A *DPYD* Variant (Y186C) in Individuals of African Ancestry Is Associated With Reduced DPD Enzyme Activity

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5-Fluorouracil (5-FU) is a component of the chemotherapy regimen used for advanced colorectal cancer and is also used for other cancers like breast, head and neck, and skin. Adjuvant therapy with 5-FU and leucovorin has been shown to significantly prolong overall survival as well as progression-free survival after colon cancer resection. The *DPYD*-Y186C variant was unique to individuals of African ancestry, and DPD activity was 46% lower in carriers as compared with noncarriers (279 ± 35 vs. 514 ± 168 pmol 5-FU min⁻¹ mg⁻¹; *P* = 0.00029). In this study, 26% of the African Americans with reduced DPD activity were carriers of Y186C. In the African-American cohort, after excluding Y186C carriers, homozygous carriers of C29R showed 27% higher DPD activity as compared with noncarriers (609 ± 152 and 480 ± 152 pmol 5-FU min⁻¹ mg⁻¹, respectively; *P* = 0.013).

5-Fluorouracil (5-FU) is used to treat many aggressive cancers, such as those of the colon, breast, and head and neck. The responses to 5-FU, with respect to both toxicity and efficacy, vary among racial groups, potentially because of variability in the activity levels of the enzyme dihydropyrimidine dehydrogenase (DPD, encoded by the *DPYD* gene). In this study, the genetic associations between *DPYD* variations and circulating mononuclear-cell DPD enzyme activity were evaluated in 94 African-American and 81 European-American volunteers. The *DPYD*-Y186C variant was unique to individuals of African ancestry, and DPD activity was 46% lower in carriers as compared with noncarriers (279 ± 35 vs. 514 ± 168 pmol 5-FU min⁻¹ mg⁻¹; *P* = 0.00029). In this study, 26% of the African Americans with reduced DPD activity were carriers of Y186C. In the African-American cohort, after excluding Y186C carriers, homozygous carriers of C29R showed 27% higher DPD activity as compared with noncarriers (609 ± 152 and 480 ± 152 pmol 5-FU min⁻¹ mg⁻¹, respectively; *P* = 0.013).

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However, a breast cancer study investigating the use of 5-FU as a component of neoadjuvant and adjuvant therapies showed that Asian patients experienced higher rates of hematologic toxicity (ranging from severe to life-threatening) but lower rates of nonhematologic toxicity, as compared with Caucasian, African-American, and Hispanic patients.16

Given the pivotal role of DPD in determining 5-FU pharmacokinetics, we hypothesized that ethnicity-related differences in DPD enzyme activity resulting from differences in DPYD genotype contribute to the observed variability in responses to 5-FU. Previous studies by our laboratory showed that DPD activity in peripheral blood mononuclear cells was significantly lower (by 12%) in the African-American population as compared with the European-American population.17 The goal of the current study was to identify the specific genetic variants in DPYD that may explain the variable responses to 5-FU. We identified a nonsynonymous DPYD variant (Y186C) that was present in the African-American population but not in the European-American group. DPD enzyme activity was significantly lower in individuals carrying this allele. Within the African-American study group, 26% of individuals with reduced DPD activity carried the variation, suggesting that it may be a risk allele for 5-FU toxicity in individuals of African ancestry.

RESULTS
Study population characteristics
The subjects in this study were a subset of a population that had previously been used to characterize DPD enzyme activity in an African-American population relative to a European-American population.17 A total of 94 African-American and 81 European-American volunteers participated in this study. It was ensured that all the subjects were free of medical conditions that may have interfered with DPD enzyme activity in circulating blood cells, as detailed in the Methods section. Women were represented in greater numbers, comprising 74% of the African-American subjects and 63% of the European-American subjects. The mean ages of the African-American and European-American populations were similar (31.9 and 32.2 years, respectively). The mean ages of female participants and male participants were also similar within both populations. Population data are summarized in Table 1.

