Polymorphisms in MIR27A associated with early-onset toxicity in fluoropyrimidine-based chemotherapy

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Supplementary Figure: 1
Abstract

Purpose: The microRNA miR-27a was recently shown to directly regulate dihydropyrimidine dehydrogenase (DPD), the key enzyme in fluoropyrimidine catabolism. A common polymorphism (rs895819A>G) in the miR-27a genomic region (MIR27A) was associated with reduced DPD activity in healthy volunteers, but the clinical relevance of this effect is still unknown. Here, we assessed the association of MIR27A germline variants with early-onset fluoropyrimidine toxicity.

Experimental Design: MIR27A was sequenced in 514 cancer patients receiving fluoropyrimidine-based chemotherapy. Associations of MIR27A polymorphisms with early-onset (cycles 1-2) fluoropyrimidine toxicity were assessed in the context of known risk variants in the DPD gene (DPYD) and additional covariates associated with toxicity.

Results: The association of rs895819A>G with early-onset fluoropyrimidine toxicity was strongly dependent on DPYD risk variant carrier status (interaction p=0.0025). In patients carrying DPYD risk variants, rs895819G was associated with a strongly increased toxicity risk (OR: 7.6; 95% CI: 1.7-34.7; p=0.0085). Overall, 71% (12 of 17) of patients who carried both rs895819G and a DPYD risk variant experienced severe toxicity. In patients without DPYD risk variants, rs895819G was associated with a modest decrease in toxicity risk (OR: 0.62; 95% CI: 0.43-0.9; p=0.012).

Conclusions: These results indicate that miR-27a and rs895819A>G may be clinically relevant for further toxicity risk stratification in carriers of DPYD risk variants. Our data suggest that direct suppression of DPD by miR-27a is primarily relevant in the context of fluoropyrimidine toxicity in patients with reduced DPD activity. However, miR-27a regulation of additional targets may outweigh its effect on DPD in patients without DPYD risk variants.
Translational relevance

Early-onset toxicity from fluoropyrimidine-based chemotherapy necessitates reduction, delay, or cessation of treatment and, in severe cases, can result in patient death. Some cases of fluoropyrimidine toxicity have been linked to reduced activity of the fluoropyrimidine metabolizing enzyme dihydropyrimidine dehydrogenase (DPD) due to deleterious genetic variants in the encoding gene (DPYD). In the present manuscript, we show that genetic variation in an oncogenic and DPYD-regulatory microRNA, miR-27a, is strongly associated with early-onset toxicity in fluoropyrimidine-based chemotherapy in patients carrying known risk variants in DPYD. MiR-27a and genetic polymorphisms that affect its expression may thus serve as novel markers for improved risk stratification in patients receiving fluoropyrimidine-based chemotherapy.

Introduction

Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme in the catabolism of the fluoropyrimidines 5-fluorouracil (5-FU) and capecitabine and, as such, is a key determinant of adverse effects from fluoropyrimidine-based chemotherapy (1,2). In particular, deleterious genetic variants in the gene encoding DPD (DPYD) are known to increase the risk of severe and potentially fatal toxicity due to increased fluoropyrimidine exposure (1,3,4). However, not all severe fluoropyrimidine toxicity episodes can be explained by currently known DPYD risk variants, and some DPYD risk variant carriers tolerate standard doses of fluoropyrimidine-based chemotherapy without major toxicity (3,5). Therefore, additional factors with a regulatory effect on DPYD expression and DPD activity may impact fluoropyrimidine catabolism and toxicity both in patients with and without DPYD risk variants. Identification of such modulating factors may prevent life-threatening toxicity episodes by improving the sensitivity and positive predictive value of pharmacogenetic testing to identify patients at increased risk of severe fluoropyrimidine toxicity.
MicroRNAs are short (17-21 nucleotide) single stranded RNAs that downregulate protein expression through recruitment of RNA-induced silencing complex (RISC) proteins. MicroRNAs have been shown to act as both oncogenes and tumor suppressors (6). Dysregulation of microRNA expression has also been associated with multidrug resistance in cancer cell lines, suggesting that microRNAs may serve as potential drug targets to enhance the anticancer activity of chemotherapy (7). The relevance of microRNAs in the context of toxicity from chemotherapy is less well known.

