

# Polymorphisms in *MIR27A* Associated with Early-Onset Toxicity in Fluoropyrimidine-Based Chemotherapy

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## 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 patients with cancer 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 ( $P_{\text{interaction}} = 0.0025$ ). In patients carrying *DPYD* risk variants, rs895819G was associated with a strongly increased toxicity risk [OR, 7.6; 95% confidence interval (CI), 1.7–34.7;  $P = 0.0085$ ]. Overall, 71% (12/17) 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. *Clin Cancer Res*; 21(9); 2038–44. ©2015 AACR.

## Introduction

Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme in the catabolism of the fluoropyrimidines 5-fluorouracil (5FU) 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 affect fluoropyrimi-

dine 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

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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### 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 article, 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.

(*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 patients with cancer 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 patients with cancer 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 because of 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'-GTCCCAAATCT-CATTACCTCCTT-3' (forward) and 5'-GGTCTGATTCTGAGTCCT-CATCTC-3' (reverse). The 555 bp fragment was amplified using the QIAGEN Multiplex PCR Kit (Qiagen) in a total reaction volume of 25  $\mu$ L containing 3  $\mu$ L of genomic DNA (to a maximum of 300 ng), 2.5  $\mu$ L Q-Solution (Qiagen), 2.5  $\mu$ L primer mix (2  $\mu$ mol/L each), and 12.5  $\mu$ L 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, and grade  $\geq 3$ ) using the rms package (23) implemented in the

**Table 1.** Patient demographics and *DPYD* risk variant frequencies

	Toxicity in chemotherapy cycles 1 and 2			<i>P</i> <sup>a</sup>
	Grade 0–1	Grade 2	Grade $\geq 3$	
<i>n</i> (%)	281 (55)	148 (29)	85 (17)	
Age [median (range)]	62 (29–99)	61 (18–88)	66 (44–81)	n.s.
Sex [female/male (%)]	93/188 (45/61)	69/79 (33/26)	44/41 (21/13)	<0.0001
Concomitant cis-/carboplatin [ <i>n</i> (%)]	23 (23)	47 (46)	32 (31)	<0.0001
<i>DPYD</i> risk variants [ <i>n</i> carriers (%)]	6 (2)	14 (9)	17 (20)	<0.0001
c.1129-5923C>G/hapB3	4 (1)	8 (5)	13 (15) <sup>b,c</sup>	
c.1679T>G	0 (0)	1 (1)	2 (2) <sup>b</sup>	
c.1905+1G>A	1 (0)	3 (2)	3 (4)	
c.2846A>T	1 (0)	2 (1)	0 (0)	

NOTE: Additional details on the patient cohort are published in ref. (3).

Abbreviation: n.s., not significant.

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

<sup>b</sup>Compound heterozygous in 1 patient.

<sup>c</sup>Homozygous in 1 patient.

**Table 2.** Associations of *MIR27A* variants with early-onset toxicity in fluoropyrimidine-based chemotherapy

	Genotype	Overall n (%)	Toxicity in chemotherapy cycles 1 and 2			P (univariate; adjusted)	OR (95% CI) (univariate; adjusted)	P <sub>interaction</sub> (unadjusted; adjusted)
			Grade 0-1 n (%)	Grade 2 n (%)	Grade ≥3 n (%)			
<b>rs895819</b>								
No <i>DPYD</i> risk variant	A/A	224 (47)	118 (43)	66 (49)	40 (59)	<b>0.028;</b>	0.73 (0.56-0.97);	<b>0.01; 0.0025<sup>a</sup></b>
	A/G	205 (43)	126 (46)	57 (43)	22 (32)	<b>0.022<sup>a</sup></b>	0.71 (0.54-0.95) <sup>a</sup>	
	G/G	48 (10)	31 (11)	11 (8)	6 (9)			
<i>DPYD</i> risk variant carriers	A/A	20 (54)	4 (67)	11 (79)	5 (29)	<b>0.014;</b>	5.2 (1.4-19.6);	
	A/G	16 (43)	2 (33)	3 (21)	11 (65)	<b>0.0073<sup>a</sup></b>	7.4 (1.7-31.9) <sup>a</sup>	
	G/G	1 (3)	0 (0)	0 (0)	1 (6)			
<b>rs11671784</b>								
Female	C/C	195 (95)	85 (91)	67 (97)	43 (98)	0.069;	0.30 (0.08-1.1);	<b>0.027; 0.016<sup>b</sup></b>
	C/T	10 (5)	7 (8)	2 (3)	1 (2)	0.063 <sup>b</sup>	0.28 (0.07-1.1) <sup>b</sup>	
	T/T	1 (0)	1 (1)	0 (0)	0 (0)			
Male	C/C	296 (96)	182 (97)	77 (97)	37 (90)	0.20;	2.1 (0.68-6.7);	
	C/T	12 (4)	6 (3)	2 (3)	4 (10)	0.16 <sup>b</sup>	2.4 (0.71-8.1) <sup>b</sup>	
	T/T	0 (0)	0 (0)	0 (0)	0 (0)			

NOTE: Significant *P* values are indicated in bold.<sup>a</sup>OLR using additive genetic model; adjusted for cis-/carboplatin coadministration and sex.<sup>b</sup>OLR using additive genetic model; adjusted for cis-/carboplatin coadministration and *DPYD* risk variants.

