Golestan University of Medical Sciences

Journal of Clinical and Basic Research

Online ISSN: 2538-3736

Frequency of dihydropyrimidine dehydrogenase and UDP-glucuronosyltransferase 1A1 variants in cancer patients requiring chemotherapy: A single-center study in southern India

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Article History

Received: 21 February 2023
Received in revised form: 2 May 2023
Accepted: 18 June 2023
Published online: 02 July 2023

DOI: 10.29252/JCBR.7.1.6

Keywords:
Neoplasms
Chemotherapy
Drug therapy
Pharmacogenetics
5-Fluorouracil
Irinotecan
Enzyme

Article Type: Original Article

Abstract

Background: Cancer treatment using drugs metabolized by the enzymes dihydropyrimidine dehydrogenase (DPYD) and UDP-glucuronosyltransferase 1A1 (UGT1A1) results in adverse effects for some patients. This is frequently reported in cancer patients undergoing therapy with 5-fluorouracil, capecitabine, and irinotecan who have polymorphisms in the genes coding for DPYD and UGT1A1. The present study assessed the DPYD*2A and UGT1A1*28 polymorphisms in cancer patients before starting chemotherapy to identify the individuals at risk of developing an adverse drug reaction.
Methods: Genomic DNA was isolated from patients and subjected to PCR amplification using specific primers to study DPYD*2A and UGT1A1*28 polymorphisms. The PCR products were assessed by Sanger sequencing for establishing the genotype.
Results: Of 75 cancer patients requiring treatment with drugs metabolized by DPYD and UGT1A1, 2 (2.66%) and 12 (29.27%) were likely to have adverse reactions based on DPYD*2A and UGT1A1*28 genotyping, respectively.
Conclusion: Our findings indicate that carrying out genotyping for these two polymorphisms will help a large number of patients requiring treatment with 5-fluorouracil, irinotecan, and capecitabine.

Highlights:

What is current knowledge?
Patients with variants in DPYD and UGT1A1 genes are at risk of experiencing drug toxicities when treated with 5-fluorouracil, capecitabine, and irinotecan
Not all physicians involve testing for these genetic variants in their treatment protocols

What is new here?
The prevalence of DPYD and UGT1A1 variants is high among the Indian subpopulation
A high percentage of Indian patients will benefit from testing for these variants
Implementing pharmacogenomic testing as a part of routine oncological care will improve patient outcome

Introduction

Current-day oncology practice is focusing on targeted therapies and personalized medicine to achieve better patient outcomes. However, it is extremely important to assess variants of enzymes involved in the metabolism of chemotherapeutic drugs (1). The use of pharmacogenomics to detect variants will allow physicians to alter treatment regimens for superior patient outcomes. Patients with altered enzyme function due to genetic variations are at higher risk of experiencing varying degrees of drug toxicity with the use of certain chemotherapeutic medications (2). Identifying such patients by genetic testing and subsequently planning individualized treatment is the essence of precision medicine.

Two enzymes, dihydropyrimidine dehydrogenase (DPYD) and uridine 5'-diphospho-glucuronosyltransferase (UGT) are involved in the metabolism of several drugs used in oncology practice (3). The enzyme DPYD is involved in the breakdown of uracil and thymine, utilizing nicotinamide adenine dinucleotide phosphate as a cofactor, and plays an important role in the pharmacokinetics of fluoropyrimidine-related anticancer drugs (4). It is coded by a gene located on chromosome 1p21.3 and has several polymorphisms. Variants in the DPYD gene interfere with the metabolism of 5-fluorouracil (5-FU) and capecitabine (5) and are used in the treatment of solid tumors, including colorectal, gastric, breast, pancreatic, and head and neck cancers (6). The DPYD*2A is a splice variant that affects the expression of DPYD (7). Hence, this loss-of-function variation results in DPYD deficiency, thereby leading to altered metabolism and adverse medication reactions (8). Around 80% of administered standard dose of 5-FU is rapidly degraded by DPYD to inactive compounds, followed by excretion within 24 hours. The metabolism is similar for capecitabine, which is converted into 5-FU by the enzyme thymidine phosphorylase intracellularly (4).

