A pharmacogenetic predictive model for paclitaxel clearance based on the DMET platform

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TRANSLATIONAL RELEVANCE

During the last 20 years, paclitaxel has become a standard cytotoxic drug in the treatment of several malignancies, such as breast, ovarian and non-small cell lung cancer. However, due to its wide inter-patient variability in plasma exposure, efficacy and toxicity profiles of this compound are still quite unpredictable for the individual patient. Dose-limiting toxicities resulting from high systemic concentrations could lead to prematurely discontinuation of treatment or to treatment-related hospitalisations. It is therefore important to identify patients at risk for (extremely) high exposures to the drug, allowing these patients to be monitored more closely or to apply dose reductions. In the current study, we explored the potential association of 1,936 genetic variants in 225 drug metabolizing enzyme and drug transporter genes (DMET-platform) with paclitaxel unbound clearance.
ABSTRACT

PURPOSE: Paclitaxel is used in the treatment of solid tumors and displays high inter-individual variation in exposure. Low paclitaxel clearance (CL) could lead to increased toxicity during treatment. We present a genetic prediction model identifying patients with low paclitaxel CL, based on the Drug-Metabolizing Enzyme and Transporter (DMET)-platform, capable of detecting 1,936 genetic variants in 225 metabolizing enzyme and drug transporter genes.

EXPERIMENTAL DESIGN: In 270 paclitaxel-treated patients, unbound plasma concentrations were determined and pharmacokinetic parameters were estimated from a previously developed population pharmacokinetic model (NONMEM). Patients were divided into a training and validation set. Genetic variants determined by the DMET platform were selected from the training set to be included in the prediction model when they were associated with low paclitaxel CL (1 SD below mean CL) and subsequently tested in the validation set.

RESULTS: A genetic prediction model including 14 SNPs was developed on the training set. In the validation set, this model yielded a sensitivity of 95%, identifying most patients with low paclitaxel CL correctly. The positive predictive value of the model was only 22%. The model remained associated with low CL after multivariate analysis, correcting for age, gender and hemoglobin levels at baseline ($P=0.02$).

CONCLUSIONS: In this first large-sized application of the DMET-platform for paclitaxel, we identified a 14 SNP model with high sensitivity to identify patients with low paclitaxel CL. However, due to the low positive predictive value we conclude that genetic variability encoded in the DMET-chip alone does not sufficiently explain paclitaxel CL.
INTRODUCTION

Paclitaxel is a highly active anti-cancer drug with a broad spectrum of activity. It is used in the treatment of several solid tumors, such as breast, ovarian, and non-small-cell lung cancer. Paclitaxel stabilizes cellular microtubules and thereby blocking chromosomal segregation and mitosis, eventually inducing apoptosis (1).

Paclitaxel is metabolized in the liver by cytochrome P450 (CYP) CYP2C8 and 3A4. The anion organic transporting polypeptide OATP1B3 was identified as an important influx transporter (2), while the efflux of paclitaxel was shown to be mediated by ABCB1 (P-glycoprotein) and ABCC2 transporters (3, 4). The pharmacokinetics of this agent are known for its large inter-individual variability, which could have important safety consequences and may also affect treatment outcome. The source of this large variability remains to be elucidated.

It has been suggested that genetic variation in the genes involved in the metabolism of paclitaxel could explain part of the mentioned variability. As a candidate gene approach, SNPs in \textit{CYP2C8}*3, \textit{CYP3A5} and \textit{ABCB1} (3435C>T, 2677G>T and 1236C>T) have been tested, but these studies yielded contradictory results (5-10).

Another approach to study genetic variants that could potentially influence paclitaxel pharmacokinetics and toxicity is by using a more broad approach, such as the Drug-Metabolizing Enzyme and Transporter (DMET) genetic platform (Affymetrix). This platform includes a large amount of potentially important genetic variants for drug metabolism, and investigates 1,936 variants in 225 genes involved in drug metabolism and transport. The aim of this study was to develop a predictive signature for paclitaxel pharmacokinetics by use of the DMET genotyping platform.
PATIENTS AND METHODS

Patients

Patients treated with paclitaxel for different tumor types were included in a prospective trial studying pharmacokinetics, pharmacodynamics and pharmacogenetics (registered at www.trialregister.nl as NTR2311, study number MEC 03.264).

