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SHORT COMMUNICATION

Prevalence of the *DPYD* variant (Y186C) in Brazilian individuals of African ancestry

Geraldo Felício Cunha-Junior[1,2] · Luciana Bastos-Rodrigues[3,5] · Pedro G. Azevedo[5] · Maria Aparecida Bicalho[1,5] · Luiz Alexandre V. Magno[5] · Luiz De Marco[4,5] · Luiz Gonzaga Coelho[1]

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Abstract

Purpose The presence of deleterious variants of dihydropyrimidine-dehydrogenase gene (DPYD) is associated with 5-Fluorouracil toxicity. Most of the data are based on findings in Caucasian populations. The variant Y186C (rs115232898) is found almost exclusively in African populations and is related to low DPD function. Its prevalence may vary among African subpopulations and in African Americans. There is no information in other populations. Brazil has the biggest African population outside Africa. We studied for the first time the frequency of this mutation in African Brazilians.

Methods We amplified exon 6 of DPYD extracted from genomic DNA of 79 healthy volunteers of genetically defined African ancestry from Southeast Brazil and 36 self-reported African descendants from Northeast Brazil in order to determine the prevalence of the variant Y186C in Brazilians of African ancestry.

Results The variant Y186C was found in heterozygosity in two samples from Southeast (2.53%) and one from Northeast (2.77%) Brazil. Overall, the prevalence of this mutation in the 115 African Brazilians was 2.6%.

Conclusions The variant Y186C is prevalent among Brazilians of African ancestry and should be taken in account in targeted genotyping for fluoropyrimidine risk variants.

Keywords *DPYD* variant · Y186C · 5-Fluorouracil

Abbreviations

DPD	Dihydropyrimidine-dehydrogenase
DPYD	The gene encoding DPD
FP	Fluoropyrimidines
5FU	Fluorouracil

Introduction

The fluoropyrimidines (FP) 5-fluorouracil (5FU) and its oral prodrug capecitabine are the mainstay of systemic therapy of colorectal cancer and play an important role in the treatment of breast, head and neck and other gastrointestinal malignancies [1]. Severe toxicity, mainly mucositis, diarrhea, hand–foot syndrome and neutropenic fever, will occur in 16–31% of patients [2, 3], being lethal in 0.5–1.0%. The rate of FP catabolism is the main determinant of side effects and is dictated by the enzyme dihydropyrimidine-dehydrogenase (DPD), which is implicated in about 50% of cases of severe toxicity.

The activity of DPD can be determined phenotypically by several methods such as DPD activity in peripheral mononuclear leukocytes (DPD radioassay), the uracil/dihydrouracil ratio and the ^{13}Carbon-uracil breath test, which measures the entire 5FU catabolic pathway [4]. However, DPD deficiency is found in only 50% of fluoropyrimidine-associated toxicity, and association with *DPYD* genotyping is important to increase sensitivity in predicting FP toxicity.

✉ Luiz De Marco
Ldemarco@ufmg.br

1 Department of Internal Medicine, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

2 Department of Oncology, Hospital da Baleia, Fundação Benjamim Guimarães, Belo Horizonte, Brazil

3 Department of Nutrition, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

4 Department of Surgery, Federal University of Minas Gerais, Av. Professor Alfredo Balena 190, Belo Horizonte 30130-100, Brazil

5 Molecular Medicine Technology Center, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

The deleterious mutations in the *DPYD* gene, IVS14 + 1G > A (rs3918290, c.1905 + 1G > A, *DPYD*2A*); c.2846A > T (rs67376798, D949 V) and c.1679T > G (rs55886062, I560S, *DPYD*13*), have been strongly associated with toxicity in Caucasians [5, 6], but those variants are present in less than 2% of the population. A synonymous mutation in exon 11 (c.1236G > A) in strong linkage with the intronic variant c.1129-5923C > G (Haplotype B3) has also been implicated in 5FU toxicity in Caucasians, being present in 4% of population [7]. Therefore, less than 10% of Europeans harbor a mutant deleterious allele of *DPYD*. The prevalence of each of the above variants in non-European populations is less than 0.1% according to publicly available genotype databases [8], and, thus, the genetic determinants of DPD deficiency in other ethnic groups such as individuals of African descent remain to be fully elucidated.

