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Meta-analysis of epidemiological studies demonstrates significant association of PTGS2 polymorphism rs689470 and no significant association of rs20417 with prostate cancer

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ABSTRACT. Evidence is accumulating that chronic inflammation has an important role in prostate cancer. Two common polymorphisms in the prostaglandin-endoperoxide synthase 2 (PTGS2) gene, rs20417 and rs689470, have been found to alter the risk for prostate cancer, but the various studies are not in agreement. To derive a more precise estimation of this association, all available studies were considered in a meta-analysis, with 10,700 patients and 13,021 controls for rs20417 and 4087 patients and 3761 controls for rs689470. We used odds ratios (ORs) to assess the strength of the association, and 95% confidence intervals (CIs) to determine the precision of the estimate. When all groups were pooled, we did not detect a

significant association of rs20417 polymorphism with prostate cancer risk. Similarly, no associations were found in the subgroup analysis. However, we found that rs689470 was significantly associated with a trend towards increased prostate cancer risk when using both additive (OR = 2.15, 95%CI = 1.04-4.44, P = 0.04) and recessive models (OR = 2.07, 95%CI = 1.07-4.03, P = 0.03) to analyze the data. In subgroup analyses stratified by ethnicity, there was no evidence that rs689470 has a significant association with prostate cancer in Caucasians. Based on our meta-analysis, rs689470 polymorphism is significantly associated with prostate cancer risk in the overall population. Nevertheless, we suggest that further studies should be made to confirm these findings.

Key words: PTGS2; Genetic polymorphisms; Prostate cancer; Meta-analysis

INTRODUCTION

Prostate cancer (PCa) is one of the most common types of cancer affecting men in the USA and is the second leading cause of cancer death among men, after lung cancer. Identifying potential risk factors for PCa is critically important to develop interventions and to expand our understanding of the biology of PCa. Despite the fact that the complex etiology of PCa remains obscure, various risk factors play an important role in PCa development, such as advanced age, tobacco use, alcohol consumption (Hiatt et al., 1994), and a diet poor in fresh fruits and vegetables (Hori et al., 2011). However, not all of those who have been exposed to these risk factors will develop PCa, suggesting that genetic factors may contribute to the carcinogenic mechanisms and the inter-individual differences in susceptibility. Results have shown that a strong association exists between states of chronic inflammation and cancer, and it is believed that mediators of inflammation may be responsible for this phenomenon (Shacter and Weitzman, 2002). A previous study suggested that chronic inflammation may lead to tumorigenesis through DNA damage by reactive oxygen and nitrogen species, enhancing cell proliferation, and stimulating angiogenesis (McArdle et al., 2006). Genetic polymorphisms of genes involved in the inflammatory pathway, including prostaglandin-endoperoxide synthase 2 (PTGS2), may impact susceptibility to PCa.

PTGS, also known as cyclooxygenase (COX), catalyzes the rate-limiting step in the formation of inflammatory prostaglandins. COX is an integral membrane bifunctional enzyme, which metabolizes arachidonic acid to many biologically active eicosanoids. The PTGS2 gene, located on chromosome 1q25.2-q25.3, is a candidate gene for PCa susceptibility (Kosaka et al., 1994). PTGS2 is an inducible enzyme that converts arachidonic acid to prostaglandins, which play a role in cell proliferation and are potent mediators of inflammation. A meta-analysis suggested that aspirin use was associated with a decreasing trend of PCa risk (Mahmud et al., 2004). Data suggest that COX-2 is overexpressed in PCa tissue compared to benign tissue from the same patient in several studies (Gupta et al., 2000; Madaan et al., 2000; Yoshimura et al., 2000; Kirschenbaum et al., 2000; Lee et al., 2001; Uotila et al., 2001). Some previous studies suggested that COX-2 may influence

carcinogenesis by inhibiting apoptosis (Tsujii and DuBois, 1995), inducing angiogenesis (Masferrer et al., 2000) and by chronic activation of immune responses (O'Byrne and Dalgleish, 2001).

Several studies were conducted to investigate the associations of PTGS2 rs20417 and rs689470 polymorphisms with PCa risk (Panguluri et al., 2004; Shahedi et al., 2006; Cheng et al., 2007; Danforth et al., 2008; Murad et al., 2009; Balistreri et al., 2010; Dossus et al., 2010; Wu et al., 2011). However, molecular epidemiological studies have yielded contradictory results concerning the potential roles of rs20417 and rs689470 polymorphisms in PCa. Individual studies might have been underpowered to detect the overall effects. Some studies are limited by their sample size and subsequently suffer from power that is too low to detect effects that may exist. Given the amount of accumulated data, we deemed it important to perform a quantitative evaluation of the evidence. Therefore, we performed this meta-analysis study to determine whether rs20417 and rs689470 polymorphisms in the PTGS2 gene are associated with PCa susceptibility in men.