The inclusion criteria in this study were (i) a histological or cytological confirmed diagnosis of cancer treated with paclitaxel, (ii) aged 18 years or older, (iii) World Health Organization (WHO) performance score of 0 or 1 and (iv) adequate hematopoietic, hepatic and renal functions according to the product information of paclitaxel (11). CYP3A4 and CYP2C8 inducers and inhibitors were not allowed during the course of paclitaxel treatment (12). All patients provided written informed consent prior to study participation. The trial was approved by the medical ethical committee of the Erasmus University Medical Center.

Pharmacokinetic analysis

Paclitaxel pharmacokinetic limited sampling with 4 to 5 samples in up to approximately 24 hours after the start of infusion was performed, and was repeated once or twice in following cycles according to each patient’s individual consent. For all samples lithium heparin was used as anticoagulant. A validated UV detection high-performance liquid chromatography (HPLC) method (13) or a validated LC-MS/MS method -- based on a method used for docetaxel (14) -- was used to quantitate paclitaxel in plasma. Measured samples for total paclitaxel plasma concentrations and a previously developed population pharmacokinetic model (15-17) were used to calculate paclitaxel pharmacokinetic parameters in each individual patient. It is known that the total plasma concentrations of paclitaxel do not show linear pharmacokinetics in contrast to the “free” plasma concentrations.. This is caused by the formulation vehicle of paclitaxel, Cremophor EL, which is responsible for disproportionate
drug accumulation in the plasma fraction, and therefore causes a decrease in free circulating
drug (18, 19). For this reason, pharmacokinetic model parameters were parameterized to be
based on unbound concentrations in the modeling, and “unbound” instead of total paclitaxel
clearance was used in the covariate analyses of this study. The individual pharmacokinetic
parameters were estimated as Empirical Bayes estimates within the non-linear mixed-effect
modeling software NONMEM (version 7, Icon Development Solutions, Ellicott City, MD).

Genetic variant analysis using the DMET platform

DNA of 293 patients was extracted from whole blood using MagnaPure LC (Roche
Diagnostics GmbH) according to manufacturer’s instructions. The Affymetrix DMET Plus
Premier Pack (Affymetix, CA, USA) was used to genotype genomic DNA of all paclitaxel
treated patients as described by Dumaual et al (20). Genotypes of all single-nucleotide
polymorphisms (SNPs) on the DMET assay were reported either as “call” or as “no call”.
Markers on the DMET assay with call rates less than 90% were excluded from analysis. After
removal of duplicate measurements, patients with missing clearance data and removal of low-
call rate assays, data for 270 patients were eligible for data analysis.

Marker selection

As shown in the flowchart (Figure 1), genetic variants were excluded from the
analysis if the genotype was identical in all patients, leaving 1,048 variant reporting different
genotypes for analysis. The remaining genotypes were tested for Hardy-Weinberg equilibrium
and SNPs deviating from this equilibrium ($P > 0.05$) were excluded. This left 770 variants for
the prediction analysis. The cohort of patients was split into a training and a validation set. To
guarantee a sufficient number of minimal observations for analysis, each genotype (wild-type,
heterozygote, or homozygote variant) of a particular SNP should occur in at least 5% of
patients, which equals 7 patients in the training set. Identification of variants which were significantly associated with low clearance was performed using a Naïve Bayes formula in the training set. The conditional probability was estimated for each SNP:

\[ P(A|B) = \frac{P(A \& B)}{P(B)}. \]

Here, A is clearance, while B is the genotype. This expresses the chance of a patient having low clearance (coded as 1) while having a homozygote wild-type, a heterozygote, or a homozygote variant genotype. Each of the possible genotypes has a probability of occurring, which is used as a weight in the prediction analysis. To select variants associated with paclitaxel clearance, a wild type or variant genotype should be present in at least half of the patients with low clearance in the training-set, and to have a \( P(A|B) > 0.2 \).

