Hematopoietic prostaglandin D synthase (HPGDS): a high stability, Val187Ile isoenzyme common among African Americans and its relationship to risk for colorectal cancer

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Abstract

Intestinal tumors in $Apc^{Min/+}$ mice are suppressed by over-production of HPGDS, which is a glutathione transferase that forms prostaglandin D_2 (PGD_2). We characterized naturally occurring HPGDS isoenzymes, to see if HPGDS variation is associated with human colorectal cancer risk. We used DNA heteroduplex analysis and sequencing to identify HPGDS variants among healthy individuals. HPGDS isoenzymes were produced in bacteria, and their catalytic activities were tested. To determine *in vivo* effects, we conducted pooled case-control analyses to assess whether there is an association of the isoenzyme with colorectal cancer. Roughly 8% of African Americans and 2% of Caucasians had a highly stable Val187lle isoenzyme (with isoleucine instead of valine at position 187). At 37 °C, the wild-type enzyme lost 15% of its activity in one hour, whereas the Val187Ile form remained >95% active. At 50 °C, the half life of native HPGDS was 9 minutes, compared to 42 minutes for Val187Ile. The odds ratio for colorectal cancer among African Americans with Val187Ile was 1.10 (95% CI, 0.75–1.62; 533 cases, 795 controls). Thus, the Val187Ile HPGDS isoenzyme common among African Americans is not associated with colorectal cancer risk. Other approaches will be needed to establish a role for HPGDS in occurrence of human intestinal tumors, as indicated by a mouse model.

Keywords

glutathione transferase; HPGDS; prostaglandin D_2; colon cancer

1. Introduction

D series prostaglandins were discovered 40 years ago [1], and prostaglandin D_2 (PGD_2) has since proven to be a mediator of sleep [2, 3], platelet aggregation, inflammation, smooth muscle contraction, and allergic asthma [4]. Hematopoietic prostaglandin D synthase (HPGDS; NCBI Gene ID 27306) converts PGH_2 to PGD_2, and the enzyme is also a σ class glutathione transferase [5, 6]. HPGDS was originally found in the rat spleen [7–11] and later in other tissues [12, 13]. The 3-dimensional structure of HPGDS has been defined for possible drug targeting, due to involvement of HPGDS in allergic asthma and inflammation [14].

Tumor-prone $Apc^{Min/+}$ mice deficient in HPGDS developed 50% more intestinal adenomas compared to controls. Conversely, $Apc^{Min/+}$ mice that over-produced the enzyme from *HPGDS* transgenes had 70% fewer tumors [15]. The concept of PGD_2 as a tumor suppressing molecule is supported by faster growth of Lewis lung cancer cells implanted onto mice that lack PTGDR [16]. Here we describe a high stability, Val187Ile HPGDS isoenzyme among African Americans and assess its impact on colorectal tumors.

2. Materials and methods

2.1 Identification of gene variants [17]

We used blood specimens from the UCLA Tissue Typing Laboratory to identify common *HPGDS* variants. One mm squares of dried blood on blotter paper served as PCR templates (903 paper; Whatman GE Healthcare; Piscataway, NJ). We used DNA heteroduplex analysis to screen for variants in exons and flanking intron regions in 47 African American and 47 white subjects. DNA sequencing identified the base substitutions. Last, we used allele-specific PCR to search for these substitutions in other ethnic groups [Chinese (Hong Kong), Filipino, Hispanic, Indian (Asian), Japanese, Korean, and Samoan). PCR primers are shown in Table S1.

2.2 HPGDS expression vectors and mutagenesis

We prepared a human HPGDS coding fragment by use of PCR, with human spleen cDNA as the template (Clontech; Mountain View, CA). PCR primers were: 5'-TAT ACA TAT GCC AAA CTA CAA ACT CAC T-3' (HPGDS-L14B) and 5'-TAT AGG ATC CCT AGA GTT TGG TTT GGG G-3' (HPGDS-R12). Primer L14B contained an NdeI restriction site (CATATG) and a total of 21 nucleotides of the *HPGDS* sequence, beginning with the ATG start site. Primer R12 contained 18 nucleotides of *HPGDS* (up to and including the TAG stop codon), followed by a BamHI site (GGA TCC). We subcloned the PCR product between NdeI and BamHI restriction sites in a bacterial plasmid (pET-5a; Novagen EMD Biosciences, Inc.; San Diego, CA), introduced selected mutations (QuikChange site-directed mutagenesis; Stratagene; La Jolla, CA), and checked the final plasmids by sequencing. Mutagenic oligonucleotides were: 5'-GGA ACA ATG TCA TGT TGA TGC TGT TGT GGA CAC TCT GG-3' and 5'-CCA GAG TGT CCA CAA CAG CAT CAA CAT GAC ATT GTT CC-3' (for Ile91Val); 5'-GCT CAC GTA TAA TGC GCC TCA TCT TAC GCA AGA CTT GG-3' and 5'-CCA AGT CTT GCG TAA GAT GAG GCG CAT TAT ACG TGA GC-3' (for Met128Thr); 5'-CCA AGC CAT TCC TGC CAT CGC TAA CTG GAT AAA ACG-3' and 5'-CGT TTT ATC CAG TTA GCG ATG GCA GGA ATG GCT GG-3' (for Val187Ile).

