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Associations between variants in the cyclooxygenase 2 enzyme gene (*PTGS2*) and development of benign prostate enlargement

Jennifer L. St Sauver^{*}, Michael M. Lieber[†], Susan L. Slager[‡], Debra J. Jacobson[‡], Michaela E. McGree[‡], and Steven J. Jacobsen[§]

^{*}Division of Epidemiology, Department of Health Sciences Research, Mayo Clinic, Rochester, MN

[†]Department of Urology, Mayo Clinic, Rochester, MN

[‡]Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, Mayo Clinic, Rochester, MN

[§]Department of Research and Evaluation, Southern California Permanente Medical Group, Pasadena, CA, USA

Abstract

OBJECTIVE—To determine if polymorphisms in the cyclooxygenase 2 (COX-2) enzyme gene (prostaglandin synthase 2; *PTGS2*) were associated with development of benign prostate enlargement (BPE), and whether associations were modified by use of NSAIDs. Participants were men residing in Olmsted County, MN, who were between 40 and 79 years of age in 1990 ($N = 356$). Prostate volume was measured by transrectal ultrasound and men reported all the medication that they were taking at the time of the examination. Men were followed biennially for 16 years. Ten tagging single nucleotide polymorphisms (SNPs) in the *PTGS2* gene were typed using the Illumina GoldenGate[™] Assay. Associations between SNPs and development of BPE (volume > 30 mL) were assessed by Cox proportional hazards models. Models were also stratified by NSAID use.

RESULTS—We observed significant associations between four polymorphisms in the *PTGS2* gene and development of BPE (all $P < 0.05$). These associations were not observed among men who used NSAIDs.

CONCLUSION—Variants in the *PTGS2* gene may increase the risk of prostate enlargement, but the increased risk may be minimized by use of NSAIDs.

INTRODUCTION

Benign prostatic enlargement (BPE) is common among aging men, and is associated with the development of poor urological outcomes [1–4]. Although the underlying pathogenesis remains unclear, a growing body of evidence suggests that inflammation may play an important role in the development of BPE [5–9].

A key mediator of inflammatory processes is the cyclooxygenase enzyme 2 (COX-2) which catalyses the production of prostaglandins from arachidonic acid [10]. Higher levels or increases in function in the COX-2 enzyme may contribute to higher levels of prostatic inflammation, and an increased risk of BPE. Increases in COX-2 levels or enzymatic activity

Correspondence: Jennifer St Sauver, Department of Health Sciences Research, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA. stsauver.jennifer@mayo.edu.

CONFLICT OF INTEREST

Dr Steven Jacobsen is an employee of Kaiser Permanente South.

may arise because of variation in the COX-2 gene (*PTGS2*). *PTGS2* is highly conserved, and functional genetic variants in the coding regions of the gene have not been described [11]. However, several investigators have found that variation in the promoter region may contribute to changes in mRNA expression and stabilization [12–15]. Higher levels of *PTGS2* expression could therefore be associated with higher levels of prostaglandin production, increased inflammation, and an increased risk of BPE.

Nonsteroidal anti-inflammatory drugs (NSAIDs) target the COX-2 enzyme and reduce inflammation by inhibiting COX-2 activity. We have previously found that men who took daily NSAIDs were less likely to develop enlarged prostates compared with men who did not take these drugs [16]. These results suggest that COX-2 activity may contribute to the development of BPE, but NSAID use may minimize an increased risk conferred by the COX-2 enzyme.

To better understand the role of the COX-2 enzyme in the development of BPE, we examined associations between polymorphisms in the COX-2 gene (*PTGS2*) and development of BPE as part of a candidate gene study examining associations between variants in nine genes and prostate enlargement. We also determined whether associations were modified by use of NSAIDs.

