms (6, 13).

Recent resequencing of the ABCG2 transporter has revealed a number of allelic variants that affect activity of the gene product in vivo. Some of these genetic variants may potentially modulate the ABCG2 phenotype in patients and therefore affect their predisposition to toxicity and response to substrate drug treatment. In particular, a single nucleotide polymorphism in exon 5 of the ABCG2 gene has been described in which a 421C>A transversion results in an amino acid change of glutamine to lysine at codon 141 (14–16). Although a detailed analysis of the potential functional consequences of this ABCG2 variant has not yet been evaluated, in vitro studies indicated altered substrate specificity and function of the mutant protein relative to the wild-type protein (14, 17). In the present study, we evaluated the allele frequency of the ABCG2 421C>A polymorphism in a large number of individuals from different ethnic populations as well as its effect on the pharmacokinetics of irinotecan.
MATERIALS AND METHODS

Patient and Population Samples. A total of 84 patients (43 women and 41 men) diagnosed with a histologically confirmed malignant solid tumor was assessed for irinotecan pharmacokinetics (Table 1). The most common tumor types in this population were of gastrointestinal or pulmonary origin. All patients were of Caucasian descent, were between 34 and 75 years old (median, 54 years), and were treated between January 1997 and June 2003 at the Erasmus University MC – Daniel den Hoed Cancer Center (Rotterdam, the Netherlands). The inclusion and exclusion criteria, premedication schedules (i.e., 8 mg of the S-hydroxyxymethylpyrimidine-3-receptor antagonist ondansetron, administered i.v., combined with 10 mg of dexamethasone), and protocols for treatment of drug-induced side effects were documented previously (18). Irinotecan (Aventis Pharma, Ho)velaken, the Netherlands) was given once every 3 weeks as a 90-minute intravenous infusion. The median dose was 600 mg (range, 260 to 875 mg). None of the patients received any other protocols for treatment of drug-induced side effects were documented previously (20). Concentrations of irinotecan, SN-38, and SN-38G were determined using a validated method based on liquid chromatography with fluorescence detection, as described elsewhere (23). Previously developed population models were used to predict the pharmacokinetic parameters of irinotecan, SN-38, and SN-38G (24). Because of the linear relationship between irinotecan dose and area under the plasma concentration-time curve (AUC; Ref. 25), the AUC normalized to the recom-
mended single-agent dose of 350 mg/m² was simulated for irinotecan and its metabolite in all patients from time 0 to 100 h after start of infusion. This analysis was performed using NON-
MEM version VI (Stuart L. Beal and Lewis B. Sheiner, San Francisco, CA). No differences were found in the simulated AUC from time 0 to 100 hours after start of infusion between patients who had been sampled up to 48 hours (n = 28) and those who had been sampled up to 500 hours (n = 56). Metabolic conversion ratios were calculated as previously described (26) and included the AUC ratio of SN-38 to irinotecan (REC), the AUC ratio of SN-38G to SN-38 (REG), and the AUC ratio of SN-38 and SN-38G to irinotecan.

Isolation of Genomic DNA and Genotype Analysis. Whole blood or plasma was used to isolate genomic DNA according to the manufacturers instructions using the Gentra PureGene Blood kit (Gentra, Minneapolis, MN) and the QIAamp DNA Blood midi kit (Qiagen, Inc., Valencia, CA), respectively. Variations in ABCG2 421C>A were analyzed by Pyrosequencing, using the Pyrosequencing AB PSQ96MA instrument and software (Uppsala, Sweden). PCR primers (forward, 5’-biotin-ATGATGTGTTGATGGGCACCT-3’, and reverse, 5’-CAGACCTAAGCTTGAATGAC-3’) were designed using Primer Express version 1.5 (ABI, Foster City, CA), and the Pyrosequencing primer (internal, 5’-GAGTGGACACTTGGG-3’) was designed using the Pyrosequencing Single-Nucleotide Polymorphism Primer Design version 1.01 software. PCR was carried out using AmpliTaq Gold PCR master mix (ABI), 5 pmol of each primer (IDT, Coralville, IA), and 1 ng of DNA. The PCR reaction was performed on a DNA engine (MJ Research, Reno, NV) at an annealing temperature of 68°C.