This resulted in the selection of 20 SNPs. Four SNPs (rs2359612, rs8050894, rs9934438, and rs9923231) were in complete linkage disequilibrium and belonged to the same gene (VKORC1). Of these SNPs, the SNP with the highest allelic frequency, rs9923231, was selected. Similarly, two other SNPs (rs7793861 and rs7797834) were in complete linkage disequilibrium in the CYP51A1 gene. Here, rs7797834 was selected because this SNP had the highest allelic frequency. Next, the remaining 16 SNPs were included in a multivariate logistic regression model. Each of these SNPs was added to the model one by one, and SNPs were included in the signature if they increased the likelihood ratio of the fitted model, leaving 14 SNPs for final analysis.

**SNP signature**

In developing the predictive model patients were divided in having either low clearance, thus assumed to have an increased risk of toxicity during paclitaxel therapy, or
normal (or high) clearance. Low clearance was defined as a clearance one SD below the mean clearance of the entire cohort. Low clearance was coded as 1, the other clearances as 0. The 14 selected SNPs (Table 3) were used to build the SNP signature predicting low clearance in paclitaxel treated patients. For each of these SNPs, the conditional probabilities estimated in the training-samples were used. For each individual sample a total probability weighted score was calculated based on the sum of all 14 probabilities of having the selected SNPs. The scores of the samples in the training-cohort were associated with clearance in a ROC-curve. From this ROC-curve, we selected the threshold where all patients with truly low clearance were identified correctly (i.e. 100% sensitivity). Finally, the probability weighted scores of the samples from the validation-cohort were calculated and compared to the selected threshold. If the score was above the threshold the sample was predicted as “low clearance”, otherwise as “rest”. The predicted calls were then compared with the actual clearance data.

Statistics

Data are presented as median with ranges, unless stated otherwise. Differences between validation and training set were tested with the chi-square test for binary covariables and differences between validation and training set in continuous variables were tested with the Mann-Whitney test. Logistic regression was used to study the influence of covariables on the association between the SNP signature and a patient having low clearance. Variables tested in this model were age, gender and hemoglobin (Hb) levels before start of therapy because these variables have previously been shown to influence paclitaxel treatment. For example, male patients have higher paclitaxel metabolism than female patients (21). Paclitaxel elimination is also negatively correlated with age (21). Furthermore, Hb levels have been shown to be a prognostic factor in cancer treatment (22-24). P-values were all two-sided and P-values <0.05 were considered statistically significant. Analysis were performed with
STATA version 11 (StataCorp LP, College Station, TX) and SPSS version 20.0 (SPSS Inc, Armonk, NY).

RESULTS

Patients

In the analysis, 270 Caucasian patients treated with paclitaxel were included. This group was divided in a training (n=140) and a validation set (n=130), so that the numbers of patients in the low clearance category are equally distributed between the training and validation set. The training and validation set displayed the same patient characteristics (Table 1). The median age of the whole cohort was 61 years (range: 18-82 years) and esophageal cancer was the main diagnosis (49%). Patients were treated with a median dose of 170 mg (range: 50-560 mg). Patients received paclitaxel weekly or every 3 weeks in different combination regimens. Patients receiving a weekly dose of 50 mg/m² paclitaxel in combination with radiotherapy, as a preoperative regimen for resectable esophageal cancer, were also included (23). There was no statistical significant difference between the training and validation-set with respect to clearance (P = 0.66), gender (P = 0.64), tumor type (P = 0.98), smoking status (P = 0.56), hemoglobin levels (P = 0.31) or platelets counts (P = 0.72) at start of therapy, excluding potential selection bias between the training and validation set.

