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ORIGINAL ARTICLE

Pharmacogenetic variants associated with outcome in patients with advanced gastric cancer treated with fluoropyrimidine and platinum-based triplet combinations: a pooled analysis of three prospective studies

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The main treatment for advanced gastric cancer is fluoropyrimidine and platinum-based chemotherapy. We investigated the clinical validitiy of 19 candidate pharmacogenetic variants in *ENOSF1* (enolase superfamily member 1), *TYMS*, *CDA*, *MTHFR*, *TYMP*, *DPYD*, *ERCC1*, *ERCC2*, *GSTP1*, *GSTT1*, *GSTM1*, *CYP3A4* and *CYP3A5* in relation to overall survival (OS), progression-free survival, objective response rate (ORR) and toxicity in 185 patients receiving triplet chemotherapy. The formal significance threshold was $P < 0.0026$. *TYMS* VNTR (variable number of 28-bp tandem repeats) 3 R/3 R genotype was formally associated with inferior ORR (odds ratio (OR) 0.3, $P = 0.0025$), whereas *ENOSF1* rs2612091 G/G was nominally associated with OS after adjustment for *TYMS* 3 R/3 R (hazard ratio (HR) 1.5, $P = 0.041$). In a subgroup analysis of patients with locally advanced disease ($n = 33$), *ENOSF1* rs2612091 was strongly associated with OS (HR 6.5, $P = 0.001$). *CYP3A4**22/*CYP3A5**3 genotype was nominally associated with grade 3/4 toxicity in patients receiving docetaxel-containing chemotherapy ($P = 0.0175$). This is the first study suggesting that *ENOSF1* rs2612091 is prognostic or predictive of OS in gastric cancer. This finding requires prospective validation.

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INTRODUCTION

Gastric cancer (GC) has a poor prognosis, with a cancer-related mortality of 75%.[1] For patients with advanced disease, chemotherapy is the main treatment option available. Standard treatment regimens include a fluoropyrimidine (mainly 5-fluorouracil or capecitabine) and a platinum agent, either cisplatin or oxaliplatin, often combined with a third agent such as docetaxel or epirubicin, as triplet combinations have demonstrated superior antitumor activity compared with doublet combinations.[2–4] Nevertheless, treatment response is highly variable, both in terms of effectiveness and risk of experiencing severe treatment-related toxicity.[5,6] There is a growing body of evidence showing that germline genetic polymorphisms in genes of drug-metabolizing and target enzymes contribute substantially to interpatient variability in response and toxicity in patients with GC.[7–14] Fluoropyrimidines act by inhibiting thymidylate synthase (TS), an enzyme that is crucial for DNA replication and repair. Higher tumor expression of TS has been associated with inferior outcome in GC and colorectal cancer patients who are treated with fluoropyrimidines.[15,16] Tumor TS expression is influenced by polymorphisms in the promoter-enhancer region of *TYMS*, the gene encoding TS. These polymorphisms have been associated with response and survival in GC and colorectal cancer patients treated with fluoropyrimidine-based chemotherapy.[7,15,17–23] A variable number of 28-bp tandem repeats (VNTR) in the 5′-untranslated region (UTR) of *TYMS*, with the most common alleles having two or three repeats (2 R or 3 R, respectively), has been associated with TS expression and was shown to affect survival.[7,18–22] Additionally, a 6-bp insertion/deletion polymorphism in the 3′-UTR of *TYMS* has been associated with response and survival in GC patients.[7–10,13,14,24] However, despite a substantial effort to determine the relationship between *TYMS* polymorphisms and outcome in GC, the role of *TYMS* polymorphisms has thus far remained controversial.[7–14,25–27] Recently, Rosmarin *et al.*[28] proposed that not *TYMS*, but rather enolase superfamily member 1 (*ENOSF1*; chr18: 683 607), a gene adjacent to *TYMS*, might explain previously observed associations between *TYMS* and outcome in patients receiving fluoropyrimidine-based chemotherapy.[28] Higher expression of *ENOSF1*, also known as reverse TS (rTS), has been found to reduce TS expression via production of an antisense RNA to *TYMS* mRNA and, in addition, via production of a protein product named rTS-β that results in a reduction of TS protein levels.[29,30] A G > A polymorphism in the intronic regions of *ENOSF1*, rs2612091, was found to affect the expression of *ENOSF1* mRNA, and was associated with the risk of capecitabine-associated toxicity.[28] Of note, rs2612091 was found to fully explain the observed

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association with toxicity that was previously thought to originate from *TYMS*, suggesting that *ENOSF1* might affect the cell's sensitivity to the cytotoxic effects of fluoropyrimidines more than *TYMS*.[28,31] In view of these recent findings, the relationship between *ENOSF1* rs2612091 and treatment response or survival after treatment with fluoropyrimidines is of interest, but has so far not been investigated in any cancer.

Polymorphisms in other genes related to the pharmacology of fluoropyrimidines, platinum and docetaxel have been associated with outcome of patients with GC.[32] These include polymorphisms in excision repair cross-complementation group 1 and 2 (*ERCC1* and *ERCC2*), which are associated with platinum sensitivity by affecting clearance of DNA-platinum adducts.[33–36] Also, polymorphisms in genes coding for drug-metabolizing enzymes, which result in reduced metabolism of chemotherapy have been associated with patients' risk of experiencing treatment-related toxicity. For taxane drugs such as docetaxel, the combination of polymorphisms in *CYP3A4* and *CYP3A5*, the main metabolic enzymes inactivating taxanes, might be associated with patients' risk of toxicity.[37,38] The clinical relevance of germline pharmacogenetic variants in patients with GC receiving standard first-line fluoropyrimidine and platinum-based doublet or triplet combinations remains poorly established, and genetic variation in enzymes affecting the pharmacokinetics or pharmacodynamics of either of these agents may affect treatment outcome in terms of survival and/or treatment-related toxicity. We performed a pharmacogenetic analysis to determine the clinical validity of 19 candidate pharmacogenetic variants in genes related to the pharmacology of fluoropyrimidines, platinum agents and docetaxel in 185 GC patients who were treated with triplet chemotherapy regimens during three previously conducted prospective clinical studies.

PATIENTS AND METHODS

Study population

The basis for this analysis were patients enrolled in three prospective clinical studies investigating triplet chemotherapy in advanced GC, which were conducted between 2003 and 2014. Briefly, the first study investigated pharmacogenetics in patients receiving ECC, that is, epirubicin 50 mg m^{-2} on day 1, cisplatin 60 mg m^{-2} on day 1 and capecitabine 1000 mg m^{-2} twice daily on days 1–14, as reported previously.[25] The second study was a phase Ib study investigating the safety and efficacy of docetaxel 50 mg m^{-2} on day 1, oxaliplatin 100 mg m^{-2} on day 1 and capecitabine 850 mg m^{-2} twice daily on days 1–14 (DOC)[39] and the third study was a multicenter phase II study investigating the efficacy of DOC plus bevacizumab (B-DOC[40]) and in case of HER2-positivity trastuzumab (B-DOCT[41]) (Meulendijks *et al.*, submitted). Genomic DNA from peripheral blood was obtained from all patients before treatment. In all three studies, pharmacogenetic analyses were defined as a secondary objective in the study protocol. A small number of pharmacogenetic markers have been investigated previously in subsets of patients.[25] However, none of the markers was previously analyzed in the entire population. Any findings in the current analysis that overlap with findings reported previously are explicitly referred to as such. All participating patients fulfilled the following inclusion criteria: histologically or cytologically confirmed irresectable and/or metastatic primary or recurrent adenocarcinoma of the stomach or gastroesophageal junction, age 18 years or older, measurable or evaluable disease according to the RECIST (Response Evaluation Criteria in Solid Tumors) guidelines,[42] WHO (World Health Organization) performance status 0–2 and adequate bone marrow, liver and renal function. All studies were approved by the medical ethics committees of the participating institutions and conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent.