SUBJECTS AND METHODS

Literature search

Relevant publications were identified through a literature search using the key words "COX-2" or "PTGS2", "prostate", "carcinoma" or "cancer" or "tumor", and "polymorphism" or "variation" in the following electronic databases: PubMed, Embase, and the Cochrane Library (last search was updated on June 28, 2011). We evaluated all associated publications to retrieve the most eligible papers. The reference lists of reviews and retrieved articles were hand searched at the same time. If more than one article was published by the same author using the same case series, we selected the study with the largest series. Articles were limited to English language papers.

Inclusion and exclusion criteria

The following inclusion criteria were used to select articles for the meta-analysis: 1) information on the evaluation of PTGS2 (rs20417 and rs689470) polymorphisms and PCa risk; 2) case-control study, and 3) sufficient genotype data were presented to calculate the odds ratio (OR) with 95% confidence interval (95%CI). Major reasons for exclusion of studies were: 1) no controls; 2) overlapping study populations, and 3) no usable data reported.

Data extraction

All data were independently extracted by two investigators according to the prespecified selection criteria. Disagreement was resolved by discussion. The following data were extracted: the name of the first author, publication year, ethnicity of the population, available genotype, number of prostate cancer cases and controls studied, and results of studies. Different ethnic descents were categorized as Caucasian, Asian, and African. For case-control studies nested within different cohorts, data were extracted separately for each group whenever possible.

Statistical analysis

The effect measure of choice was OR with its corresponding 95%CI. The significance of the summary OR was determined with a Z-test and $P < 0.05$ was considered to be statistically significant. Heterogeneity assumption was checked by the Q-test. $P \geq 0.10$ for the Q-test indicated lack of heterogeneity among the studies. Either a random-effects model or fixed-effects model was used to calculate pooled effect estimates in the presence or absence of heterogeneity (Mantel and Haenszel, 1959; DerSimonian et al., 1986).

First, we examined rs20417 genotypes using additive (CC *vs* GG), recessive (CC *vs* GC + GG) and dominant (CC + GC *vs* GG) genetic models. The relationship between the allele and susceptibility to PCa was then examined. For rs689470 polymorphism, we evaluated the same effects. Stratified analyses were also performed by ethnicity. Publication bias was assessed by visual inspection of funnel plots in which the standard error of log (OR) of each study was plotted against its log (OR). An asymmetric plot indicates a possible publication bias. The symmetry of the funnel plot was further evaluated by the Egger linear regression test. The significance of the intercept was determined by the *t*-test suggested by Egger et al., 1997 ($P < 0.05$ was considered to be representative of statistically significant publication bias). All statistical tests were performed with Review Manage, version 5.0, except the publication bias test. The publication bias test was performed with Stata 10.0 (Stata Corporation, College Station, TX, USA).

RESULTS

According to the inclusion criteria defined above, we identified 12 independent studies in 8 eligible reports (Panguluri et al., 2004; Shahedi et al., 2006; Cheng et al., 2007; Danforth et al., 2008; Murad et al., 2009; Balistreri et al., 2010; Dossus et al., 2010; Wu et al., 2011), including 14,282 cases and 16,277 controls. All 12 studies included were written in English. Twelve independent studies consisted of 8 Caucasian, 1 Asian, and 3 African populations. The main characteristics for all eligible studies are listed in Table 1.

Meta-analysis databases

PTGS2 rs20417

There are 9 studies (10,700 cases and 13,021 controls) analyzing the relationship between PTGS2 rs20417 polymorphism and the risk of PCa. In the overall population, there was significant heterogeneity in rs20417 polymorphism for the dominant model comparison, and the C allele *vs* G allele, except for the additive model and recessive model comparisons. After subgroup analyses by ethnicity, significant heterogeneity was only detected in Africans. We did not detect an association between rs20417 polymorphism and PCa risk in the overall population when examining the contrast of CC *vs* GG, CC *vs* GC + GG, CC + GC *vs* GG, and C allele *vs* G allele (OR = 1.00, 95%CI = 0.86-1.17, P = 0.96; OR = 1.00, 95%CI = 0.86-1.16, P = 1.00; OR = 0.92, 95%CI = 0.75-1.12, P = 0.40, and OR = 0.92, 95%CI = 0.76-1.10, P = 0.34, respectively). In subgroup analyses stratified by ethnicity, no noteworthy associations were observed in Caucasians and Africans. The detailed data are presented in Table 2.

Table 1. Main characteristics of studies included in this meta-analysis.

