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A *DPYD* variant (Y186C) in individuals of African ancestry associated with reduced DPD enzyme activity

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Abstract

5-fluorouracil (5-FU) is used to treat many aggressive cancers, such as those of the colon, breast, and head & neck. The responses to 5-FU, both toxicity and efficacy, vary between racial groups, potentially due to variability in enzyme activity of dihydropyrimidine dehydrogenase (DPD, encoded by *DPYD*). In the present 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% reduced in carriers compared to non-carriers (279±35 compared to 514±168 pmol 5-FU min^{-1} mg^{-1}; *P*=0.00029). 26% of the African Americans with reduced DPD activity in this study carried Y186C. In the African American cohort, following exclusion of Y186C carriers, homozygous carriers of C29R showed 27% higher DPD activity compared to non-carriers (609±152 and 480±152 pmol 5-FU min^{-1} mg^{-1}, respectively; *P*=0.013).

Keywords

dihydropyrimidine dehydrogenase; 5-fluorouracil; pharmacogenetics

INTRODUCTION

5-Fluorouracil (5-FU) is an important component of the chemotherapy regimen used for the management of advanced colorectal cancer, and is widely used for the treatment of other cancers, such as those of the breast, head & neck, and skin. Adjuvant therapy with 5-FU and leucovorin has been shown to significantly prolong overall and progression free survival following colon cancer resection (1, 2). The addition of oxaliplatin was later shown to

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CONFLICT OF INTEREST/DISCLOSURE

The authors have no conflicts of interest to declare.

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.

provide additional benefit in advanced colorectal cancer (3–5). In certain parts of the world, such as Japan, 5-FU is also commonly used to treat lung cancer (6).

5-FU is converted to active metabolites by the enzymes of the uracil anabolic pathway. These metabolites block cell cycle progression by inhibiting thymidylate synthase and incorporating into RNA and DNA (7). Some degree of tumor targeting occurs as the effects are most pronounced in rapidly dividing cancer cells, however effects are felt system-wide, primarily in tissues with a high degree of cell turnover. These off-target effects can manifest as severe toxicities (hematological, cutaneous, mucosal, and digestive tract) or death in extreme cases (8). Whereas the uracil anabolic pathway is responsible for converting 5-FU to active metabolites, the opposing uracil catabolic pathway converts approximately 85% of administered 5-FU to inactive metabolites (9). Dihydropyrimidine dehydrogenase (DPD, encoded by the *DPYD* gene) is the initial and rate limiting enzyme of the catabolic pathway, and as such, deficiency of DPD, either due to disruptive variations of the *DPYD* gene or decreased expression, has been shown to be highly predictive of adverse toxicity (10, 11).

The contribution of genetic variations to 5-FU toxicity is further underscored by the variable responses to 5-FU-based treatments reported for different racial groups. Colon cancer patients of African ancestry have approximately 5% lower survival following surgery and treatment with 5-FU-containing adjuvant therapy relative to those of European ancestry (12). An additional study confirmed that African patients experienced a lower response rate and reduced overall survival following therapy, and suggested that adverse toxicity to therapy may vary between African and European American patients (13). In a large randomized phase 3 clinical trial in which patients received 5-FU in conjunction with leucovorin and/or levamisole, African Americans experienced a higher incidence of anemia and leukopenia while Caucasians were more likely to suffer from gastrointestinal toxicities like nausea, vomiting, stomatitis and diarrhea (14). The incidence of 5-FU toxicity was reported to be similar for Asians and Western populations (15), 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 hematological toxicity (severe to life-threatening), but lower rates of non-hematological toxicity, compared to Caucasian, African American, or Hispanic patients (16).

Given the pivotal role of DPD in determining 5-FU pharmacokinetics, we hypothesized that population differences in DPD enzyme activity due to differences in *DPYD* genotype contribute to the variable responses to 5-FU observed. Previous studies by our lab have shown that DPD activity in peripheral blood mononuclear cells (PBMC's) was significantly lower (12%) in the African American population compared to the European American population (17). The goal of the present study was to identify the specific genetic variants in *DPYD* that may explain the variable responses to 5-FU. We identified a nonsynonymous *DPYD* variation (Y186C) that was found only in the African American population, but not in the European American group. DPD enzyme activity was significantly reduced 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 with African ancestry.