The highly homologous microRNAs miR-27a and miR-27b have recently been shown to downregulate DPD expression by directly targeting RISC proteins to *DPYD* (8,9). MiR-27a is thought to be oncogenic (10) and shows increased expression in tumors (11). Elevated expression of miR-27a has been associated with poor cancer prognosis (12,13), chemotherapy resistance (13,14), and increased risk of metastasis (15). Several studies have identified miR-27a as an indirect regulator of the drug efflux pump multidrug resistance protein 1 (MDR1), suggesting a possible mechanism for its observed role in chemotherapy resistance (14,16,17). A common polymorphism (rs895819A>G) in the genomic region encoding miR-27a (*MIR27A*) has been associated with cancer risk (15,18) and survival in patients with gastric and lung cancer (19,20). In recent functional studies, the G allele was associated with higher levels of miR-27a and reduced DPD levels (8). Given the location of this polymorphism within the hairpin loop of the miR-27a pre-microRNA, the higher levels of active microRNA observed in rs895819G carriers was suggested to result from more efficient pre-microRNA processing.

The clinical relevance of genetic variation in the *DPYD*-regulatory *MIR27A* in the context of toxicity from fluoropyrimidine-based chemotherapy is unknown at present. Given the key role of DPD in fluoropyrimidine pharmacokinetics and the reported impact of rs895819 on DPD activity in healthy volunteers (8), this study investigated the association between polymorphisms in *MIR27A* and early-onset toxicity in Caucasian cancer patients receiving fluoropyrimidine-based chemotherapy. This is the first
study to investigate regulatory microRNA germline genetic variants in a clinical context as a potential predictor of fluoropyrimidine toxicity, and MIR27A polymorphisms in the context of chemotherapy toxicity in general.

**Patients and Methods**

*Patients*

Variants in MIR27A were assessed in a patient cohort that was previously investigated for DPYD variants and their association with early-onset fluoropyrimidine toxicity (3). In brief, DNA samples were collected and fluoropyrimidine-related toxicity (hematologic including infections, gastrointestinal, and dermatologic) in the first two chemotherapy cycles was assessed in 514 cancer patients receiving fluoropyrimidine-based chemotherapy (Table 1). This cohort included 500 patients recruited prospectively (i.e. without prior knowledge of toxicity) and 14 additional patients with known fluoropyrimidine toxicity resulting in therapy delay or cessation in the first two chemotherapy cycles. One patient with known fluoropyrimidine toxicity included in the previous study (3) was excluded due to the unavailability of DNA for the analysis of MIR27A. Patient characteristics, observed early-onset fluoropyrimidine toxicities, and identified DPYD risk variants have been described previously (3). The study was approved by the ethics committees of both participating centers (3).

*Genotyping and sequencing of MIR27A*

The complete genomic region encompassing MIR27A was sequenced in all patients using the primers 5’-GTCCCCAAATCTCATTACCTCCTT-3’ (forward) and 5’-GGTCTGGATTCTGAGTCCTCCTTC-3’ (reverse). The 555 bp fragment was amplified using the QIAGEN Multiplex PCR kit (Qiagen) in a total reaction volume of 25 µl containing 3 µl of genomic DNA (to a maximum of 300 ng), 2.5 µl Q-Solution (Qiagen), 2.5 µl primer mix (2 µM each), and 12.5 µl Qiagen Multiplex Master Mix (2X). PCR
amplification was performed in a GeneAmp 9800 Fast Thermal Cycler using the Universal Multiplex Cycling Protocol (Qiagen) with an annealing temperature of 58°C and 35 amplification cycles. Sanger sequencing was performed using the same primers and the Big Dye Terminator v3.1 Cycle Sequencing kit (Life Technologies) on an ABI Prism 3130xl Genetic Analyzer (Life Technologies). Hydrolysis probe-based methods were not used for genotyping due to the close proximity of the rs895819 and rs11671784 genetic variants within MIR27A, which led to incorrect genotyping results, consistent with a previous report (21).