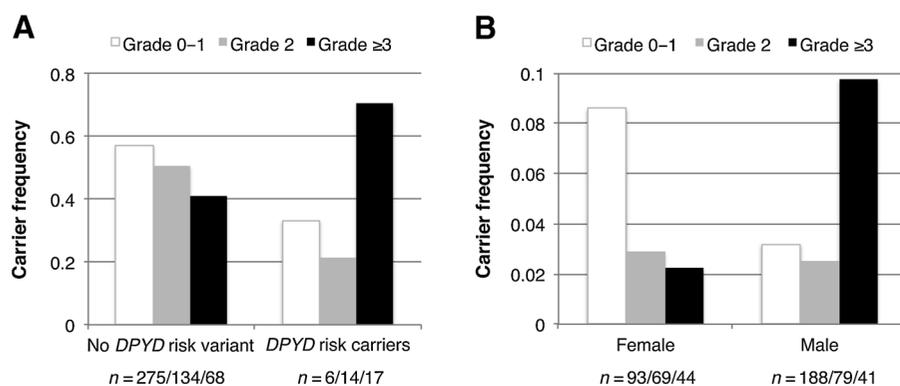
statistical software R (24). The proportional odds assumption for OLR models was assessed using the test of parallel lines in SPSS (IBM). Initial association tests were performed using an additive genetic model; other genetic models were subsequently tested only for significant associations. Associations 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, and c.2846A>T; ref. 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 5FU versus capecitabine (3). Nonadditive 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 (ROC) 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 5,000 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, and infections), mechanism-related gastrointestinal (diarrhea and mucositis), other gastrointestinal (nausea, vomiting, and dehydration), and dermatologic toxicities (hand foot syndrome, dry skin, and 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 1,000 Genomes Project (MAF EUR = 0.33 and 0.024, respectively; ref. 26).

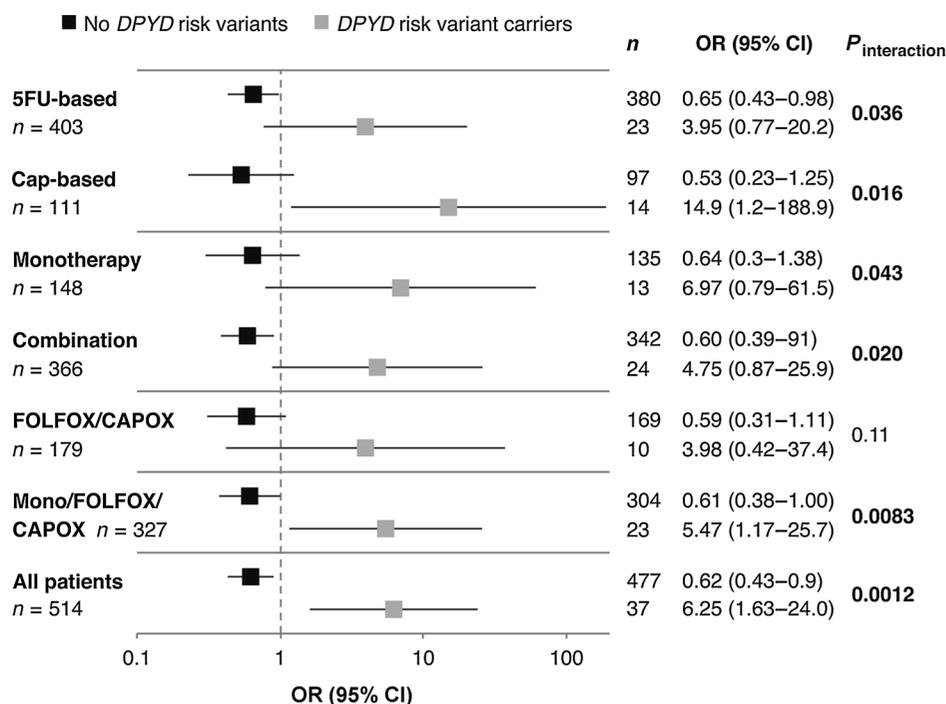
An association was noted between rs895819 and early-onset fluoropyrimidine toxicity; however, this association was strongly dependent on *DPYD* risk variant carrier status (OLR *P*<sub>interaction</sub> = 0.0025; Table 2). Within the group of patients carrying *DPYD* risk variants, rs895819 was associated with an increased risk of fluoropyrimidine toxicity (OLR *P*<sub>adj</sub> = 0.0073; Table 2), whereas in patients without *DPYD* risk variants, the SNP was associated with a reduced risk of toxicity (OLR *P*<sub>adj</sub> = 0.022; Table 2). The same effect was observed when a dominant genetic model for rs895819 was used [Fig. 1; *DPYD* risk variant carriers *P* = 0.0085; OR, 7.6;

