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Mutational spectrum of dihydropyrimidine dehydrogenase gene (DPYD) in the Tunisian population

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Abstract

Dihydropyrimidine dehydrogenase enzyme (DPD) deficiency is a pharmacogenetic syndrome leading to severe side-effects in patients receiving therapies containing the anticancer drug 5-fluorouracil (5-FU). The aim of this population study is to evaluate gene variations in the coding region of the dihydropyrimidine dehydrogenase gene (DPYD) in the Tunisian population. One hundred and six unrelated healthy Tunisian volunteers were genotyped by denaturing HPLC (DHPLC). Twelve variants in the coding region of the DPYD were detected. Allele frequencies of DPYD*5 (A1627G), DPYD*6 (G2194A), DPYD*9A (T85C), A496G, and G1218A were 12.7%, 7.1%, 13.7%, 5.7%, and 0.5%, respectively. The DPYD alleles DPYD*2A (IVS 14 + 1g > 1), DPYD*3 (1897 del C) and DPYD*4 (G1601A) associated with DPD deficiency were absent from the examined subjects. We describe for the first time a new intronic polymorphism IVS 6–29 g>t, found in an allelic frequency of 4.7% in the Tunisian population. Comparing our data with that obtained in Caucasian, Egyptian, Japanese and African–American populations, we found that the Tunisian population resembles Egyptian and Caucasian populations with regard to their allelic frequencies of DPYD polymorphisms. This study describes for the first time the spectrum of DPYD sequence variations in the Tunisian population. ***To cite this article: R. Ben Fredj et al., C. R. Biologies 330 (2007).***
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Résumé

Analyse génétique du gène de la dihydropyrimidine déhydrogénase (DPYD) dans la population tunisienne. Le déficit enzymatique en dihydropyrimidine déhydrogénase est un syndrome pharmacogénétique entraînant de sévères toxicités chez les patients cancéreux traités par le 5-fluorouracile (5-FU). L'objectif de notre étude est de déterminer les variations génétiques touchant le gène de la DPD (DPYD) chez la population tunisienne. L'ADN de 106 volontaires a été analysé par *denaturing HPLC* (DHPLC). Douze variations génétiques ont été observées dans la région codante de la DPYD. Les fréquences alléliques de DPYD*5 (A1627G), DPYD*6 (G2194A), DPYD*9A (T85C), A496G et G1218A étaient de 12,7%, 7,1%, 13,7%, 5,7% et 0,5 %, respectivement. Les allèles DPYD*2A (IVS 14 + 1g > 1), DPYD*3 (1897 del C) et DPYD*4 (G1601A), causant des déficits en DPD, n'ont

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pas été observés. On a trouvé un nouveau polymorphisme intronique IVS 6–29 g>t, la fréquence allélique étant de 4,7%. Grâce à la comparaison des fréquences alléliques, on peut conclure que la population tunisienne ressemble à ses homologues égyptienne et caucasienne. ***Pour citer cet article : R. Ben Fredj et al., C. R. Biologies 330 (2007).***
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Keywords: 5-Fluorouracile; Tunisian population; Dihydropyrimidine dehydrogenase gene; DHPLC

Mots-clés : 5-Fluorouracile ; Population tunisienne ; Gène de la dihydropyrimidine déhydrogenase ; DHPLC

1. Introduction

The enzyme dihydropyrimidine dehydrogenase (DPD; EC 1.3.1.2) is a rate-limiting enzyme in the metabolic pathway involved in the breakdown of pyrimidine bases and the antineoplastic agent 5-fluorouracil [1–4]. As more than 80% of administered drug at standard dose are rapidly degraded by DPD to inactive compounds, the catabolic pathway of 5-FU plays a key role in drug response and 5-FU-induced toxicity [1–5].

A DPD-deficient phenotype manifesting as 5-FU-induced toxicity in cancer patients is at least in part due to inherited variations in the dihydropyrimidine dehydrogenase gene (DPYD) [4,6–10].