Using the new method of calculating DPD activity described in the Methods section of this article, the mean enzyme activity in African-American volunteers was 499 pmol 5-FU min−1 mg−1, 10% lower than the average in European-American volunteers, 553 pmol 5-FU min−1 mg−1 ($P = 0.012$, Supplementary Figure S1a online and Table 1). The ethnicity-related difference in enzyme activity was more pronounced in women (P = 0.011, Supplementary Figure S1b online) than in men (P = 0.54, Supplementary Figure S1c online). Overall, DPD activity tended to be lower in women than in men, although the difference was not statistically significant (P = 0.25, Supplementary Figure S1d–f online). No correlation between age and DPD activity was noted in either population (Supplementary Figure S2a,b online). The results noted in this study are similar to the reported characterization of DPD activity in the earlier study with a larger study population.17

Genotyping of the DPYD gene
Genotyping was performed by targeted resequencing of the 23 exons encoding DPYD. A summary of the single-nucleotide polymorphisms (SNPs) detected, including RefSeq (rs) numbers and HGVS names, is presented in Supplementary Table S1 online. In this article, coding region variants are referred to in terms of the amino acid change they encode, and noncoding variants by location within the intron relative to the nearest exon. Of the 30 variants detected, 16 were located within exons (11 nonsynonymous and 5 synonymous), and 14 were in introns. Six SNPs were detected only in the African-American population (IVS2–69, S175S, Y186C, IVS8–31, P453R, and N457N), and three variants were unique to the European-American population (I560S, N635N, and D949Y; Table 2). The rare variant most commonly reported in DPD deficiency, DPYD*2A (rs3918290, c.1905+1G>A, also known as IVS14+1G>A), was not detected in any of the study participants (Table 2). The allele frequencies of all SNPs except IVS11–106 were in Hardy–Weinberg equilibrium (Supplementary Table S1 online). In this study, the allele frequencies of detected variants were similar to those reported in the 1000 Genomes Project18–20 and the Exome Sequencing Project21 (Supplementary Table S2 online).

Genetic variants associated with altered DPD activity
In an effort to identify variations that perturb enzyme function, SNPs were tested for quantitative trait association with DPD activity (Table 2). In addition, DPD enzyme activity was summarized

| Table 1 Study population demographics |
|--------------------------------------|
|                                      |
| Total      | African-American | European-American |
| Sample size | 94               | 81                  |
| Mean age (years) ± SD | 31.9 ± 9.1      | 32.2 ± 10.6         |
| Mean DPD activitya ± SD | 499 ± 173       | 553 ± 136           |
| Range      | 118–986          | 213–809             |

5-FU, 5-fluouracil; DPD, dihydropyrimidine dehydrogenase; F, female; M, male.
6-FU catabolism (pmol min−1 mg−1).
by single-marker genotype for each SNP. The complete data for all SNPs are presented in Supplementary Table S3 online; the data relating to associated variants are summarized in Figure 1. The strongest association was noted for the I560S variant with respect to the European-American cohort (\(P = 0.00057\), false-discovery rate \(P = 0.014\); Table 2). The I560S variant was detected as a heterozygote in a single subject; this subject had the lowest enzyme activity in the European-American cohort (Figure 1a).

In the African-American population, five SNPs showed significant single-marker associations (IVS2-69, Y186C, I543V, IVS15+75, and V732I; Table 2). Of these, Y186C was most strongly associated with altered enzyme activity (\(P = 0.00096\), false-discovery rate \(P = 0.026\), Table 2). The 6.4% of African-Americans who were carriers of Y186C had significantly lower DPD enzyme activity as compared with noncarriers (\(P = 0.00029\); Figure 1b). The average DPD enzyme activity in Y186C carriers was 279 pmol 5-FU min\(^{-1}\) mg\(^{-1}\), which is 46% lower than the average for noncarriers (514 pmol 5-FU min\(^{-1}\) mg\(^{-1}\)). The Y186C variant was not detected in any of the European Americans in this study.