Candidate pharmacogenetic variants

Candidate pharmacogenetic variants in genes encoding enzymes involved in the pharmacokinetics (i.e. absorption, distribution, metabolism and excretion) or pharmacodynamics of fluoropyrimidines, platinum agents or docetaxel were identified and selected based on a computerized literature search in PubMed (Supplementary Appendix). Pharmacogenetic variants were determined using either TaqMan real-time PCR assays from Applied Biosystems (Foster City, CA, USA), PCR followed by Sanger sequencing or PCR followed by restriction fragment length polymorphism analysis and visualization on agarose gel (Supplementary Appendix).

End points and statistical analysis

Patient and treatment characteristics were described. The primary end point was overall survival (OS). Secondary end points were progression-free survival (PFS), objective response rate (ORR) and associations between pharmacogenetic variants and treatment-related toxicity.

OS and PFS were estimated using the Kaplan–Meier method. OS was defined as the time between the first treatment day and the day of death from any cause. PFS was defined as the time between first treatment day and the day of progression or death, whichever came first. OS and PFS were compared between genotypes in univariable analysis using log-rank tests, and in multivariable analysis using Cox regression models, with adjustment for factors associated with OS, that is, extent of disease (locally advanced vs metastatic), WHO performance status (0 vs 1–2) and treatment regimen (ECC, DOC, B-DOC or B-DOCT).

Response was evaluated according to RECIST (version 1.0 or 1.1, depending on the study).[42,43] Only patients with measurable disease at baseline were included in the response evaluation. Associations between pharmacogenetic variants and ORR were tested in univariable analysis using Fisher's exact test or χ^2 tests and in multivariable analysis using logistic regression, with adjustment for extent of disease, WHO performance status and treatment regimen. Treatment-related toxicity was monitored and recorded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTC-AE, v.3.0 or v.4.03, depending on the study). Maximum toxicity during treatment was dichotomized as absent to moderate (grade 0–2) vs severe (grade $\geqslant 3$). Associations with severe toxicity were investigated only in multivariable analysis, using logistic regression with adjustment for age (continuous), gender and treatment regimen. Results are reported as hazard ratios (HR) for OS and PFS, and as odds ratios (OR) for ORR and toxicity, with corresponding 95% confidence intervals (CIs) and *P*-values.

Pharmacogenetic variants were analyzed assuming dominant or recessive models, based on previous studies regarding their functional effect in preclinical studies as well as their effect in pharmacogenetic association studies (Table 2). Unless stated otherwise, association analyses reported in the text are performed according to the genetic model reported in the tables. For *ENOSF1* rs2612091, a largely uncharacterized polymorphism, a dominant model as well as a log-additive (multiplicative) model were investigated. In view of the known functional interaction between *TYMS* and *ENOSF1*,[28,44] variants in these genes were analyzed alone, and in combination by including variants from both genes in the same model. The predictive value of *CYP3A4**22 and *CYP3A5**3 genotypes for docetaxel-associated toxicity was also analyzed in combination, as the combined activity of these variants is responsible for metabolism of CYP3A4/CYP3A5 substrates.[45]

All pharmacogenetics variants were tested for deviation from Hardy–Weinberg equilibrium using the exact test.[46] Allele and genotype frequencies were compared with frequencies reported in literature and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). Linkage disequilibrium was analyzed for variants in *TYMS* and *ENOSF1*, which are both located on chromosome 18. D' and r were calculated using Haploview.[47]

To determine whether the effects of pharmacogenetic variants differed in subgroups of patients, interactions were investigated between genotypes and patient and disease characteristics.

A Bonferroni correction was applied to control for type I error as a result of testing for 19 pharmacogenetic variants. The threshold for formal significance was set at $P < 0.0026$ (0.05/19) for all analyses. Statistical tests resulting in $P < 0.0026$ are referred to as formally significant, and test achieving only $P < 0.05$ are referred to as nominally significant. The same threshold was applied to study statistical interactions with clinical covariates and effects in subgroups. All statistical tests were two-sided. Statistical analyses were performed using R v.3.1.0. and SPSS v.17.0 (SPSS, Chicago, IL, USA).

RESULTS

Study population

A total of 196 patients was treated in the three prospective studies combined, of which 185 patients (95%) were included in this

Table 1. Patient characteristics and associations with overall survival

Characteristic	*N*	*(%)*	*HR (95%CI)*	P *value*
Age				
Years (range)	59	(27–77)	1.00 (0.99–1.02)	0.826
Sex				
Female	50	(27)	1.00	0.894
Male	135	(73)	0.98 (0.68–1.41)	
WHO performance status				
0	97	(52)	1.00	0.117
1–2	88	(48)	1.31 (0.94–1.83)	
Site of primary cancer				
Gastric	97	(52)	1.00	0.968
Gastroesophageal junction	88	(48)	1.01 (0.72–1.41)	
Previous surgery				
No	154	(83)	1.00	0.796
Yes	31	(17)	1.06 (0.70–1.60)	
Previous chemotherapy				
No	170	(92)	1.00	0.646
Yes	15	(8)	1.15 (0.64–2.08)	
Extent of disease				
Locally advanced	33	(18)	1.00	0.001
Metastatic	152	(82)	2.29 (1.42–3.70)	
Treatment regimen				
ECC	75	(41)	1.00	0.039
DOC	31	(17)	1.01 (0.66–1.54)	
B-DOC[a]	56	(30)	0.79 (0.51–1.22)	
B-DOCT[b]	23	(12)	0.40 (0.19–0.84)	

Abbreviations: B-DOC, bevacizumab, docetaxel, oxaliplatin, capecitabine; B-DOCT, bevacizumab, docetaxel, oxaliplatin, capecitabine, trastuzumab; CI, confidence interval; DOC, docetaxel, oxaliplatin, capecitabine; ECC, epirubicin, cisplatin, capecitabine; HR, hazard ratio; WHO, World Health Organization. [a]All patients treated with B-DOC were screened for tumor HER2 status prior to treatment and had HER2-negative disease. [b]All patients treated with B-DOCT were screened for tumor HER2 status prior to treatment and had HER2-positive disease.

analysis. The remaining nine patients (5%) did not consent to pharmacogenetic analysis. The clinical characteristics of the included patients are summarized in Table 1. The median follow-up was 2 years and 6 months. An association with OS was observed for the extent of disease and treatment regimen. None of the other clinical characteristics were associated with treatment outcome.