RESULTS

Study population characteristics

Individuals used for this study were a subset of a population previously used to characterize DPD enzyme activity in an African American population relative to a European American population (17). Ninety-four African American and 81 European American volunteers participated in this study. All study 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 African American and 63% of European American participants. The mean ages for the African American and European American populations were similar (31.9 and 32.2 years, respectively). The mean ages for female and male participants were also similar within both populations. Population data is summarized in Table 1.

Using the new method of calculating DPD activity described in the methods section of this manuscript, the mean enzyme activity of African American volunteers was 499 pmol 5-FU min^{-1} mg^{-1}, 10% lower than the average of European American volunteers, 553 pmol 5-FU min^{-1} mg^{-1} (P=0.012, Supplementary Figure S1a and Table 1). The racial difference in enzyme activity was more pronounced in women (P=0.011, Supplementary Figure S1b) than in men (P=0.54, Supplementary Figure S1c). 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). No correlation between age and DPD activity was noted in either population (Supplementary Figure S2a-b). The results noted for this study are similar to the previously reported characterization of DPD activity in the 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 detected SNPs, including RefSeq (rs) numbers and HGVS names, is presented in Supplementary Table S1. In this manuscript, coding region variants are referred to by 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 were unique to the European American population (I560S, N635N, and D949V, 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). In this study, the allele frequencies of detected variants were similar to those reported for the 1000 Genomes Project (18–20) and the Exome Sequencing Project (21) (Supplementary Table S2).

Genetic variants associated with altered DPD activity

SNPs were tested for quantitative trait association with DPD activity in an effort to identify variations that perturb enzyme function (Table 2). Additionally, DPD enzyme activity was

summarized by single marker genotype for each SNP. Full data for all SNPs is presented in Supplementary Table S3; data is summarized for associated variants in Figure 1. The strongest association was noted for the I560S variant in the European American cohort (*P*=0.00057, FDR *P*=0.014; Table 2). I560S was detected as a heterozygote in a single individual, who had the lowest enzyme activity in the European American cohort (Figure 1a).

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

The nonsynonymous SNPs V732I and I543V showed weak evidence for 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% reduction in DPD activity compared to non-carriers (*P*=0.049; Figure 1c). The European American population also contained 8 carriers of V732I, 7 heterozygous and 1 homozygous (Supplementary Table S3). No significant difference in activity was noted in European Americans (Figure 1c). Heterozygous and homozygous African American carriers of I543V tended to have DPD activity that was lower than that of non-carriers, however the reductions were not significant (*P*=0.11 and *P*=0.19, respectively; Figure 1d). Heterozygous carriers of the intronic SNPs IVS2-69 and IVS15+75 also had reduced enzyme activity within the African American population (*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). Overall, the model parameters predicted 16% of the variation observed in this study. Individually, Y186C and I560S predicted 8.5% and 4.3% of the observed variation (*P*=0.00027 and *P*=0.0017, respectively). Covariates were not shown to significantly contribute to the observed variance.

Potential effect of C29R on DPD activity

Because Y186C, which showed strong association with decreased DPD activity, was present at a relatively high frequency in the African American cohort, analyses were repeated on a subpopulation in which carriers of Y186C were removed. A summary of DPD enzyme activity by single SNP genotype in this restricted cohort is presented in Supplementary Table S5. 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 not to a significant level (Figure 2, left). In the restricted cohort, volunteers that were homozygous for C29R showed 27% higher DPD enzyme activity compared to those that did not carry the SNP (*P*=0.013; Figure

2, right). No additional significant differences in enzyme activity between single marker genotype groups were noted for the restricted cohort (Supplementary Table S5).

Linkage disequilibrium with Y186C in the African American cohort

We noted that carriers of Y186C often carried additional *DPYD* variants, prompting 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 SNP, suggesting that some of the weak associations we observed could be due to LD with another associated allele. The degree of LD between variants in the African American population is presented in Figure 3. IVS2-69 and V732I showed evidence of co-transmission with Y186C (LOD=5.5 and LOD=2.2, respectively). No recombination was detected between Y186C and IVS2-69 (D′=1). Moderate recombination was noted between Y186C and V732I (D′=0.47). Little evidence for linkage to Y186C was noted for I543V or IVS15+75. There is limited evidence to suggest linkage between IVS2-69, I543V, IVS15+75, and V732I.