A study in 258 health volunteers demonstrated that the prevalence of DPD deficiency as measured by DPD radioassay is threefold greater in African Americans as compared to Caucasian population (8 vs. 2.8%) [9]. Subsequently the same group assessed phenotypically 219 health volunteers with DPD radioassay and uracil breath test and demonstrated concordance between both tests in 208 cases. In 11 cases, DPD radioassay was normal but breath test was positive, suggesting deficiency in enzymes downstream of DPD (dihydropyrimidinase or *b*-ureidopropionase). Eight out eleven of discordant cases were African Americans suggesting that this population might have, at least in some cases, poor 5FU catabolism due to deleterious mutations in other catabolic enzymes [10, 11]. A subsequent study [12] used a subset of the population from a previous study [9] comprising 94 African Americans and 81 European Americans to search for specific deleterious mutations in each ethnic group. A nonsynonymous variant in exon 6, c.557A > G (rs115232898, Y186C), previously described in one DPD-deficient African Americans [13] by the same group, but not suspected at that time, was found in heterozygosity in six out of 94 African Americans (6.4%). Correlation with DPD radioassay in the subgroup of African Americans revealed a 46% reduction in DPD activity in carriers compared to non-carriers of Y186C. This mutation was unique to individuals of African ancestry and was present in 26% of African Americans with DPD deficiency. Subsequently the impact of Y186C in DPD function was tested in an in vitro system [14, 15] which demonstrated a 15–29% relative decrease in activity compared with wild type DPD. Interestingly, there have been only two reported cases of severe toxicity to 5FU in patients harboring Y186C [16, 17].

Recent studies pointed out to variable prevalence of Y186C in African subpopulations inside Africa [7] and even among African Americans [18]. Data regarding the prevalence of this variant in highly admixed populations such as South American are scarce. Brazil has an admixed population composed of Europeans, Africans and Amerindians, and have the largest black population outside Africa, comprising 30–40% of Brazilians. According to the latest self-declared ethnicity data by the Brazilian census agency (https://www.ibge.gov.br/pt/inicio.html) approximately 43% of the population regards themselves as Caucasians. Therefore, we sought to determine the prevalence of Y186C in a setting of healthy Brazilian volunteers of African ancestry which comprise most of the Brazilian population.

Materials and methods

Subjects

The study comprises two cohorts of Brazilian subjects of African ancestry. The first cohort encompassed 79 genetically defined African Brazilian healthy volunteers from Belo Horizonte, Minas Gerais State, Southeast Brazil. Thirty-two were women and 47 were men. The second cohort was composed by 36 self-reported African Brazilians (nine women and 27 men), healthy blood donors from Ilheus, Bahia State, Northeast Brazil.

All subjects signed a written informed consent. The Ethics Committee of Federal University of Minas Gerais approved the storage and use of DNA for genetic studies.

Genetic ancestry

The first cohort group ($n = 79$) came from an original cohort of 520 volunteers from an outpatient geriatric clinic from Federal University of Minas Gerais, for whom genomic DNA was available in a genetic bank. All individuals had genetic ancestry determined by typing DNA for a panel of 40 validated ancestry-informative biallelic short insertion/deletion DNA polymorphisms (*InDels*) [19]. Amplicons were sized using an ABI3130 DNA Sequencer (Applied Biosystems) and analyzed using the GeneMapper Software version 3.7. As a population clustering algorithm, we used the *Structure* program version 2.3, available at <http://pritchh.bsd.uchicago.edu/structure.html> to infer the structure of each population [20] and allocate individuals to the three different ethnic groups: Africans, Europeans and Amerindians. The selection criteria were an African proportion above 0.45. For a highly miscegenous population such as Brazilian, an ancestry proportion of 0.45 was considered high enough to characterize ethnicity.

Sequencing of the DPYD–Variant Y186C

Genomic DNA of the patients was isolated from blood samples according to a proteinase K-based standard protocol, using the high salt method. After DNA isolation, genotyping

through Sanger sequencing analysis was carried out. Exons 6 of *DPYD* were amplified by PCR with primers specific for the region comprising the variant Y186C. No other *DPYD* variant was tested in this study. Primers used were as follows: Forward: 5'- ACTGAAAATGTACTGCTCATTGCT -3'; Reverse: 5'- TGCTCCATCATTTCTGACACT -3'.

For PCR reactions, 2 µL of DNA at 30 ng/µL were mixed with 2.5 µL of 10X IIB Buffer (40 mM NaCl; 10 mM Tris HCl pH 8.4; 0.1% Triton X-100; 1.5 mM $MgCl_2$), 2.5 µL of 0.2-mM dNTP, 0.5 µL of each primer at 10 pmol/µL and 0.25 µL of Taq polymerase (Invitrogen, Brazil) 0.625 U, on a final volume of 25 µL. Samples were placed on a Eppendorf Mastercycler (Hamburg, Germany) at 94 °C for 3 min and then 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s and a final extension time at 72 °C for 5 min. PCR products were purified using Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, São Paulo, Brazil) following manufacturer's protocol and visualized on a silver-stained 6.5% polyacrylamide gel. Sequences were obtained on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Bidirectional sequence data were analyzed using Sequencher 4.9 software.

Results

For the first cohort, among 79 volunteers with genetically defined African ancestry from Southeast Brazil, the mean African proportion was 0.7 (0.45–0.98). The variant Y186C was found in heterozygosity in two men, one of them with 0.58 and the other 0.9 African proportion and the minor allele frequency (MAF) was 1.26%.

