Severe Capecitabine Toxicity Associated With a Rare *DPYD* Variant Identified Through Whole-Genome Sequencing

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INTRODUCTION

Fluoropyrimidine drugs, both fluorouracil (FU) and its prodrug capecitabine, are widely used in the treatment of solid tumors such as breast, colorectal, and gastric cancers.[1] Over 2 million patients newly diagnosed with cancer are treated each year with fluoropyrimidines.[2] Between 10% and 40% of these patients develop severe, sometimes life-threatening toxicities, which may include mucositis, neutropenia, nausea, severe diarrhea, vomiting, stomatitis, and hand-foot syndrome.[2] These toxicities can be caused by genetic variants in *DPYD*, the gene that encodes for dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme responsible for FU catabolism.[1,2]

Clinical *DPYD* genotypic testing typically includes the following known toxicity-associated *DPYD* variants: c.1905+1G>A (*2A, rs3918290), c.1679T>G (*13, rs55886062), c.2846A>T (rs67376798), and c.1129-5923T>G (rs75017182). Guidelines for using these results to guide fluoropyrimidine therapy have been published by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group.[2,3] However, targeted genotyping is limited to testing known toxicity-associated *DPYD* variants and may not include novel or rare variants, which can also be deleterious to DPD function and contribute to severe toxicity.[4-9]

Here, we present a case of a patient with a rare variant of unknown significance in *DPYD* who had a severe, life-threatening capecitabine toxicity. The variant was predicted to be deleterious using a recently reported in silico tool (DPYD-Varifier),[10] and impaired function was confirmed in vitro.

METHODS

This study was approved by the Indiana University Institutional Review Board. Informed consent was obtained from the patient to have patient-derived specimens and medical records used for research and reporting. Patient germline DNA was obtained from whole blood and used for whole-genome sequencing (WGS), targeted *DPYD* genotyping, and Sanger sequencing. Detailed methods can be found in the Appendix for WGS and Sanger sequencing. Targeted genotyping was performed in the following Clinical Laboratory Improvement Amendments–certified laboratories: ARUP Laboratories (Salt Lake City, UT) and the Indiana University Pharmacogenomics Laboratory (Indianapolis, IN).

Integrated Genomics Viewer Version 2.4.10 (Broad Institute, Cambridge, MA)[11] was used to visualize WGS data, and Ingenuity Variant Analysis (Qiagen, Germantown, MD) was used for variant identification and annotation. DPYD-Varifier[10] was used to evaluate the functional impact of p.R235Q on DPD function. The effect of p.R235Q on DPD enzyme activity was determined in vitro as previously described.[10]

CASE REPORT

A 59-year-old Indian woman was diagnosed with metastatic colon cancer and started on a neoadjuvant treatment regimen of capecitabine (3 500-mg tablets orally twice a day), oxaliplatin, and bevacizumab (a treatment time line is presented in Fig 1). After 9 days of treatment, the patient developed grade 4 mucositis at the ileum and was hospitalized, at which time all chemotherapy was stopped. Hospital records indicate the presence of febrile neutropenia at admission; however, WBC counts were not available to report. While hospitalized, the patient was found to have an ileal obstruction. In all, the patient was hospitalized for 3 weeks. Because of the severity of toxicity, the patient was believed to be DPD deficient, and commercial testing for 3 toxicity-associated *DPYD* variants (c.1905+1G>A, c.1679T>G, and c.2846A>T) was performed. The patient was found to be wild-type for all 3 variants.