Influence of variables on paclitaxel pharmacokinetics

In the total cohort, we tested the influence of the variables age, gender and Hb levels before start of therapy on unbound paclitaxel clearance because these variables have previously been shown to influence paclitaxel treatment. Age was not significantly correlated with paclitaxel pharmacokinetics in our data set (R = -0.09; P = 0.16). Gender had a significant effect on paclitaxel unbound clearance. In our cohort males had higher median
clearance than females (mean 541 L/h vs. 432 L/h; \(P < 0.00001\)). We found a significant but weak correlation between Hb levels at start of paclitaxel therapy and paclitaxel unbound clearance (\(R = 0.2; P = 0.001\)).

Genetic signature predicting low metabolism

The mean clearance of all patients was 488 ± 149 L/h. Therefore, the threshold for having low clearance was 339 L/h, which was 1 SD below the mean of the total cohort. In total 14 SNPs located on 11 different chromosomes were selected for the prediction model (Table 3). Included in the model were SNPs in the genes: SLC22A11 (rs1783811), GSTZ1 (rs7975), SLC28A2 (rs1060896), VKORC1 (rs9923231), PGAP3 (rs2952151), CDA (rs1048977), EPHX1 (rs1051740), CYP20A1 (rs1048013), SLC6A6 (rs2341970), CRIP3 (rs2242416), GSTA4 (rs13197674), AKAP9 (rs7785971), CYP51A1 (rs7797834), and CYP2D7P1 (rs28360521). The probabilities of having a homozygote wild-type, heterozygote, or homozygote variant for each SNP are listed in Table 3. The sum of these probabilities gives each patient a probability score and these scores were used to generate a ROC curve. From this curve a threshold was selected (2.12) at which point a 100% sensitivity was achieved to identify low-clearance patients in the training set. All patients with a probability score higher than 2.12 were scored as having low clearance and all patients with a probability score lower than 2.12 were scored as not having low clearance. Subsequently, we validated this predictive model in the validation cohort. Figure 2 shows paclitaxel clearance levels according to the predicted group; the median paclitaxel clearance in the group predicted as low clearance (n=93) was significantly lower than the group predicted as “rest” (n=37) (461 vs. 525 L/h; \(P = 0.01\)). With the cut off score of 2.12, 20 out of 21 patients with truly low clearance were predicted by the signature as having low clearance, yielding the model a sensitivity of 95% and a positive predictive value of 22% (Table 4, Figure 2). Patients with a positive SNP
prediction model had an OR of 9.9 (95%CI 1.3-76.4; \( P = 0.028 \)) of having low clearance. When tested in a multivariate logistic regression model to correct for the influence of age, gender and hemoglobin levels at start of therapy, the SNP prediction model was independently associated with low clearance (OR = 10.9; 95%CI 1.4-86.3; \( P = 0.024 \)). None of the other tested variables significantly improved the model.
DISCUSSION

During the early years of the 21st century, emphasis has been on somatic tumor mutations that can predict disease course and treatment outcome and could therefore aid in selecting the appropriate therapy for an individual patient. However, germline genetic variation, as present in normal tissue can influence the pharmacokinetics and pharmacodynamics of an anti-cancer drug regardless of tumor type and therefore also affect treatment outcome and toxicity (26). Knowledge of this germline variation could therefore significantly contribute to a truly individualized pharmacotherapy of anti-cancer drugs.

In the current study, we present the findings of a pharmacokinetic-pharmacogenetic study that relates multiple genetic variants in metabolic enzymes and transporters to the unbound clearance of paclitaxel, in order to identify patients with low clearance who are potentially at risk for increased toxicity. This analysis resulted in the development of a genetic signature, predictive for low paclitaxel clearance, containing 14 SNPs, which yielded a high sensitivity, but a low positive predictive value, when tested in a validation cohort.

To the best of our knowledge, this is the first large-scale application of the DMET platform to explain the pharmacokinetic variability of a commonly used anti-cancer drug. The traditional candidate gene approach is most often used in pharmacogenetic cancer research (27-29). However, this method is only able to identify a limited number of genetic variants which are plausible candidates within the current knowledge of the field. The DMET platform enables us to study genetic variants in all currently known drug metabolizing enzymes and transporters simultaneously, making this a tool with high potential for pharmacogenetic research (26).