2.3 Biochemical characterization of HPGDS isoenzymes

HPGDS isoenzymes were produced in *E. coli* strain BL21 (DE3; Stratagene). Cells were grown in L-broth with ampicillin to an absorbance of 0.7 at 600 nm and then induced with IPTG. Cells were suspended in phosphate buffered saline (PBS) with 5 mM dithiothreitol (DTT) and lysed with lysozyme (0.5 mg/ml) while stirring for 30 minutes at 4 °C. We recovered soluble proteins by centrifugation (12,000 rpm in a GSA rotor at 4 °C). Ammonium sulfate was added to a concentration of 60% saturation. Pellets were resuspended in PBS with 5 mM DTT and 5 mM $MgCl_2$ (buffer 2) and then loaded onto a Sephadex G-75 column (GE Healthcare). Peak fractions containing HPGDS were pooled and loaded onto a glutathione-agarose column (Sigma-Aldrich; St. Louis, MO), washed with 3 column volumes of buffer 2, and eluted with 2 column volumes of fresh 50 mM Tris-Cl pH 9.0 with 10 mM reduced glutathione. Peak fractions were dialyzed into 50 mM $NaPO_4$ (pH 7.0) containing 10% glycerol at 4 °C, aliquoted, and stored at −80 °C.

We measured glutathione transferase activity of HPGDS enzymes with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate [18]. PGD_2 synthesizing activity was measured with $[1-^{14}C]PGH_2$ as substrate [19].

2.4 Modeling of the 3-dimensional structure of the HPGDS Val187Ile variant

Molecular modeling of the variant enzyme was based on the crystal structure of native, Mg^{2+}-bound HPGDS at 1.8 Å resolution (PDB entries 1IYI and IIYH; [14, 20]). We used the software package, 'O,' to select the most stable rotamer of residue Ile187 [21]. CNS programs [20] were used to obtain an energy-minimized model of the mutant enzyme, without unfavorable contacts associated with the C_δ atom of Ile187.

2.5 Epidemiologic studies of colorectal neoplasia

We used data on African Americans in 6 published case-control studies in analyses of the HPGDS Val187Ile variant. For adenomas, we used the University of Southern California/Kaiser sigmoidoscopy study [USC/Kaiser; 22] and the University of North Carolina Diet and Health Study III [UNC DHS III; 23]. For cancer, we used the Multiethnic Cohort Study [MECS; 24], the Women's Health Initiative observational study [WHI; 25], and the North Carolina Colorectal Cancer Study 1 [NCCCS1; 26, 27] and Study 2 [NCCCS2; 28]. For the

WHI subjects, we matched the available African American colorectal cancer cases (50) to 3 controls each (from among a total of 6,534 controls), according to baseline age and follow-up time.

Dried blood spots on blotter paper or DNA samples in solution were tested for Val187Ile, by use of allele-specific PCR. Samples from USC/Kaiser, UNC DHS III, MECS, and WHI were tested in duplicate and then re-tested, if initial results were unclear. Samples from NCCCS1 and NCCCS2 had an initial test and then re-testing, if the initial result was unclear or to confirm presence of the Val187Ile variant. A randomly chosen quality control subset (10% of NCCCS1) showed 100% consistency with assigned genotypes.

Additionally, we analyzed a variant of prostaglandin-endoperoxide synthase 2 (PTGS2) -- an enzyme that precedes HPGDS in PGD_2 biosynthesis. We used the 4 cancer studies listed above and a genetic epidemiology study of colorectal cancer in Baltimore [29].