The enzyme, UGT, metabolizes a variety of lipophilic compounds, including bilirubin, steroids, toxins, and therapeutic medications. This enzyme controls the phase II metabolic pathway known as glucuronidation, which consists of glucuronic acid being conjugated to particular substrates, producing water-soluble metabolites that can be excreted from the body. The UGT1A gene complex is on chromosome 2q37 and is involved in the metabolism of anti-cancer drugs like irinotecan and 5-FU. The common variant of the UGT1A1 gene is UGT1A1*28, which has an altered number of TA repeats in its promoter, containing six TA repeats within the TATA box promoter region. Extra (TA) repeats decrease the rate of transcription initiation of the UGT1A1 gene, leading to reduced gene expression, resulting in irinotecan toxicity that presents as severe neutropenia, fever, and diarrhea, and limits the usage of

[DOI: 10.61186/jcbr.7.1.6]

irinotecan. This occurs in 20-25% of patients, and approximately 7% of them die from these complications (9).

The present study aimed to evaluate DPYD*2A and UGT1A1*28 polymorphisms in cancer patients requiring chemotherapy with 5-FU, capecitabine, and irinotecan to identify individuals with altered metabolism of these chemotherapeutic drugs, which will ultimately enable oncologists to improve patient outcomes by altering drug dosage.

Methods

This study was carried out on 89 cancer patients who were referred to the Department of Genetics and Molecular Medicine, Kamineni Hospitals in Hyderabad, India for molecular testing of DPYD *2A and UGT1A1*28 variants. Patients were tested for one or both polymorphisms according to their treatment plan.

- ***DNA Isolation and PCR***

Approximately 2 ml of blood were collected in ethylenediaminetetraacetic acid containers and genomic DNA was isolated for all the samples using the salting out method (10).

Three-step PCR amplification was carried out in a thermal cycler using specific primers (Table 1) for DPYD and UGT1A1 polymorphisms. The primers were synthesized by Bioserve Biotechnologies (India) Private Limited, Hyderabad, India.

The PCR reaction was set up in a reaction volume of 25 µL containing 1 µL of the isolated DNA. The reaction was carried out according to the protocol described in our previous paper (11). PCR products were visualized after agarose gel electrophoresis and then sent for Sanger sequencing.

Table 1. Sequences of the primers used for DPYD and UGT1A1 gene sequencing

Gene	Primers	Annealing temperature	Product size (bp)
DPYD*2A	Forward: CTAAAGGCTGACTTTCCAGACTAC Reverse: CAGCAAAGCAACTGGCAGATTC	58 °C	155
UGT1A1	Forward: TCCCTGCTACCTTTGTGGAC Reverse: AGCAGGCCCAGGACAAGT	60 °C	186

Results

The study included all patients who were referred to the Department of Genetics for DPYD and/or UGT1A1 polymorphism testing with diagnoses of colon, breast, stomach, pancreatic, brain, liver, or other types of cancer. Gender distribution analysis revealed 33 female patients and 56 male patients, for a total of 89 patients. Of 89 patients, the majority of patients (47.19%) were diagnosed with colon cancer. In addition, 29 patients were diagnosed with other types of cancer that were less common in the study group, such as Ewing sarcoma, ovarian cancer, etc. (Table 2).

Table 2. Frequency of different types of cancer

Type of cancer	Number of patients	Percentage
Colon	42	47.19%
Breast	6	6.74%
Stomach	4	4.49%
Pancreas	3	3.37%
Brain	2	2.25%
Liver	5	5.62%
Other/Unknown	27	30.34%

Less frequently encountered types of cancer in our study, such as ovarian, renal, bone, esophageal, and mediastinal tumors were grouped.