Our genetic prediction model yielded a sensitivity of 95% when tested in the validation cohort. However, the model had a positive predicting value of only 22%. This means that this model identifies almost all patients with truly low clearance, at the cost of a high percentage
of false positives. Also, 36 out of 37 patients (97%) have truly no ‘low-clearance’ after testing negative for the genetic prediction model, meaning that they do not have an increased toxicity risk. To be clinically applicable, we reasoned that a prediction model should have besides a high sensitivity also a high positive predictive value. The latter is not reached in our study.

In previous studies several SNPs have been associated with paclitaxel clearance or toxicity, but contradictory findings have been reported. For example, $ABCB1\ 3435\ C>T$ was associated with paclitaxel clearance (30) and $ABCB1\ 2677\ G>T/A$ was associated with response to paclitaxel (31). The combination of $ABCB1\ 3435\ C>T$ and $ABCB1\ 2677\ G>T/A$ has been linked to neutropenia and patients with at least one $ABCB1\ 3435\ C>T$ showed a trend towards more development of neurotoxicity during paclitaxel therapy (6). Also, SNPs in $CYP2C8$ have been related to paclitaxel therapy response. For example, $CYP2C8*3$ carriers had higher rates of complete response than non-carriers (32). On the same note, $CYP2C8*3$ carriers were found to have lower paclitaxel clearance than non-carriers (8) and a higher risk of neurotoxicity (9, 10). However, none of these previous identified associations were found in other studies (5,33).

Interestingly, in the currently developed model, none of the SNPs that were previously associated with paclitaxel clearance or toxicity were included. However, this may partly be due to the fact that even this DMET-chip is not fully covering all existing drug metabolizing enzyme and transporter genes known today. For instance, $CYP3A4*22$, that we recently correlated with paclitaxel-induced neurotoxicity (34) is not (yet) available on the chip. In addition, epoxide hydrolase 1 (EPHX1) and glutathione S-transferase alpha 4 (GSTA4) have previously been associated with docetaxel clearance in a small study which related selected genes in metabolism and signaling of reactive oxygen species (ROS) (35, 36). Also, it was found that EPHX1 was upregulated in gemcitabine resistant non-small cell lung cancer cells (37). None of the other 12 SNPs that were selected for the prediction model have previously
been associated with clearance or toxicity of taxanes. Because of the discrepancy between the previously associated candidate SNPs with paclitaxel clearance and the outcome of this DMET analysis, we presume it is unlikely that common inherited genetic variability in drug metabolizing enzymes and transporters will contribute enough to explain the (large) interpatient variability in paclitaxel clearance. Several non-inherited factors may mask the pharmacogenetic effects (38). However, Peters et al described the response to paclitaxel treatment as having a high heritability when assessing heritable drug-induced cell-killing on 125 lymphoblastoid cell lines derived from 14 families (39). If paclitaxel-induced toxicity and treatment outcome are more heritable than pharmacokinetics, remains to be elucidated. Our group is currently working on a study associating paclitaxel-induced toxicity to genetic variability as encoded on the DMET platform. To conclude, we developed a validated genetic prediction model in a large cohort of paclitaxel-treated patients to identify patients at risk of low clearance. Although this validated prediction model for paclitaxel clearance had a high sensitivity, its positive predictive value was too low to be of direct clinical use. Strikingly, the genes that are reported to influence paclitaxel pharmacokinetics were not identified in this analysis; we therefore conclude that genetic variability in DMET genes does not substantially contribute in explaining a large part of the interpatient variability in paclitaxel clearance. If these genes can explain the interpatient variability in the pharmacokinetics of other drugs should be explored in further studies.