MATERIALS AND METHODS

Details related to the development of the study cohort have been published previously [17,18]. Briefly, a randomly sampled, population-based group of white men between 40 and 79 years of age residing in Olmsted County, MN, in 1990 was identified through the Rochester Epidemiology Project [19]. Men with a history of prostate or bladder surgery, urethral surgery or stricture, or medical or other neurological conditions that could affect normal urinary function were excluded. Subsequently, 3874 men were asked to join the study and 2115 agreed to participate (55%). A 25% random subsample was invited to participate in a detailed clinical urological examination including providing a blood sample. A total of 476 (89%) men agreed to participate in this more intensive examination. Prostate size was measured by transrectal ultrasound (Bruel and Kjaer type 8551 7.0 MHz. endosonic multiplane transducer).

The cohort was actively followed on a biennial basis for 16 years using a similar protocol to the initial examination. During the second and third round of visits, men who did not participate in the follow-up were replaced by men randomly selected from the community, after being screened for the exclusion criteria used at baseline ($n = 332$). Of the replacement men, 158 were added to the clinic subset. Additionally, in round 8, 133 men from the larger cohort became part of the in-clinic cohort, for a total of 767 randomly selected in-clinic men. Of these men, 356 (46%) had sufficient DNA available for typing, had consented to allow their DNA to be used for this study, and had prostate volume data available. The study was reviewed and approved by the Institutional Review Boards of Mayo Clinic and Olmsted Medical Center.

The study participants were asked (by structured interview) to report all prescribed and over-the-counter medications that were taken on a daily basis at the time of the initial visit. The dosage, unit of administration, starting date and directions for use of each medication were recorded when such information was available. When possible, medication information was recorded directly from the label of the bottle. Medication use was again ascertained by questionnaire in round 4 (1996), round 6 (2000), and during each biennial follow-up round thereafter. Most NSAID users (80%) took daily aspirin, and 7% of all the NSAID users took a low-dose aspirin. Other NSAIDs used included ibuprofen, naproxen and diclofenac.

sodium. Participants who reported NSAID use before the development of BPE were considered exposed.

Associations between variants in the gene *PTGS2* and prostate enlargement were examined as part of a larger candidate gene study examining 27 genes, nine of which were relevant to this study (Appendix). DNA was extracted from the buffy-coat cell layer of blood samples and stored at -70°C . DNA samples were quality checked using pico green. Tagging single nucleotide polymorphisms (SNPs) [20] were selected for the genes using Hapmap, Seattle SNPs, and the National Institute of Environmental Health Sciences SNPs Caucasian population data. Tagging SNPs covered 5 kb upstream and downstream of each gene with a minor allele frequency of 5% and a pairwise r^2 threshold of 0.90. Genotyping was conducted at the Mayo Clinic Genotyping Shared Resource using the Illumina GoldenGate™ Assay. We attempted to obtain genotype data for 482 men; two failed initial typing, but were successfully re-run. No men were excluded because of a low call rate ($< 95\%$). Overall, 672 markers were typed. Of these, 30 (4.5%) were complete failures, and 14 (2.1%) SNPs were removed for quality control reasons (two were monomorphic, two were excluded because of poor clustering and 10 failed Hardy–Weinberg equilibrium; $P < 0.001$).

For statistical analysis, a principal components analysis was used to determine the association of each candidate gene with the risk of developing BPE (prostate volume > 30 mL). As a global gene test, principal components were used to create uncorrelated components that were linear combinations of the SNPs from each gene. The components were ranked according to the amount of the total SNP variance explained. The smallest subset of components that accounted for at least 90% of the variability among the SNPs was included in a multivariable logistic regression model, and a likelihood ratio test was used to jointly test the significance of the selected principal components while reducing the number of independent degrees of freedom [21].

For those genes that were significant at the gene level test, we evaluated the effects of individual SNPs for predicting development of prostate enlargement (prostate volume > 30 mL and > 40 mL) using a proportional hazard regression model. Each polymorphism was modelled as a gene-dosage effect (P trend). People with two copies of the rare variant allele were assigned scores of 2, whereas those with one or zero copies were assigned scores of 1 and 0, respectively. SNPs with a P for trend < 0.05 were considered significant.