Statistical Considerations. All pharmacological parameters are presented as mean values, unless stated otherwise. To relate pharmacokinetic parameters with the polymorphism, a nonparametric Kruskal-Wallis one-way ANOVA test was used, followed by a comparison of all means with a Tukey-Kramer test.

Table 1 Patient demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline screening</td>
<td>84</td>
</tr>
<tr>
<td>Age (y)</td>
<td>54 (34–75)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>41/43</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.85 (1.29–2.36)</td>
</tr>
<tr>
<td>Length (m)</td>
<td>1.70 (1.51–1.92)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.0 (38.6–113.5)</td>
</tr>
<tr>
<td>WHO performance status</td>
<td>1 (0–1)</td>
</tr>
</tbody>
</table>

Pretherapy clinical chemistry

Aspartate aminotransferase (units/L) 28 (6–185)
Alanine aminotransferase (units/L) 20 (4–225)
Total bilirubin (µmol/L) 8 (3–22)
Serum creatinine (µmol/L) 78 (45–132)
Serum albumin (g/L) 41 (30–51)
Total serum protein (g/L) 77 (62–82)

Pretherapy hematology

White blood cell count (×10⁹/L) 7.80 (2.80–27.0)
Absolute neutrophil count (×10⁹/L) 5.38 (1.54–24.0)
Platelets (×10⁹/L) 304 (132–966)
Hemoglobin (mmol/L) 7.55 (5.20–10.4)
Hematocrit (L/L) 0.37 (0.27–0.48)

NOTE. Continuous data are given as median with range in parentheses; categorical data as number of patients.

4 Internet address: http://medicine.iupui.edu/lockhart/table.htm.

5 Internet address: http://coriell.umdnj.edu/ccr/ccrsumm.html.

multiple-comparison test (NCSS v2001; J. Hintze, Number Cruncher Statistical Systems, Kaysville, UT). A \( \chi^2 \) test was used to compare the distribution of the polymorphic allele in the given world populations. All test results with \( P < 0.05 \) were regarded as statistically significant.

**RESULTS**

**ABCG2 Genotyping.** Significant differences in allele frequencies were observed between the given world populations (\( P < 0.001 \); Table 2). In the Han Chinese population, the variant allele appears to be very common with a found frequency of 34%. Fifty-seven percent of the studied Han Chinese were carrying at least one variant allele. In contrast, in the sub-Saharan African population, the variant allele appeared to be very rare with a frequency < 1%. Approximately 5% of studied African-American alleles were found to be variant, whereas the frequency of variant alleles in American Caucasians was 12%. The frequency distributions of all populations are in Hardy-Weinberg equilibrium.

In the 84 European Caucasian cancer patients, the frequency of the variant \( ABCG2 \) 421A allele was found to be 10.7%, and the genotype distribution showed 68 patients carrying the wild-type sequence (81%). A total of 16 patients (19%) carried at least one variant allele, 14 of whom were carrying the heterozygous sequence (17%), and 2 the homozygous variant sequence (2%).

