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CYP3A5 rs776746 is the causal CYP3A variant of hypertension

CHAPTER 6

CYP3A5 rs776746, rather than CYP3A4 rs4646437, is the causal CYP3A variant in the association with sunitinib-induced hypertension

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Submitted
ABSTRACT

Both CYP3A5 rs776746 and CYP3A4 rs4646437 have been associated independently with sunitinib-induced hypertension in patients with clear cell metastatic renal cell carcinoma (cc-mRCC). The objective of this study is to investigate which single nucleotide polymorphism (SNP) is the causal genetic factor in the association with sunitinib-induced hypertension. A two-SNP interaction analysis was performed in a cohort of 286 sunitinib-treated cc-mRCC patients. Both CYP3A5 rs776746 and CYP3A4 rs4646437 showed significant associations with the risk of hypertension (odds ratio (OR) =3.4, 95% CI: 1.5-7.8, P=0.003; OR=2.5, 95% CI: 1.2-5.2, P=0.020, respectively, GG-genotype as reference). When both SNPs were entered into a model simultaneously, the OR of CYP3A5 rs776746 changed only marginally (OR=2.9, 95% CI: 0.8-11.3, P=0.115) while the OR of CYP3A4 rs4646437 changed substantially (OR=1.2, 95% CI: 0.3-4.1, P=0.785), indicating that CYP3A5 rs776746 is the causal variant. In conclusion, not the CYP3A4 rs4646437, but the CYP3A5 rs776746 is the causal CYP3A variant in the association with sunitinib-induced hypertension.
INTRODUCTION

Sunitinib, an oral multi-targeted tyrosine kinase inhibitor, has been approved as first-line treatment for patients with metastatic renal cell carcinoma (mRCC). It inhibits multiple receptor tyrosine kinases in the angiogenesis pathway, including vascular endothelial growth factor (VEGF) receptors. Physiologically, inhibition of VEGF signaling causes a decrease in the production of nitric oxide, resulting in vasoconstriction. Thus, hypertension is an on-target side effect of patients treated by sunitinib. It has been reported repeatedly that the inter-individual response to sunitinib is variable. Sunitinib-induced hypertension has been proposed as a potential biomarker to predict treatment outcome. To date, several single nucleotide polymorphisms (SNPs) in VEGFA, VEGFR-2 and eNOS genes, involved in the angiogenesis pathway, have been associated with sunitinib-induced hypertension in mRCC patients. Since there is a relationship between sunitinib exposure and disease outcome as well as hypertension, SNPs in genes encoding sunitinib-metabolizing enzymes (such as CYP3A4 and CYP3A5) might also be potential predictors of sunitinib-induced hypertension.

CYP3A5 rs776746, located in intron 3 of CYP3A5, is a well-studied SNP especially in the metabolism of tacrolimus. The G-allele of CYP3A5 rs776746 (also known as CYP3A5*3) is a non-functional allele. Patients with a GA or AA genotype are regarded as CYP3A5 expressers. Early in 2011, Van der Veldt et al. have performed a retrospective pharmacogenetics association study in 136 clear cell mRCC (cc-mRCC) patients treated with sunitinib and demonstrated that progression-free survival was significantly improved in patients carrying the A-allele in CYP3A5 rs776746 A/G. Later, in an observational, prospective study including 101 patients with cc-mRCC, García-Donás et al. have reported that the A-allele of CYP3A5 rs776746 was associated with an increased risk of dose reduction due to toxicity. Hereafter, the association of CYP3A5 rs776746 with dose reduction was confirmed by Diekstra et al. in a cohort of 333 cc-mRCC sunitinib-treated patients. In this cohort, Diekstra et al. also identified that patients carrying the A-allele of CYP3A5 rs776746 had a higher risk of hypertension (odds ratio (OR)=4.7, 95% CI: 1.47-15.0, P=0.009).