### Table 2 Association between \textit{DPYD} allelic variants and DPD enzyme activity in the volunteer populations

| Amino acid | Ref allele/ | Allele counts | \(P^a\) | FDR\(^b\) | Allele counts | \(P^a\) | FDR\(^b\) |
|------------|-------------|---------------|-------|-------|---------------|-------|-------|
| change     | var allele  | (ref/var)     |       |       | (ref/var)     |       |       |
| C29R       | T/C         | 110/70        | 0.38  | 0.68  | 115/43        | 0.90  | 0.97  |
| IVS2-69    | G/A         | 184/4         | 0.096 | 0.083 | 162/0         | —     | —     |
| IVS5+18    | G/A         | 183/1         | 0.098 | 0.29  | 158/4         | 0.33  | 0.67  |
| M160V      | A/G         | 176/6         | 0.76  | 0.86  | 147/15        | 0.15  | 0.59  |
| S175S      | G/A         | 183/1         | 0.41  | 0.68  | 162/0         | —     | —     |
| Y186C      | A/G         | 182/6         | 0.00096\(^c\) | 0.026 | 162/0         | —     | —     |
| IVS6-8     | A/G         | 170/10        | 0.62  | 0.80  | 161/1         | 0.81  | 0.97  |
| IVS7-118   | A/G         | 166/16        | 0.051 | 0.23  | 145/17        | 0.32  | 0.67  |
| IVS8+41    | T/C         | 169/15        | 0.53  | 0.75  | 159/1         | 0.82  | 0.97  |
| IVS8-31    | C/T         | 175/13        | 0.98  | 0.98  | 162/0         | —     | —     |
| IVS9+36    | A/G         | 184/4         | 0.39  | 0.68  | 161/1         | 0.21  | 0.67  |
| IVS9+134   | T/G         | 153/33        | 0.067 | 0.26  | 140/16        | 0.15  | 0.59  |
| IVS10-15   | T/C         | 180/8         | 0.46  | 0.68  | 145/17        | 0.14  | 0.59  |
| M406I      | G/A         | 171/11        | 0.74  | 0.86  | 155/1         | 0.83  | 0.97  |
| E412E      | G/A         | 183/1         | 0.096 | 0.29  | 157/3         | 0.41  | 0.76  |
| IVS11-106  | T/A         | 186/2         | 0.15  | 0.36  | 141/21        | 0.90  | 0.97  |
| P453R      | C/G         | 183/1         | 0.56  | 0.76  | 162/0         | —     | —     |
| N457N      | C/T         | 178/4         | 0.21  | 0.47  | 162/0         | —     | —     |
| S534N      | G/A         | 182/2         | 0.13  | 0.35  | 161/1         | 0.78  | 0.97  |
| I543V      | A/G         | 162/22        | 0.048 | 0.23  | 139/23        | 0.97  | 0.97  |
| I560S      | T/G         | 186/0         | —     | —     | 157/1         | 0.00057\(^c\) | 0.014 |
| IVS13+39   | C/T         | 174/14        | 0.43  | 0.68  | 143/19        | 0.78  | 0.97  |
| IVS13+40   | A/G         | 74/114        | 0.96  | 0.98  | 74/88         | 0.34  | 0.67  |
| F632F      | T/C         | 177/11        | 0.45  | 0.68  | 154/8         | 0.64  | 0.97  |
| N635N      | C/T         | 188/0         | —     | —     | 161/1         | 0.30  | 0.67  |
| *2A        | G/A         | 184/0         | —     | —     | 162/0         | —     | —     |
| IVS15+75   | A/G         | 165/23        | 0.027 | 0.23  | 126/36        | 0.11  | 0.59  |
| V732I      | G/A         | 182/6         | 0.043 | 0.23  | 153/9         | 0.34  | 0.67  |
| IVS18-39   | G/A         | 176/6         | 0.77  | 0.86  | 146/16        | 0.94  | 0.97  |
| D949V      | A/T         | 184/0         | —     | —     | 161/1         | 0.69  | 0.97  |
| P1023T     | C/A         | 180/8         | 0.89  | 0.96  | 161/0         | 0.06  | 0.59  |

DPD, dihydropyrimidine dehydrogenase; FDR, false-discovery rate; ref, reference; var, variant.