Given the evidence for linkage between Y186C and two weakly associated variants (V732I and IVS2-69), pairwise proxy associations were performed with Y186C using PLINK (22). None of the proxies yielded a more significant *P* value than Y186C alone, suggesting that the observed weak associations were due to linkage with Y186C (data not shown). Additionally, following removal of Y186C carriers from the AA cohort, significant differences in enzyme activity were not detected for any of the weakly associated SNPs (IVS2-69, I543V, IVS15+75, and V732I; Supplementary Table S5).

DISCUSSION

In a study of healthy volunteers, we show that carriers of the *DPYD* variations Y186C and I560S have significantly diminished DPD enzyme activity compared to non-carriers. In this study, 6 out of 94 African Americans were heterozygous for Y186C while no European Americans carried the variation. Within the African American population, the enzyme activity of Y186C carriers was approximately half (54%) of that of non-carriers, suggesting that the amino acid change may negatively impact protein function. Y186C carriers represented 26% of individuals (6 of 23) with the lowest quartile of DPD activity in African Americans. This SNP has only been mentioned in one other report, in which our lab detected the variant in a single partially DPD deficient African American (23). Previous reports from large pharmacogenetic studies of 5-FU toxicity did not likely include Y186C since the SNP was not genotyped as part of any of the three phases of the HapMap project (24) and is only a recent addition to public SNP databases. Two additional non-synonymous variants, I543V and V732I, showed weak associations with reduced DPD enzyme activity in African Americans. V732I was shown to be in LD with Y186C, and exclusion of Y186C carriers from analysis produced insignificant *P* values for either I543V or V732I. These results support previous conclusions that I543V and V732I are benign polymorphisms that do not contribute significantly to 5-FU toxicity (25–27).

A comparison of genotype frequencies in publically available data from the 1000 Genomes Project (18) showed that Y186C was most prevalent in the African super-population

(Supplementary Figure S3). The YRI (Yoruba in Ibadan, Nigera) population had the highest percentage of carriers of this SNP, 9.1%. Within the ASW (Americans of African Ancestry in southwest USA) and LWK (Luhya in Webuye, Kenya) populations, 1.6%, and 2.1% of individuals were heterozygous for the SNP, respectively. Outside of African populations, the SNP was only detected in a single individual from the PUR (Puerto Ricans from Puerto Rico) population. Y186C was not detected in any of the European (CEU, FIN, GBR, IBS, or TSI), Asian (CHB, CHS, or JPT), or other two American (CLM or MXL) 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 dataset, 0.55% of individuals are heterozygous carriers of *2A. For comparison, 1.1% of the overall 1000 Genomes population is heterozygous for Y186C. *2A is detected most frequently in the FIN (Finish in Finland) population, of which 3.2% are heterozygous for the SNP. Y186C was detected as a heterozygote at a greater frequency in our population (6.4%), and within the YRI population of the 1000 Genomes dataset (9.1%). Additionally, with the exception of a single heterozygote in the ASW population, *2A is absent from the African super-population. The only *DPYD* allele showing association with DPD deficiency in the European American population of our study, I560S, is widely considered a rare variant as well. I560S was detected in a single FIN sample within the 1000 Genomes dataset (0.092% of the overall population).

A comparison of DPD activity in the cohort of African American individuals that did not carry Y186C revealed that homozygous carriers of C29R had higher enzyme activity than volunteers who were wildtype for C29R. C29R was previously identified in DPD deficient patients as a compound heterozygous SNP with R886H (rs1801267) and *2A (28, 29). Recombinant DPD protein harboring the C29R amino acid substitution expressed in *E. coli* lacked detectible enzyme activity (29), however Numerous clinical studies have since failed to show association of C29R with DPD deficiency, e.g. (25–27). Additional 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 compared to the mean of the entire population studied (30). A subsequent study showed that carriers of C29R (combined heterozygous and homozygous) had a 2-times lower risk for developing serious gastrointestinal toxicity following 5-FU treatment compared to non-carriers (31). Recently our lab reported a functional characterization of the C29R variant using a mammalian system of expression (32). The enzyme activity of recombinantly expressed DPD containing the C29R substitution was significantly higher (13%) than wildtype.