Statistical analyses

Deviations of the observed genotype frequencies from Hardy-Weinberg equilibrium (HWE) were assessed using the exact test implemented in GENEPOP on the web v4.2 (22) with complete enumeration of alleles. Associations of rs895819 and rs11671784 with fluoropyrimidine toxicity were assessed by ordinal logistic regression (OLR) with three toxicity groups (grade 0-1, grade 2, grade ≥3) using the rms package (23) implemented in the statistical software R (24). The proportional odds assumption for OLR models was assessed using the test of parallel lines in SPSS (IBM). Initial association tests were performed using an additive genetic model; other genetic models were subsequently tested only for significant associations. Associations were evaluated using both univariate OLR and by adjusting for coadministration of cisplatin or carboplatin (grouped as one variable), sex, and the number of DPYD risk alleles (c.1129-5923C>G/hapB3, c.1905+1G>A, c.1679T>G, c.2846A>T) (3). Female sex and coadministration of cis- or carboplatin were previously shown to be associated with increased early-onset toxicity in this cohort, whereas no effect was observed for other concomitant chemotherapeutics (e.g. oxaliplatin, anthracyclines), or for 5-FU vs. capecitabine (3). Non-additive effects were evaluated between MIR27A polymorphisms and included covariates. Associations were investigated for overall toxicity (i.e. the maximum CTCAE grade across all assessed categories in the first two chemotherapy cycles). Receiver operating characteristics curves were compared between different OLR models using the R package pROC (25). Confidence intervals and one-sided p-values for comparison of areas under the curve were
determined using bootstrap with 5000 replicates. For an assessment of rs895819 and rs11671784 allele frequencies in relation to different toxicities, individual toxicity categories were grouped as hematologic (leukopenia, thrombopenia, anemia, infections), mechanism-related gastrointestinal (diarrhea, mucositis), other gastrointestinal (nausea, vomiting, dehydration), and dermatologic toxicities (hand foot syndrome, dry skin, hair loss). P-values <0.05 were considered statistically significant.

Results
Two variants were detected in the MIR27A genomic region, rs895819 and rs11671784. Neither variant deviated from HWE (p = 0.24 and p = 1.00, respectively), and observed allele frequencies (minor allele frequency, MAF = 0.31 and 0.023, respectively) were similar to those reported for European populations (EUR) in the 1000 Genomes Project (MAF EUR = 0.33 and 0.024, respectively) (26).

An association was noted between rs895819 and early-onset fluoropyrimidine toxicity; however, this association was strongly dependent on DPYD risk variant carrier status (OLR interaction p = 0.0025; Table 2). Within the group of patients carrying DPYD risk variants, rs895819 was associated with an increased risk of fluoropyrimidine toxicity (OLR p_adj = 0.0073; Table 2), whereas in patients without DPYD risk variants, the SNP was associated with a reduced risk of toxicity (OLR p_adj = 0.022; Table 2). The same effect was observed when a dominant genetic model for rs895819 was used (Figure 1; DPYD risk variant carriers p = 0.0085; OR = 7.6, 95% CI: 1.7-34.7; DPYD risk variant non-carriers p = 0.012; OR = 0.62, 95% CI: 0.43-0.9; OLR interaction p = 0.0023). This association was observed independently in patients receiving 5-FU-based or capecitabine-based therapies, and when considering patients receiving fluoropyrimidine monotherapy (with or without folinic acid) or combination therapies separately (Figure 2). The association was also similar to that observed in the full cohort when considering a more homogenous subgroup including only patients receiving fluoropyrimidine monotherapy, FOLFOX or
CAPOX regimens (n=327; Figure 2), suggesting no major confounding effect of other combination therapies.