ACKNOWLEDGEMENTS

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REFERENCES


Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients</th>
<th>Training set</th>
<th>Validation set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>270</td>
<td>140</td>
<td>130</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>61 (18-82)</td>
<td>61 (18-79)</td>
<td>61 (18-82)</td>
</tr>
<tr>
<td>Gender, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>139 (51.5)</td>
<td>74 (52.9)</td>
<td>65 (50)</td>
</tr>
<tr>
<td>Female</td>
<td>131 (48.5)</td>
<td>66 (47.1)</td>
<td>65 (40)</td>
</tr>
<tr>
<td>Median dose, mg (range)</td>
<td>170 (50-560)</td>
<td>170 (50-560)</td>
<td>168 (50-490)</td>
</tr>
<tr>
<td>Primary tumor site, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>131 (49)</td>
<td>68 (49)</td>
<td>63 (49)</td>
</tr>
<tr>
<td>Ovary</td>
<td>37 (14)</td>
<td>19 (14)</td>
<td>18 (14)</td>
</tr>
<tr>
<td>Cervix</td>
<td>20 (7)</td>
<td>10 (7)</td>
<td>10 (8)</td>
</tr>
<tr>
<td>Endometrial</td>
<td>13 (5)</td>
<td>8 (6)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Breast</td>
<td>18 (7)</td>
<td>8 (6)</td>
<td>10 (8)</td>
</tr>
<tr>
<td>Lung</td>
<td>9 (3)</td>
<td>5 (4)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Head/Neck</td>
<td>10 (4)</td>
<td>5 (4)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>A(CUP)</td>
<td>8 (3)</td>
<td>4 (3)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Testis</td>
<td>6 (2)</td>
<td>2 (1)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Other</td>
<td>18 (7)</td>
<td>11 (8)</td>
<td>7 (5)</td>
</tr>
</tbody>
</table>

*Continuous data are given as median with range in parentheses, and categorical data are given as number of patients with percentage of the total population in parentheses.*

*Abbreviations: N, number; A (CUP), (adenoma) carcinoma of unknown origin.*
Table 2. Paclitaxel clearance in both setsa

<table>
<thead>
<tr>
<th>Paclitaxel clearance (L/h)b</th>
<th>All patients</th>
<th>N</th>
<th>Training set</th>
<th>N</th>
<th>Validation set</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>480 (138-1,037)</td>
<td>270</td>
<td>474 (138-1,037)</td>
<td>140</td>
<td>494 (239-618)</td>
<td>130</td>
</tr>
<tr>
<td>Low clearance-group</td>
<td>286 (138-339)</td>
<td>44</td>
<td>258 (138-328)</td>
<td>23</td>
<td>302 (239-339)</td>
<td>21</td>
</tr>
<tr>
<td>Not low clearance-group</td>
<td>512 (340-1,037)</td>
<td>226</td>
<td>505 (340-1,037)</td>
<td>117</td>
<td>518 (345-858)</td>
<td>109</td>
</tr>
</tbody>
</table>

aData are represented as median with ranges

b Paclitaxel clearance in based on unbound concentrations

Abbreviations: N, number of patients
Table 3. Selected SNPs for the conditional probability gene signature and probability score

| Genetic Variation | Gene (Chr) | SNP value =0<sup>a</sup> | SNP value =1<sup>a</sup> | SNP value =2<sup>a</sup> | P(SNP=0|low clearance)<sup>b</sup> | P(SNP=1|low clearance)<sup>c</sup> | P(SNP=2|low clearance)<sup>d</sup> |
|-------------------|-----------|-----------------|----------------|----------------|----------------|----------------|----------------|
| rs1783811         | SLC22A11 (Chr11) | AA              | AG            | GG             | 0.154          | 0.106          | 0.230          |
| rs7975            | GSTZ1 (Chr 14)  | CC              | CT            | TT             | 0.205          | 0.16           | 0              |
| rs1060896         | SLC28A2 (Chr 15) | AA              | AC            | CC             | 0.213          | 0.133          | 0.105          |
| rs9923231         | VKORC1 (Chr16)  | CC              | CT            | TT             | 0.235          | 0.119          | 0.136          |
| rs2952151         | PGAP3 (Chr17)   | CC              | CT            | TT             | 0.203          | 0.136          | 0.111          |
| rs1048977         | CDA (Chr1)      | CC              | CT            | TT             | 0.210          | 0.127          | 0.071          |
| rs1051740         | EPHX1 (Chr1)    | CC              | CT            | TT             | 0.091          | 0.119          | 0.214          |
| rs1048013         | CYP20A1 (Chr2)   | AA              | AG            | GG             | 0.226          | 0.106          | 0.2            |
| rs2341970         | SLC6A6 (Chr3)   | CC              | CT            | TT             | 0.208          | 0.115          | 0.111          |
| rs22242416        | CRIP3 (Chr6)    | AA              | AG            | GG             | 0.174          | 0.090          | 0.26           |
| rs13197674        | GSTA4 (Chr6)    | CC              | CT            | TT             | 0.111          | 0.103          | 0.234          |
| rs7785971         | AKAP9 (Chr7)    | AA              | AT            | TT             | 0.111          | 0.109          | 0.241          |
| rs7797834         | CYP51A1 (Chr7)   | AA              | AG            | GG             | 0.241          | 0.095          | 0.167          |
| rs28360521        | CYP2D7P1 (Chr22)| CC              | CT            | TT             | 0.207          | 0.098          | 0.143          |