Candidate pharmacogenetic variants

The pharmacogenetic variants that were selected for analysis are listed in Table 2. All variants were in Hardy–Weinberg equilibrium ($P > 0.050$, results are detailed in the Supplementary Appendix), and allele frequencies corresponded with previously reported frequencies in similar Caucasian populations (details are provided in the Supplementary Appendix).

Associations of *TYMS* VNTR and *ERCC2* 2251A > C genotypes with ORR

A total of 173/185 patients (94%) was evaluable for response, the remaining patients had non-measurable disease (8 patients, 4%) or went off study before the first tumor evaluation (4 patients, 2%). Eleven patients (6%) had a complete response, 85 (49%) partial response, 66 (38%) stable disease and 11 (6%) had progressive disease as best response.

Two variants were nominally associated with ORR in multivariable analysis, the *TYMS* 5′-UTR VNTR 3R/3R genotype (OR 0.4, $P = 0.006$) and *ERCC2* 2251A > C C/C genotype (OR 3.3, $P = 0.031$; Table 3). When the G > C substitution in the 3R allele was considered in addition to *TYMS* VNTR, patients with the 3 RG/3 RG genotype appeared to have the lowest ORR (OR 0.2, $P = 0.049$).

Considering the known functional interaction between *ENOSF1* and *TYMS*, we investigated the combined effects of *TYMS* VNTR and *ENOSF1* rs2612091 on ORR. There was moderate linkage between *TYMS* VNTR and rs2612091 ($D' = 0.76$, $r^2 = 0.47$). Multivariable analysis (with adjustment for clinical covariates) showed that there was some evidence for the *ENOSF1* rs2612091 G/G genotype to be associated with ORR (OR 0.5, 95% CI: 0.88–4.22, $P = 0.102$) after correction for *TYMS* VNTR genotype. Importantly, after correction for *ENOSF1* rs2612091, the *TYMS* VNTR 3R/3R genotype was formally associated with ORR (OR 0.3, 95% CI: 0.14–0.65, $P = 0.0025$). There were no statistical interactions between *TYMS* or *ERCC2* genotypes and clinical characteristics (not shown).

Associations between *ENOSF1* and survival end points

At clinical data cutoff, 145/185 patients (78%) had progressed, and 139/185 patients (75%) had deceased. The *ENOSF1* rs2612091 G/G genotype was found to be nominally associated with shorter OS compared with non-G/G genotypes in univariable analysis (Table 3). The effect of rs2612091 was not maintained in multivariable analysis. However, when the combined effects of *ENOSF1* rs2612091 and the *TYMS* VNTR on OS were investigated by including both variants in a multivariable model (with adjustment for clinical covariates), the *ENOSF1* rs2612091 G/G genotype was nominally associated with inferior OS (HR 1.5, 95% CI: 1.0–2.3, $P = 0.041$), whereas 3R/3R genotype showed a trend toward inferior OS (HR 1.5, 95% CI: 1.0–2.3, $P = 0.076$). Similar results were obtained when both variants were analyzed assuming an additive model (HR 1.4, $P = 0.031$ for *ENOSF1* rs2612091 and HR 1.4, $P = 0.072$ for *TYMS* VNTR).

Associations between *ENOSF1* rs2612091 and outcome in relation to the extent of disease

We further investigated the effect of *ENOSF1* rs2612091 on survival end points by determining interactions with clinical covariates. There was a strong interaction between rs2612091 and the extent of disease in relation to OS (HR 4.3 for locally advanced disease vs metastatic disease, $P = 0.0003$) and PFS (HR 3.3, $P = 0.0019$). These findings suggested that the effect of *ENOSF1* rs2612091 on OS was stronger in patients with locally advanced, non-metastasized disease. We therefore further investigated the effect of *ENOSF1* rs2612091 in the subgroup of patients with locally advanced disease and the subgroup with metastatic disease. In locally advanced disease, *ENOSF1* rs2612091 was strongly predictive for OS (Figure 1a). The median OS for patients with the G/G genotype of rs2612091 was 6.5 months, whereas patients who carried G/A had a much longer OS of 17.5 months. Patients with the A/A genotype had exceptionally good outcome (median OS not reached). In contrast, no effect of rs2612091 on OS was observed in patients with metastatic disease (Figure 1b). In multivariable analysis, the effect of *ENOSF1* rs2612091 remained formally significant in patients with locally advanced disease (HR 6.5 for G/G vs A/A or A/G, 95% CI: 2.1–20.0, $P = 0.001$). In patients with metastatic disease, no association with OS was observed (HR 1.1, 95% CI: 0.74–1.69, $P = 0.597$). Similar findings were obtained for PFS (HR 4.3, $P = 0.005$ for locally advanced disease, vs HR 1.0, $P = 0.921$ for metastatic disease). Addition of the *TYMS* VNTR to the model did not reveal a significant association between the VNTR and outcome in patients, which locally advanced disease (HR 1.8, 95% CI: 0.7–5.0, $P = 0.256$), and the *TYMS* VNTR alone was not