We also examined potential interactions between use of NSAIDs and the *PTGS2* SNPs by incorporating interaction terms between NSAID use and the SNPs into the proportional hazard models. As a result of the limited number of men who developed a prostate volume > 40 mL, interactions were examined only for the outcome of prostate volume > 30 mL.

To assess the robustness of the results, multiple testing was accounted for in two ways. In addition to reporting nominal P values (without adjustment for multiple testing), we also calculated the Bonferroni adjusted P values. These adjusted P values are considered conservative because they are intended to control for the chance of making an erroneous conclusion. An alternative is to control the false discovery rate (FDR) [22]. The FDR controls the proportion of erroneous significant tests and can have greater power than a Bonferroni-adjusted P value. Analyses were conducted using SAS (SAS Institute, Cary, NC, Version 9.1).

RESULTS

Overall, the 356 men in this study had a median baseline age of 48.6 years (25th, 75th percentiles: 42.9, 57.4 years). Men were followed for a median of 8.7 years (25th, 75th percentiles: 1.5, 14.4 years), and 239 (67.1%) men developed a prostate volume > 30 mL

during follow-up. Results of the principal components analyses for the nine genes examined are shown in the Appendix. In the gene-level principal components analysis, *PTGS2* was significant at $P < 0.05$. After adjusting for multiple comparisons, *PTGS2* remained significant (FDR $P < 0.001$). As only *PTGS2* was significant after adjusting for multiple comparisons, only the results from *PTGS2* are reported in this paper. The gene structure of *PTGS2* and the locations of the genotyped SNPs are shown in Fig. 1 (which is adapted from Seattle SNPs [23].)

The SNP-level results for *PTGS2* are shown in Table 1. Overall, four SNPs were significantly associated with developing a prostate volume > 30 mL (rs5275: OR 1.24, 95% CI 1.03, 1.51; rs4648261 OR 2.97, 95% CI 1.32, 6.68; rs689466 OR 1.28, 95% CI 1.02, 1.61; rs2745559 OR 1.44, 95% CI 1.14, 1.81). Additionally, rs4648261 and rs2745559 remained associated with developing a prostate volume > 40 mL (FDR $P < 0.05$), whereas rs5275 and rs689466 remained marginally significant (FDR $P \leq 0.075$; Table 1). These SNPs were not highly correlated with each other, as the maximum pairwise r^2 value among the four SNPs was 0.13.

As NSAID use was previously associated with a decreased risk of developing an enlarged prostate in this group of men, it was possible that NSAID use could mask associations between *PTGS2* SNPs and development of an enlarged prostate. Therefore, we examined associations between the *PTGS2* SNPs in men who used and did not use NSAIDs before the development of prostate volume > 30 mL. Overall, 178 (50%) men in this study used an NSAID before the development of a prostate volume > 30 mL. Significant associations were present only among men who did not use NSAIDs, and significant interactions were observed between NSAID use and two SNPs (Table 2).

DISCUSSION

In this study, we observed significant associations between polymorphisms in the *PTGS2* gene and development of BPE. Additionally, we found that NSAID use modified associations between SNPs in the *PTGS2* gene and BPE. Our results suggest that variants in the *PTGS2* gene may increase the risk of prostate enlargement, but that this increased risk may be minimized by use of NSAID medications.

The COX-2 enzyme is a known mediator of systemic inflammation, and our results are consistent with previous studies, which have found that inflammation in the prostate gland is associated with prostate enlargement [6–8]. These data are also consistent with previous studies that have suggested a direct role for COX-2 in prostate enlargement. In the prostate epithelium, Wang *et al.* found that increased COX-2 expression was associated with increased cellular proliferation [24]. Minnery and Getzenberg [25] also found that blocking COX-2 activity could reduce cell viability and induce apoptosis in a benign prostatic hyperplasia cell line. In an observational cohort study, we found that men who took NSAIDs were less likely to develop an enlarged prostate [16]. Together, these studies suggest that COX-2 may play a role in prostate enlargement, and that NSAID use may prevent prostate enlargement, presumably by blocking COX-2 activity. The results presented in this article are consistent with these previous lines of evidence, as the risk of prostate enlargement among men with specific *PTGS2* SNPs was significant only among men who did not take NSAIDs. Our data therefore suggest that men with particular variants in the *PTGS2* gene may be at increased risk for developing prostate enlargement, but that this risk could potentially be reduced by taking medications that inhibit the COX-2 enzyme.

