Capecitabine HFS Supplementary Material

Clinical, Dose-Exposure, and Pharmacogenetic Factors Associated with Clinically Significant Capecitabine-Induced Hand-Foot Syndrome in Iraqi Cancer Patients: An Observational Study

SUPPLEMENTARY METHODS

Methods S1 Inflammatory Index Definitions

The neutrophil-to-lymphocyte ratio (NLR) denotes the absolute neutrophil count divided by the absolute lymphocyte count. The platelet-to-lymphocyte ratio (PLR) denotes the platelet count divided by the absolute lymphocyte count. The systemic immune-inflammation index (SII) denotes the platelet count multiplied by NLR [1]. Blood-cell counts expressed on the same concentration scale yield dimensionless NLR and PLR values; the numerical scale of SII follows that of the platelet count. CRP is expressed in mg/L. These definitions describe the indices summarized in Table **S1**.

Methods S2 Specimen Processing and Genotyping

Genomic DNA was isolated from 200 µL of EDTA-anticoagulated peripheral blood with the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany), using the manufacturer's silica-membrane spin-column procedure [2]. DNA concentration was measured fluorometrically and purity was assessed by ultraviolet spectrophotometry. Aliquots for subsequent testing were kept at −20°C, with separate working aliquots used to limit repeated freezing and thawing. Specimens and DNA aliquots carried coded study identifiers.

Single-nucleotide variants were tested in singleplex reactions by TaqMan allelic discrimination on a QuantStudio 3 real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). Each 10-µL reaction contained 1× TaqMan Genotyping Master Mix, 1× allele-specific primer/probe mixture, 10 ng genomic DNA and nuclease-free water. Amplification and fluorescence acquisition used the assay-specific manufacturer program. Endpoint fluorescence clusters were assessed with the instrument's genotyping software, followed by manual review of uncertain calls [3]. This platform has been used for capecitabine-related candidate-gene investigations [4].

The DPYD panel included c.1905+1G>A (rs3918290; *2A), c.1679T>G (rs55886062; *13), c.2846A>T (rs67376798) and the HapB3-associated c.1129−5923C>G (rs75017182) [5]. MTHFR rs1801133 was reported using both its traditional C677T designation and the current transcript notation NM_005957.5:c.665C>T (p.Ala222Val) [6]. The CDA promoter SNP rs532545 was described using its conventional −451C>T designation [7]. Marker identities and analytical category definitions are summarized in Table S3.

The TYMS 5′ regulatory-region repeat was evaluated separately from the SNP assays. The region containing the 28-bp tandem repeat was amplified with forward primer 5′-TTCCCGGGTTTCCTAAGACT-3′ and reverse primer 5′-TGGATCTGCCCCAGGTACT-3′ [7]. A fluorescent label on the forward primer enabled capillary fragment sizing. Fragment lengths were interpreted against sequence-characterized repeat controls to assign the common 2R and 3R alleles. Selected representative genotypes and ambiguous patterns were evaluated by bidirectional Sanger sequencing. Unresolved or atypical repeat patterns were treated as unclassified. The repeat-based grouping did not incorporate the 3RG/3RC suballele distinction.

Methods S3 Genotyping Quality Control and Analysis Categories

Extraction blanks were processed with DNA-extraction batches. Genotyping plates included no-template controls and sequence-characterized genotype controls. A randomly selected 10% subset of DNA samples was tested in duplicate under coded identifiers, and laboratory personnel were blinded to HFS classification. Genotype clusters, control behavior and duplicate agreement were reviewed before category assignment. Duplicate testing with a TaqMan/QuantStudio workflow has been described in capecitabine pharmacogenetic research [4].

Calls with low fluorescence, poor cluster separation, discordant duplicates or uncertain repeat patterns underwent repeat testing from a separate DNA aliquot. Persistent failures remained missing and were not assigned

to the reference category. The per-marker call rate was defined as the proportion of tested samples with an assigned genotype, and duplicate concordance as the proportion of repeated genotype pairs with identical calls. The denominators for these measures distinguish samples tested from successfully classified samples, consistent with genetic-association reporting recommendations [8].