In patients carrying \textit{DPYD} risk variants, adding rs895819 genotype significantly improved the area under the receiver operating characteristics curve (AUROC) to identify patients at increased risk of severe early-onset toxicity in comparison with an OLR model that included only cis-/carboplatin coadministration and sex (grade 0-2 vs. ≥3 AUROC = 0.82 vs. 0.66, p = 0.019; Figure 3). Overall, 46% (17 of 37) of \textit{DPYD} risk variant carriers experienced grade ≥3 early-onset toxicity; this proportion was only 25% (5 of 20) in patients not carrying rs895819G (Table 2). Conversely, 71% (12 of 17) of patients carrying both rs895819G and a \textit{DPYD} risk variant experienced severe early-onset fluoropyrimidine toxicity (Table 2), resulting in improved patient stratification when including rs895819 in the OLR model. In patients not carrying \textit{DPYD} risk variants, rs895819 only marginally improved the identification of patients at risk of severe early-onset toxicity (grade 0-2 vs. ≥3 AUROC = 0.68 vs. 0.66, p = 0.16; Figure 3) or the identification of patients at low risk of toxicity (grade 0-1 vs. ≥2 AUROC = 0.71 vs. 0.69, p = 0.030), suggesting a clinical relevance of rs895819 for fluoropyrimidine toxicity primarily in \textit{DPYD} risk variant carriers.

A majority of \textit{DPYD} risk variant carriers (25 of 37) were carriers of the c.1129-5923G/hapB3 variant (3) (Table 1). An interaction with rs895819 was also observed when considering c.1129-5923C>G/hapB3 alone (OLR interaction $p_{	ext{adj}} = 0.011$). For the other \textit{DPYD} risk variants, the number of carriers was small (Table 1), resulting in insufficient statistical power to assess a potential non-additive effect with rs895819 individually. However, among c.1905+1A carriers (n = 7), all three patients who experienced grade ≥3 early-onset toxicity also carried rs895819G. Of the remaining c.1905+1A carriers, two patients who also carried rs895819G experienced grade 2 toxicity. The remaining two patients who did not carry rs895819G developed grade 1 and grade 2 toxicity. These observations are in agreement with an increased toxicity
risk conferred by rs895819G in carriers of c.1905+1A as observed for the combined risk variants and for c.1129-5923C>G/hapB3 alone.

No overall association with early-onset fluoropyrimidine toxicity was observed for rs11671784 (Table 2). Similarly, no effect dependent on DPYD risk variants was detected (OLR interaction p_{adj} = 0.9). However, only three carriers of rs11671784 also carried DPYD risk variants; thus an evaluation of such an effect was limited by extremely low statistical power. A sex-dependent effect on fluoropyrimidine toxicity was observed for rs11671784 (OLR interaction p = 0.027; Table 2). Specifically, female carriers of rs11671784T experienced less fluoropyrimidine toxicity; however, the variant was associated with increased toxicity risk in male patients (Figure 1). This non-additive effect was also observed when using a dominant genetic model (OLR interaction p_{adj} = 0.016) and when excluding DPYD risk variant carriers (OLR interaction p_{adj} = 0.013). As to be expected due to the moderate effect size and the low population frequency of the variant, inclusion of rs11671784 did not improve the predictive accuracy of an OLR model with cis-/carboplatin coadministration, DPYD risk variants and sex as covariates (grade 0-2 vs. ≥3 AUROC = 0.72 vs. 0.70, p = 0.17), or when analyzing female and male patients separately (Female: grade 0-2 vs. ≥3 AUROC = 0.65 vs. 0.64, p = 0.20; Male: grade 0-2 vs. ≥3 AUROC = 0.72 vs. 0.69, p = 0.11).