The *PTGS2* SNPs that were associated with prostate enlargement were located throughout the gene and in both the 3' and 5' untranslated regions. Functional data for the four

significant SNPs are limited. However, the rs5275 SNP has been previously associated with changes in *PTGS2* expression [14]. This SNP is located in the 3' untranslated region of the gene, and may be involved in stabilization of post-translational mRNA. Lee and Jeong [12] have previously shown that there are elements in the 3' untranslated region of the *PTGS2* gene that may interact with β -catenin, a transcriptional regulator in the Wnt signalling pathway. β -Catenin appears to control *PTGS2* expression at both the transcriptional and post-transcriptional levels by interacting with AU-rich elements in the 3' untranslated region of the *PTGS2* gene to stabilize *PTGS2* mRNA. This stabilization results in the upregulation of the COX-2 enzyme. Higher expression may then lead to increased activity, a higher inflammatory response, and an increased risk of prostate enlargement. Men who have variants associated with *PTGS2* stabilization may therefore be more prone to higher inflammatory responses, and subsequent prostate enlargement. However, if these men also took NSAIDs, the higher expression levels could be balanced by decreased function. Our data are in agreement with this hypothesis because men who had these 'risk' alleles but also took NSAIDs did not have an increased risk of prostate enlargement.

This study suggests that NSAID use may be especially useful among men with particular alleles in the *PTGS2* gene. However, NSAIDs can cause significant adverse health consequences, including a risk of gastrointestinal bleeding [26]. Therefore, recommending routine NSAID use to prevent BPE for all men is inappropriate. However, if men at high risk of BPE can be identified, possibly through identification of genetic factors that place them at higher risk of BPE, the costs and benefits of NSAID treatment to prevent BPE for an individual patient can be discussed. A number of risk factors for developing NSAID complications have been identified, and men at high risk of complications may benefit from additional therapies, such as use of proton pump inhibitors, to prevent NSAID complications [27].

Limitations of our study include the relatively small sample size examined, and the lack of replication in an independent sample. Additionally, the prostate volumes of this population are relatively small compared with those of men who typically seek urological care because of BPE. As a result, we had limited power to examine associations between *PTGS2* polymorphisms and development of a prostate volume > 40 mL. Our results in Table 1 indicate that the associations observed between specific polymorphisms in *PTGS2* and developing a prostate > 40 mL were in the same direction, and of similar magnitude, to the associations between the same polymorphisms and a prostate volume > 30 mL. These results were often no longer significant because of the small number of men who developed a prostate volume > 40 mL, but they do suggest that these polymorphisms may be associated with prostate enlargement in general. However, it is necessary to replicate this study in an independent sample with a more diverse range of prostate volumes to conclude that these *PTGS2* polymorphisms are associated with BPE.

An additional limitation of our study is that BPE does not always lead to clinically significant LUTS or other adverse urological outcomes. Men with enlarged prostates are more likely to develop progressively worsening LUTS, decreased maximum urinary flow rates, and acute urinary retention, compared with men with small prostates [1–4]. Pressure flow studies also clearly document the adverse effect of prostate enlargement on urinary flow [28]. Additionally, medical or surgical therapies to reduce prostate volume can substantially improve LUTS and reduce the incidence of other adverse urological outcomes, such as acute urinary retention [29,30]. However, correlations between BPE and LUTS are modest [1,31–34], and it is possible for men to experience significant LUTS even in the absence of BPE [35]. This lack of complete correlation between BPE and adverse urological outcomes indicates that multiple aetiological factors contribute to adverse urological outcomes. BPE will play an important role in these outcomes in some men, but not all men.