**Irinotecan Pharmacokinetics.** In the entire population, the mean dose-normalized AUC of irinotecan was 19,851 ±

### Table 2 Genotype and allele frequencies for \( ABCG2 \) 421 C>A in different world populations

<table>
<thead>
<tr>
<th>Population</th>
<th>( n )</th>
<th>C/C</th>
<th>C/A</th>
<th>A/A</th>
<th>( p )</th>
<th>( q )</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Caucasian</td>
<td>88</td>
<td>68 (77)</td>
<td>19 (22)</td>
<td>1 (1)</td>
<td>0.88</td>
<td>0.12</td>
</tr>
<tr>
<td>European Caucasian</td>
<td>84</td>
<td>68 (81)</td>
<td>14 (17)</td>
<td>2 (2)</td>
<td>0.89</td>
<td>0.11</td>
</tr>
<tr>
<td>African American</td>
<td>94</td>
<td>85 (90)</td>
<td>8 (9)</td>
<td>1 (1)</td>
<td>0.95</td>
<td>0.05</td>
</tr>
<tr>
<td>African (sub-Sahara)</td>
<td>938</td>
<td>923 (98.4)</td>
<td>14 (1.5)</td>
<td>1 (0.1)</td>
<td>0.99</td>
<td>0.01</td>
</tr>
<tr>
<td>Han Chinese</td>
<td>95</td>
<td>41 (43)</td>
<td>43 (45)</td>
<td>11 (12)</td>
<td>0.66</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Abbreviations: \( n \), number of patients studied; C/C, homozygous wild-type frequency; C/A, heterozygous frequency; A/A, homozygous variant frequency.

* Number represents number of patients, with percentage in parentheses.
† Data are given as relative frequency.
‡ \( p \) and \( q \) represent standard Hardy-Weinberg nomenclature for allele frequencies.

### Table 3 Summary of pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg)</td>
<td>600</td>
<td>602</td>
<td>260–875</td>
</tr>
<tr>
<td>Infusion duration (h)</td>
<td>1.50</td>
<td>1.51</td>
<td>0.75–2.50</td>
</tr>
<tr>
<td>AUC irinotecan (ng × h/mL)</td>
<td>18,350</td>
<td>19,851</td>
<td>10,350–46,079</td>
</tr>
<tr>
<td>AUC SN-38 (ng × h/mL)</td>
<td>566</td>
<td>639</td>
<td>216–2,605</td>
</tr>
<tr>
<td>AUC SN-38G (ng × h/mL)</td>
<td>2,911</td>
<td>4,105</td>
<td>962–29,961</td>
</tr>
<tr>
<td>REC (%)</td>
<td>3.01</td>
<td>3.24</td>
<td>0.79–8.80</td>
</tr>
<tr>
<td>(AUC SN-38 + SN-38G)/AUC irinotecan</td>
<td>0.199</td>
<td>0.241</td>
<td>0.067–2.04</td>
</tr>
<tr>
<td>REG</td>
<td>5.97</td>
<td>7.25</td>
<td>1.70–37.5</td>
</tr>
</tbody>
</table>

Abbreviations: REC, relative extent of conversion (AUC ratio of SN-38 to irinotecan); REG, relative extent of glucuronidation (AUC ratio of SN-38G to SN-38).

### Table 4 Pharmacokinetic parameters as a function of \( ABCG2 \) 421C>A genotype

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Wt( ^\ast ) (( n = 68 ))</th>
<th>Het/Var( ^\ast ) (( n = 16 ))</th>
<th>( P )†</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC irinotecan (ng × h/mL)</td>
<td>19,979 (2.280–37,678)</td>
<td>19,305 (7.239–31,392)</td>
<td>0.715</td>
</tr>
<tr>
<td>AUC SN-38 (ng × h/mL)</td>
<td>627 (0–2,029)</td>
<td>692 (0–1,776)</td>
<td>0.669</td>
</tr>
<tr>
<td>AUC SN-38G (ng × h/mL)</td>
<td>3,836 (0–9,979)</td>
<td>5,083 (0–18,777)</td>
<td>0.555</td>
</tr>
<tr>
<td>AUC SN-38 + SN-38G (ng × h/mL)</td>
<td>4,382 (0–10,751)</td>
<td>5,775 (0–19,687)</td>
<td>0.485</td>
</tr>
<tr>
<td>REC (%)</td>
<td>3.17 (0.02–6.11)</td>
<td>3.52 (0.02–6.84)</td>
<td>0.466</td>
</tr>
<tr>
<td>(AUC SN-38 + SN-38G)/AUC irinotecan</td>
<td>0.218 (0.0436)</td>
<td>0.325 (0.1255)</td>
<td>0.559</td>
</tr>
<tr>
<td>REG</td>
<td>7.00 (0–16.1)</td>
<td>8.16 (0–25.6)</td>
<td>0.803</td>
</tr>
</tbody>
</table>