To quantify the heterogeneity between studies, we used Cochran's Q parameter and the associated *P*-value, by the use of Stata 10 (StataCorp LP, College Station, TX). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression, comparing Val187Ile carriers to wild-type subjects (Val/Val) for HPGDS analyses and Val511Ala carriers to wild-type subjects (Val/Val) for PTGS2 analyses (SAS version 9.1; SAS Institute; Carey, NC). We adjusted ORs for age (within 5-year intervals) and gender in the individual studies (except WHI) and for age, gender, and study site in combined analyses. Variables were retained in logistic regression models if omitting them changed the OR estimate by at least 10%. Adjustment for body mass index gave the same results as those without adjustment. Therefore, we omitted body mass index from the analyses. All *P*-values were 2-sided, and values <0.05 were considered statistically significant.

3.0 Results and discussion

3.1 HPGDS variants (Table 1)

Six DNA variants were identified, and 3 of them changed the amino acid sequence: Ile91Val, Met128Thr, and Val187Ile (reported in [6]). One substitution in the coding region did not change an amino acid (c.597 C>G), and there were 2 intron variants (IVS2+11 A>C, IVS3+13 T>C [30]). Variants that changed the amino acid sequence were evaluated further.

Val187Ile allele frequencies were 0.044 among healthy African Americans (106/2,386) and 0.010 among healthy whites (3/294; Table 1). Val187Ile was not detected among small samples of Chinese (Hong Kong), Hispanics, Asian Indians, Japanese, Koreans (25 individuals each), or Samoans (24 individuals). Three white specimens (from the UCLA Tissue Typing Laboratory), 2 African American specimens (from the North Carolina DHS III study), and one Filipino specimen (out of 25 from the UCLA Tissue Typing Laboratory) had all 3 substitutions (Ile91Val, Met128Thr, and Val187Ile), indicating an allele with at least 2 of the nucleotide changes.

3.2 Stabilities of HPGDS isoenzymes

We observed weak affinity of human HPGDS for glutathione agarose during purification [6, 31], leading to low recovery of enzymes from *E. coli* despite high expression from plasmid vectors. However, we obtained adequate amounts of purified enzymes by increasing the amounts of $MgCl_2$ and DTT in the binding buffer.

Glutathione transferase specific activities at room temperature were: wild-type, 5.8 ± 0.2; Val187Ile, 4.7 ± 0.5; Ile91Val, 4.2 ± 0.1; Met128Thr, 3.0 ± 0.1 μmol/min/mg of protein

(mean ± standard error; 3 determinations). Prostaglandin D synthase specific activities followed the same order: wild-type, 17.3; Val187Ile, 20.4; Ile91Val, 18.6; Met128Thr, 10.5 μmol/min/mg of protein (single measurements with radio-labeled PGH_2 at 37 °C). Met128Thr converts a hydrophobic thioether side chain to a hydrophilic side chain. The change occurs between 2 conserved residues in a hydrophobic cavity at the dimer interface (Leu127, Asp130) [5, 6].

Thermal stabilities of HPGDS variants were first evaluated by use of glutathione transferase assays. The half-life of the Val187Ile variant increased >4-fold to 42 minutes at 50 °C, compared to 9 minutes for the wild-type (Fig. 1A). High stability was found with 3 independent enzyme preparations of the wild type and Val187Ile variant and thus was not due to purification differences.

Similarly, Val187Ile had higher stability when PGD_2 synthesis activity was measured. Specifically, at 50 °C, the half-life for wild-type PGD_2 synthesis activity was 14 minutes, whereas the Val187Ile variant retained 95% of its activity over this interval (Fig. 1B). In contrast, the Ile91Val and Met128Thr variants lost half of their activities by 6 minutes at 50 °C (Fig. 1B). At 37 °C, the wild-type enzyme lost 15% of its activity in one hour, whereas Val187Ile remained >95% active (Fig. 1C). The Val187Ile allele was in the *HPGDS* clone expressed by Jowsey et al. [6], but enzyme stability was not reported.

3.3 3-Dimensional structure of the Val187Ile isoenzyme

Residue 187 lies in the N-terminal portion of helix-9, far from the catalytic and dimerization domains of the enzyme. In native HPGDS, helix-9 and its adjoining C-terminal loop are held in place by one van der Waals and 7 hydrogen bonding interactions. However, there are 3 more van der Waals interactions in the variant enzyme (Figs. 1D–E). These interactions are likely to reduce mobility of helix-9 and of the outer regions of the enzyme.

3.4 Case-control analyses of the HPGDS Val187Ile variant and colorectal tumors

Highly expressed transgenic HPGDS enzymes inhibit intestinal adenomas in $Apc^{Min/+}$ mice [15]. Therefore, we did case-control analyses to see if the highly stable HPGDS isoenzyme prevents colorectal tumors in humans. As described in section 2.5, we used DNA and data from 6 published studies of colorectal tumors. Subjects in these studies were characterized by age, gender, body mass index, and NSAID usage (Table S2).