When combining the non-additive effects of rs895819 and rs11671784 in a multivariate OLR model adjusting for cis-/carboplatin coadministration, both associations remained significant (rs895819*DPYD risk variants: interaction p_{adj} = 0.0034; rs11671784*sex: interaction p_{adj} = 0.023), suggesting independent effects. Allele frequencies of rs895819 in patients grouped according to the severity of early-onset fluoropyrimidine toxicity were similar for hematologic and gastrointestinal toxicities, suggesting a similar effect of the variant (Figure 4; Supplementary Figure). On the other hand, no clear trend was observed when considering only dermatologic toxicities (Figure 4; Supplementary Figure).
Discussion

Known risk variants in \textit{DPYD} are well accepted as predictive markers of fluoropyrimidine-related toxicity (1,4,27). However, due to their limited predictive power, there is a need to identify additional factors that contribute to inter-individual variability in sensitivity towards fluoropyrimidine-based chemotherapy to enable more effective patient stratification for toxicity risk (27,28). Here, we provide the first evidence that polymorphisms in \textit{MIR27A} may influence the susceptibility to early-onset fluoropyrimidine toxicity. In particular, our data strongly suggest that the common \textit{MIR27A} variant rs895819G may lead to an increased risk of severe fluoropyrimidine toxicity in individuals that also carry \textit{DPYD} risk variants. Within the group of \textit{DPYD} risk variant carriers, rs895819 genotype significantly improved the identification of patients at high risk of severe toxicity.

In the present study, >70\% of patients carrying both rs895819G and a \textit{DPYD} risk variant experienced severe early-onset fluoropyrimidine toxicity (Table 2). In contrast, only 25\% of carriers that were homozygous for rs895819A experienced severe toxicity. This suggests that rs895819 genotype may enable additional patient stratification among \textit{DPYD} risk variant carriers for fluoropyrimidine toxicity risk prediction. Due to the low population frequency of \textit{DPYD} risk variants, most variants are present in the heterozygous state, leaving one functional gene copy. Interestingly, only approximately 50\% of these heterozygous carriers experience severe toxicity from fluoropyrimidine-based chemotherapy (1), suggesting that additional mechanisms influence DPD activity and thus contribute to fluoropyrimidine toxicity. Our results suggest such a potential mechanism through altered regulation of DPD by miR-27a. In carriers of \textit{DPYD} risk variants, the increased toxicity risk associated with rs895819G is consistent with previous functional studies where rs895819G was associated with increased levels of miR-27a and reduced DPD enzyme function (8). Specifically, our data support a model in which rs895819G downregulates DPD expression through increased miR-27a expression. In individuals with impaired DPD enzyme function due to \textit{DPYD} risk variants, this suppression may exacerbate the enzymatic impairment to a sub-critical level, resulting in a prolonged half-life of administered fluoropyrimidines and increased toxicity.
susceptibility to severe early-onset toxicity. Admittedly, this study contained a limited number of DPYD risk variant carriers. Therefore, this finding requires validation in additional patient cohorts.

The opposite effect of rs895819G in patients not carrying a DPYD risk variant was unexpected in light of the direct regulation of DPYD by miR-27a and the suggested effect of rs895819 on miR-27a levels. The effect size of this association was moderate and only marginally improved the stratification of patients according to toxicity risk. In the absence of DPYD genetic variation reducing DPD activity, this variant may thus not be of major clinical relevance in the context of early-onset fluoropyrimidine toxicity. Nevertheless, this finding points towards additional effects of miR-27a in the context of fluoropyrimidine chemotherapy, which may be unrelated to DPD. Such a mechanism has been suggested through Bcl-2 and Bax, two members of the Bcl-2 protein family involved in maintaining the balance between cell proliferation and cell death, and the regulation of apoptosis in response to cell death stimuli (29,30). Knockdown of miR-27a has previously been shown to reduce Bcl-2 expression and increase Bax expression (29). Bcl-2 has an anti-apoptotic function whereas Bax is thought to be pro-apoptotic (30), suggesting a pro-apoptotic effect of reduced miR-27a expression mediated via Bcl-2 and Bax (29). Correspondingly, increased miR-27a levels in rs895819G may thus result in an anti-apoptotic effect in response to cytotoxic drugs, potentially explaining the negative association with early-onset toxicity from fluoropyrimidine-based chemotherapy in patients without DPYD risk variants noted in the present study.