Table 2. Pharmacogenetic variants included in the analysis

Gene	*SNP/variation*	*RS number*	*Location*	*Amino acid substitution*	*Functional effects on enzyme function*	*Number genotyped (%)*	*Allele frequency*	*Studies investigating clinical relevance*
Genes related to fluoropyrimidine pharmacology								
ENOSF1	G > A	rs2612091	Intronic	–	Increased *ENOSF1* mRNA expression.[28] Has been found to regulate thymidylate synthase activity and has been associated with 5-FU resistance.[44,54]	185 (100%)	0.532	28
TYMS	28-bp VNTR (2R/3R)	rs34743033 (rs45445694)	5'UTR	–	The 3 R allele results in a higher level of translational activity and mRNA stability than the 2 R allele, and increased TS expression.[19]	185 (100%)	0.481	7,8,11–14,26
TYMS	G > C in 3 R	rs2853542	5'UTR	–	Disruption of an upstream stimulating factor binding site, resulting in reduced transcriptional activity.[20]	185 (100%)	0.200	7,8,10–12,25–27
TYMS	c.1494del6bp	rs16430	3'UTR	–	Reduced mRNA stability and decreased tumor *TYMS* mRNA expression.[22]	185 (100%)	0.303	7–10,13,14
MTHFR	c.677C > T	rs1801133	Exon 4	$Ala^{222}Val$	Increased thermoliability and reduced enzyme activity, leading to increased levels of 5,10-methylenetetrahydrofolate.[63]	185 (100%)	0.338	7,8,13,14,26
CDA	c.79A > C	rs2072671	Exon 1	$Lyc^{27}Gln$	Decreased enzyme activity, hypothetically leading to reduced activation of capecitabine.[64]	185 (100%)	0.346	31,65,66
TYMP	c.1412C > T	rs11479	Exon 10	$Ser^{471}Leu$	Functional relevance unknown, but previously associated with fluoropyrimidine-associated toxicity.[66]	185 (100%)	0.078	31,66
DPYD[a]	c.1236G > A	rs56038477	Exon 11	$Glu^{412}Glu$	In complete linkage with c.1129-5923C > G in intron 10 which causes a pre-mRNA splicing defect, resulting in decreased enzyme activity.[67]	185 (100%)	0.041	31,65–69
DPYD[a]	c.1601G > A	rs1801158	Exon 13	$Ser^{534}Asn$	Has been associated with reduced and increased DPD enzyme activity.[70,71]	185 (100%)	0.021	31,65,68,72,73
DPYD[a]	c.2846A > T	rs67376798	Exon 22	$Asp^{949}Val$	Reduced enzyme activity due to interference with cofactor binding or electron transport.[71]	185 (100%)	0.014	31,70,74,75
Genes related to platinum pharmacology								
GSTP1	c.313A > G	rs1695	Exon 5	$Ile^{105}Val$	Alteration in substrate affinity and less detoxification capacity.[76]	185 (100%)	0.403	7,8,10,25–27,60
GSTT1	Gene deletion	–	–	–	Null genotype results in absent enzyme activity.[77]	185 (100%)	0.216	7,8,26,27,60
GSTM1	Gene deletion	–	–	–	Null genotype results in absent enzyme activity.[77]	185 (100%)	0.519	7,8,26,27,60
ERCC1	c.354C > T	rs11615	Exon 4	$Asn^{118}Asn$	Reduced translation, decreased mRNA levels.[78]	185 (100%)	0.646	7,8,10,12,26,27,35,60
ERCC1	c.*197G > T	rs3212986	3'UTR	–	Thought to be associated with altered DNA repair capacity.[79,80]	185 (100%)	0.235	7,10,12,25,27,35,60,81
ERCC2	c.2251A > C	rs13181	Exon 23	$Lys^{751}Gln$	Altered DNA repair capacity.[82]	185 (100%)	0.370	7,8,10,26,35,60
ERCC2	c.934C > T	rs1799793	Exon 10	$Asp^{312}Asn$	Altered DNA repair capacity.[82]	184 (99%)	0.381	7,8,10,35,60,81
Genes related to docetaxel pharmacology								
CYP3A4[b]	c.522-191C > T (*CYP3A4**22)	rs35599367	Intronic	–	Reduced CYP3A4 enzyme activity, leading to reduced metabolic clearance of taxanes.[38]	110 (100%)	0.055	37
CYP3A5[b]	c.219-237G > A (*CYP3A5**3)	rs776746	Intronic	–	Induces a splicing defect which results in reduced CYP3A5 enzyme activity, potentially leading to reduced clearance of taxanes.[83]	110 (100%)	0.095	84

Abbreviations: *CDA*, cytidine deaminase (gene); *CYP3A4*, cytochrome P450 3A4 (gene); *CYP3A5*, cytochrome P450 3A5 (gene); *DPYD*, dihydropyrimidine dehydrogenase (gene); *ENOSF1*, enolase superfamily member 1 (gene); *ERCC1*, excision repair cross-complementation group 1 (gene); *ERCC2*, excision repair cross-complementation group 2 (gene); 5-FU, 5-fluorouracil; *GSTM1*, glutathione S-transferase mu 1 (gene); *GSTP1*, glutathione S-transferase pi 1 (gene); *GSTT1*, glutathione S-transferase theta 1 (gene); *MTHFR*, methylenetetrahydrofolate reductase (gene); SNP, single nucleotide polymorphism; TS, thymidylate synthase; *TYMS*, thymidylate synthase (gene); *TYMP*, thymidine phosphorylase (gene); UTR, untranslated region; VNTR, variable number of 28- bp tandem repeats. [a]The *DPYD**2 A variant was not selected for analysis of associations with fluoropyrimidine-associated toxicity because most patients had been screened prior to treatment for this mutation and variant allele carriers received a reduced dose of the fluoropyrimidine to avoid treatment-related toxicity. The *DPYD* c.1679 T > G was selected and analyzed but not detected in any of the patients and therefore not reported in the table. [b]The *CYP3A4* and *CYP3A5* variants were investigated only in the subgroup of patients treated with docetaxel-containing chemotherapy (*n*, 110).

Table 3. Pharmacogenetic associations with efficacy endpoints

Genotype	*Response*					*Progression-free survival*					*Overall survival*			
	N	*ORR*	*P*[a]	*OR [95%CI]*	*P*[b]	*N*	*Months*	*P*[a]	*HR [95%CI]*	*P*[b]	*Months*	*P*[a]	*HR [95%CI]*	*P*[b]
ENOSF1 rs2612091														
A/A, G/A	135	56%	0.71	1.0	0.530	142	7.8	0.063	1.0	0.33	12.9	**0.013**	1.0	0.123
G/G	38	53%	5	0.8 [0.4–1.6]		43	7.1		1.2 [0.8–1.8]	0	9.8		1.4 [0.9–2.0]	
TYMS 5′UTR VNTR														
2R/2R, 2R/3R	126	61%	**0.017**	1.0	**0.006**	138	7.8	0.261	1.0	0.15	12.0	0.370	1.0	0.292
3R/3R	47	40%		0.4 [0.2–0.7]		47	7.2		1.3 [0.9–1.9]	2	12.1		1.2 [0.8–1.9]	
TYMS 5′UTR 3R G>C														
2R/2R, 2R/3RC	91	58%	0.237	1.0		101	7.7	0.341	1.0		10.8	0.134	1.0	
2R/3RG, 3RC/3RG	53	58%		0.9 [0.5–1.9]	0.887	54	6.9		0.8 [0.6–1.2]	0.339	12.6		0.8 [0.5–1.2]	0.271
3RC/3RC	18	50%		0.6 [0.2–1.7]	0.322	19	9.3		1.0 [0.6–1.8]	0.934	13.8		0.9 [0.5–1.6]	0.632
3RG/3RG	11	27%		0.2 [0.1–1.0]	**0.049**	11	6.4		1.4 [0.7–3.0]	0.324	8.7		1.5 [0.7–3.4]	0.286
TYMS 3′UTR														
Ins/ins,	83	59%	0.444	1.0	0.592	90	7.7	0.797	1.0	0.625	10.9	0.486	1.0	0.433
Ins/del, del/del	90	52%		0.8 [0.5–1.6]		95	7.3		0.9 [0.7–1.3]		12.0		0.9 [0.6–1.2]	
MTHFR 677C>T														
C/C, C/T	157	55%	1.000	1.0	0.799	165	7.8	0.071	1.0	**0.040**	12.5	0.094	1.0	**0.041**
T/T	16	56%		0.9 [0.3–2.4]		20	5.7		1.7 [1.0–2.7]		8.2		1.7 [1.0–2.7]	
TYMP 1412C>T														
C/C	149	55%	0.827	1.0	0.491	159	7.4	0.884	1.0	0.616	12.2	0.774	1.0	0.973
C/T, T/T	24	58%		1.4 [0.6–3.4]		26	7.8		0.9 [0.5–1.4]		10.7		1.0 [0.6–1.6]	
CDA 79A>C														
A/A, A/C	148	57%	0.515	1.0	0.740	158	7.8	0.114	1.0	0.300	12.6	**0.036**	1.0	0.174
C/C	25	48%		0.9 [0.4–2.1]		27	5.7		1.3 [0.8–2.0]		9.0		1.4 [0.9–2.2]	
ERCC1 *197G>T														
C/C	106	54%	0.638	1.0	0.574	110	7.7	0.513	1.0	0.751	12.9	0.401	1.0	0.703
C/A, A/A	67	58%		1.2 [0.6–2.3]		75	7.7		0.9 [0.7–1.3]		10.7		1.1 [0.8–1.5]	
ERCC1 354C>T														
C/T, T/T	154	55%	0.626	1.0	0.709	164	7.8	0.549	1.0	0.650	12.5	**0.040**	1.0	**0.047**
C/C	19	63%		1.2 [0.4–3.3]		21	7.1		1.1 [0.7–1.9]		9.5		1.7 [1.0–3.1]	
ERCC2 934C>T														
C/C	74	55%	1.000	1.0	0.905	78	7.9	0.588	1.0	0.895	12.2	0.475	1.0	0.564
C/T, T/T	98	56%		1.0 [0.6–1.9]		106	7.1		1.0 [0.7–1.4]		11.5		1.1 [0.8–1.4]	
ERCC2 2251A>C														
A/A, A/C	153	53%	0.092	1.0	**0.031**	163	7.4	0.721	1.0	0.284	11.5	0.805	1.0	0.974
C/C	20	75%		3.3 [1.1–9.8]		22	9.2		0.8 [0.5–1.3]		13.3		1.0 [0.6–1.7]	
GSTT1 deletion														
No deletion	136	59%	0.097	1.0	0.075	145	7.4	0.656	1.0	0.813	11.5	0.215	1.0	0.831
Deletion	37	43%		0.7 [0.5–1.0]		40	7.9		1.1 [0.7–1.6]		13.7		1.1 [0.7–1.6]	
GSTM1 deletion														
No deletion	84	51%	0.287	1.0	0.331	89	7.8	0.837	1.0	0.902	12.1	0.790	1.0	0.902
Deletion	89	60%		1.4 [0.7–2.5]		96	7.4		1.0 [0.7–1.4]		11.7		1.0 [0.7–1.4]	
GSTP1 313A>G														
A/A, A/G	146	55%	0.833	1.0	0.831	153	7.7	0.602	1.0	0.585	11.7	0.715	1.0	0.585
G/G	27	59%		0.9 [0.4–2.2]		32	7.9		0.9 [0.6–1.4]		13.7		0.9 [0.6–1.4]	