In addition to rs776746 in CYP3A5, polymorphisms in CYP3A4 have also been associated with sunitinib-induced hypertension. At the American Society of Clinical Oncology annual
meeting in 2012, Urun et al.\textsuperscript{9,10} have reported in a cohort including 159 mRCC patients, that the A-allele of \textit{CYP3A4} rs4646437 A/G showed a protective role in sunitinib-induced toxicity. A-allele carriers had a four-fold lower risk of grades 3–4 toxicity compared to patients with the GG-genotype (OR=0.27, 95% CI: 0.08–0.88, P=0.03). No association between \textit{CYP3A4} rs4646437 and hypertension was observed.\textsuperscript{9,10} In a subsequent validation study in a cohort of 287 cc-mRCC patients, we identified that the A-allele of \textit{CYP3A4} rs4646437 was associated with an increased risk of hypertension (OR =2.4, 95% CI: 1.1–5.2, P = 0.021).\textsuperscript{11} No association of \textit{CYP3A4} rs4646437 with other sunitinib-induced toxicities were detected.\textsuperscript{11} The inconsistent results were attributed to varying A-allele frequency, differences of covariates in multivariate analysis and heterogeneity of patient characteristics such as prior treatment in two studies. Unlike \textit{CYP3A5} rs776746, the functionality of \textit{CYP3A4} rs4646437 has not been studied mechanistically. Located in intron 7, \textit{CYP3A4} rs4646437 is not a coding region of known \textit{CYP3A4} transcripts. It has been reported that the A-allele is associated with increased or decreased activity of the \textit{CYP3A4} enzyme and activity may also depend on gender.\textsuperscript{12–15} In these studies, the impact of this genetic variant was conducted by the assessment of \textit{CYP3A4} enzymatic activity on the pharmacokinetics of a \textit{CYP3A4}-metabolized drug, but not a specific \textit{CYP3A4}-substrate. One could speculate that inaccurate prediction of SNP function may occur if the influences from other enzymes involving in the drug metabolism process are not taken into account. Therefore, the effect of this genetic variant on \textit{CYP3A4} enzyme activity in aforementioned studies should be interpreted carefully.

The \textit{CYP3A4} and \textit{CYP3A5} genes lie in close proximity (136 kb) to one another on chromosome 7 (chr. 7q22.1).\textsuperscript{16} As a result, some effects originally thought to be due to a \textit{CYP3A4} allele may actually be caused by a \textit{CYP3A5} allele. As mentioned above, \textit{CYP3A4} rs4646437 and \textit{CYP3A5} rs776746 have been associated with the risk of sunitinib-induced hypertension in separate studies.\textsuperscript{3,11} The objective of the present study is to investigate which SNP is the causal genetic factor in the association with sunitinib-induced hypertension.
CYP3A5 rs776746 is the causal CYP3A variant of hypertension

METHODS

Study population

Sunitinib-treated cc-mRCC patients from The Netherlands, Spain and The United States were taken into consideration in the present study. Patients with clinical information and genotyping data for both CYP3A4 rs4646437 and CYP3A5 rs776746 were included in the statistical analysis. This cohort has been evaluated for associations of several genetic variants with sunitinib efficacy and toxicities previously. In brief, patients received 50 mg, 37.5mg or 25 mg sunitinib for at least one cycle in a 4-week on/2-week off schedule or a continuous dosing regimen. Blood pressure was measured at baseline, week 4 and week 6 of each cycle during 4 cycles of sunitinib treatment. Hypertension grade was scored according to the National Cancer Institute-Common Terminology Criteria for Adverse Events (version 3.0 or 4.0). Final score of hypertension was corrected by subtracting baseline score from the maximum score in four cycles and dichotomized as hypertension grades 0-2 versus grades 3-4.