After surgical debulking, the patient enrolled into the Indiana University Health Precision Genomics Clinic for genome-guided cancer therapy (Fig 1). WGS was performed on germline and tumor DNA. As part of care, noncarrier status for c.1905+1G>A was confirmed. The patient remained off chemotherapy until computed tomography scan revealed disease progression. As a result of the previously observed toxicity, the patient was started on a modified leucovorin, FU,

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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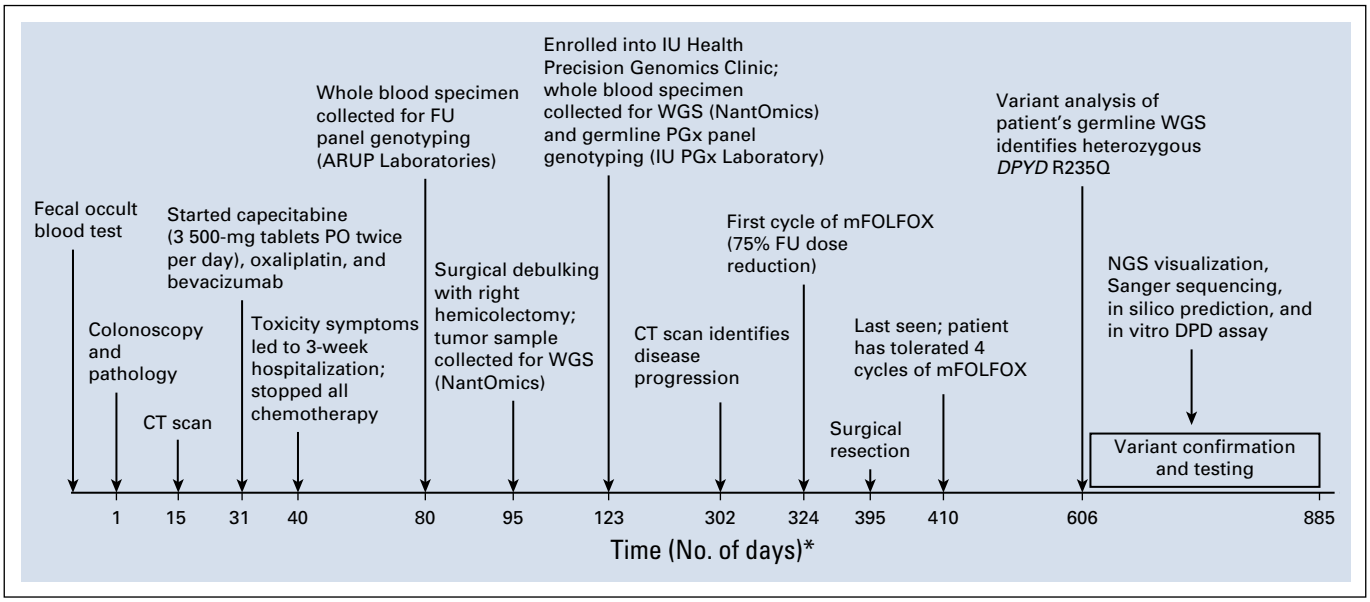

FIG 1. Time line of patient's case report. This diagram shows the time line of key events in the diagnosis, treatment, and laboratory testing of *DPYD* variants. The horizontal line represents the number of days from initial diagnosis to the patient's last encounter after modified infusional fluorouracil, leucovorin, and oxaliplatin (mFOLFOX) treatment. (*) Not drawn to scale. CT, computed tomography; DPD, dihydropyrimidine dehydrogenase; FU, fluorouracil; IU, Indiana University; NGS, next-generation sequencing; PGx, pharmacogenomics; PO, oral; WGS, whole-genome sequencing.

and oxaliplatin (mFOLFOX) regimen with a specific 75% FU dose reduction. The patient has tolerated 4 cycles of mFOLFOX therapy with no reported toxicity to date.

On the basis of the extreme observed toxicity, results of targeted *DPYD* genotyping, and tolerance to the reduced mFOLFOX regimen, we hypothesized that the patient might carry a deleterious *DPYD* variant that was not covered by the testing panel. Review of the germline WGS data confirmed noncarrier status for c.1905+1G>A, c.1679T>G, c.2846A>T, and c.1129-5923T>G. However, the patient carried a heterozygous rare missense variant in *DPYD*, rs755416212 (NM_000110.3:c.704G>A; NP_000101.2: p.Arg235Gln; referred to as p.R235Q; Fig 2A). Genotype was confirmed using Sanger sequencing (Fig 2B). Data in the Genome Aggregation Database and Exome Aggregation Consortium database indicate that this variant has a minor allele frequency of 0.00001.