DPYD status compared the reference category at the four tested loci with heterozygosity for a panel variant. Homozygous or multiple-variant genotypes require separate characterization and are not equivalent to a single heterozygous category. TYMS 3R/3R, 2R/3R and 2R/2R were assigned to the low, intermediate and high categories, respectively; these were exploratory research labels. MTHFR was represented by CC, CT and TT genotypes. CDA rs532545 used dominant coding, with CT and TT grouped together and CC as the reference. The adjusted TYMS contrast compared the high category with the combined low and intermediate categories. These targeted categories do not imply whole-gene coverage or validated HFS-directed prescribing rules.

SUPPLEMENTARY TABLES

Table S1: Inflammatory Indices According to Recorded HFS Classification

Variable	Total (N=180)	No/grade 1 HFS (n=97)	Clinically significant HFS (n=83)	P value
C-reactive protein, mg/L	7.41 ± 4.73	7.26 ± 4.58	7.57 ± 4.91	0.669
Neutrophil-to-lymphocyte ratio	2.79 ± 0.89	2.75 ± 0.94	2.83 ± 0.84	0.531
Platelet-to-lymphocyte ratio	170.73 ± 57.23	168.15 ± 57.40	173.75 ± 57.23	0.514
Systemic immune-inflammation index	817.37 ± 339.63	810.81 ± 360.70	825.04 ± 315.22	0.778

Data are mean ± standard deviation. P values are from Welch independent-samples t-tests. CRP, C-reactive protein; HFS, hand-foot syndrome; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index. Groups use the recorded classification described in the main Methods, including six cases with a nonstandard severity code.

Table S2: Exploratory Single-Marker ROC Summaries for Recorded Clinically Significant HFS

Variable	AUC	Exploratory cut-off	Sensitivity	Specificity
Initial capecitabine dose, mg/m²/day	0.602	2125.00	0.687	0.505
Recorded cumulative capecitabine dose, g	0.683	289.10	0.627	0.680
CRP, mg/L	0.531	7.20	0.470	0.629
NLR	0.518	1.66	0.940	0.155
eGFR, mL/min/1.73 m² (inverse risk)	0.464	84.20	0.952	0.093

AUC, area under the receiver operating characteristic curve; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HFS, hand-foot syndrome; NLR, neutrophil-to-lymphocyte ratio; ROC, receiver operating characteristic. Cut-offs use the units in the first column. Sensitivity and specificity are proportions. These are apparent, same-sample estimates for individual variables, not a combined-model AUC. The eGFR row retains the recorded inverse-risk orientation. The operating points are exploratory and do not constitute validated clinical decision thresholds. AUC confidence intervals, calibration, and validation estimates are not part of these summaries. The cumulative-dose operating point uses the recorded prescription-based treatment-course total and is not a threshold derived from dose accrued exclusively before HFS onset.

Table S3: Pharmacogenetic Markers and Category Definitions

Gene	Marker and designation	Technique	Analytical category
DPYD	rs3918290 c.1905+1G>A; *2A rs55886062 c.1679T>G; *13 rs67376798 c.2846A>T rs75017182 c.1129−5923C>G; HapB3 [5]	TaqMan allelic discrimination	Reference at the four loci versus a heterozygous panel-variant category
TYMS	rs34743033 28-bp tandem repeat 2R/3R [9]	PCR and fragment sizing; sequence assessment of uncertain calls	3R/3R: low 2R/3R: intermediate 2R/2R: high Exploratory repeat-based labels
MTHFR	rs1801133 C677T; NM_005957.5:c.665C>T p.Ala222Val [6]	TaqMan allelic discrimination	CC: reference CT: heterozygous TT: homozygous variant
CDA	rs532545 Promoter −451C>T [7]	TaqMan allelic discrimination	CC: reference CT/TT: variant carriers Dominant model

rs, reference SNP identifier; 2R/3R, two/three copies of the TYMS promoter repeat. A reference genotype applies only to the loci tested. TYMS labels describe the exploratory coding used in this analysis and do not define a validated pharmacogenetic prescribing algorithm. The functional DPYD HapB3 variant rs75017182 is distinguished from the linked synonymous marker rs56038477.