Abbreviations: *CDA*, cytidine deaminase (gene); CI, confidence interval; *CYP3A4*, cytochrome P450 3A4 (gene); *CYP3A5*, cytochrome P450 3A5 (gene); *DPYD*, dihydropyrimidine dehydrogenase (gene); *ERCC1*, excision repair cross-complementation group 1 (gene); *ERCC2*, excision repair cross-complementation group 2 (gene); *ENOSF1*, enolase superfamily member 1 (gene); *GSTM1*, glutathione S-transferase mu 1 (gene); *GSTP1*, glutathione S-transferase pi 1 (gene); *GSTT1*, glutathione S-transferase theta 1 (gene); HR, hazard ratio; *MTHFR*, methylenetetrahydrofolate reductase (gene); ORR, objective response rate (defined as the proportion of patients with complete and partial response as best response); OR, odds ratio; *TYMS*, thymidylate synthase (gene); *TYMP*, thymidine phosphorylase (gene). [a]Univariable *P* value; *P* values in bold represent *P* values significant at the nominal significance level. The threshold for formal significance was $P < 0.0026$. [b]Multivariable *P* value; *P* values in bold represent *P* values significant at the nominal significance level. The threshold for formal significance was $P < 0.0026$.

predictive either in patients with locally advanced disease or metastatic disease (Figures 1c and d).

Associations of *MTHFR*, *CDA* and *ERCC1* with survival end points

There was a nominally significant association between *MTHFR* 677C>T T/T genotype and inferior PFS, as well as a nominal association with inferior OS (Table 3). There were no associations between *MTHFR* genotypes and patient characteristics (not shown).

Three other variants were nominally associated with OS: *CDA* 79A>C, *ERCC1* 354C>T and *ENOSF1* rs2612091. Patients with the C/C genotype of *CDA* 79A>C had significantly shorter OS compared with non-C/C genotypes (9.0 vs 12.6 months, $P = 0.036$). However, patients carrying C/C also had metastatic disease more often than patients carrying non-C/C genotypes (100% vs 79%, $P = 0.005$). As a

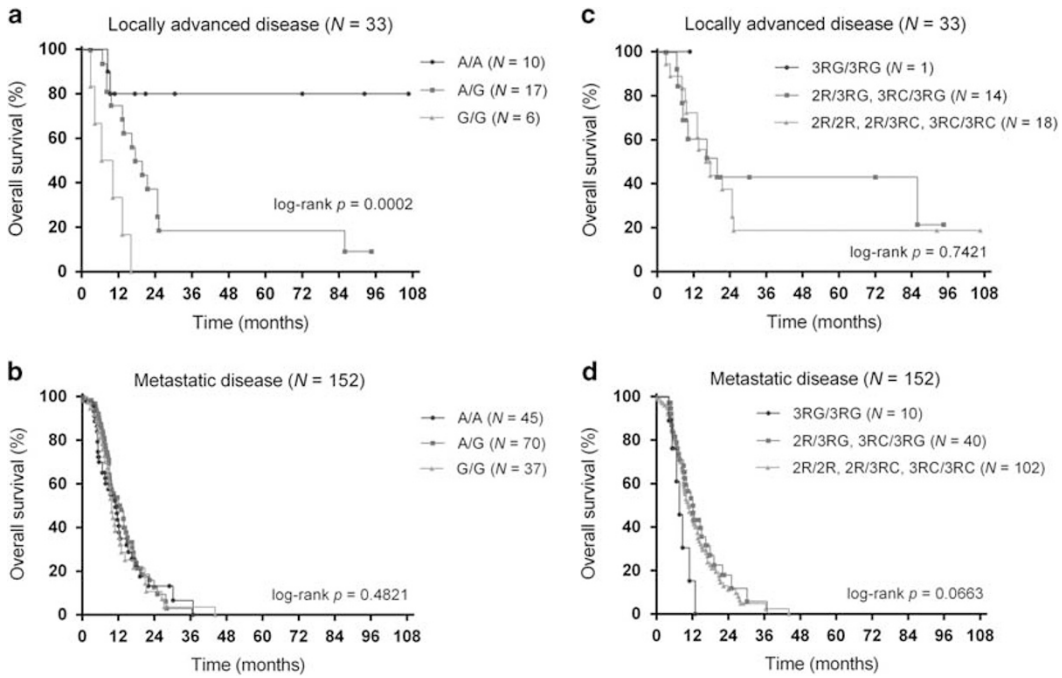

Figure 1. Associations of *ENOSF1* (enolase superfamily member 1) and *TYMS* variants with overall survival. Associations between *ENOSF1* rs2612091 and overall survival in patients with (**a**) locally advanced disease and (**b**) metastatic disease at baseline. Pairwise comparisons of the groups shown in (**a**) revealed significant differences between *ENOSF1* rs2612091 AA and AG genotypes ($P=0.0246$), AA and GG genotypes ($P=0.0023$) and AG and GG genotypes ($P=0.0021$). Pairwise comparisons of groups in (**b**) did not reveal significant differences. Associations between *TYMS* haplotypes of rs45445694 and rs2853542 and overall survival in patients who had locally advanced disease at baseline are shown in (**c**); patients with metastatic disease are shown in (**d**). Pairwise comparisons of groups in (**c**) and (**d**) did not reveal significant differences ($P>0.1$).