Abbreviations: Wt, homozygous wild-type patients (C/C); Het/Var, combined data of heterozygous and homozygous variant patients (C/A or A/A); \( n \), number of patients studied; REC, relative extent of conversion (AUC ratio of SN-38 to irinotecan); REG, relative extent of glucuronidation (AUC ratio of SN-38G to SN-38).

* Data are presented as mean values with the 95% confidence interval in parentheses.
† Numbers represent \( P \) values from a non parametric Kruskal-Wallis test.
6,461 ng × hour/mL. The AUCs of SN-38 and SN-38G were 639 ± 386 and 4,105 ± 4,155 ng × hour/mL, respectively, which is in line with earlier findings (Table 3; Ref. 25).

**Irinotecan Disposition/ABCG2 421C>A Genotype Relationships.** Pharmacokinetic parameters were observed as a function of the ABCG2 421C>A genotype (Table 4). The AUC of irinotecan (P = 0.72) and its active metabolite SN-38 (P = 0.67) did not differ significantly between patients carrying the wild-type sequence and patients carrying at least one variant allele. On average, the AUC of irinotecan was somewhat higher in patients carrying the wild-type sequence, whereas the AUCs of SN-38 and SN-38G were slightly higher in patients carrying at least one variant allele (Fig. 1), which contributed to a minor increase in the overall systemic exposure to SN-38. However, variability in the extent of conversion of irinotecan to SN-38 and the extent of SN-38 glucuronidation was similar in both groups (Table 4).

**DISCUSSION**

Irinotecan is registered for the first- and second-line treatment of unresectable colorectal cancer and is also active in various other human malignancies (27, 28). It is a prodrug of the topoisomerase I inhibitor SN-38, which is formed through a carboxylesterase-mediated cleavage of the parent drug. The interindividual variability in irinotecan and SN-38 pharmacokinetic parameters is large and has been associated with variation in its clinical outcome and toxicity profiles (25). Previous investigations have demonstrated that body surface area is an unimportant factor in explaining this variability (29). The interindividual variability in SN-38 pharmacokinetics is more likely related to a host of other factors, including hepatic function (30) and use of concomitant medication (31) in addition to multiple polymorphic pathways involved in the excretion and biotransformation of irinotecan. The latter include esterases regulating cleavage of irinotecan, cytochrome P450 3A4- and 3A5-mediated oxidations of the parent drug (32), and glucuronic acid conjugation of SN-38 by members of the UGT1A family (33). It has been suggested previously that the metabolism of SN-38 is substantially influenced by a promoter polymorphism in the TATA-box sequence of the UGT1A1 gene (i.e., UGT1A1*28; Ref. 34). Indeed, an extra (7th) TA repeat in both alleles results in a substantial reduction in transcriptional and functional UGT1A1 activity compared with patients carrying the heterozygous or wild-type sequence (35, 36). Because SN-38 is also a known substrate for the ABC transporter ABCG2 (8–11), we here evaluated the potential functional significance of the ABCG2 421C>A polymorphism in cancer patients receiving irinotecan to identify additional mechanisms underlying interindividual variation in SN-38 pharmacokinetics.