Of 75 patients assessed for DPYD*2A (1905+1 G>A) gene polymorphism, 2 (2.66%) were found to be homozygous (AA), 20 patients (26.66%) were found to be heterozygous (AG), while 53 patients (70.66%) were found to be homozygous (GG) (Table 3). Moreover, UGT1A1(TA)n genotyping in 41 patients showed that 9 patients (21.95%) were homozygous for the TA6/6 repeats, 12 patients (29.27%) carried TA7/7 or TA7/8, while 20 patients (48.78%) were TA6/7 (Table 3 and Figures 1 and 2).

Table 3. Results of genetic testing for DPYD*2A and UGT1A1*28 variant

Gene	DPYD*2A		UGT1A1	
	No. of patients/percentage	Genotype	No. of patients/percentage	Genotype
Homozygous wild type	53 (70.66%)	GG	9 (21.95%)	TA6 / TA6
Heterozygous	20 (26.66%)	AG	20 (48.78%)	TA6 / TA7
Mutant type	2 (2.66%)	AA	12 (29.27%)	TA7 / TA7, TA7 / TA8
Allele Frequency	G = 126 (84%) A = 24 (16%)		TA6 = 38 (46%) TA7/8 = 44 (54%)	

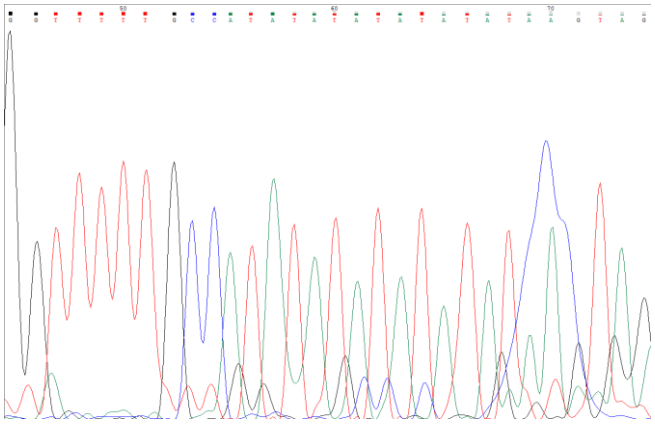

Figure 1. Chromatogram for UGT1A1 TA6/TA6 showing the number of TA repeats visualized by Sanger sequencing

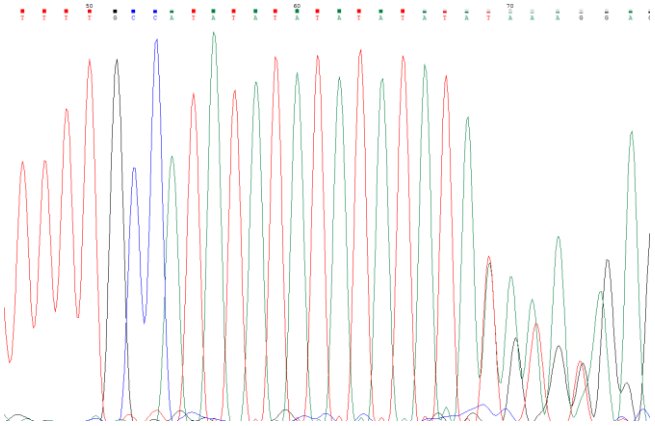

Figure 2. Chromatogram for UGT1A1 TA6/TA7 showing the number of TA repeats visualized by Sanger sequencing