Statistical analysis

A dominant genetic model for both SNPs was used to re-analyze their genetic effects on the risk of hypertension between patients with a GG-genotype and all A-allele carriers, because the number of patients with an AA-genotype was rare. CYP3A5 rs776746 and CYP3A4 rs4646437 were tested separately against hypertension using binary logistic regression corrected for age, gender and study center. Then both SNPs together with age, gender and study center were entered simultaneously into the model. All tests were two sided and carried out using SPSS Statistical Package for Windows (version 23.0; IBM, Armonk, NY, USA).

RESULTS

A total of 286 patients with available clinical and genotyping data were included in the analysis. Patient characteristics have been described in detail previously. Briefly, all patients had cc-mRCC; 97% were Caucasian and 68% were male. The patients’ age ranged from 34-87 years at the start of sunitinib and 94% initiated the standard dose regimen (50 mg daily dose, 4-weeks on and 2-weeks off). A total of 39 (13.6%) patients experienced
grade 3 or 4 hypertension. The genotype distribution and linkage disequilibrium between CYP3A5 rs776746 and CYP3A4 rs4646437 are shown in Table 1.

**Table 1** The genotype distribution and linkage disequilibrium of CYP3A5 rs776746 and CYP3A4 rs4646437 in 286 patients with metastatic renal cell carcinoma

<table>
<thead>
<tr>
<th>SNP</th>
<th>CYP3A4 rs4646437 A/G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
</tr>
<tr>
<td>CYP3A5 rs776746 A/G</td>
<td>GG</td>
</tr>
<tr>
<td></td>
<td>GA</td>
</tr>
<tr>
<td></td>
<td>AA</td>
</tr>
<tr>
<td>Linkage disequilibrium</td>
<td>(D')</td>
</tr>
</tbody>
</table>

**Table 2** The genetic association of CYP3A5 rs776746 and CYP3A4 rs4646437 with sunitinib-induced hypertension

| Genetic association | Hypertension | | P-value * | OR | 95%CI |
|--------------------|--------------|-------------------------------------------------|
| Grade 0-2          | Grade 3-4    | P-value * | OR | 95%CI |
| CYP3A5 rs776746    | GG           | 221 | 28 | 0.003 | 3.4* | 1.5-7.8 |
|                    | GA+AA        | 26  | 11 |        |      |       |
| CYP3A4 rs4646437   | GG           | 202 | 25 | 0.020 | 2.5* | 1.2-5.2 |
|                    | GA+AA        | 45  | 14 |        |      |       |
| CYP3A5 rs776746    | GG           | 221 | 28 | 0.115 | 2.9* | 0.8-11.3 |
|                    | GA+AA        | 26  | 11 |        |      |       |
| CYP3A4 rs4646437   | GG           | 202 | 25 | 0.785 | 1.2* | 0.3-4.1 |
|                    | GA+AA        | 45  | 14 |        |      |       |

*P-value calculated by multivariate analysis after correction for age, gender and study center, *GG-genotype as reference group, OR: odds ratio.
Both CYP3A5 rs776746 and CYP3A4 rs4646437 were significantly associated with the risk of hypertension after correction for age, gender and study center (OR = 3.4, 95% CI: 1.5-7.8, P = 0.003; OR = 2.5, 95% CI: 1.2-5.2, P = 0.020, respectively, GG-genotype as reference). When both SNPs were entered into a model simultaneously, the OR of CYP3A5 rs776746 changed only marginally (OR = 2.9, 95% CI: 0.8-11.3, P = 0.115) while the OR of CYP3A4 rs4646437 changed substantially (OR = 1.2, 95% CI: 0.3-4.1, P = 0.785), indicating that CYP3A5 rs776746 is the main effect contributor. Table 2 shows the results of the genetic association analyses.