\(^a\)Asymptotic \(P\) value calculated by Wald test. \(^b\)Step-up Benjamini and Hochberg (1995) false-discovery rate. \(^c\)Significant \(P\) value after correcting for multiple testing (Bonferroni method; threshold for significance: \(P = 0.0016\)).
The nonsynonymous SNPs, V732I and I543V, showed weak evidence of association with reduced DPD activity in the African-American cohort \((P = 0.043\) and \(P = 0.048;\) Table 2). The six heterozygous carriers of V732I showed a 29% lower DPD activity than noncarriers \((P = 0.049;\) Figure 1c). The European-American population also had eight carriers of V732I, seven heterozygous and one homozygous (Supplementary Table S3 online). No significant difference in activity was noted in European Americans who carried this variant (Figure 1c). African-American carriers of I543V, both heterozygous and homozygous, tended to have DPD activity that was lower than those of noncarriers; however, these differences were not significant \((P = 0.11\) and \(P = 0.19,\) respectively; Figure 1d). Within the African-American population, heterozygous carriers of the intronic SNPs, IVS2-69 and IVS15+75, also had lower enzyme activity \((P = 0.0030\) and \(P = 0.040;\) Figure 1e,f).

The additive effects of the two most strongly associated single variants, Y186C and I560S, and the covariates age, race, and gender, were assessed in the overall population, using a linear regression model (Supplementary Table S4 online). Overall, the model parameters predicted 16% of the variation observed in this study. Individually, Y186C and I560S predicted 8.5 and 4.3%, respectively, of the observed variation \((P = 0.00027\) and \(P = 0.0017,\) respectively). The covariates were not shown to make any significant contribution to the observed variance.

**Potential effect of C29R on DPD activity**

Because Y186C (which showed strong association with lower levels of DPD activity) was present at a relatively high frequency in the African-American cohort, the analyses were repeated in data from a subpopulation from which carriers of Y186C had been removed. A summary of DPD enzyme activity associated with single SNP genotype in this restricted cohort is presented in Supplementary Table S5 online. In the restricted cohort, significant differences in DPD activity were noted for a single SNP, C29R. In the full African-American cohort, DPD activity was higher in heterozygous and homozygous carriers of C29R but did not reach a significant level (Figure 2, left). In the restricted cohort, those who were homozygous for C29R showed 27% higher DPD enzyme activity than those who did not carry the SNP \((P = 0.013;\) Figure 2, right). No additional significant differences in enzyme activity between single-marker genotype groups were observed in the restricted cohort (Supplementary Table S5 online).

**Linkage disequilibrium with Y186C in the African-American cohort**

We noted that carriers of Y186C often carried additional DPYD variants. This finding prompted us to evaluate the contribution of linkage disequilibrium (LD) to the observed results. Ranking of volunteers by enzyme activity revealed that many individuals with low DPD activity carried more than one associated
SNPs associated with reduced DPD enzyme activity. DPD, dihydropyrimidine dehydrogenase; LD, linkage disequilibrium; SNP, single-nucleotide polymorphism; wt, wild-type.

Figure 2 Boxplot histograms show the distribution of DPD enzyme activity by C29R genotype in the African-American (AA) population as a whole (left) and in the African-American population who are noncarriers of Y186C (right). Significantly elevated DPD enzyme activity is noted for homozygous carriers of the C29R SNP variant (CC genotype) when data relating to individuals carrying Y186C are excluded from the analysis. Boxplots present population means (filled dots), medians (dark black lines), 25–75% confidence intervals (boxes), 5–95% confidence intervals (dashed lines and whiskers), and outliers (open dots). P values are presented for significant differences (P < 0.05) in enzyme activity within a population, and are not shown if P ≥ 0.05.

DPD, dihydropyrimidine dehydrogenase; SNP, single-nucleotide polymorphism; wt, wild-type.

Figure 3 The degree of LD among the DPYD SNPs in the African-American population is presented. A D' value of 1 indicates that no recombination was observed between the markers tested. Logarithm of odds (LOD) scores ≥2 suggest that SNPs are inherited together. A high degree of LD between SNPs (D' = 1, LOD ≥ 2) is indicated in red, and the lowest degree of LD (D' < 1, LOD < 2) is indicated in white. Recombination rates <1 are presented as a percentage. The LD block that includes Y186C is outlined in yellow. Asterisks are used to highlight the LD between Y186C and other SNPs (IVS2-69, IVS2-69, IVS15+75, and V732I; Supplementary Table S5 online).