DPYD-Varifier, a gene-specific in silico variant classifier,[6,10] predicted that p.R235Q was deleterious to DPD function. This prediction was validated using a previously reported in vitro assay for measuring variant function,[10] which showed that the p.R235Q variant significantly reduced DPD activity by 88% compared with wild-type DPD ($P = 6.26 \times 10^{-7}$; Fig 3). We conclude that p.R235Q likely contributed to the patient's severe, life-threatening capecitabine toxicity.

DISCUSSION

This case study illustrates the limitation of using targeted *DPYD* genotyping and the benefit of using sequence-based approaches to identify individuals at risk for severe fluoropyrimidine toxicity. The 4 known toxicity-associated *DPYD* variants (c.1905+1G>A, c.1679T>G, c.2846A>T, and c.1129-5923T>G) have been well characterized in the White population and have recommendations for genotype-guided dosing.[2,3] However, several studies have identified novel rare *DPYD* variants associated with fluoropyrimidine toxicity using sequencing.[4-9] Our study illustrates that sequencing may be beneficial for detecting unique, common, and rare variants, especially in individuals of non-White ancestry for whom there is limited pharmacogenetic knowledge.

The p.R235Q missense variant was previously observed in a patient presenting with hematuria who was diagnosed with DPD deficiency.[12] The patient was compound heterozygous for the p.R235Q missense and p.C79X nonsense variants. *DPYD* analysis of the parents revealed that the father carried p.C79X and the mother carried p.R235Q. DPD activity in the mother was approximately 50% below the population average, as measured ex vivo using peripheral-blood mononuclear cells. This observation corroborates our in vitro finding of impaired DPD function of the p.R235Q variant. To our knowledge, this is the first report of an association between *DPYD* p.R235Q and capecitabine toxicity. Collectively, these data support the clinical actionability of p.R235Q.

Interestingly, p.R235Q's effect was similar to the previously reported p.R235W variant (Fig 3), which had a significant 86% decrease in DPD activity compared with wild-type DPD ($P = 6.44 \times 10^{-7}$).[13] This variant was found in a patient with DPD deficiency like the p.R235Q variant.[14] Furthermore, one study examining the role of amino acid 235 on