DISCUSSION

Previously, CYP3A5 rs776746 and CYP3A4 rs4646437 have been reported independently for their association with sunitinib-induced hypertension in cc-mRCC patients.\(^3,11\) Due to their close position on chromosome 7, it was yet unknown which SNP is causal to this adverse event. Therefore, we performed the present study to answer this question. Even though neither of SNPs met the significance threshold in the simultaneous analysis, the effect size of CYP3A5 rs776746 remained similar when compared to the results from individual analysis, indicating that CYP3A5 rs776746, rather than CYP3A4 rs4646437, is the causal genetic factor in the association with sunitinib-induced hypertension in Caucasian patients with cc-mRCC.

In this study, there were 37 A-allele carriers of CYP3A5 rs776746, 36 of whom also carried the A-allele of CYP3A4 rs4646437. There is evidence showing that CYP3A4 rs4646437 is in high linkage disequilibrium (LD) with CYP3A5 rs776746 (\(r^2\) from 0.781 to 0.913, D’ was not available), based on a population consisting of Caucasian and African-American persons.\(^17\) Thus, it is reasonable to hypothesize that the effect of CYP3A4 rs4646437 on sunitinib-induced hypertension is most likely driven by CYP3A5 rs776746. However, Li et al.\(^12\) and Liu et al.\(^18\) have individually reported a lower LD between CYP3A5 rs776746 and CYP3A4 rs4646437 in Chinese population (\(r^2 = 0.244\) and \(r^2 = 0.22\), respectively, no D’ data were given). When we calculated the LD of two SNPs using the genotyping data from our 286 cc-mRCC patients, a moderate LD was present (D’ = 0.914, \(r^2 = 0.512\)), which was similar as that calculated by data from the 1000 Genomes Project (D’ = 1, \(r^2 = 0.558\), calculated by SNP Annotation and Proxy Search (SNAP)\(^19\) in CEU population (Utah
residents with Northern and Western European ancestry), but different from YRI (African) population ($D^2=0.557$, $r^2=0.273$).

The contradictory LD among different populations is common, because measurement depends in part on the distribution of SNP allele frequencies. Of note, the minor allele frequencies of $CYP3A5$ rs776746 and $CYP3A4$ rs4646437 vary widely across ethnicities. In Caucasian populations, the G-allele frequency of $CYP3A5$ rs776746 is 0.82-0.95, whereas this is 0.33 in African American, 0.65 in Chinese and 0.85 in Japanese populations. With regard to $CYP3A4$ rs4646437, the G-allele frequency is as follows: European, 0.9; African American, 0.29; Chinese and Japanese, 0.88. It has to be mentioned that the precise measurement of LD requires a substantial sample size. Unfortunately, a large variation in sample size (ranging from 220 to 2845 persons) exists in the aforementioned studies.

There are several limitations in our study. Despite our cohort is one of the largest mRCC cohort available for pharmacogenetics studies, the number of patients may be considered relatively small when patients are divided into different groups according to genotype and hypertension grade, which could influence the statistical power. In addition, we did not take other genetic variants into account, such as SNPs in $VEGFA$ and $eNOS$ genes, even though their genetic associations with hypertension have been reported. This was because the main research question of the current study was to identify the causal genetic variant from $CYP3A5$ rs776746 and $CYP3A4$ rs4646437. Moreover, due to the ethnic differences in allele frequency and degree of LD, the admixture based on ancestry could be a concern in the present study. However, merely 10 (3%) patients were not originally from Caucasian region, which could be considered negligible. Finally, the interpretation and conclusion of the present study could be only confined in Caucasian population, rather than, for example, African population, in which $CYP3A4$ rs4646437 has lower LD with $CYP3A5$ rs776746 and the impact of $CYP3A4$ rs4646437 should not be ignored.

In conclusion, $CYP3A5$ rs776746, rather than $CYP3A4$ rs4646437, is the causal $CYP3A$ variant of the genetic association with sunitinib-induced hypertension in Caucasian cc-mRCC patients. The LD between $CYP3A5$ rs776746 and $CYP3A4$ rs4646437 in Caucasian population should be taken into account in future studies examining the pharmacogenetics of other drugs which are substrates of these metabolizing enzymes.
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REFERENCES