<sup>a</sup> SNP values as coded on the DMET platform

<sup>b</sup> Probability that a patient is homozygote wild type for each SNP, given that this patient has a clearance < 339.6 L/h

<sup>c</sup> Probability that a patient is heterozygote for each SNP, given that this patient has a clearance < 339.6 L/h

<sup>d</sup> Probability that a patient is homozygote for the variant allele for each SNP, given that this patient has a clearance < 339.6 L/h

Abbreviations: SLC22A1; solute carrier family 22 (organic anion/urate transporter) member 11, GSTZ1; glutathione transferase zeta 1, SLC28A2; solute carrier family 28, (sodium-coupled nucleoside transporter) member 2, VKORC1; Vitamin K epoxide reductase complex subunit 1, PGAP3; post-GPI attachment to proteins 3, CDA; cytidine deaminase, EPHX1; epoxide hydrolase 1 microsomal (xenobiotic), CYP20A1; cytochrome P450 family 20 subfamily a polypeptide 1, SLC6A6; solute carrier family 6 (neurotransmitter transporter taurine) member 6, CRIP3; cysteine-rich protein 3, GSTA4; glutathione S-transferase alpha 4, AKAP9; A kinase anchor protein 9, CYP51A1; cytochrome P450 family 51 subfamily A polypeptide 1, CYP2D7P1; cytochrome P450 family 2 subfamily D polypeptide 7 pseudogene 1.
Table 4. Results of validation of SNP prediction model in validation set

<table>
<thead>
<tr>
<th>SNP prediction model</th>
<th>Truly low-clearance</th>
<th>Truly no low-clearance</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted low-clearance</td>
<td>20 (15)</td>
<td>73 (56)</td>
<td>93 (72)</td>
</tr>
<tr>
<td>Predicted no low-clearance</td>
<td>1 (1)</td>
<td>36 (28)</td>
<td>37 (28)</td>
</tr>
<tr>
<td>N</td>
<td>21 (16)</td>
<td>109 (84)</td>
<td>130 (100)</td>
</tr>
</tbody>
</table>

*a cells represent absolute number of patients with percentage of total patients in parentheses

Abbreviations: N, number of patients
FIGURE LEGEND

Figure 1
Flowchart of genetic marker selection.

Figure 2
Presentation of the individual clearance values (L/h), y-axis) according to the genetic variants prediction group. The horizontal line represents the current cutpoint (Mean – 1 standard deviation in all samples) for unbound clearance.
270 patients: 1936 SNPs

Excluded: same genotype
Excluded: not in HWE

270 patients: 770 SNPs

Training set

Identify SNPs using conditional probability

Excluded SNPs: too few observations, too low P(A|B), colinear

Signature 14 SNPs:
Calculate clearance-score

Validation set

Apply signature:
Predict clearance status
A pharmacogenetic predictive model for paclitaxel clearance based on the DMET platform


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