Taken together, our findings indicate that the relevance of the MIR27A rs895819 polymorphism in early-onset toxicity from fluoropyrimidine-based chemotherapy may depend on the cellular context, such as the baseline expression or activity of the involved miR-27a targets. The increased fluoropyrimidine toxicity risk associated with rs895819G in carriers of DPYD risk variants suggests that in patients with reduced baseline DPD activity, the direct regulatory effect of miR-27a on DPD outweighs anti-apoptotic effects mediated through other targets. Conversely, in patients with normal DPD activity, the moderate reduction in DPD expression associated with rs895819G may be outweighed by effects on other targets that reduce
chemotherapy cytotoxicity, thereby reducing the clinical relevance of rs895819 for early-onset toxicity prediction in this group of patients.

However, in the absence of deleterious DPYD variants, miR-27a and rs895819 may be of potential relevance for the anticancer activity of fluoropyrimidine-based chemotherapy. Consistent with this supposition, the high-expression miR-27a variant rs895819G has been associated with reduced survival in lung and gastric cancer patients (19,20). Furthermore, increased miR-27a levels have been observed in chemotherapy resistant tumors (12-14) and suppression of miR-27a has been shown to increase the sensitivity of cancer cell lines to 5-FU and other chemotherapy drugs (14,17,29,31). Given that no outcomes related to chemotherapy effectiveness (e.g. survival) were assessed in our study, further investigation of the effects of rs895819 genotype on survival and chemotherapy response in colorectal cancer is warranted.

Interestingly, opposite effects in relation to early-onset fluoropyrimidine toxicity were observed in our cohort between male and female patients for the rare miR-27a variant rs11671784. Although the observed sex-dependent effect was robust with respect to the inclusion or exclusion of other associated covariates, these findings should be interpreted with caution due to the low population frequency of this variant and the limited number of carriers studied. The functional implications of this variant, which is also located in the stem loop of the miR-27a pre-microRNA, have not been investigated. Whereas the expression of some microRNAs is affected by sex steroids (32), no such effects have been reported for miR-27a to our knowledge. Therefore, a potential sex-dependent impact of rs11671784 on pri-microRNA processing cannot be excluded and requires further investigation.

In conclusion, our results suggest that the common MIR27A variant rs895819 may be of clinical relevance to improve the prediction of severe fluoropyrimidine toxicity in patients carrying risk variants in DPYD. It should be noted that while the present study included multiple treatment regimens, all of which were
fluoropyrimidine based, the generalizability of this finding to specific regimens will require additional validation. Regardless, consideration of rs895819 genotype may permit further patient stratification and thus allow improved tailoring of fluoropyrimidine dose reductions to those DPYD risk variant carriers at highest risk of toxicity (4). Based on indirect evidence of a potential detrimental effect of MIR27A rs895819G on clinical outcome in patients receiving FP-based chemotherapy, the potential relevance of this polymorphism in the context of fluoropyrimidine-based chemotherapy in patients without DPYD risk variants requires further investigation. Our findings support the important regulatory role of microRNAs in cancer chemotherapy and highlight, for the first time, the potential of miR-27a and rs895819 as a modulator of chemotherapy toxicity in a clinical context. Our results strongly suggest that further investigation of this microRNA and its genetic variants is needed in the context of fluoropyrimidine-based chemotherapy for an improved understanding of factors that contribute to inter-patient variability in fluoropyrimidine toxicity and effectiveness.

Acknowledgements

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References


Tables

**Table 1.** Patient demographics and *DPYD* risk variant frequencies. Additional details on the patient cohort are published in (3).