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 (33), and biochemical studies of tyrosine to cysteine mutations in another protein have suggested that the amino acid substitution may cause aberrant dimer crosslinking (34). Individuals with Y186C were also noted to carry additional nonsynonymous *DPYD* variants. There is limited evidence that these amino acid changes

contribute to DPD deficiency on their own, however we cannot rule out the possibility that multiple (possibly benign) amino acid changes can exert an additive effect resulting in impaired enzyme function.

Racial differences in DPD activity have been previously suggested as evidence that genetics have a key role in determining a person's sensitivity to 5-FU, however there is limited information on the contribution of population-specific SNPs to DPD deficiency (35). Hepatic DPD activity was shown to be 13% lower in African Americans than European Americans, although the difference was not statistically significant (36). A study of breast cancer patients suggested that the enzymatic activity of DPD was similar in African American and European American populations, however only limited number of healthy controls were tested (37). A subsequent study showed that DPD activity was similar for British Caucasian, Southwest Asian, and Kenyan populations, but significantly reduced in the Ghanaian population (38). 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 reduced DPD activity relative to Asian, European, and eastern African populations. Reduced DPD activity alone is likely not the sole cause for the discrepancy in toxicity and response to 5-FU between racial groups. Later tumor stage at diagnosis, comorbid disease, and race-related polymorphisms in genes such as p53 and 5,10-methylenetetrahydrofolate reductase have been suggested to contribute to variable outcomes and toxicities following 5-FU treatments (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. Further complicating the interpretation of genetic variants, as we observed in this study, many individuals often carry more than one SNP within the *DPYD* gene. Based on our data showing that Y186C is significantly associated with decreased DPD activity, testing for this SNP prior to 5-FU therapy may potentially avoid severe toxicity in those populations of patients that are at high risk of carrying the allele. Overall, these findings further support the individualization of anti-cancer therapies utilizing genetic markers prior to therapy.

METHODS

Study populations

The sample population used in this study consisted of individuals who had been characterized for DPD activity (17). In the present study, the *DPYD* coding region was sequenced in individuals for whom DNA was available. The population in the present study included 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 manuscript, the designation "African American" indicates that the individual self-reported their ethnicity as primarily "African American / Black." "European American" indicates that the individual self-reported their 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 with samples 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# F020610007 and X000830002) and Mayo Clinic (IRB# 09-007080).

Collection of protein lysates from circulating cells

To minimize variations from known circadian expression pattern for DPD (40), 60 mL of whole blood was collected at approximately 12:00 pm prior to consumption of the mid-day meal. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood, suspended in buffer A (35 mM potassium phosphate, 2.5 mM magnesium chloride, and 10 mM 2-mercaptoethanol; pH 7.4) and lysed by sonication as previously reported (41). Lysates were cleared by centrifugation and total protein concentration determined by Bradford assay (Bio-Rad Protein Assay, Bio-Rad Laboratories Inc., Hercules, CA).

Measurement of DPD Enzyme Activity

Enzyme activity was measured using 500 μg of total protein in a reaction containing 200 μM NADPH, 8.2 μM [6-^{14}C] 5-FU (56 mCi/mmol), 35 mM potassium phosphate, 2.5 mM magnesium chloride, and 10 mM 2-mercaptoethanol; pH 7.4). Reactions were incubated for 30 minutes at 37°C with constant agitation. Aliquots were removed at 5 minute intervals and immediately transferred to reaction termination tubes containing an equivalent volume of ice-cold 100% ethanol. Protein was precipitated at −80°C for 16 hours and removed by centrifugation followed by filtration through 0.2 μm PVDF membrane.

In the present study, calculation of DPD activity was performed utilizing a modification of a method previously reported (41) that represents an improved means of calculating enzyme activity. Conversion of [6-C^{14}]-FU to [6-C^{14}]-5-Dihydrofluorouracil ([6-C^{14}]-5-DHFU) was determined using two reverse-phase C18 HPLC columns (Grace) connected in serial to a Hewlett-Packard 1050 equipped with a Radiomatic FLO-ONE Beta flow scintillation analyzer. For each time point collected, the percent conversion of 5-FU to 5-DHFU was determined as ([6-C^{14}]-5-DHFU) / ([6-C^{14}]-5-FU + [6-C^{14}]-5-DHFU). Picomoles of 5-FU per mg protein was determined by multiplying the percent conversion by the input amount of 5-FU per reaction. This data was plotted relative to the time at which the aliquot was 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 *DPYD* coding regions to identify genetic variants