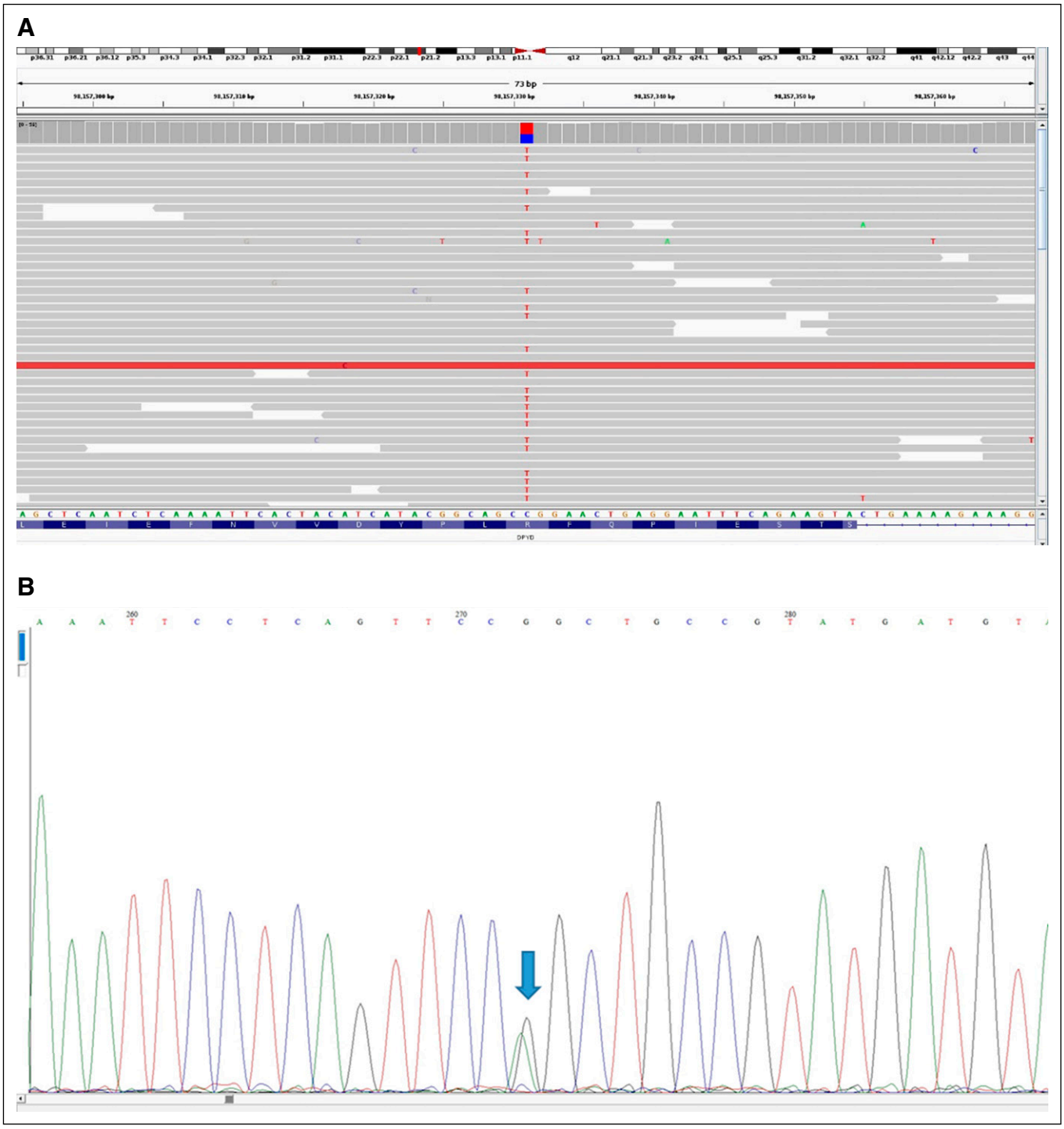

FIG 2. Presence of the heterozygous *DPYD* p.R235Q (c.704G>A) variant in the patient's germline genome. (A) Whole-genome sequencing reads identified the heterozygous single-nucleotide variant (C>T on the + DNA strand depicted) in *DPYD*. Sequencing reads matching the reference sequence are shown in gray. Data were displayed using Integrated Genomics Viewer. (B) Sanger sequencing confirmed the presence of the heterozygous single-nucleotide variant (G>A on the – coding strand) in *DPYD* as identified by the arrow.

DPD function reported that additional missense mutations (p.R235A and p.R235K) deplete DPD activity in vitro.[15] Amino acid 235 is well conserved and important for flavin adenine dinucleotide (FAD) binding.[15,16] Deleterious variants in DPD have been found enriched at conserved residues and in close proximity to important domains such as the FAD domain.[10] Altogether, our findings and these studies indicate this amino acid position does not tolerate missense changes and should be considered clinically actionable.