<table>
<thead>
<tr>
<th></th>
<th>Toxicty in chemotherapy cycles 1-2</th>
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<tbody>
<tr>
<td></td>
<td>Grade 0-1</td>
</tr>
<tr>
<td>n (%)</td>
<td>281 (55)</td>
</tr>
<tr>
<td>Age [median (range)]</td>
<td>62 (29-99)</td>
</tr>
<tr>
<td>Sex [f/m (%)]</td>
<td>93/188 (45/61)</td>
</tr>
<tr>
<td>Concomitant cis-/carboplatin [n (%)]</td>
<td>23 (23)</td>
</tr>
<tr>
<td><em>DPYD</em> risk variants [n carriers (%)]</td>
<td>6 (2)</td>
</tr>
<tr>
<td>c.1129-5923C&gt;G/hapB3</td>
<td>4 (1)</td>
</tr>
<tr>
<td>c.1679T&gt;G</td>
<td>0 (0)</td>
</tr>
<tr>
<td>c.1905+1G&gt;A</td>
<td>1 (0)</td>
</tr>
<tr>
<td>c.2846A&gt;T</td>
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</table>

<sup>a</sup> OLR p-value, adjusted for other significant covariates (cis-/carboplatin, sex, *DPYD* risk variants)

<sup>b</sup> Compound heterozygous in one patient

<sup>c</sup> Homozygous in one patient

n.s.: not significant
Table 2. Associations of MIR27A variants with early-onset toxicity in fluoropyrimidine-based chemotherapy.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Overall n (%)</th>
<th>Grade 0-1 n (%)</th>
<th>Grade 2 n (%)</th>
<th>Grade ≥3 n (%)</th>
<th>p-value (univariate; adjusted)</th>
<th>odds ratio (95% CI)</th>
<th>interaction p (unadjusted; adjusted)</th>
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<tr>
<td><strong>rs895819</strong></td>
<td></td>
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<tr>
<td>No DPYD risk variant</td>
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<tr>
<td>A/A</td>
<td>224 (47)</td>
<td>118 (43)</td>
<td>66 (49)</td>
<td>40 (59)</td>
<td>0.028; 0.73 (0.56-0.97);</td>
<td></td>
<td>0.01; 0.0025^a</td>
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<tr>
<td>A/G</td>
<td>205 (43)</td>
<td>126 (46)</td>
<td>57 (43)</td>
<td>22 (32)</td>
<td>0.022^a 0.71 (0.54-0.95)^a</td>
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<tr>
<td>G/G</td>
<td>48 (10)</td>
<td>31 (11)</td>
<td>11 (8)</td>
<td>6 (9)</td>
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<tr>
<td>DPYD risk variant carriers</td>
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<tr>
<td>A/A</td>
<td>20 (54)</td>
<td>4 (67)</td>
<td>67 (97)</td>
<td>66 (98)</td>
<td>0.04; 5.2 (1.4-19.6);</td>
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<td>A/G</td>
<td>16 (43)</td>
<td>2 (33)</td>
<td>3 (21)</td>
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<td>0.0073^a 7.4 (1.7-31.9)^a</td>
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<td>G/G</td>
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<td><strong>rs11671784</strong></td>
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<tr>
<td>C/C</td>
<td>195 (95)</td>
<td>85 (91)</td>
<td>67 (97)</td>
<td>43 (98)</td>
<td>0.069; 0.30 (0.08-1.1);</td>
<td></td>
<td>0.027; 0.016^b</td>
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<tr>
<td>C/T</td>
<td>10 (5)</td>
<td>7 (8)</td>
<td>2 (3)</td>
<td>1 (2)</td>
<td>0.063^b 0.28 (0.07-1.1)^b</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>C/C</td>
<td>296 (96)</td>
<td>182 (97)</td>
<td>77 (97)</td>
<td>37 (90)</td>
<td>0.20; 2.1 (0.68-6.7);</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>12 (4)</td>
<td>6 (3)</td>
<td>2 (3)</td>
<td>4 (10)</td>
<td>0.16^b 2.4 (0.71-8.1)^b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^aOLR using additive genetic model; adjusted for cis-/carboplatin coadministration and sex

^bOLR using additive genetic model; adjusted for cis-/carboplatin coadministration and DPYD risk variants

Significant p-values are indicated in bold.
**Figure legends**

**Figure 1.** Carrier frequencies of (A) rs895819 in patients without and with *DPYD* risk variants (c.1129-5923C>G/hapB3, c.1905+1G>A, c.1679T>G, c.2846A>T) and (B) rs11671784 in female and male patients according to NCI CTCAE toxicity grade in chemotherapy cycles 1-2.