The OR for colorectal cancer with the Val187Ile variant (versus wild-type Val/Val) was 1.10 (95% CI, 0.75–1.62; 533 cases and 795 controls). Similarly, the OR for colorectal adenomas was 0.93 (95% CI, 0.46–1.86; 161 cases and 219 controls). Thus, there was no change in risk for colorectal tumors with Val187Ile (Table 2).

Strengths of the analyses were population-based cases and controls in the Multiethnic Cohort, North Carolina, and WHI studies. The USC/Kaiser sigmoidoscopy-based study screened asymptomatic individuals for a first diagnosis of adenomas, but 15–20% of individuals with no polyp by sigmoidoscopy may have adenomas more proximally [32]. A limitation was low prevalence of Val187Ile. Specifically, the combined colorectal cancer studies had 80% power to detect an OR of ~0.5. Larger samples are needed to exclude weaker associations.

We also measured excretion of 11,15-dioxo-9α-hydroxy-2, 3, 4, 5-tetranorprostan-1,20-dioic acid (tetranor PGDM) in relation to Val187Ile in a pilot study of healthy African American volunteers. Tetranor PGDM is the most abundant urinary metabolite of PGD_2 [33]. Median values of urinary tetranor PGDM (in ng/mg of creatinine) were 5.16 for controls (18 individuals; mean age, 35.4 years) and 6.68 for Val187Ile carriers (5

individuals; mean age, 39.2). The results indicate no difference in tetranor PGDM excretion for Val187Ile carriers and controls ($P = 0.48$, Mann-Whitney; data not shown). However, the study was limited by difficulties in recruiting non-smokers (i.e., subjects with undetectable urine cotinine). Smoking induces PTGS2 [34], which in turn produces PGH_2 (the substrate for HPGDS) -- thereby potentially raising PGD_2 metabolite levels. Larger studies are needed for a definitive interpretation.

3.5 Case-control analyses of the PTGS2 Val511Ala variant

We considered whether variants of phospholipase A_2 (group IVA; PLA2G4A) and PTGS2 -- 2 enzymes that precede HPGDS in PGD_2 biosynthesis -- might influence HPGDS effects. We found no DNA variant that changes the PLA2G4A amino acid sequence among 45 blood specimens from healthy African Americans (Table S3).

For PTGS2, Fritsche et al. [35] reported a variant -- alanine replacing valine at residue 511 -- that has the potential to alter the cyclooxygenase active site (Fig. S1; rs5273; [17]). Enzyme assays showed no differences in catalytic function of the Val511Ala variant, when measured with arachidonic acid, linoleic acid, or 2-arachidonyl glycerol as substrates. However, PTGSs can also convert various endogenous or dietary fatty acids to different prostanoid products [36], such as series 1 and 3 prostaglandins. Moreover, PTGS2 that has been acetylated by aspirin converts arachidonic acid into 15-epi-lipoxin, rather than PGH_2 [37]. Several mutations in the cyclooxygenase active site of PTGSs can also result in production of non-prostanoid compounds [38].

The Val511Ala PTGS2 isoenzyme occurs among 8–10% of African Americans. There were (non-statistically significant) inverse associations for colon cancer or adenomas among individuals who were Val511Ala carriers and/or users of nonsteroidal anti-inflammatory drugs (NSAIDs) [17, 26].

Therefore, we produced new data from WHI and NCCCS2 subjects and combined them with all published data in Tables S4 and S5 [17, 26, 29]. However, we found no association with colorectal cancer in relation to the PTGS2 variant (Val511Ala carriers versus wild-type Val/Val subjects; overall OR, 0.77; 95% CI, 0.52–1.13; 704 cases and 1,038 controls; Table S5A).

We also analyzed Val511Ala apart from NSAID usage and in combination with NSAID use, in order to assess for interaction between Val511Ala and NSAIDs. Among all subjects, the OR for colorectal cancer among NSAID users was 0.80 (95% CI, 0.63–1.01; Table S5B), indicating no preventive effect in this study population. Results were similar when individuals with Val511Ala were excluded (OR, 0.85; 95% CI, 0.66–1.09; Table S5B). Among NSAID non-users, the OR for subjects with Val511Ala versus wild-type subjects was 0.95 (95% CI, 0.57–1.57; Table S5C), indicating no effect of Val511Ala apart from NSAID use. Finally, compared to wild-type individuals who were non-users, the OR was 0.41 (95% CI, 0.18–0.92; Table S5D) for individuals who had Val511Ala and were users. However, numbers of subjects in this group were small (8 cases and 27 controls), and there was no statistical interaction between NSAID use and Val511Ala ($P = 0.17$).