**Figure 1.**

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

**Figure 2.**

Association of rs895819 with early-onset toxicity in different patient subgroups and the full cohort; 5FU-based, 5FU-based therapies; Cap-based, capecitabine-based therapies; Monotherapy/Mono, 5FU or capecitabine monotherapy (with or without folinic acid); combination, combination therapies, including any other cytotoxic medication; FOLFOX, 5FU + oxaliplatin + folinic acid; CAPOX, capecitabine + oxaliplatin; OR, odds ratio with 95% confidence interval for rs895819.  $P_{interaction}$ ,  $P$  value for the nonadditive 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.



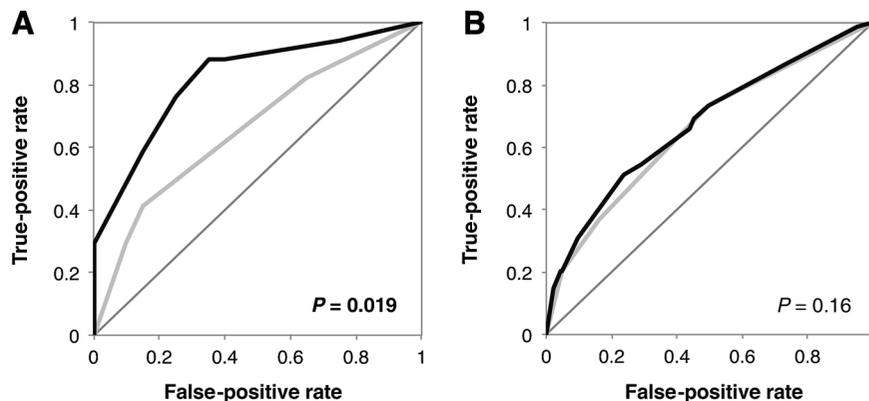
95% confidence interval (CI), 1.7–34.7; *DPYD* risk variant non-carriers  $P = 0.012$ ; OR, 0.62; 95% CI, 0.43–0.9; OLR  $P_{interaction} = 0.0023$ ]. This association was observed independently in patients receiving 5FU-based or capecitabine-based therapies, and when considering patients receiving fluoropyrimidine monotherapy (with or without folinic acid) or combination therapies separately (Fig. 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 ( $n = 327$ ; Fig. 2), suggesting no major confounding effect of other combination therapies.

In patients carrying *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 coadminis-

tration and sex (grade 0–2 vs.  $\geq 3$  AUROC = 0.82 vs. 0.66,  $P = 0.019$ ; Fig. 3). Overall, 46% (17/37) of *DPYD* risk variant carriers experienced grade  $\geq 3$  early-onset toxicity; this proportion was only 25% (5/20) in patients not carrying rs895819G (Table 2). Conversely, 71% (12/17) of patients carrying both rs895819G and a *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 *DPYD* risk variants, rs895819 only marginally improved the identification of patients at risk of severe early-onset toxicity (grade 0–2 vs.  $\geq 3$  AUROC = 0.68 vs. 0.66,  $P = 0.16$ ; Fig. 3) or the identification of patients at low risk of toxicity (grade 0–1 vs.  $\geq 2$  AUROC = 0.71 vs. 0.69,  $P = 0.030$ ), suggesting a clinical relevance of rs895819 for fluoropyrimidine toxicity primarily in *DPYD* risk variant carriers.

**Figure 3.**

ROC curves for rs895819 to identify patients at risk of grade  $\geq 3$  early-onset toxicity in patients with (A) and without (B) *DPYD* risk variants. Black line, model, including rs895819 and covariates cis-/carboplatin and sex; Grey line, model, including only cis-/carboplatin and sex. Significant  $P$  values for differences in AUROC between models are indicated in bold.