DPYD, located on chromosome 1p22, consists of 23 exons, spanning a genomic region of around 950 kb, and includes a 3-kb coding sequence with introns of average size of about 43 kb [11,12]. More than 39 different DPYD alleles have so far been identified. The DPYD*2A allele, due to a point mutation within the 5′-splice site of intron 14, results in the deletion of 55 amino acids and is the most frequent mutation in patients with partial or complete DPD deficiency [13,14]. In addition, a series of other mutations has been reported to be responsible for DPD protein alterations, leading to its reduced catalytic activity [13,15,16].

Among Caucasians populations, DPD activity is highly variable, influencing the individual response of cancer patients to fluoropyrimidine drugs, and has been shown to be related to DPYD gene sequence variations [17,18]. Few studies on the frequency of variant alleles of the DPYD gene in general populations have been performed. This study is one such contribution towards the DPYD gene database, reporting, for the first time, the frequency and type of sequence variations in the DPYD gene among the Tunisian population.

2. Materials and method

2.1. Study subjects

One hundred and six unrelated Tunisian subjects participated in this study; all subjects were of Arab origin. The individuals were of different ages (between 21 and 72 years) and consisted of 47 women and 59 men.

Genomic DNA was isolated from blood samples using the Wizard Genomic DNA purification kit (cat. #A1120) (Promega, Madison, USA) according to the instructions of the manufacturer.

2.2. PCR amplification of DPYD exons

Primer pairs utilised for the amplification of DPYD exons and exons–intron boundaries were as reported previously [19]. Amplicons were generated with the expand high-fidelity PCR system (Roche, Mannheim, Germany). The PCR reactions were performed in a volume of 50 μl with 33 cycles consisting of a denaturation step at 94 °C for 35 s, primer annealing for 30 s and an elongation step at 72 °C for 1 min, unless otherwise stated. The final step extension at 72 °C was performed during 5 min. Primer sequences and PCR conditions are listed in Table 1.

2.3. Mutations analysis of the DPYD gene by DHPLC

DHPLC analysis was carried out using a semi-automated WAVE® DNA Fragment Analysis System equipped with a DNASep™ column (Transgenomic, Inc., Omaha, NE). The mutation analysis was performed under partially denaturing conditions to separate heteroduplex molecules in the case of heterozygous samples, as described previously [19]. Optimal heteroduplex recognition on the DNASep™ column occurs at a critical temperature that was predicted for each exon by melt software available either through the website http://insertion.stanford.edu/cgi-bin/melt.pl [20] or by the WaveMaker calculation software. In order to avoid missing any mutation, additional runs were carried out at one degree below and above the calculated temperature(s). Homozygous sequence alterations were detected by adding a known wild-type PCR sample to an unknown sample before heteroduplex generation. The peak profiles were compared to those of control samples possessing a known validated variation. DHPLC running conditions for each amplicon are summarized