These analyses show no association between Val511Ala and risk for colorectal cancer. Strengths of the analyses include the high quality of subject ascertainment and data collection, including 3 population-based studies. However, more subjects are needed to exclude low level associations. The pooled data had 80% power to detect an OR for cancer of ~0.5. Other limitations were lack of NSAID data for 20% of individuals (119 cases; 222 controls) and varying definitions of NSAID use.

We also evaluated prostaglandin production in relation to Val511Ala among healthy African Americans and found no change in PGE_2 production (by peripheral blood monocytes) or excretion of PGE-M and PGI-M by 4 healthy Val511Ala carriers (not shown) [39]. Taken together, the data support an interpretation that the PTGS2 Val511Ala variant is not phenotypically important.

3.6 Comparison of the Val187Ile variant to HPGDS transgenes and knockouts in mice

As mentioned in the introduction, $Apc^{Min/+}$ mice with human *HPGDS* transgenes had 70% fewer intestinal adenomas. In these mice, the human *HPGDS* transgene (driven by a strong constitutive promoter) produced 375 times more mRNA transcripts than were found for the endogenous mouse *Hpgds* genes [15]. Conversely, $Apc^{Min/+}$ mice with heterozygous or homozygous *Hpgds* gene knockouts had 50% more tumors compared to controls. These mice should produce at least 50% less HPGDS in expressing cells.

In contrast to these mutations introduced in mice, the Val187Ile variant should mainly affect removal of HPGDS, rather than production. Historically, rates of heat inactivation of enzymes *in vitro* correlated roughly with enzyme half-lives *in vivo* [40]. A 15% loss of wild-type HPGDS activity in one hour at 37 °C (Fig. 1C) corresponds to a half-life for unfolding of 4.3 hours. The half-life for unfolding of the Val187Ile enzyme at 37 °C may then be 20 hours, as estimated from comparison of the half-lives at 50 °C (42 minutes for Val187Ile versus 9 minutes for wild-type HPGDS; Fig. 1A).

During the first half-life after HPGDS production in cells that are heterozygous for Val187Ile, the average ratio of the level of the Val187Ile isoenzyme to the level of wild-type HPGDS may be only 1.3 to 1, as estimated from half-lives for unfolding and exponential decay. This ratio corresponds to only a 1.15-fold increase in the HPGDS level [or $(1 + 1.3)/2$]. Thus, the amount of lingering HPGDS is expected to be much less than produced from a 375-fold overexpressed *HPGDS* transgene in mice.

Degradation of intracellular proteins can also be regulated by the ubiquitin/proteasome system [41, 42]. For example, PTGS2 is removed by the ubiquitin system and has a half-life of 3.5 to 8 hours in colon cancer cells [43]. A 19-amino acid segment located 6 residues from the C-terminus targets PTGS2 for proteasomal degradation [44]. On the other hand, PTGS1 is constitutively produced in many tissues and has a long half-life (>12 hours). (PTGS1 is the major source of PGH_2 for PGD_2 production under normal conditions [33].) However, there are no data on ubiquitin and HPGDS.

It seems likely that the lack of a tumor suppressing effect of the Val187Ile isoenzyme may be due to inadequate amounts of HPGDS, compared to amounts found in *HPGDS* transgenic mice. Thus, results of our case-control studies on Val187Ile are compatible with an interpretation that high levels of PGD_2 may be needed to suppress intestinal tumors, assuming that the mouse results represent PGD_2 effects in humans. However, because our data can only exclude protective effects with ORs < 0.5 (section 3.5), low statistical power may also be a reason for lack of a detectable effect of the variant.

3.7 Other approaches to studies of PGD_2 and colorectal cancer

Other bioactive molecules that influence PGD_2 effects include prostaglandin catabolizing enzymes and PGD_2 receptors. 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) is recognized as a major enzyme for prostaglandin degradation, but PGD_2 is a poor substrate for the enzyme [45]. Alternatively, the PGD_2 receptor, PTGDR (also known as DP1; Gene ID 5729) appears to be involved in growth of intestinal tumors. For example, Gustaffson et al. found 5-fold lower expression of PTGDR in colorectal cancers, compared to normal tissues (62 tumors and 43 normal tissues, from 62 patients [46]). Galamb et al. observed

decreasing PTGDR expression going from normal tissues, to adenomas, to early cancers (Dukes A, B), and to advanced cancers (Dukes C, D; [47]).