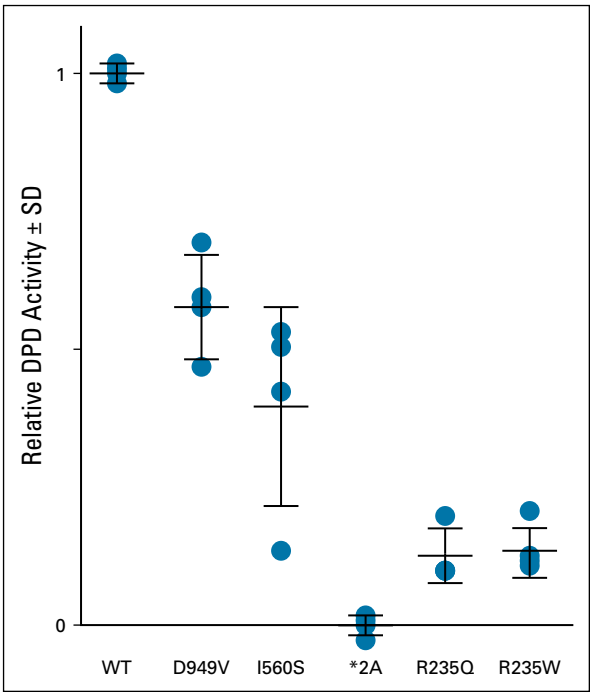

FIG 3. Functional assessment of p.R235Q *DPYD* variant. The in vitro enzyme activity of recombinantly expressed *2A (c.1905+1G>A), *13 (c.1679T>G, p.I560S), p.D949V (c.2846A>T), p.R235Q (c.704G>A), and p.R235W (c.703C>T) variants was compared with reference (wild-type [WT]) dihydropyrimidine dehydrogenase (DPD). For each variant, the mean of 4 independent biologic replicate experiments is presented as a horizontal bar ± standard deviation (SD). Each biologic replicate was measured in triplicate (ie, 3 technical replicates each).

The p.R235W variant has been incorporated into CPIC's guidelines for *DPYD* genotype-guided dosing with an assigned haploid activity score of 0 as a nonfunctional allele.[2] Given the similar function of p.R235Q and p.R235W (Fig 3), we feel it may be appropriate to consider p.R235Q similarly with a haploid activity score of 0. As such, the patient in this study would be considered an intermediate metabolizer with a calculated activity score of 1 according to CPIC guidelines. The recommended FU dose reduction with an activity score of 1 is 50%. The fact that the patient tolerated FU with a 75% specific FU dose reduction with no reported toxicity suggests that CPIC's recommendation of a 50% FU dose reduction might be sufficient to avoid toxicity while preserving drug efficacy for treatment. However, dose titration with therapeutic drug monitoring to prevent underdosing is recommended when available.

The patient was also tested for *TYMS* germline polymorphisms (rs11280056, rs45445694, and rs34743033) as part of the targeted genotyping panel. The patient was homozygous for the 6 base pair (bp) deletion (rs11280056) in the 3′ untranslated region (UTR; DEL/DEL), which is predictive for low *TYMS* expression.[17,18] The patient also had 3 copies (3R) of the 28-bp tandem repeat in the 5′ UTR promoter enhancer region (rs45445694) with the absence of a variant (rs34743033, G>C) within the second tandem repeat of the 3R allele. Altogether, the 3RG/3RG genotype is associated with increased *TYMS* expression.[19,20] The clinical association and utility of these *TYMS* polymorphisms with fluoropyrimidine toxicity are unclear.[21-25] A recent meta-analysis found no significant association with severe GI or hematologic toxicities for *TYMS* (rs45445694 and rs11280056) or *ENOSF1* (rs2612091) variants.[26] On the basis of the literature, the *TYMS* DEL/DEL genotype was unlikely to contribute to the patient's severe toxicity.

In conclusion, this case demonstrates the potential benefits of sequence-based *DPYD* genotyping to fluoropyrimidine dose individualization. One challenge to using sequence-level data is the classification of novel and understudied variants. We overcame this challenge using DPYD-Varifier, an in silico classifier, to predict whether the variant was deleterious to DPD function. The prediction was then validated by assessing the variant's impact on DPD activity in vitro. Collectively, this approach of using genomic sequencing along with DPYD-Varifier and functional testing can help to identify rare toxicity-linked *DPYD* variants and improve genotype-guided therapy in patients treated with fluoropyrimidines.