The results of our study have focused only on BPE, and further characteristics of these men will need to be identified to determine which of these men will progress to adverse urological outcomes.

False-positive and false-negative associations must be considered in all association studies. To address false-positive results because of multiple testing, we calculated the Bonferroni adjusted *P* value at both the gene and SNP-level and found that the *PTGS2* gene and one SNP remained significant ($P < 0.05$). We also calculated FDR *P* values for the individual *PTGS2* SNPs and two remained significant (FDR $P < 0.05$).

False-negative results are also possible, particularly for the SNPs that are uncommon in this population. Additionally, there may be other mechanisms (such as variance in copy number) by which *PTGS2* may influence the development of prostate enlargement, and these alternative genetic associations were not examined in this study.

Finally, all participants in our study were white, and results may not be generalizable to men of other races or ethnicities. However, this homogeneous sample population should minimize spurious results caused by population stratification.

Our results suggest that variants in the COX-2 enzyme gene (*PTGS2*) may increase the risk of prostate enlargement, and that this increased risk may be minimized by NSAID use. It is currently difficult to identify men at high risk of developing BPE or other urological complications for clinical trials. If these results are replicated in an independent sample, these data could be used to identify men at higher risk of BPE, and these men may represent a useful study population for preventive clinical trials.

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Abbreviations

BPE	benign prostate enlargement
COX-2	cyclooxygenase-2
SNP	single nucleotide polymorphism
FDR	false discovery rate

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FIG. 1. *PTGS2* gene structure and location of the tagSNPs (figure adapted from Seattle SNPs [23])

*Indicates a significant association between the SNP and development of BPE among men who did not use NSAIDs

TABLE 1

Associations between *PTGS2* SNPs and risk of developing a prostate volume > 30 mL and > 40 mL

SNP ID	Frequency of the high risk allele	Per copy of variant allele HR (95% CI)	One copy of variant allele HR (95% CI)	Two copies of variant allele HR (95% CI)	P trend
Volume > 30 mL					
rs2206593	0.06	0.98 (0.67, 1.43)	0.95 (0.64, 1.39)	16.44 (2.22, 121.95)	0.92
rs5275	0.65	1.24 (1.03, 1.51)	1.56 (1.01, 2.40)	1.24 (0.96, 1.61)	0.03*
rs5277	0.14	1.26 (0.99, 1.60)	1.38 (1.04, 1.83)	0.94 (0.39, 2.27)	0.06
rs4648261	0.97	2.97 (1.32, 6.68)	-	2.97 (1.32, 6.68)	0.009[†]
rs20417	0.84	1.03 (0.80, 1.32)	1.05 (0.43, 2.55)	1.03 (0.78, 1.36)	0.83
rs689466	0.81	1.28 (1.02, 1.61)	1.65 (0.85, 3.22)	1.31 (0.99, 1.72)	0.03*
rs12042763	0.24	1.13 (0.93, 1.38)	1.26 (0.97, 1.62)	0.96 (0.59, 1.57)	0.20
rs2745559	0.17	1.44 (1.14, 1.81)	1.52 (1.16, 1.98)	1.59 (0.75, 3.38)	0.002[‡]
rs10911905	0.13	1.08 (0.83, 1.41)	1.07 (0.80, 1.42)	1.49 (0.55, 4.00)	0.55
rs2143416	0.84	1.03 (0.80, 1.32)	1.05 (0.43, 2.55)	1.03 (0.78, 1.36)	0.83
Volume > 40 mL					
rs2206593	0.06	0.83 (0.48, 1.41)	0.77 (0.44, 1.33)	43.90 (5.49, 350.82)	0.49
rs5275	0.65	1.17 (0.90, 1.51)	1.26 (0.72, 2.19)	1.21 (0.86, 1.70)	0.23*
rs5277	0.14	1.05 (0.75, 1.47)	1.03 (0.70, 1.52)	1.30 (0.48, 3.52)	0.76
rs4648261	0.97	8.47 (1.18, 60.59)	-	8.47 (1.18, 60.59)	0.03[†]
rs20417	0.84	1.08 (0.77, 1.51)	3.03 (0.42, 21.70)	1.02 (0.71, 1.48)	0.66
rs689466	0.81	1.07 (0.80, 1.44)	1.46 (0.60, 3.58)	1.03 (0.72, 1.47)	0.64*
rs12042763	0.24	1.03 (0.80, 1.34)	1.06 (0.76, 1.50)	0.99 (0.53, 1.83)	0.80
rs2745559	0.17	1.26 (0.92, 1.73)	1.27 (0.89, 1.82)	1.59 (0.59, 4.32)	0.15 [‡]
rs10911905	0.13	1.08 (0.77, 1.54)	1.14 (0.78, 1.66)	0.52 (0.07, 3.74)	0.65
rs2143416	0.84	1.08 (0.77, 1.51)	3.03 (0.42, 21.70)	1.02 (0.71, 1.48)	0.66