Several PTGDR variants have been cataloged, including nonsense mutations (W48X and Y177X [48]). Screening for PTGDR nonsense variants may be a potential way to analyze colorectal cancer in relation to PGD_2 effects by a genetic epidemiology approach. However, frequencies of the currently known nonsense alleles are very low.

4.0 Conclusions

A highly stable Val187Ile HPGDS variant occurs in 8% of African Americans. There was no protective effect of the variant found against colorectal cancer. Inhibition of intestinal tumors by transgenic HPGDS in $Apc^{Min/+}$ mice was associated with 375-fold over-expression of HPGDS [15], which most likely produces more HPGDS than is found with Val187Ile. Therefore, although the naturally occurring Val187Ile isoenzyme differs biochemically from the wild-type enzyme and is fairly common among African Americans, other studies will be needed to establish a role for PGD_2 in development of intestinal tumors in humans, as predicted from mouse models. Analysis of PGD_2 receptor (PTGDR) variants may be another approach.

Highlights

> We conducted a systematic survey of naturally occurring HPGDS enzyme variants. > A high stability Val187Ile variant occurs in 8% of African Americans and 2% of Caucasians. > Val187Ile does not protect against colon cancer. > Other methods will be needed to establish a role for HPGDS in development of human intestinal tumors, as shown by mouse model data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Apc	adenomatous polyposis coli
CDNB	1-chloro-2,4-dinitrobenzene
CI	confidence interval
dbSNP	the National Center for Biotechnology Information database for single nucleotide polymorphisms
HPGDS	hematopoietic prostaglandin D synthase
Min	a mouse *Apc* allele that causes predisposition to multiple intestinal neoplasia
NSAID	nonsteroidal anti-inflammatory drug
OR	odds ratio
PGD$_2$	prostaglandin D$_2$
PGH$_2$	prostaglandin H$_2$
PLA2G4A	phospholipase A$_2$, group IVA (cytosolic, calcium-dependent)
PTGDR	PGD$_2$ receptor, also known as DP1
PTGS2	prostaglandin-endoperoxide synthase 2