DISCUSSION

In a study of healthy volunteers, we show that carriers of the DPYD variations Y186C and I560S have significantly diminished DPD enzyme activity as compared with noncarriers. In this study, 6 of the 94 African Americans were heterozygous for Y186C, whereas no European Americans were heterozygous for Y186C, whereas no European Americans carried the variation.
Within the African-American population, the enzyme activity in Y186C carriers was approximately half (54%) of that in non-carriers, suggesting that the amino acid change may negatively impact protein function. Carriers of Y186C represented 26% of individuals (6 of 23) with the lowest quartile of DPD activity in the African-American population. This SNP has been mentioned in only one other report, wherein our laboratory detected the variant in a single partially DPD-deficient African American. Y186C is a recent addition to the public SNP databases and was not genotyped as part of the HapMap project. As such, the SNP was probably not evaluated in many previous pharmacogenetic studies of 5-FU toxicity. Two additional nonsynonymous variants, I543V and V732I, showed weak associations with reduced levels of DPD enzyme activity in African Americans. Of these, V732I was shown to be in LD with Y186C, and exclusion of data from Y186C carriers from the analysis produced insignificant P values for I543V and V732I. These results support earlier conclusions that I543V and V732I are benign polymorphisms that do not contribute significantly to 5-FU toxicity.

A comparison of genotype frequencies in publicly available data from the 1000 Genomes Project showed that Y186C was most prevalent in the African super-population (Supplementary Figure S3 online). The YRI (Yoruba in Ibadan, Nigeria) population had the highest percentage of carriers of this SNP, 9.1%. Within the ASW (Africans of African Ancestry in southwest USA) and LWK (Luhya in Webuye, Kenya) populations, 1.6 and 2.1% of individuals, respectively, were heterozygous for the SNP. Apart from African populations, the SNP was detected in only a single individual from the PUR (Puerto Ricans from Puerto Rico) population. Y186C was not detected in any of the European (CEU, Utah Residents with Northern and Western European ancestry; FIN, Finnish in Finland; GBR, British in England and Scotland; IBS, Iberian population in Spain; or TSI, Toscani in Italia), Asian (CHB, Han Chinese in Beijing, China; CHS, Southern Han Chinese; or JPT, Japanese in Tokyo, Japan), or other two American (CLM, Colombians from Medellin; or MXL, Mexican Ancestry from Los Angeles, USA) populations. No homozygous carriers of Y186C have been reported to date.

The *2A variant is generally considered to be the most prevalent DPYD allele associated with DPD deficiency, although the penetrance of the variant is very low. In the overall 1000 Genomes Project data set, 0.55% of individuals are heterozygous carriers of *2A. In comparison, 1.1% of the overall 1000 Genomes population is heterozygous for Y186C. The *2A variant is detected most frequently in the FIN population, 3.2% of whom are heterozygous for the SNP. The variant Y186C was detected as a heterozygote at a greater frequency in our study population (6.4%), and within the YRI population of the 1000 Genomes data set (9.1%). In addition, with the exception of a single heterozygote in the ASW population, *2A is absent from the African super-population. The only DPYD allele showing an association with DPD deficiency in the European-American population of our study, I560S, is also widely considered to be a rare variant. The variant I560S was detected in a single FIN sample within the 1000 Genomes data set (0.092% of the overall population).

A comparison of DPD activity in the cohort of African-American individuals who did not carry Y186C revealed that homozygous carriers of C29R had higher enzyme activity than individuals who had wild-type C29R. The presence of C29R was earlier identified in DPD-deficient patients as a compound heterozygous SNP with R886H (rs1801267) and *2A. The recombinant DPD protein harboring the C29R amino acid substitution expressed in Escherichia coli lacked detectible enzyme activity. However, numerous subsequent clinical studies have failed to show any association between C29R and DPD deficiency. Other studies have suggested that C29R may be protective against 5-FU toxicity. A study by Seck et al. showed that carriers of a haplotype containing C29R, but no additional detected variants, had elevated DPD enzyme activity as compared with the mean of the entire population studied. A subsequent study showed that carriers of C29R (combined heterozygous and homozygous) had a twofold lower risk than noncarriers for developing serious gastrointestinal toxicity after 5-FU treatment. Recently our lab reported a functional characterization of the C29R variant, using a mammalian system of expression. The enzyme activity of recombinantly expressed DPD containing the C29R substitution was significantly higher (13%) than that of the wild-type.