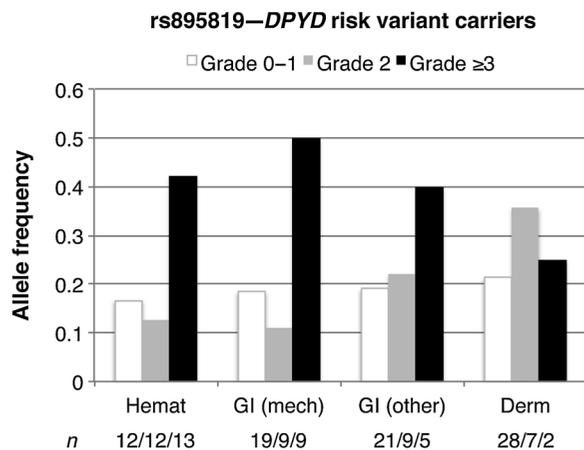
A majority of *DPYD* risk variant carriers (25/37) were carriers of the c.1129-5923C/hapB3 variant (Table 1; ref. 3). An interaction with rs895819 was also observed when considering c.1129-5923C>G/hapB3 alone (OLR interaction  $P_{\text{adj}} = 0.011$ ). For the other *DPYD* risk variants, the number of carriers was small (Table 1), resulting in insufficient statistical power to assess a potential nonadditive effect with rs895819 individually. However, among c.1905+1A carriers ( $n = 7$ ), all 3 patients who experienced grade  $\geq 3$  early-onset toxicity also carried rs895819G. Of the remaining c.1905+1A carriers, 2 patients who also carried rs895819G experienced grade 2 toxicity. The remaining 2 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  $P_{\text{adj}} = 0.9$ ). 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  $P_{\text{interaction}} = 0.027$ ; Table 2). Specifically, female carriers of rs11671784T experienced less fluoropyrimidine toxicity; however, the variant was associated with increased toxicity risk in male patients (Fig. 1). This nonadditive effect was also observed when using a dominant genetic model (OLR interaction  $P_{\text{adj}} = 0.016$ ) and when excluding *DPYD* risk variant carriers (OLR interaction  $P_{\text{adj}} = 0.013$ ). To be expected because of 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.  $\geq 3$  AUROC = 0.72 vs. 0.70,  $P = 0.17$ ), or when analyzing female and male patients separately (female: grade 0–2 vs.  $\geq 3$  AUROC = 0.65 vs. 0.64,  $P = 0.20$ ; male: grade 0–2 vs.  $\geq 3$  AUROC = 0.72 vs. 0.69,  $P = 0.11$ ).

When combining the nonadditive effects of rs895819 and rs11671784 in a multivariate OLR model adjusting for cis-/carboplatin coadministration, both associations remained significant (rs895819\**DPYD* risk variants: interaction  $P_{\text{adj}} = 0.0034$ ; rs11671784\*sex: interaction  $P_{\text{adj}} = 0.023$ ), 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 (Fig. 4 and Supplementary Fig. S1). On the other hand, no clear trend was observed when considering only dermatologic toxicities (Fig. 4 and Supplementary Fig. S1).

## Discussion

Known risk variants in *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 interindividual variability in the sensitivity toward fluoropyrimidine-based chemotherapy to enable more effective patient stratification for toxicity risk (27, 28). Here, we provide the first evidence that



**Figure 4.**

Allele frequencies of rs895819 in *DPYD* risk variant carriers for different toxicity types according to toxicity grade in chemotherapy cycles 1 and 2. Hemat, hematologic toxicities; GI (mech), mechanism-related gastrointestinal toxicities (diarrhea and mucositis); GI (other), other gastrointestinal toxicities (nausea, vomiting, and dehydration); Derm, dermatologic toxicities.

polymorphisms in *MIR27A* may influence the susceptibility to early-onset fluoropyrimidine toxicity. In particular, our data strongly suggest that the common *MIR27A* variant rs895819G may lead to an increased risk of severe fluoropyrimidine toxicity in individuals that also carry *DPYD* risk variants. Within the group of *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 *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 *DPYD* risk variant carriers for fluoropyrimidine toxicity risk prediction. Because of the low population frequency of *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 *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 *DPYD* risk variants, this suppression may exacerbate the enzymatic impairment to a subcritical level, resulting in a prolonged half-life of administered fluoropyrimidines and increased 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 toward 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 antiapoptotic function whereas Bax is thought to be proapoptotic (30), suggesting a proapoptotic 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 antiapoptotic 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 antiapoptotic 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 patients with lung and gastric cancer (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 5FU 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 pre-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, although 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). On the basis of indirect evidence of a potential detrimental effect of *MIR27A* rs895819G on clinical outcome in patients receiving fluoropyrimidine-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 interpatient variability in fluoropyrimidine toxicity and effectiveness.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Authors' Contributions

**Conception and design:** U. Amstutz, M. Joerger, C.R. Largiadèr

**Development of methodology:** R.B. Diasio

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** U. Amstutz, M. Joerger, C.R. Largiadèr

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** U. Amstutz, S.M. Offer, J. Sistonen, M. Joerger, C.R. Largiadèr

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