All 23 exons of *DPYD* (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. 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 *DPYD*. The full genomic sequence for the region encoding DPYD 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 manuscript by the amino acid change for which they encode. Intronic variations are reported by 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 (FDR) was used to correct for multiple testing bias for each SNP. Exact *P* values for deviation from Hardy-Weinberg equilibrium (HWE) were calculated as described (43). 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 1.07 (22) was used to calculate Wald, FDR, HWE, and proxy *P* values. Multivariate analyses were performed using a general linear model using PLINK and R.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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STUDY HIGHLIGHTS

What is the current knowledge on the topic?

Only a few rare *DPYD*variants have actually been linked with reduced DPD function and 5-FU toxicity, primarily the *2A/IVS14+1G>A variant. Racial differences in 5-FU response have been reported, however the genetic factors contributing to these differences have not been identified.

What question this study addressed?

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 significantly associated with reduced DPD activity in the African American population. 26% 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 largely developed using populations of European descent. These data 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.

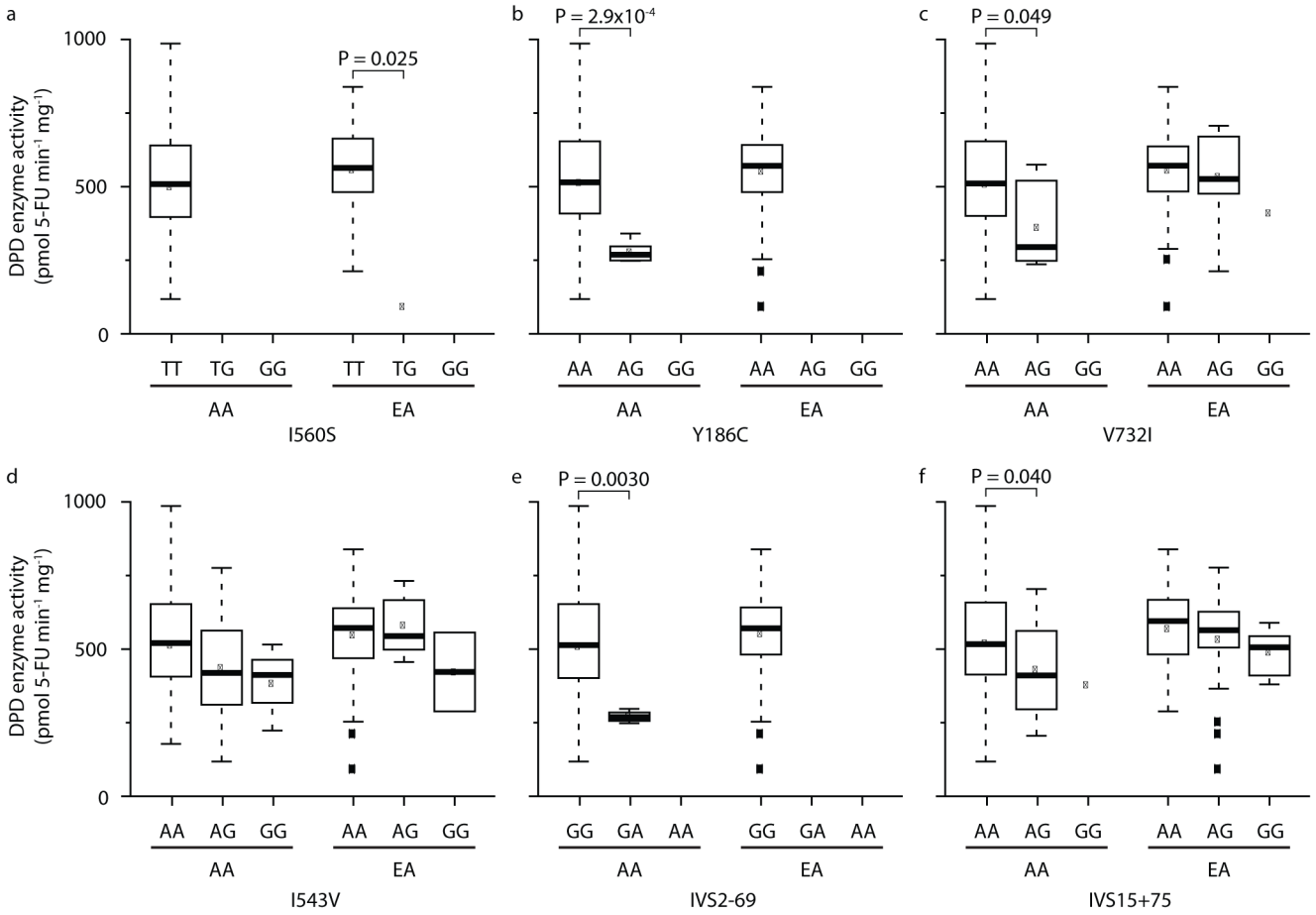

Figure 1.

DPD enzyme activity for carriers and non-carriers of I560S (**a**), Y186C (**b**), V732I (**c**), I543V (**d**), IVS2-69 (**e**), and IVS15+75 (**f**) is reported for African American (AA) and European American (EA) populations. Boxplots presenting 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) are presented. If a single volunteer carried a given genotype, the value is presented as a single filled dot. *P* values are presented for significant differences ($P<0.05$) in enzyme activity within a population, and are not shown if $P\geq0.05$. A full summary of enzyme activity by genotype is presented in Supplementary Table S3.

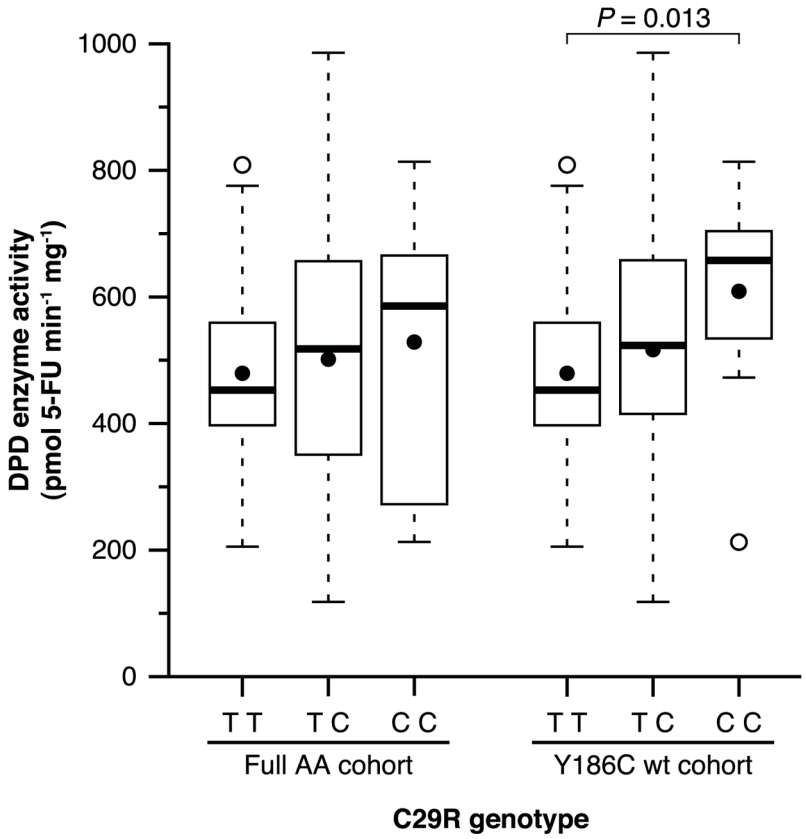

Figure 2.

Boxplot histograms showing 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 not carrying Y186C (right) are shown. Significantly elevated DPD enzyme activity is noted for homozygous carriers of the C29R SNP (CC genotype) when individuals carrying Y186C are excluded from analysis. Boxplots depict variables as detailed for Figure 1.