Functionally, the Y186C amino acid change may lead to the observed phenotype by affecting DPD dimer formation. Tyrosine contains a bulky aromatic side chain, whereas the side chain of cysteine occupies a much smaller volume. Amino acid 186 is located near the surface of the protein, and biochemical studies of tyrosine-to-cysteine mutations in another protein have suggested that the amino acid substitution may cause aberrant dimer cross-linking. Individuals with Y186C were also noted to carry additional nonsynonymous DPYD variants. The evidence that these amino acid changes contribute to DPD deficiency on their own is limited; however, one cannot rule out the possibility that multiple (possibly benign) amino acid changes can exert an additive effect, resulting in impaired enzyme function.

Ethnicity-related differences in DPD activity have earlier been suggested as evidence that genetics has a key role in determining a person’s sensitivity to 5-FU; however, the information on the contribution of population-specific SNPs to DPD deficiency is limited. Hepatic DPD activity was shown to be 13% lower in African Americans than in European Americans, although the difference was not statistically significant. A study of breast cancer patients suggested that the enzymatic activity of DPD was similar in African-American and European-American populations; however, only a limited number of healthy controls were tested in that study. A subsequent study showed that DPD activity was similar for British Caucasian, Southwest Asian, and Kenyan populations but significantly lower in the Ghanaian population. In our study, DPD enzyme activity was 10% lower in African Americans than in European Americans. Taken together, these reports suggest that western African and African-American populations may have lower DPD activity as compared with Asian, European, and eastern African populations. Lower DPD
activity alone is not likely to be the sole cause for the discrepancies in toxicity and response to 5-FU among racial groups. Later tumor stage at diagnosis, presence of comorbid disease, and ethnicity-related polymorphisms in genes such as p53 and 5,10-methylene tetrahydrofolate reductase have been suggested to be potential contributors to variable outcomes and toxicities after 5-FU treatment.12

In summary, DPD enzyme activity is a biologically important predictor of 5-FU toxicity.39 DPD deficiency is not a monoallelic condition in which a single variation is responsible for the majority of enzyme deficiencies but rather one in which multiple variants produce a range of enzyme activities. As a further complication in interpreting the role of genetic variants, we observed that many individuals often carry more than one SNP within the DPDYD gene. In light of our finding that Y186C is significantly associated with decreased DPD activity, testing for this SNP before initiating 5-FU therapy may potentially avoid severe toxicity in populations of patients who have a higher probability of carrying the allele. Overall, these findings further support the need to individualize anticancer therapies through the use of genetic markers before starting therapy.

METHODS

Study populations. The sample population in this study consisted of individuals who had already been characterized for DPD activity.17 The DPDYD coding region was sequenced in individuals for whom DNA was available. The population comprised 94 of 149 African Americans and 81 of 109 European Americans from the original study.17 Participants were asked to provide their age, gender, and primary ethnicity. In this article, the term “African-American” indicates that the individual self-reported his/her ethnicity as primarily “African-American/black”. “European-American” indicates that the individual self-reported his/her ethnicity as primarily “European-American/Caucasian/white (non-Hispanic).” Individuals were excluded from the study if they had respiratory, gastric, or metabolic diseases; if they had previously been diagnosed with cancer; or if they were receiving prescription medication that may have affected white blood cell counts. Study participants were recruited from student and staff populations whose samples had been collected at the University Hospital of the University of Alabama at Birmingham. This study was approved by the institutional review boards of the University of Alabama at Birmingham (IRB no. F020610007 and X000830002) and Mayo Clinic (IRB no. 09-007080).