In the second cohort, among 36 self-reported African Brazilians from Northeast Brazil, the variant Y186C was found in heterozygosity in one woman, with a MAF of 1.39%. For the whole population ($n = 115$), the prevalence of Y186C was 2.6% and MAF was 1.30%.

Discussion

The genetic basis for FP poor catabolism in individuals of African ancestry has not been fully clarified. *DPYD*2A* and D949 V (canonical mutations in Caucasians) are present in only 0.1% of African Americans [8, 18] and 0.8% in Americans of African ancestry in the South West USA (http://grch37.ensembl.org/index.html, accessed 16th August 2019), respectively. In addition, the polymorphism Y186C is present in 2% (MAF) of African population [8], reaching 4% (MAF) in the subpopulations Mandinka in the Gambia and Yoruba in Ibadan, Nigeria. These subpopulations share similar genetic characteristics [21] and have their origins in the same geographic area, Western Africa, which was the main source of African diaspora in the first 150 years of Portuguese transatlantic slave trade, when most of them were sent to North America, Caribbean Islands and North/Northeast Brazil. It is estimated that African Americans have a mean West African ancestry of 50–77% [21, 22] which confers a high probability of finding Y186C in those populations. A study reports a high prevalence of around 6% in African Americans, estimating Y186C as the putative cause of at least one forth to one-third of cases of DPD deficiency in African Americans [12]. However, even inside USA, different African subpopulations show diverse prevalence of this variant. In a Mayo Clinic study with 588 healthy African Americans volunteers of Somali ($n = 548$) or Kenyan ($n = 40$) ancestry from the State of Minnesota, USA [18], Y186C was detected in 0.2% of Somali and 7.5% of Kenyans. The low frequency in Somali probably reflects their distinct ancestry from Western Africans [21].

Data regarding Y186C as well as other *DPYD* mutations associated with 5FU toxicity in South America are scarce. We have previously shown that the Uracil breath test has moderate accuracy in discriminating individuals who manifested severe toxicity from those who had mild or no toxicity to 5FU [23]. In this report, we extended our data, demonstrating the value of DPYD genotyping in the Brazilian population. To our knowledge, the only published report was carried out in 60 gastrointestinal cancer patients from Southern Brazil [24]. The variant Y186C was not found, but this region is mainly populated by European immigrants, and ethnicity of this cohort was not described.

Brazil was the destiny of 40% of Africans deported to Americas [25, 26]. Due to changes in African slave trade over 300 years, it has two African ancestry components: the first associated with Western Africans (more evident in the Northeast and similar to North America) and the other with progressively more representativity of Bantus of Central, South and East Africa (more represented in the Southeast/South). These historical data are in line with recent genetic proof for the diverse African ancestry in different areas of Brazil coming from the Epigen Project [27] which studied genomic DNA from 6487 Brazilians from Northeast, Southeast and South. They found among African Brazilians a proportion of Western/Eastern African ancestry of 3.00 in Northeast, 1.79 in Southeast and 1.30 in South Brazil, as compared to 4.85 for a control of African Americans. The different patterns of slave trade and the high miscegenation of Indians, Europeans and Africans make it difficult to infer the prevalence of Y186C mutation in Brazilian population, although it could be presumed that Y186C is found more frequently in African Americans, followed by African Brazilian from the Northeast, Southeast and South, respectively.

Interestingly, our study found similar proportions of Y186C between Southeast and Northeast populations. Some weakness is the low number of individuals from the Northeast and the

self-reported (but not genetically verified) African ancestry of this population although, according to Epigen Project [26, 27], in Bahia state the vast majority (97%) of the self-reported black individuals was at the highest quartile of African ancestry. The prevalence of this variant is less than that observed in African Americans population but is far from being negligible when studying *DPYD* deleterious variants.

DPD deficiency is not routinely tested in the Brazil. Phenotypic tests are not available outside research laboratories although genetic tests for *DPYD*2A*, D949 V and *DPYD*13* are available in the private health system. With over 200 million people, Brazil has the biggest African population outside Africa, and unlike North America, miscegenation is highly prevalent; about 47% of Brazilian people define themselves as of mixed race and 8% as black (https://www.ibge.gov.br/; accessed 12th August 2019). Taking this into account, a genetic test for DPD deficiency in Brazil should include Y186C, which is probably the first or second most prevalent deleterious *DPYD* variant in general population (assuming Haplotype B3 is present in 4% of Caucasians). This practice should be adopted in all countries of large African diaspora populations such as USA and some Latin American countries. In a global perspective, given the migration crisis, even Western European countries should think about testing other variants not frequent in non-Caucasian populations.

Conclusion

The variant Y186C is prevalent among Brazilians of African ancestry and this finding is in line with previous reports in African and African American populations. This mutation should be taken in account in targeted genotyping for fluoropyrimidine risk variants especially in American continent and Africa but even in Western European countries with predominant Caucasian ethnicity, given the current migrant crisis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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