Table 1
Amplification and DHPLC conditions for DPYD mutation analysis

Exon	Size (bp)	5′-Primer	3′-Primer	PCR $T°$ (°C)	$MgCl_2$ (mM)	DHPLC $T°$ (°C)
1	184	gctgtcacttggctctct	cacctacccgcagagca	56	2	64.63
2	285	gtgacaaagtgagagagaccgtgtc	gccttacaatgtgtggagtgagg	62	1.5	56.58
3	330	caattttaatgaatgctacccaat	ctgaatggtggcaatgaact	55	2	56.58
4	245	ggtagaaaatagattatctc	gatttgctaagacaagctg	50	2	53
5	284	gtttgtcgtaatttggctg	atttgtgcatggtgatgg	52	1.5	53.58
6	357	gaggatgtaagctagtttc	ccatttgtgtgcgtgaagttc	52	2	56
7	360	gtcctcatgcatatcttgtgtg	gcttctgcctgcctgatgtagc	57	1.5	56
8	324	ttttggctgacttttcattc	tggccaatcatttctatctg	53	2	54
9	242	ccctcctcctgctaatg	gaacaatgtgctgctgag	54	2	55.57
10	342	gagagtgacacttcatcag	ctgttggtgtacaactcc	51	1.5	60
11	442	actggtaaactcag	caattccctgaaagctag	51	1.5	55.57
12	453	ttcctgtatgtgaggtgta	gaagcacttatccattgg	51	1.5	55.57
13	440	cggatgactgtgttgaagtg	tgtgtaatgataggtcgtgtc	56	1.5	52.58
14	410	tgcaaaaatgtgagaaggacc	cagcaaagcaactggcagattc	60	1.5	54.58
15	358	tatctttgtgtacaactgga	tgtgaaatccaaggacc	51	1.5	52
16	223	aacggtgaaagcctattgg	tagtaactatccatacggggg	55	1.5	55.58
17	238	cacgtctccagctttgctgttg	cgggcaactgattcaagtcaag	60	1.5	55.60
18	220	ttgaatgggttttaactatcgtgtctt	aagaaagcacaatgcaag	Touch down 60–50	1.5	55.60
19	300	tgtccagtgacgctgtcatcac	cattgcatttgtgagatggag	56	1.5	55.58
20	399	gagaagtgacgctgtcatcac	cacagacccatcatatggctg	52	1.5	58
21	228	tctgacctaacatgcttc	ccagtaaagtaggcatac	51	2	56
22	291	gagcttgctaagtaattcagtggc	agagcaatatgtggcacc	56	1.5	58
23	269	ggggacaatgatgatgacctatgtgg	ggtgacatgaaagttcacagcac	55	2	59

in Table 1. Samples exhibiting atypical profiles were further subjected to sequence analysis.

2.4. DNA sequencing

Identification of DNA variations was achieved by sequencing the respective PCR products using an Applied Biosystem model 310 DNA sequencing system.

2.5. Statistical analysis

Differences in allele frequencies between Tunisian and other ethnic populations were measured by the Fisher exact test. A P value below 0.05 was considered statistically significant throughout the population comparisons.

3. Results

A high-throughput mutation analysis of the coding region and the flanking intronic regions of the DPYD gene were performed by DHPLC. Twelve different variant alleles were found in 106 healthy Tunisian volunteers (212 chromosomes). Eighty-six alleles were identical with the reference sequence and were assigned as wild type and 126 alleles revealed sequence variations.

Many of the sequence variations were found clustered in exons 2, 6, 11, 13, 14 and 18 with allelic frequencies between 0.5% and 13.7% attesting the copresence of rare variants and common polymorphisms. The DPYD variations identified by multiple peaks in the DHPLC chromatograms are listed in Table 2. Two subjects displayed G1236A nucleotide substitution corresponding to a silent E412E change. Another T1896C substitution, again a silent F632F change, was detected at an allelic frequency of 2.4%. A total of 82 alleles found in heterozygous state corresponded to nonsynonymous amino acid substitutions. One among them was also found in homozygous state in a single subject and concerned the DPYD*9A allele (85T>C, C29R). Since this allele was found in heterozygous state in 27 subjects, it can be considered a common polymorphism in the Tunisian population. Interestingly, a DHPLC profile distinct from that of the wild type was found in intron 6 in 10 subjects. Sequence analysis revealed a novel intronic variation IVS 6–29 g>t at an allelic frequency of 4.7%, which is not uncommon in the studied Tunisian population.

4. Discussion

Recent studies investigating the molecular mechanisms of DPD deficiency have indicated that genetic alterations in DPYD, including exon skipping [21], deletion [22], and missense mutations [14,23,24] may contribute to the DPD-deficient phenotype. The analysis

Table 2
DPYD mutations in the Tunisian population

Exon	Mutation	Allele	Effect	Heterozygotes/106 subjects	Homozygotes/106 subjects	Allelic frequency (%)
2	T85C	9A	C29R	27	1	13.7
6	A496G		M166V	12	0	5.7
7	**IVS 6–29g>t** *		–	10	0	4.7
8	IVS 8+113c>t		–	1	0	0.5
11	G1218A		M406T	1	0	0.5
	IVS 10–15 t>c		–	9	0	4.2
	G1236A		E412E	2	0	0.9
12	IVS 11–119 a>g		–	14	0	6.6
	IVS 11–106 t>a		–	1	0	0.5
13	A1627G	5	I543V	27	0	12.7
14	T1896C		F632F	5	0	2.4
18	G2194A	*6	V732I	15	0	7.1

* New intronic variation.