result, the association between *CDA* 79A>C and OS was not maintained in multivariable analysis (HR 1.4, 95% CI: 0.9–2.2, $P=0.174$). Patients with the *ERCC1* 354C>T C/C genotype had shorter OS than patients with non-C/C genotypes in univariable and multivariable analysis. There were no differences in patient or disease characteristics between *ERCC1* 354C>T genotypes and no statistical interactions with clinical covariates (not shown).

Associations of *CYP3A4, CYP3A5* and *ENOSF1* genotypes with treatment-related toxicity

Results of the associations between pharmacogenetic variants and treatment-related toxicity are shown in Table 4, and frequencies of severe treatment-related toxicity per treatment regimen are summarized in the Supplementary Appendix. Two nominally significant associations were detected: *DPYD* 2846A>T was associated with hand-foot syndrome and *CYP3A4**22 was associated with gastrointestinal toxicity. None of the individual variants were associated with global severe treatment-related toxicity. However, *CYP3A4**22 and *CYP3A5**3 genotypes combined were nominally associated with global severe toxicity in patients treated with docetaxel in univariable analysis (Figure 2a). A nominally significant association was maintained in multivariable analysis (OR 2.7, $P=0.030$).

For *ENOSF1* rs2612091, a nominally significant association with gastrointestinal toxicity was noted when an additive model was assumed (Figure 2b), and this association was maintained in multivariable analysis (OR 2.3 per additional A allele, $P=0.038$). No associations with toxicity at the formal significance level were present.

DISCUSSION

We investigated the clinical validity of 19 candidate pharmacogenetic variants as predictors of outcome in patients with advanced GC treated with fluoropyrimidine and platinum-based triplet chemotherapy. The *TYMS* VNTR 3RG/3RG genotype was significantly associated with inferior ORR, in line with previous findings.[23,8] *TYMS* VNTR was not associated with our primary end point OS, and could therefore not be clinically validated. Furthermore, previous studies have shown inconsistent associations between *TYMS* variants and OS (Supplementary Appendix).[8,12,7,14,13,11,10,9,25–27] As OS is the most clinically relevant end point in the palliative setting in GC, the overall evidence does not support the clinical validity of the investigated *TYMS* variants, and they are therefore unlikely to have clinical utility. Data on the association between the *TYMS* VNTR 3RG allele and ORR appear to be more consistent.[23,8] Importantly, in the neoadjuvant setting in GC it has been shown that ORR before surgery, that is, tumor downstaging, is the strongest independent predictor of OS (HR 0.43) after correction for other covariables.[48] Therefore, the *TYMS* VNTR variant may be of clinical value in this setting, but this needs to be further assessed. Overall, the current evidence does not support the use of *TYMS* variants as biomarkers in the palliative setting.

A recent study by Rosmarin *et al.*[28] suggests that *ENOSF1*, a gene adjacent to *TYMS*, might have an important additional role in regulating the cell's sensitivity to fluoropyrimidines. We found that in the overall population, *ENOSF1* rs2612091 G/G genotype was nominally associated with shorter OS in univariable analysis (Table 3). The effect of rs2612091 was not maintained in multivariable analysis. However, when adjusting for *TYMS* VNTR, which functionally interacts with *ENOSF1*, rs2612091 G/G genotype was nominally associated with inferior OS (HR 1.5, $P=0.041$), and 3R/3R genotype showed a trend toward inferior OS (HR 1.5, $P=0.076$). These results, while not formally confirming clinical validity, do suggest that *TYMS* and *ENOSF1* variants, combined, may be predictive and/or prognostic of OS in the palliative treatment of GC.