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The Indiana University School of Medicine Pharmacogenomics Laboratory is a fee-for-service clinical laboratory that offers clinical pharmacogenetic testing (V.M.P.).

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Henricks LM, Lunenburg CATC, de Man FM, et al: DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: A prospective safety analysis. Lancet Oncol 19:1459-1467, 2018
2. Amstutz U, Henricks LM, Offer SM, et al: Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing: 2017 update. Clin Pharmacol Ther 103:210-216, 2018
3. Lunenburg CATC, van der Wouden CH, Nijenhuis M, et al: Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene-drug interaction of DPYD and fluoropyrimidines. Eur J Hum Genet 28:508-517, 2020
4. Del Re M, Quaquarini E, Sottotetti F, et al: Uncommon dihydropyrimidine dehydrogenase mutations and toxicity by fluoropyrimidines: A lethal case with a new variant. Pharmacogenomics 17:5-9, 2016
5. van Kuilenburg AB, Meijer J, Maurer D, et al: Severe fluoropyrimidine toxicity due to novel and rare DPYD missense mutations, deletion and genomic amplification affecting DPD activity and mRNA splicing. Biochim Biophys Acta Mol Basis Dis 1863:721-730, 2017
6. Shrestha S, Tapper EE, Trogstad-Isaacson CS, et al: Dose modification for safe treatment of a compound complex heterozygous *DPYD* variant carrier with 5-fluorouracil. JCO Precis Oncol 10.1200/PO.18.00179
7. Tong CC, Lam CW, Lam KO, et al: A novel *DPYD* variant associated with severe toxicity of fluoropyrimidines: Role of pre-emptive *DPYD* genotype screening. Front Oncol 8:279, 2018
8. Bukhari N, Azam F, Alfawaz M, et al: Identifying a novel DPYD polymorphism associated with severe toxicity to 5-FU chemotherapy in a Saudi patient. Case Rep Genet 2019:5150725, 2019
9. Palmirotta R, Lovero D, Delacour H, et al: Rare dihydropyrimidine dehydrogenase variants and toxicity by fluoropyrimidines: A case report. Front Oncol 9:139, 2019
10. Shrestha S, Zhang C, Jerde CR, et al: Gene-specific variant classifier (DPYD-varifier) to identify deleterious alleles of dihydropyrimidine dehydrogenase. Clin Pharmacol Ther 104:709-718, 2018
11. Robinson JT, Thorvaldsdóttir H, Winckler W, et al: Integrative genomics viewer. Nat Biotechnol 29:24-26, 2011
12. van Kuilenburg AB, Meijer J, Dobritzsch D, et al: Identification of two novel mutations C79X and R235Q in the dihydropyrimidine dehydrogenase gene in a patient presenting with hematuria. Nucleosides Nucleotides Nucleic Acids 27:809-815, 2008
13. Offer SM, Fossum CC, Wegner NJ, et al: Comparative functional analysis of DPYD variants of potential clinical relevance to dihydropyrimidine dehydrogenase activity. Cancer Res 74:2545-2554, 2014
14. Vreken P, Van Kuilenburg AB, Meinsma R, et al: Dihydropyrimidine dehydrogenase (DPD) deficiency: Identification and expression of missense mutations C29R, R886H and R235W. Hum Genet 101:333-338, 1997
15. Lohkamp B, Voevodskaya N, Lindqvist Y, et al: Insights into the mechanism of dihydropyrimidine dehydrogenase from site-directed mutagenesis targeting the active site loop and redox cofactor coordination. Biochim Biophys Acta 1804:2198-2206, 2010
16. Weidensee S, Goettig P, Bertone M, et al: A mild phenotype of dihydropyrimidine dehydrogenase deficiency and developmental retardation associated with a missense mutation affecting cofactor binding. Clin Biochem 44:722-724, 2011
17. Mandola MV, Stoehlmacher J, Zhang W, et al: A 6 bp polymorphism in the thymidylate synthase gene causes message instability and is associated with decreased intratumoral TS mRNA levels. Pharmacogenetics 14:319-327, 2004
18. Pullmann R Jr, Abdelmohsen K, Lal A, et al: Differential stability of thymidylate synthase 3′-untranslated region polymorphic variants regulated by AUF1. J Biol Chem 281:23456-23463, 2006
19. Mandola MV, Stoehlmacher J, Muller-Weeks S, et al: A novel single nucleotide polymorphism within the 5′ tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 binding and alters transcriptional activity. Cancer Res 63:2898-2904, 2003