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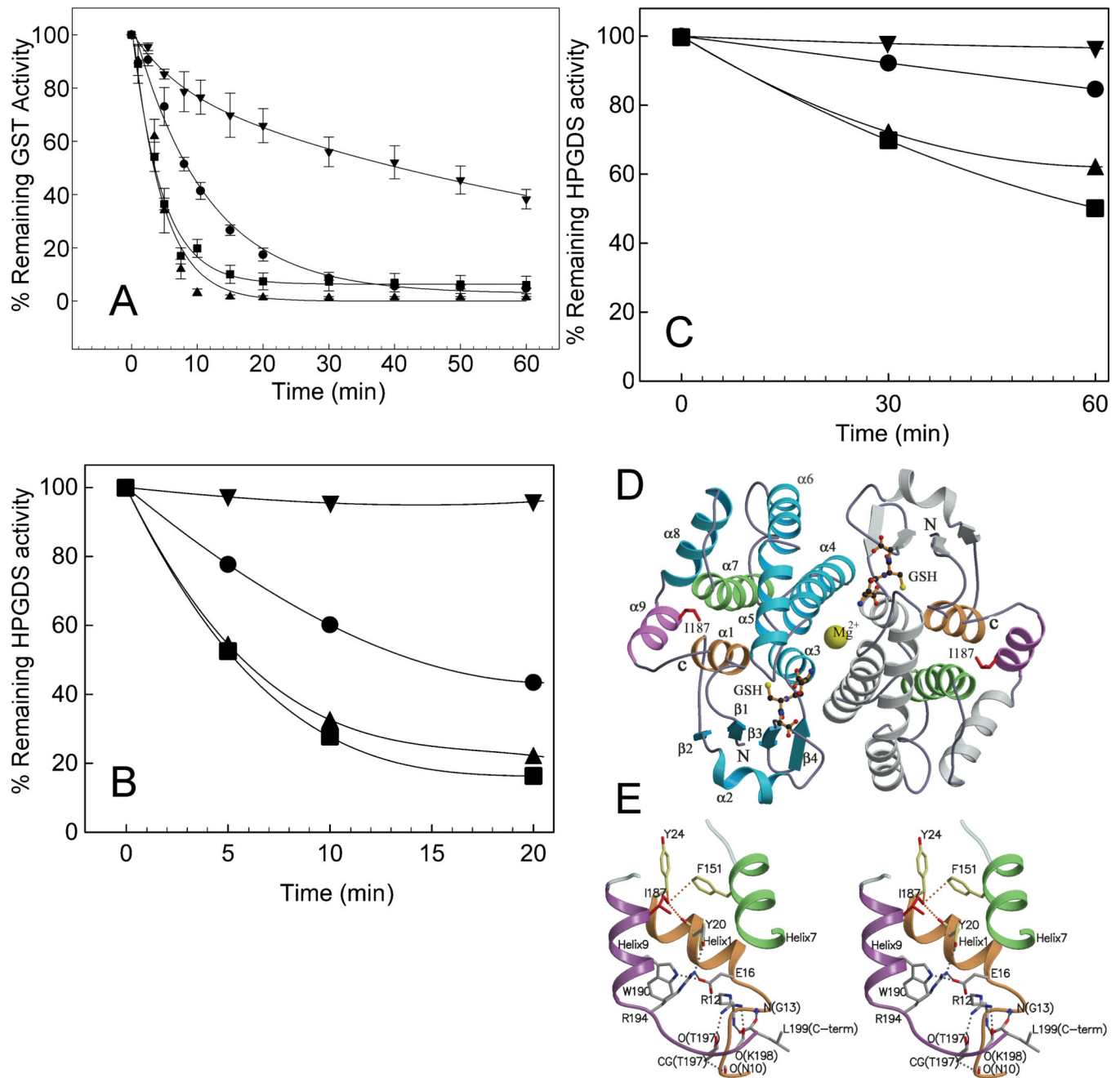
Fig. 1.
(A, B, C) Thermal stabilities of HPGDS isoenzymes. Enzymes were pre-incubated for the indicated times, followed by activity assays. Initial activities were set to 100%. Symbols: ●, wild-type; ■, Ile91Val; ▲, Met128Thr; ▼, Val187Ile. (A) Glutathione transferase (GST) activities at 50 °C with 1-chloro-2,4-dinitrobenzene (3 independent assays). Half-lives (minutes) were: wild-type, 8.6 ± 0.1; Ile91Val, 4.3 ± 1.9; Met128Thr, 2.0 ± 0.5; Val187Ile, 41.7 ± 9.3 ($P < 0.05$, ANOVA with Dunnett's method). (B) PGD_2 synthesis activities at 50 °C with radiolabeled PGH_2. (C) PGD_2 synthesis activities at 37 °C, as in B. (D, E) Structure of the Val187Ile isoenzyme, based on the crystal structure of native, Mg^{2+}-bound HPGDS at 1.8 Å resolution [PDB entries 1IYI and IIYH]. (D) Ribbon drawing of dimeric HPGDS. The

Ile187 side chain is a red stick figure in helix-9. (E) Stereo views of the region near Ile187. Added van der Waals interactions occur between the C_δ atom of Ile 187 and the O (Tyr 20, 3.73 Å), C_β (Tyr 24, 3.73 Å) and C_ς (Phe 151, 3.69 Å) atoms of nearby side chains (red dotted lines). Interaction between Ile 187 and Phe 151 is predicted to give extra stability to helix-9.

Table 1

HPGDS variants identified by screening blood specimens from healthy individuals

Variant	Gene region	Carrier[a] frequency		Allele frequency (95% CI)	
		African Americans	Whites	African Americans	Whites
IVS2+11 A>C (dbSNP ID: rs2289186)	Intron 2	15/47[b]	22/47[b]	0.80 (0.70–0.87)	0.64 (0.54–0.73)
IVS3+13 T>C (dbSNP ID: rs55672062)	Intron 3	3/47[c]	1/47[c]	0.032 (0.007–0.092)	0.011 (0–0.047)
c.271 A>G (Ile91Val) (dbSNP ID: rs61752528)	Exon 4	12/398[d]	4/147[e]	0.015 (0.008–0.026)	0.014 (0.004–0.036)
c.383 T>C (Met128Thr) (dbSNP ID: rs34124298)	Exon 5	4/398[d]	3/147[e]	0.005 (0.002–0.013)	0.010 (0.002–0.031)
c.559 G>A (Val187Ile) (dbSNP ID: rs76328980)	Exon 6	100/1,193[f]	3/147[e]	0.044 (0.035–0.052)	0.010 (0.002–0.031)
c.597 C>G (Leu199Leu)	Exon 6	0/47[g]	2/47[g]	0 (0–0.048)	0.021 (0.001–0.080)

[a]Carrier frequency refers to the heterozygote frequency [or $2q(1-q)$, where q is the allele frequency]. (See also footnote f below.)