**Figure 2.** Association of rs895819 with early-onset toxicity in different patient subgroups and the full cohort. 5FU-based: 5-FU-based therapies; Cap-based: capecitabine-based therapies; Monotherapy/Mono: 5-FU or capecitabine monotherapy (with or without folinic acid); Combination: combination therapies including any other cytotoxic medication; FOLFOX: 5-FU + oxaliplatin + folinic acid; CAPOX: capecitabine + oxaliplatin; OR: odds ratio with 95% confidence interval for rs895819. Interaction p-value: p-value for the non-additive effect between rs895819 and *DPYD* risk variant carrier status, adjusted for sex and cis-/carboplatin coadministration (if applicable) and using a dominant genetic model. Significant p-values are indicated in bold.

**Figure 3.** Receiver operating characteristics curves for rs895819 to identify patients at risk of grade ≥3 early-onset toxicity in patients (A) with and (B) without *DPYD* risk variants. Black line: Model including rs895819 and covariates cis-/carboplatin and sex; Grey line: Model including only cis-/carboplatin and sex.

**Figure 4.** Allele frequencies of rs895819 in *DPYD* risk variant carriers for different toxicity types according to toxicity grade in chemotherapy cycles 1-2. Hemat: Hematologic toxicities; GI (mech): Mechanism-related gastrointestinal toxicities (diarrhea, mucositis); GI (other): Other gastrointestinal toxicities (nausea, vomiting, dehydration); Derm: Dermatologic toxicities.
Figure 1

A

- Grade 0-1
- Grade 2
- Grade ≥3

Carrier frequency

No DPYD risk variant
n=275/134/68

DPYD risk carriers
n=6/14/17

B

- Grade 0-1
- Grade 2
- Grade ≥3

Carrier frequency

Female
n=93/69/44

Male
n=188/79/41
Figure 2

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>OR (95% CI)</th>
<th>Interaction p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5FU-based</strong></td>
<td>n=403</td>
<td>380</td>
<td>0.65 (0.43-0.98)</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>3.95 (0.77-20.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Cap-based</strong></td>
<td>n=111</td>
<td>97</td>
<td>0.53 (0.23-1.25)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>14.9 (1.2-188.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Monotherapy</strong></td>
<td>n=148</td>
<td>135</td>
<td>0.64 (0.3-1.38)</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>6.97 (0.79-61.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Combination</strong></td>
<td>n=366</td>
<td>342</td>
<td>0.60 (0.39-91)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>4.75 (0.87-25.9)</td>
<td></td>
</tr>
<tr>
<td><strong>FOLFOX/CAPOX</strong></td>
<td>n=179</td>
<td>169</td>
<td>0.59 (0.31-1.11)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3.98 (0.42-37.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Mono/FOLFOX/CAPOX n=327</strong></td>
<td>304</td>
<td>23</td>
<td>0.61 (0.38-1.00)</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>5.47 (1.17-25.7)</td>
<td></td>
</tr>
<tr>
<td><strong>All patients</strong></td>
<td>n=514</td>
<td>477</td>
<td>0.62 (0.43-0.9)</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>6.25 (1.63-24.0)</td>
<td></td>
</tr>
</tbody>
</table>

Odds ratio (95% CI)
Figure 3

A

B

$\text{True positive rate}$

$\text{False positive rate}$

$p = 0.019$

$p = 0.16$
Figure 4

rs895819 - DPYD risk variant carriers

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Grade 0-1</th>
<th>Grade 2</th>
<th>Grade ≥3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemat</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>GI (mech)</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>GI (other)</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Derm</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

n: 12/12/13  19/9/9  21/9/5  28/7/2
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Ursula Amstutz, Steven M. Offer, Johanna Sistonen, et al.

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