The allele frequency of ABCG2 421A varies highly around the diverse populations. The presented findings in the four world populations are in line with earlier findings. The ABCG2 421A allele appears to be very common in Japanese and Chinese populations, with reported allele frequencies between 26 and 35% (14, 16). To the best of our knowledge, no data have been reported previously on sub-Sahara African populations. However, the low frequency of ABCG2 421A (<1%) in this population is in line with earlier observations in Africans north of the

![Fig. 1 Boxplot of dose-normalized AUC of irinotecan (A), SN-38 (B), and SN-38G (C) as a function of the ABCG2 421C>A (Q141K) genotype. Wt (1) are homozygous wild-type patients (C/C; n = 68); Het/Var (2) are patients carrying at least one variant allele (C/A or A/A; n = 16). The box represents the difference between the 25th and 75th percentiles, whereas the horizontal line inside the box represents the median. Closed circles are defined as outliers (i.e., values between 1.5 and 3 box lengths from the 75th percentile or 25th percentile) or extremes (farther away). Whiskers are drawn from the ends of the box to the largest and smallest values that are not outliers.](clincancerres.aacrjournals.org)
Sahara and in African Americans (16) and with the relatively low variant allele frequency in the currently studied African-American population. The observed 10.7% allele frequency of ABCG2 421A in the 84 European Caucasian cancer patients is similar to that found in the Caucasian American individuals (12%) and those described previously in American Caucasian (frequency, 14%; n = 85; Ref. 16) and Swedish populations (frequency, 10%; n = 60; Ref. 15).

In our patients, pharmacokinetic parameters of irinotecan and SN-38 were not significantly different between patients wild type for ABCG2 and patients carrying at least one defective ABCG2 421C>A allele (Table 4). At first sight, this finding is inconsistent with the previous demonstration of reduced protein expression being associated with the variant allele (14) and with altered plasma pharmacokinetics of another camptothecin analogue, diflomotecan (BN80915), when given intravenously (37). However, given the complexity of irinotecan metabolism (28), it is possible that the contribution of ABCG2 421C>A to changes in drug disposition is obscured by a functional role of other polymorphic proteins involved in irinotecan elimination. In addition, because of a partial substrate overlap between ABCB1 (P-glycoprotein) and ABCG2 (14), the effect of absenteeism of functional ABCG2 may be corrected for by the presence, or even by a compensatory overexpression, of functional ABCB1.

Despite the lack of a significant association between ABCG2 421C>A genotype and irinotecan disposition, it is worth mentioning that one of two patients homozygous for the variant allele showed very extensive accumulation of SN-38 and SN-38G, with a value for the combined AUC of SN-38 and SN-38G of 30,761 ng × hour/mL and with a value for the combined AUC ratio of SN-38 and SN-38G to irinotecan that was >8-fold higher than the mean of all patients (2.041 versus 0.241). The patient developed very severe side effects, including a grade 4 leukocytopenia, a grade 4 neutropenia, and a grade 4 diarrhea, and eventually died within 9 days after the administration of irinotecan. This patient was previously shown to be heterozygous for the UGT1A1*28 polymorphism (20), ruling out a causative link between this phase II glucuronidation pathway and the observed phenotype. The only other patient homozygous for this allele did not show aberrant SN-38 pharmacokinetics. However, it cannot be excluded that patients with this rare genotype comprising two variant alleles for ABCG2 421C>A show markedly impaired ability to eliminate SN-38 through active ABCG2-mediated hepatobiliary and or intestinal secretion and demonstrate excessive toxicity. Clearly, additional investigation is required to unambiguously assess the relationship between ABCG2 421C>A homozygous variant genotype and irinotecan disposition.

In conclusion, the ABCG2 421C>A polymorphism does not seem to play a major role in vivo in the disposition of irinotecan in European Caucasian cancer patients, although it cannot be ruled out that patients with two variant alleles for ABCG2 421C>A may have impaired ability to eliminate SN-38. The ABCG2 421C>A polymorphism was found to vary highly in different ethnic groups, with <1% variant alleles found in Africans, and up to 34% in Chinese subjects. A prospective trial to corroborate the usefulness of gene diagnosis of this ABCG2 polymorphism, in combination with other polymorphisms of putative relevance in irinotecan metabolism and disposition such as UGT1A1*28 or the recently described variant UGT1A1 -3156G>A (36), before irinotecan chemotherapy in different ethnic populations seems warranted.

REFERENCES
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