[b]Data were from 47 specimens from the UCLA Tissue Typing Laboratory screened by heteroduplex analysis (primers L12 and R11). The "C" allele is more common than the "A" allele (frequencies, 0.80 and 0.20). Carrier and allele frequencies were calculated from heteroduplex analysis data on the 47 specimens, rather than from sequencing or allele-specific PCR data.

[c]Data were from 47 specimens from the UCLA Tissue Typing Laboratory screened by heteroduplex analysis (primers L6 and R6). The allele frequency was estimated from heteroduplex analysis data on the 47 specimens, rather than sequencing or allele-specific PCR data.

[d]Data were from 47 specimens from the UCLA Tissue Typing Laboratory screened by heteroduplex analysis and 351 specimens genotyped by allele-specific PCR (50 from the UCLA Tissue Typing Laboratory; 82 from our human study of genetic variants of prostaglandin metabolism; 121 controls from the University of Southern California/Kaiser study; 98 controls from the University of North Carolina DHS III study).

[e]Data were from 147 specimens from the UCLA Tissue Typing Laboratory. Forty-seven were screened by heteroduplex analysis, and 100 were genotyped by allele-specific PCR.

[f]Data were from the 398 specimens listed in footnote c in addition to 795 specimens genotyped by allele-specific PCR [255 controls from the Multiethnic Cohort Study; 147 controls from the Women's Health Initiative observational study; and controls from the North Carolina Colorectal Cancer Study 1 (NCCCS1; 284 specimens) and Study 2 (109 specimens)]. Among the 1,193 individuals genotyped for Val187Ile, there were 100 heterozygotes (carriers) and 3 individuals homozygous for Val187Ile (2 from the Multiethnic Cohort Study and one from NCCCS1).

[g]Data were from 47 specimens from the UCLA Tissue Typing Laboratory screened by heteroduplex analysis. The 2 c.597 C>G mutations occurred in specimens that also had c.559 G>A mutations, seen by DNA sequencing.

Table 2

Odds ratios for colorectal neoplasia in relation to the Val187Ile HPGDS variant for African Americans in 6 study populations[a].

A. *Association between Val187Ile and occurrence of colorectal cancer (P = 0.92[b])*

	Colorectal cancer		Controls		OR (95% CI)
	V187I+ (%)	V187I−	V187I+ (%)	V187I−	
WHI	2 (4.2)	46	9 (6.1)	138	0.66 (0.14–3.15)
MECS	9 (6.6)	128	20 (7.8)	235	0.80 (0.34–1.86)
NCCCS1	17 (9.9)	155	25 (8.8)	259	1.05 (0.54–2.04)
NCCCS2	22 (12.5)	154	12 (11.0)	97	1.12 (0.53–2.37)
All studies	50 (9.4)	483	66 (8.3)	729	1.10 (0.75–1.62)

B. *Association between Val187Ile and occurrence of colorectal adenomas (P = 0.29[b])*

	Colorectal adenomas		Controls		OR (95% CI)
	V187I+ (%)	V187I−	V187I+ (%)	V187I−	
USC/Kaiser	8 (6.7)	111	14 (11.6)	107	0.54 (0.22–1.35)
Kaiser	7 (16.7)	35	8 (8.2)	90	2.47 (0.78–7.79)
UNC DHS III	15 (9.3)	146	22 (10.0)	197	0.93 (0.46–1.86)

[a] Abbreviations: "V187I+" indicates Val/Ile or Ile/Ile genotypes; "V187I−" indicates the Val/Val genotype; WHI, Women's Health Initiative observational study [25]; MECS, Multiethnic Cohort Study [24]; NCCCS1 and NCCCS2, North Carolina Colorectal Cancer Study 1 [26, 27] and 2 [28]; USC/Kaiser, the University of Southern California/Kaiser sigmoidoscopy study of colorectal adenomas [22]; UNC DHS III, the University of North Carolina Diet and Health Study III [23]. The odds ratios (ORs) and 95% confidence intervals (CIs) represent comparison of Val187Ile to wild-type genotypes (Val/Val). Site-specific genotype effects were estimated controlling for age and sex (except for the WHI study). The OR for the combined study also controlled for study site. There were 5 Val187Ile homozygotes (Ile/Ile) in the entire data set: one case in UNC DHS III; 2 controls in MECS; and one case and one control in NCCCS1. Adjustment for body mass index gave results very close to those without adjustment. Therefore, we omitted body mass index from the analyses shown. NCCCS2 cases had rectal cancer.

[b] To quantify the heterogeneity between studies, we used Cochran's Q parameter and the associated *P*-value, by the use of Stata 10 (StataCorp LP, College Station, TX).