* FDR *P* value ≤ 0.075;

[†] FDR *P* value < 0.05;

[‡] Bonferroni and FDR *P* values < 0.05.

TABLE 2
Associations between *PTGS2* SNPs and development of a prostate volume >30 mL stratified by NSAID use

SNP number *	SNP ID	NSAID use		No NSAID use		Interaction term <i>P</i> value
		Per copy of variant allele		Per copy of variant allele		
		HR (95% CI)	<i>P</i> trend	HR (95% CI)	<i>P</i> trend	
1	rs2206593	0.53 (0.26, 1.10)	0.09	1.48 (0.95, 2.30)	0.09	0.02
2	rs5275	1.06 (0.78, 1.46)	0.69	1.47 (1.15, 1.87)	0.002	0.08
3	rs5277	1.30 (0.90, 1.87)	0.17	1.39 (1.01, 1.93)	0.047	0.67
4	rs4648261	1.29 (0.41, 4.06)	0.67	6.28 (2.00, 19.77)	0.002	0.05
5	rs20417	0.84 (0.56, 1.26)	0.39	1.26 (0.92, 1.72)	0.15	0.11
6	rs689466	1.18 (0.83, 1.68)	0.35	1.11 (0.83, 1.49)	0.48	0.79
7	rs12042763	1.07 (0.79, 1.46)	0.64	1.23 (0.95, 1.59)	0.11	0.43
8	rs2745559	1.13 (0.75, 1.73)	0.55	1.48 (1.12, 1.95)	0.006	0.26
9	rs10911905	1.34 (0.86, 2.10)	0.20	0.87 (0.62, 1.20)	0.39	0.11
10	rs2143416	0.84 (0.56, 1.26)	0.39	1.26 (0.92, 1.72)	0.15	0.11

* Note Fig. 1 for location of the SNPs

APPENDIX

ASSOCIATIONS BETWEEN CANDIDATE GENES AND DEVELOPMENT OF A PROSTATE VOLUME > 30 ML

Gene (Alias)*	HapMap SNPs (n)	Typed SNPs (n)	Gene coverage (%)	Df	P value [†]	Stepdown Bonferroni P value	False discovery rate P value
<i>ADRA1A</i>	157	70	97.45	18	0.84	1.00	0.85
<i>ADRA1B</i>	43	22	95.35	8	0.75	1.00	0.85
<i>ADRA1D</i>	23	20	95.65	6	0.60	1.00	0.85
<i>DBH</i>	49	30	85.71	10	0.04	0.30	0.17
<i>MAOA</i>	39	7	100	2	0.85	1.00	0.85
<i>PTGS2</i>	17	10	100	6	< 0.001	< 0.001	< 0.001
<i>SLC6A2</i>	80	40	97.5	11	0.19	1.00	0.58
<i>TRPAI</i>	123	34	98.4	7	0.75	1.00	0.85
<i>TRPVI</i>	49	31	94.2	8	0.68	1.00	0.85

* As defined in Entrez Gene.

[†] Likelihood ratio P values (similar results with Wald and Score P values) from Phreg models (time to ever/never outcome) using principal components explaining at least 90% of the variance