20. Yawata A, Kim SR, Miyajima A, et al: Polymorphic tandem repeat sequences of the thymidylate synthase gene correlates with cellular-based sensitivity to fluoropyrimidine antitumor agents. Cancer Chemother Pharmacol 56:465-472, 2005
21. Lecomte T, Ferraz JM, Zinzindohoué F, et al: Thymidylate synthase gene polymorphism predicts toxicity in colorectal cancer patients receiving 5-fluorouracil-based chemotherapy. Clin Cancer Res 10:5880-5888, 2004
22. Jennings BA, Kwok CS, Willis G, et al: Functional polymorphisms of folate metabolism and response to chemotherapy for colorectal cancer, a systematic review and meta-analysis. Pharmacogenet Genomics 22:290-304, 2012
23. Rosmarin D, Palles C, Pagnamenta A, et al: A candidate gene study of capecitabine-related toxicity in colorectal cancer identifies new toxicity variants at DPYD and a putative role for ENOSF1 rather than TYMS. Gut 64:111-120, 2015
24. Meulendijks D, Jacobs BA, Aliev A, et al: Increased risk of severe fluoropyrimidine-associated toxicity in patients carrying a G to C substitution in the first 28-bp tandem repeat of the thymidylate synthase 2R allele. Int J Cancer 138:245-253, 2016
25. Rosmarin D, Palles C, Church D, et al: Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: Investigation in the QUASAR2 study, systematic review, and meta-analysis. J Clin Oncol 32:1031-1039, 2014
26. Hamzic S, Kummer D, Froehlich TK, et al: Evaluating the role of ENOSF1 and TYMS variants as predictors in fluoropyrimidine-related toxicities: An IPD meta-analysis. Pharmacol Res 152:104594, 2020

APPENDIX

Whole-Genome Sequencing

Germline and somatic whole-genome sequencing (WGS) was performed by NantOmics (Culver City, CA), a Clinical Laboratory Improvement Amendments–certified laboratory, as previously described (Rabizadeh S, et al: Oncotarget 9:19223-19232, 2018). Sequencing depth for all *DPYD* exons was ≥ 30× by WGS.

Sanger Sequencing

The patient's DNA served as the template to amplify the region containing the p.R235Q variant by polymerase chain reaction (PCR). PCR was performed using Platinum SuperFi PCR Mastermix (Thermofisher Scientific, Waltham, MA) using the following primers: 5′-GCATCTTTCTGCTTCTGCCTGAT-3′ (forward 1), 5′-GTATTGAAATTGCTTTTGGCCAGTT-3′ (reverse 1), 5′-TGTCCTCATGCATATCTTGTGTG-3′ (forward 2), and 5′-TCCTTTCTTTTTGAGCAGTACACA-3′ (reverse 2). Primers were obtained from Integrated DNA Technologies (Coralville, IA). Amplification conditions were 98°C for 30 seconds, 35 cycles at 98°C for 10 seconds, 62.1°C for 10 seconds, and 72°C for 30 seconds. Final extension was at 72°C for 5 minutes. Individual PCR products from both PCR reactions were purified using the MinElute PCR purification kit (Qiagen, Germantown, MD) according to the user manual. DNA concentration was measured using the Qubit dsDNA BR assay kit according to the user manual (Invitrogen, Waltham, MA). Samples were prepared and Sanger sequenced by ACGT (Germantown, MD) using each PCR product as the template with 1 of the 4 primers individually described earlier.