Table 3
DPYD allele frequency in different populations

		Frequency of DPYD variants (*P* value vs. Tunisian)							
Population (réf)	***n***	***2 A(a)**	***3 (b)**	***4**	***5**	***6**	***9A**	**A496G**	**G1218A**
Tunisian (this study)	**212**	0	0	0	0.126	0.072	0.128	0.057	0.006
Caucasian (18)	**314**	0	0	0.016 ***NS***	0.14 ***NS***	0.002 (<0.0001)	0.19 (0.05)	0.08 ***NS***	0.01 ***NS***
Finish (12)	**180**	0.011	–	0.033 (0.02)	0.072 ***NS***	0.067 ***NS***			
British (3)	**120**	0	0	0.008 ***NS***	0.28 (0.0004)	0.058 ***NS***			
Egyptian (17)	**478**	0	0	0.028 (0.03)	0.115 ***NS***	0.09 ***NS***			
Japanese (12)	**100**	0	–	0.011	0.35 (<0.0001)	0.044 ***NS***			
Taiwanese (12)	**262**	0	–	0	0.21 (0.01)	0.014 ***NS***			
African–American (12)	**210**	0	–	0.005	0.227(0.009)	0.019 (0.01)			

n, total number of alleles.
Differences in allele frequencies were measured by the Fisher exact test.
NS: not significant ($P > 0.05$).
(a) No significant difference between the Tunisian population and the other ones.
(b) No significant difference between the Tunisian, Caucasian, British, and Egyptian populations.

of genetic variations of drug-metabolizing enzymes is thought to be a useful tool in predicting individuals being at risk for adverse drug reactions [25]. The enzyme DPD plays a critical role in the catabolism of the anticancer agent 5-FU and is involved in severe life-threatening reactions towards 5-FU in the case of DPD-deficient patients. The present study is the first report on the spectrum of DPYD variants in the Tunisian population.

Given the fact that DPYD locus is quite large and complex in terms of distribution of sequence variations over the gene stretch, we employed a high-throughput method for rapid identification of both known and unknown sequence variations in this gene [26,27].

Multiple heterozygous sequence variations in heterozygous state were detected in the studied Tunisian population, with allelic frequencies comparable to those of other populations, except for the DPYD*2A variant (Table 3). This deficient variant allele was not found in our cohort, contrary to the case of the Finnish [12] and Caucasian populations [13,14], who had allele frequencies of 0.9% and 1.1%, respectively. This could be due to the size of the cohort studied here.

The DPYD*5 allele, not associated with DPD deficiency, was observed in Tunisian samples with an allele frequency of 12.7%, similar to that found in Egyptians [17] and Caucasians [18], with a frequency of 11.5% and 14%, respectively, but at a lower frequency than in Japanese (35%), Taiwanese (21%), and African–Americans (22.7%) [12,28]. The second most common polymorphism in the Tunisian population was DPYD*9A, at a frequency of 13.7%.

For DPYD*6, a non-deficient allele, the observed frequency in our population was 7.1% and was comparable to that of Caucasians, Japanese, Africans, Finnish, and Egyptians (5%, 9.8%, 4%, 6.7%, and 9%, respectively), with the lowest frequency for the Taiwanese people (1.4%) [12,29].

The variant A496G (Met166Val) allele [4,19,30] was found at a frequency of 5.7% in our population, and the impact of amino acid change on DPD function is controversial: it is reported as associated with reduced DPD activity [30,31] as well described as an allele with normal DPD enzyme activity [32].

Some of the detected point mutations are common in all populations, but exhibited some population differences in their frequency.

However, functionally relevant frequencies are rare in populations, and this is the case with the studied Tunisian population.

In this study, we describe, for the first time, a new intronic polymorphism found with allelic frequencies of 4.8% in the Tunisian population, IVS 6–29 g>t (intron 6). Since this variation was not observed in 157 Caucasian individuals [18], we may consider it as 'restricted' to the Tunisian population and maybe to neighbouring population groups.

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