Collection of protein lysates from circulating cells. To minimize variations from known circadian expression patterns of DPD,40 60 ml of whole blood was collected at ~12:00 pm just before the midday meal. Peripheral blood mononuclear cells were isolated from whole blood, suspended in buffer A (35 mmol/l potassium phosphate, 2.5 mmol/l magnesium chloride, and 10 mmol/l 2-mercaptoethanol; pH 7.4) and lysed by sonication as previously reported.41 Lysates were cleared by centrifugation followed by filtration through 0.2 μm polyvinylidene difluoride membrane.

DPD activity was calculated using a modification of a previously reported method,41 representing an improved means of calculating enzyme activity. Conversion of [6-C14]-FU to [6-C14]-5-dihydroflourouracil ([6-C14]-5-DHFU) was determined using two reverse-phase C18 HPLC columns (Grace, Columbia, MD) connected in series to an Agilent/ Hewlett-Packard 1050 HPLC and Radiomatic FLO-ONE Beta flow scintillation analyzer (Agilent, Santa Clara, CA). For each time point of aliquot collection, the percentage conversion of 5-FU to 5-DHFU was determined as ([6-C14]-5-DHFU)/([6-C14]-5-FU + [6-C14]-5-DHFU). The calculation of picomoles of 5-FU per mg protein was made by multiplying the percentage conversion by the input amount of 5-FU per reaction. These data were plotted relative to the time points at which the aliquots were removed from the reaction. The rate of conversion (pmol 5-FU min−1 mg−1) was determined by linear regression of the plotted data.

Sequencing of the DPDYD coding regions to identify genetic variants. All 23 exons of DPDYD (NM_000110.3) were PCR-amplified using primers and reaction conditions as previously reported.42 PCR products were sequenced using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Grand Island, NY) on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Sequence analysis was performed using GeneScan and Genotyper software (Applied Biosystems) and Sequencher (Gene Codes, Ann Arbor, MI). NM_000110.3 was used as the reference sequence of DPDYD. The full genomic sequence for the region encoding DPDYD was retrieved from NC_000001.10. All positions are reported relative to the CRCh37.p10 primary references assembly of the human genome. Variations within the exon coding regions are reported in this article in terms of the amino acid change for which they encode. Intronic variations are reported in terms of their position relative to the nearest exon.

Statistical analyses. The two-tailed exact Wilcoxon rank sum test was used to compare enzyme activities between sample groups. Asymptotic P values were calculated using the Wald test to assess allelic association with DPD enzyme activity (treated as a continuous, quantitative variable). Step-up false-discovery rate was used to correct for multiple-testing bias for each SNP. Exact P values for deviation from Hardy–Weinberg equilibrium were calculated as described.33 The R Environment for Statistical Computing version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria) was used to calculate Wilcoxon tests and for general data analyses. PLINK version 1.0722 was used to calculate Wald, false-discovery rate, Hardy–Weinberg equilibrium, and proxy P values. Multivariate analyses were performed using a general linear model, using PLINK and R.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

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AUTHOR CONTRIBUTIONS

S.M.O., L.K.M., and R.B.D. designed the research. S.M.O. and R.B.D. wrote the manuscript. S.M.O. A.M.L., L.K.M., C.F., and N.J.W. performed the research. S.M.O. analyzed the data.

CONFLICT OF INTEREST

The authors declared no conflict of interest.
Study Highlights

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

- Only a few rare DPYD variants have actually been shown to be linked to reduced DPD function and 5-FU toxicity, the chief among these being the *2A/IVS14+1G>A* variant. Ethnicity-related differences in 5-FU response have been reported; however, the genetic factors contributing to these differences have not been identified.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

- This study examined DPD enzyme activity in African-American and European-American cohorts to identify changes in the coding regions of DPYD that could contribute to reduced DPD enzyme activity.

**WHAT THIS STUDY ADDS TO OUR KNOWLEDGE**

- The Y186C variant was found to be significantly associated with reduced DPD activity in the African-American population. Twenty-six percent of African Americans with low DPD activity carried the variant.

**HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS**

- Current predictive tests for 5-FU sensitivity were developed using data largely from populations of European descent. The data from our study provide a genetic marker that may be helpful in predicting 5-FU toxicity in individuals of African ancestry. The results of this study will help to facilitate the individualization of 5-FU therapy for individuals of African descent.

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