Table 4. Pharmacogenetic associations with treatment-related toxicity

Genotype	*Global toxicity*					*Hematological toxicity*				*Gastrointestinal toxicity*				*Hand-foot syndrome*			
	N	G *0–2%*	G *3–4%*	*OR (95% CI)*	P-*value*[a]	G *0–2%*	G *3–4%*	*OR (95% CI)*	P-*value*[a]	G *0–2%*	G *3–4%*	*OR (95% CI)*	P-*value*[a]	*G 0–2%*	*G 3–4%*	*OR [95%CI]*	*P*[a]
TYMS 5′UTR																	
2R/2R, 2R/3R	137	53	47	1.0	0.851	66	34	1.0	0.461	89	11	1.0	0.103	96	4	1.0	–
3R/3R	48	52	48	1.0 [0.5–2.1]		73	27	0.7 [0.3–1.6]		79	21	2.1 [0.5–2.3]		100	0	NA	
TYMS 5′UTR 3RG>C																	
2R/2R, 2R/3RC	101	53	47	1.0		67	33	1.0		89	11	1.0		96	4	1.0	
2R/3RG, 3RC/3RG	54	54	46	0.9 [0.4–1.8]	0.763	65	35	0.9 [0.4–1.9]	0.706	87	13	1.3 [0.4–3.7]	0.660	96	4	1.2 [0.2–7.5]	0.837
3RC/3RC	19	53	47	0.9 [0.3–2.7]	0.912	73	27	0.7 [0.2–2.3]	0.538	74	26	2.6 [0.7–9.6]	0.138	100	0	NA	–
3RG/3RG	11	45	55	1.4 [0.4–5.0]	0.615	74	26	1.0 [0.2–4.3]	0.955	82	18	1.6 [0.3–9.2]	0.602	100	0	NA	–
TYMS 3′UTR																	
Ins/ins	90	48	52	1.0	0.139	68	32	1.0	0.846	84	16	1.0	0.458	97	3	1.0	0.953
Ins/del, del/del	95	58	42	0.6 [0.3–1.2]		67	33	0.9 [0.5–1.8]		88	12	0.7 [0.3–1.7]		97	3	1.0 [0.2–5.0]	
MTHFR 677C>T																	
C/C, C/T	165	54	46	1.0	0.592	69	31	1.0	0.392	85	15	1.0	–	97	3	1.0	0.351
T/T	20	45	55	1.3 [0.5–3.5]		55	45	1.6 [0.6–4.4]		100	0	NA		95	5	3.3 [0.3–41.6]	
CDA 79A>C																	
A/A, A/C	158	53	47	1.0	0.592	67	33	1.0	0.825	87	13 15	1.0	0.98	97	3	1.0	0.906
C/C	27	56	44	0.8 [0.3–1.9]		70	30	0.9 [0.3–2.4]		85		1.0 [0.3–3.4]		96	4	1.1 [0.1–10.8]	
TYMP 1412C>T																	
C/C	159	54	46	1.0	0.825	66	34	1.0	0.293	87	13	1.0	0.758	96	4	1.0	–
C/T, T/T	26	50	50	1.1 [0.5–2.6]		77	23	0.6 [0.2–1.6]		85	15	1.2 [0.4–4.2]		100	0	NA	
DPYD 1236G>A																	
G/G	170	52	48	1.0	0.657	67	33	1.0	0.621	87	13	1.0	0.745	97	3	1.0	0.597
G/A, A/A	15	60	40	0.8 [0.3–2.4]		80	20	0.7 [0.2–2.9]		80	20	1.3 [0.3–5.4]		93	7	1.9 [0.2–19.2]	
DPYD 1601G>A																	
G/G	177	53	47	1.0	0.838	68	32	1.0	0.54	86	14	1.0	–	97	3	1.0	–
G/A	8	63	37	0.9 [0.2–4.0]		63	37	1.6 [0.3–8.1]		100	0	NA		100	0	NA	
DPYD 2846A>T																	
A/A	180	53	47	1.0	0.581	68	32	1.0	0.956	86	14	1.0	–	97	3	1.0	**0.024**
A/T	5	40	60	1.7 [0.3–11.2]		60	40	0.9 [0.1–6.3]		100	0	NA		80	20	38 [1.6–881]	
ENOSF1 rs2612091																	
A/A, G/A	142	50.7	49.3	1.0	0.359	66.2	33.8	1.0	0.513	83.8	16.2	1.0	0.102	97.2	2.8	1.0	0.404
G/G	43	60.5	39.5	0.7[0.3–1.5]		72.1	27.9	0.8 [0.3–1.7]		95.3	4.7	0.3 [0.1–1.3]		95.3	4.7	2.1[0.4–13.5]	
CYP3A4 *22[b]																	
*1/*1	98	63	37	1.0	0.206	81	19	1.0	0.237	85	15	1.0	0.042	96	4	1.0	0.454
*1/*22	12	42	58	2.2 [0.6–7.8]		92	8	0.3 [0.0–2.4]		58	42	4.0 [1.0–15.2]		92	8	2.4 [0.2–24.6]	
CYP3A5 *3[b]																	
*1/*1, *1/*3	20	80	20	1.0	0.065	90	10	1.0	0.355	95	5	1.0	0.121	100	0	1.0	–
*3/*3	90	57	43	3.1 [0.9–10.0]		80	20	2.1 [0.4–10.4]		79	21	5.2 [0.6–42.1]		94	6	NA	

	Global toxicity					*Hematological toxicity*				*Gastrointestinal toxicity*				*Sensory neuropathy*			
	N	G *0–2%*	*G 3–4%*	*OR [95%CI]*	*P*[a]	*G 0–2%*	*G 3–4%*	*OR [95%CI]*	*P*[a]	*G 0–2%*	*G 3–4%*	*OR [95%CI]*	*P*[a]	*G 0–2%*	*G 3–4%*	*OR [95%CI]*	*P*[a]
GSTT1 deletion																	
No deletion	145	52	48	1.0	0.419	67	33	1.0	0.507	88	12	1.0	0.234	95	5	1.0	–
Deletion	40	58	42	0.9 [0.6–1.2]		70	30	0.9 [0.6–1.3]		83	17	1.4 [0.8–2.3]		100	0	NA	
GSTM1 deletion																	
No deletion	89	56	44	1.0	0.479	70	30	1.0	0.419	87	13	1.0	0.637	96	4	1.0	0.795
Deletion	96	50	50	1.1 [0.8–1.5]		66	34	1.2 [0.8–1.6]		87	13	0.9 [0.6–1.4]		96	4	0.9 [0.4–1.9]	

Table 4. (Continued)

	Global toxicity					*Hematological toxicity*				*Gastrointestinal toxicity*				*Sensory neuropathy*			
	N	G *0–2%*	*G 3–4%*	*OR [95%CI]*	*P*[a]	*G 0–2%*	*G 3–4%*	*OR [95%CI]*	*P*[a]	*G 0–2%*	*G 3–4%*	*OR [95%CI]*	*P*[a]	*G 0–2%*	*G 3–4%*	*OR [95%CI]*	*P*[a]
GSTP1 313A>G																	
A/A, A/G	153	54	46	1.0	0.527	68	32	1.0	0.248	86	14	1.0	0.441	95	5	1.0	0.518
G/G	32	50	50	1.3 [0.6–2.9]		66	34	1.8 [0.7–4.4]		88	12	0.6 [0.2–2.1]		97	3	0.5 [0.1–4.4]	
ERCC1 *197G>T																	
C/C	110	55	45	1.0	0.692	68	32	1.0	0.722	88	12	1.0	0.677	95	5	1.0	0.242
C/A, A/A	75	51	49	1.1 [0.6–2.1]		67	33	1.1 [0.6–2.3]		84	16	1.2 [0.5–3.0]		97	3	0.4 [0.1–2.0]	
ERCC1 354C>T																	
C/T, T/T	164	54	46	1	0.617	66	34	1	0.493	88	12	1	0.583	96	5	1	0.656
C/C	21	48	52	1.3 [0.5–3.3]		81	19	0.7 [0.2–2.2]		76	24	1.4 [0.4–4.7]		95	4	0.6 [0.1–5.7]	
ERCC2 934C>T																	
C/C	78	59	41	1.0	0.26	71	29	1.0	0.511	91	9	1.0	0.136	92	8	1.0	0.055
C/T, T/T	106	48	52	1.4 [0.8–2.6]		65	35	13 [0.6–2.5]		83	17	2.1 [0.8–5.6]		98	2	0.2 [0.0–1.0]	
ERCC2 2251A>C																	
A/A, A/C	163	55	45	1	0.247	69	31	1	0.165	86	14	1	0.569	96	4	1	0.995
C/C	22	41	59	1.7 [0.7–4.5]		55	45	2.1 [0.7–5.8]		91	9	0.6 [0.1–3.1]		96	4	1.0 [0.1–9.3]	

Abbreviations: *CDA*, cytidine deaminase (gene); CI, confidence interval; G0-2/G3-5, grade 0-2/3-5 toxicity according to the Common Terminology Criteria for Adverse Events (CTC-AE); *DPYD*, dihydropyrimidine dehydrogenase (gene); *CYP3A4*, cytochrome P450 3A4 (gene); *CYP3A5*, cytochrome P450 3A5 (gene); *ENOSF1*, enolase superfamily member 1 (gene); *ERCC1*, excision repair cross-complementation group 1 (gene); *ERCC2*, excision repair cross-complementation group 2 (gene); *GSTM1*, glutathione S-transferase mu 1 (gene); *GSTP1*, glutathione S-transferase pi 1 (gene); *GSTT1*, glutathione S-transferase theta 1 (gene); *MTHFR*, methylenetetrahydrofolate reductase (gene); OR, odds ratio; NA, not available due to zero events in one of the groups; *TYMS*, thymidylate synthase (gene); *TYMP*, thymidine phosphorylase (gene). [a]Multivariable *P* value; *P* values in bold represent *P* values significant at the nominal significance level. The threshold for formal significance was $P < 0.0026$. [b]Determined in the subset of patients treated with docetaxel, oxaliplatin, and capecitabine.