Discussion

There has been an increasing amount of data indicating the importance of DPYD and UGT1A1 variants as risk factors for chemotherapy-associated toxicity in cancer patients under treatment with 5-FU, capecitabine, and irinotecan. The implementation of these pharmacogenetic tests in regular clinical practice is still not routinely carried out (12). The US oncology guidelines recommend DPYD testing for all patients requiring treatment with 5-FU (6). A variety of cancers including colon, breast, pancreatic, and gastric cancers are currently being treated with 5-FU, while colon cancers are also treated with capecitabine and irinotecan in case of failure of first-line drug therapy. Approximately 31–34% of patients develop severe toxicities with 5-FU, a prodrug that requires conversion by DPYD into cytotoxic metabolites for its anti-tumor effects (9). The risk of chemotherapy-related death was shown to be 25.6 times higher in DPYD *2A variant carriers receiving conventional dosages of 5-FU therapy (13). Another study reported that 71% of patients with severe 5-FU toxicity showed reduced DPYD activity, suggesting that impaired DPYD functioning is a contributor to 5-FU-related adverse effects (14). Drug toxicity could be controlled in DPYD-deficient patients by reducing the dose of 5-FU, or by using a U.S. Food and Drug Administration-approved antidote, uridine triacetate (15).

A previous study by Pavithran, et al. (2019) analyzed the prevalence of DPYD variants among patients receiving treatment with 5-FU or capecitabine-

[DOI: 10.61186/jcbr.7.1.6]

based regimens in a single center based in South India and identified that 31.9% of patients carried the DPYD *2A variant allele (16).

The prevalence of DPYD deficiency is approximately 3–5% in the Caucasian population and 8% in the African-American population. However, its prevalence in the Indian population has not been determined (17). Genetic testing and subsequent recalculation of drug doses in 18 patients carrying the DPYD*2A variant resulted in 11% (2 patients) experiencing grade 0 toxicity, 61% (11 patients) experiencing grade 1 to 2 toxicity, and 28% (5 patients) experiencing grade 3 or higher toxicity. Through dose adjustment, the risk of grade 3 toxicity was reduced from 73% to 28% (18).

In a study by Jada et al. (2007), the UGT1A1*28 allele was found in 65.2% of healthy Asian patients and 33.3% of Asian cancer patients (19). In a similar study by Balram et al. (2001), the frequency of the UGT1A1*28 allele in the Indian population was found to be 35.1% (20). In our study, 78% of patients were found to carry the UGT1A1*28 risk allele. Homozygous patients (TA7/7) require a 70% reduction in dose compared with the standard dose. High doses of drugs are recommended for patients with *1/*1 genotype (21).

One of the limitations of this study was the inability to follow up all patients undergoing treatment and collect more data regarding their response to therapy and adverse drug reactions.

Our study determined that a significant proportion of cancer patients will benefit from genotyping for the two polymorphisms prior to commencing chemotherapy. This study highlights the importance of implementing screening for these two variants as a part of routine care in oncology treatment to help a significant percentage of cancer patients benefit from appropriate cancer care.

Conclusion

This study provides evidence that 29.33% of patients in the study area carried the risk allele for DPYD*2A, while the UGT1A1*28 risk alleles were found in 78.05% of patients, suggesting that a very high percentage of Indian patients will benefit from genotyping prior to commencing chemotherapy with 5-FU, irinotecan, and capecitabine.

Acknowledgments

The authors are grateful to the present and past staff of the Department of Molecular Medicine and Genetics, Kamineni Hospital, Hyderabad, India

Funding source

The authors received no financial support for the research, authorship, and/or publication of this article

Ethical statement

Consent for testing was taken from all of the patients. All the tests performed were clinically required and hence ethical approval was not sought.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author contributions

KG performed data analysis and wrote the first draft of the manuscript. NB was involved in molecular analysis and writing the manuscript. SM performed the final editing of the manuscript and referred cases to our study by suggesting genetic testing. QH conceptualized the study, supervised it, and performed the final editing of the manuscript. All authors read and approved the final manuscript and agree to be accountable for the content of the work.

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How to Cite:
Gangavarapu K, Shyamali Bora N, Srinivas M, Hasan Q. Frequency of dihydropyrimidine dehydrogenase and UDP-glucuronosyltransferase 1A1 variants in cancer patients requiring chemotherapy: A single-center study in southern India. *JCBR*. 2023;7(1):6-8.

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[DOI: 10.61186/jcbr.7.1.6]