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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 Val187Ile isoenzyme (with isoleucine instead of valine at position 187). At 37 °C, the wild-type enzyme lost 15% of its activity in 1 h, whereas the Val187Ile form remained >95% active. At 50 °C, the half life of native HPGDS was 9 min, compared to 42 min 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.

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 A2, group IVA (cytosolic, calcium-dependent); PTGDR, PGD_2 receptor, also known as DP1; PTGS2, prostaglandin-endoperoxide synthase 2.

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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 millimeter 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 TGG-3′ (for Val187Ile).

2.3. Biochemical characterization of HPGDS isoenzymes

HPGDS isoenzymes were produced in *Escherichia 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 min 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 6534 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