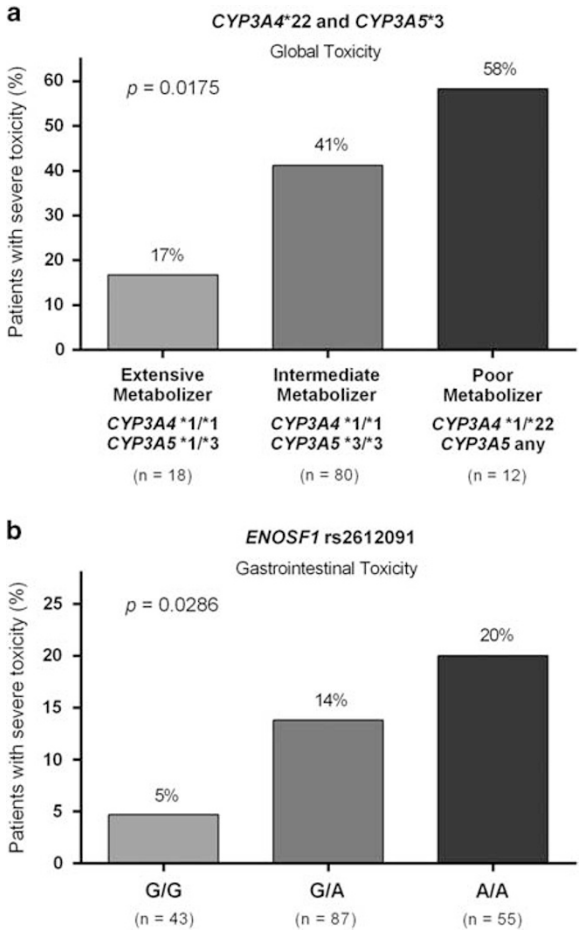

Figure 2. Associations of *ENOSF1*, *CYP3A4* and *CYP3A5* with severe treatment-related toxicity. Associations between *CYP3A4**22 and *CYP3A5**3 and global severe toxicity are shown in (**a**), and associations between *ENOSF1* rs2612091 genotypes and incidence of severe gastrointestinal toxicity are shown in (**b**). The *P*-values represent the Mantel–Haenszel test for trend.

We further investigated the effect of *ENOSF1* on OS in the subset of GC patients with locally advanced disease and patients with metastatic disease. Previous studies suggest that these subgroups may have different tumor biology, as proposed by Abramowicz *et al.*[49] who showed marked differences in serum proteome signatures in pre-treatment blood samples between patients presenting with locally advanced vs metastatic disease. Also, marked differences in treatment response have been previously observed between patients presenting with locally advanced vs metastatic disease.[50,51] In patients with locally advanced disease, we found a strong effect of *ENOSF1* rs2612091 G>A on OS, the rs2612091 G allele being formally and independently associated with inferior OS after adjustment for covariables (HR 6.5, $P=0.001$). Furthermore, increasingly poor outcome was observed with each additional rs2612091 G allele (Figure 1a). It should be acknowledged that this analysis concerned a very small number of patients and that replication studies investigating the effect of *ENOSF1* in GC are required. These studies could focus on populations of GC patients with locally advanced disease, including patients treated in the neoadjuvant setting.

With regard to toxicity, Rosmarin *et al.*[28] showed that rs2612091 explained the association with fluoropyrimidine-associated toxicity that was initially thought to originate from *TYMS* VNTR. Indeed, while we found *TYMS* genotypes not to be predictive of toxicity, there was evidence for an association between *ENOSF1* rs2612091 and gastrointestinal toxicity ($P=0.0286$; Figure 2b). These findings may indicate an association between *ENOSF1* rs2612091 and the pharmacodynamics of fluoropyrimidines. From what is known about the functional role of *ENOSF1*, the observed effect of rs2612091 might be the result of regulation of TS activity. *ENOSF1* encodes an antisense RNA to *TYMS* mRNA, which has been found to downregulate TS expression.[52,53] In addition, *ENOSF1* produces multiple protein products, including rTS-α and rTS-β, as a result of alternate splicing.[52,54] rTS-β has been found to downregulate TS protein levels by a second, largely unresolved mechanism, which supposedly acts at the post-translational level.[44] Thus, *ENOSF1* affects TS expression at the post-transcriptional and post-translational level and appears to be an important regulator of TS activity.[29] This is of interest, considering the fact that many studies have shown that higher tumor TS expression is associated with inferior outcome in GC patients treated with fluoropyrimidines (as well as in patients with colorectal cancer).[15,17,55–58] The *ENOSF1* rs2612091 G allele, which we found to be associated with inferior outcome, was previously found to be associated with decreased *ENOSF1* expression,[28] which was based on the described functional effects of *ENOSF1* could potentially explain the reduced cytotoxic effect of fluoropyrimidines.[28,44]

We found that patients with the *ERCC1* 354C>T C/C genotype had shorter OS than patients with non-C/C genotypes in univariable and multivariable analysis. This is in line with a study in 126 colorectal cancer patients treated with oxaliplatin in which patients with the C/C genotype also had inferior OS ($P=0.006$) and ORR ($P=0.02$).[59] Different previous studies in GC, however, showed no effect of this variant, indicating that this variant is most likely not useful clinically as a prognostic or predictive marker in GC.[10,26,27,60–62]

Last, a nominal association between *CYP3A4*/*CYP3A5* genotypes and gastrointestinal toxicity in patients treated with docetaxel was found. This is of interest, and can be explained by the critical role of CYP3A enzymes in the metabolism of taxanes.[37] We are not aware of other studies previously investigating the clinical validity of *CYP3A4*/*CYP3A5* genotypes as predictors of docetaxel toxicity, but are currently performing additional validation studies.

We could not validate the clinical validity of the other investigated variants. There are many factors that can lead to inconsistency in the effects of germline polymorphisms on clinical outcome, including but not limited to: discrepancy between germline and tumor genome, variation in genotype–phenotype relationship, and the effect of other genes and enzymes. For pharmacogenetic markers to be useful in the clinic, a high level of consistency in the effect on outcome is required. For this reason, the pharmacogenetic variants investigated in this study cannot currently be recommended as biomarkers to base treatment decision on.

CONCLUSION

Our findings indicate that *TYMS* VNTR is associated with ORR, in line with previous reports. However, the effect of *TYMS* VNTR did not translate into an OS benefit, questioning the clinical validity of *TYMS* variants in the palliative treatment of GC. This is the first study suggesting that *ENOSF1* rs2612091 is prognostic and/or predictive of OS in GC. Prospective validation is required to confirm this finding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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