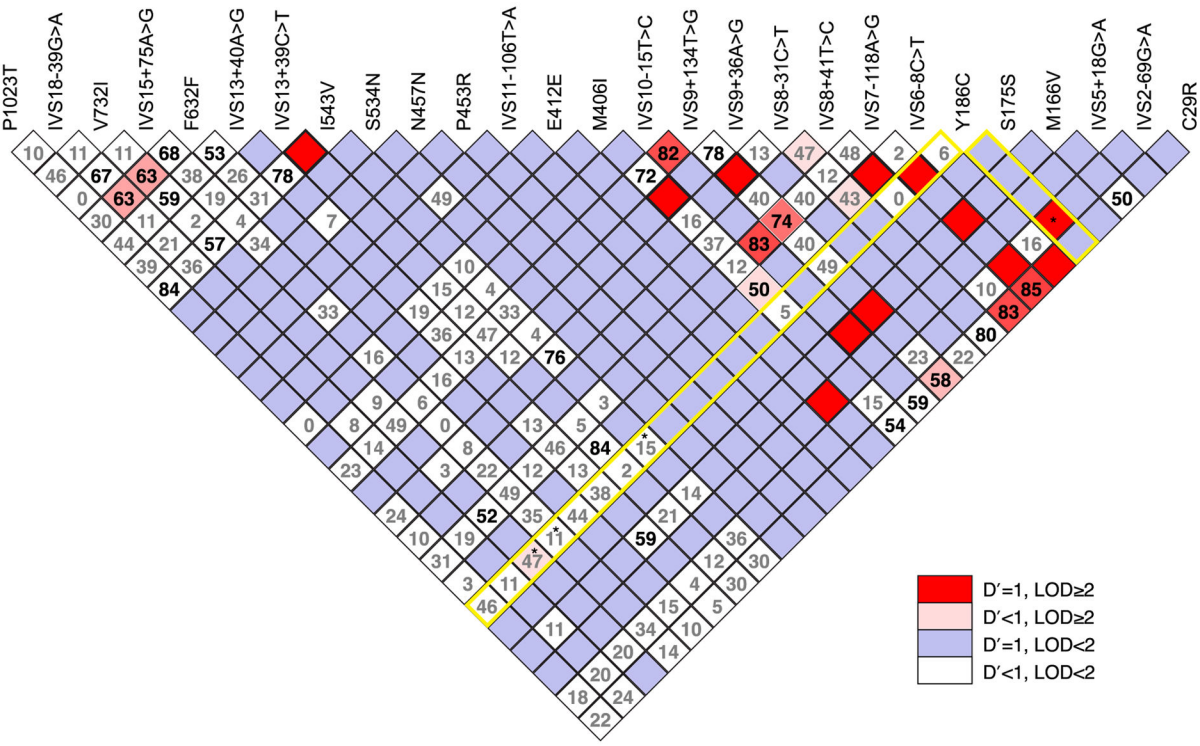

Figure 3.

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 greater than or equal to 2 suggests 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) 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 associated with reduced DPD enzyme activity.

Table 1

Study population demographics

	African American			European American		
	Total	**F**	**M**	**Total**	**F**	**M**
Sample Size	94	70	24	81	51	30
Mean Age (years)	31.9	30.5	35.8	32.2	32.0	32.6
±STDV	±9.1	±9.0	±8.4	±10.6	±10.6	±10.7
Mean DPD Activity *	499	492	521	553	551	555
±STDV	±173	±169	±184	±136	±124	±158
Range		118–986	213–809		213–757	93–839

*5-FU catabolism (pmol min^{-1} mg^{-1})

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Table 2

Allelic association between *DPYD* variants and DPD enzyme activity in the volunteer populations

		African American			European American		
Amino Acid Change	**Ref Allele / Var Allele**	**Allele Counts (Ref / Var)**	**P** *	**FDR** †	**Allele Counts (Ref / Var)**	**P** *	**FDR** †
C29R	T / C	110 / 70	0.38	0.68	115 / 43	0.90	0.97
IVS2-69	G / A	184 / 4	0.0062	0.083	162 / 0	--	--
IVS5+18	G / A	183 / 1	0.098	0.29	158 / 4	0.33	0.67
M166V	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 ‡	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 ‡	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

		African American			European American		
Amino Acid Change	**Ref Allele / Var Allele**	**Allele Counts (Ref / Var)**	**P** *	**FDR** †	**Allele Counts (Ref / Var)**	**P** *	**FDR** †
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 / 1	0.06	0.59

*Asymptotic P value calculated by Wald test.

†Step-up Benjamini and Hochberg (1995) false discovery rate.

‡Significant P value following correction for multiple testing (Bonferroni method, threshold for significance: P=0.0016).