First author	Country	Cases	Controls	Ethnicity	rs20417 cases			rs20417 controls			rs689470 cases			rs689470 controls		
					GG	GC	CC	GG	GC	CC	CC	CT	TT	CC	CT	TT
Balistreri et al., 2010	Italy	50	125	Caucasian	31	15	4	65	46	14						
Cheng et al., 2007	USA	416	417	Caucasian	294	115	7	293	113	11	397	18	1	401	15	1
Cheng et al., 2007	USA	89	88	African	38	42	9	38	38	12	25	39	25	33	43	12
Dossus et al., 2009	USA, Europe	7975	8566	Caucasian (74%)	5561	2155	259	5999	2299	268						
Murad et al., 2009	UK	1592	3028	Caucasian	1104	451	37	2137	819	72						
Panguluri et al., 2004	USA	124	163	African	106	14	4	155	7	1						
Panguluri et al., 2004	USA	90	90	Caucasian	88	2	0	90	0	0						
Panguluri et al., 2004	Nigeria	146	108	African	134	10	2	82	22	4						
Wu et al., 2011	Taiwan	218	436	Asian	198	20	0	365	71	0						
Danforth et al., 2008	USA (PLCO)	1143	1384	Caucasian							1096	46	1	1321	63	0
Danforth et al., 2008	USA (Nutrition Cohort)	1096	1103	Caucasian							1054	41	1	1066	37	0
Shahedi et al., 2006	Sweden	1343	769	Caucasian							1279	64	0	711	57	1

Table 2. Meta-analysis of PTGS2 rs20417 and rs689470 polymorphisms and prostate cancer.

Genetic model (No. of studies)	Sample size		Analysis model	Test of association		P value for heterogeneity	P value (Egger test)
	Case	Control		OR (95%CI)	P		
rs20417							
Total (9)							
CC *vs* GG (7)	7590	9151	F	1.00 (0.86-1.17)	0.96	0.38	0.411
CC *vs* GC + GG (7)	10392	12495	F	1.00 (0.86-1.16)	1.00	0.47	0.422
CC + GC *vs* GG (9)	10700	13021	R	0.92 (0.75-1.12)	0.40	0.0005	0.579
C *vs* G (9)	21400	26042	R	0.92 (0.76-1.10)	0.34	0.0002	0.554
Caucasian (5)							
CC *vs* GG (4)	7297	8859	F	1.01 (0.86-1.18)	0.90	0.62	
CC *vs* GC + GG (4)	10033	12136	F	1.01 (0.86-1.18)	0.92	0.69	
CC + GC *vs* GG (5)	10123	12226	F	1.02 (0,96-1.08)	0.52	0.55	
C *vs* G (5)	20246	24452	F	1.02 (0.97-1.07)	0.55	0.52	
African (3)							
CC *vs* GG (3)	293	292	F	0.88 (0.42-1.84)	0.73	0.11	
CC *vs* GC + GG (3)	359	359	F	0.86 (0.42-1.75)	0.67	0.15	
CC + GC *vs* GG (3)	359	359	R	0.97 (0.27-3.43)	0.96	0.0001	
C *vs* G (3)	718	718	R	0.98 (0.31-3.11)	0.97	<0.0001	
rs689470							
Total (5)							
TT *vs* CC (5)	3879	3546	F	2.15 (1.04-4.44)	0.04	0.56	0.355
TT *vs* CC + CT (5)	4087	3761	F	2.07 (1.07-4.03)	0.03	0.60	0.398
TT + CT *vs* CC (5)	4087	3761	R	0.97 (0.71-1.33)	0.86	0.07	0.112
T *vs* C (5)	8174	7522	R	1.02 (0.72-1.46)	0.90	0.01	0.403
Caucasian (4)							
TT *vs* CC (4)	3829	3501	F	1.16 (0.30-4.54)	0.83	0.55	
TT *vs* CC + CT (4)	3998	3673	F	1.17 (0.30-4.57)	0.82	0.56	
TT + CT *vs* CC (4)	3998	3673	F	0.86 (0.69-1.07)	0.18	0.12	
T *vs* C (4)	7996	7346	F	0.86 (0.70-1.07)	0.18	0.11	

OR = odds ratio; CI = confidence interval; R = random effect model; F = fixed effect model.

PTGS2 rs689470

Five independent studies with a total of 4087 cases and 3761 controls were included in the meta-analysis for the PTGS2 rs689470 polymorphism. There was no significant heterogeneity except in dominant model comparison and T *vs* C in the overall population. After subgroup analyses by ethnicity, significant heterogeneity was effectively removed in Caucasians. In overall population analyses, a significant effect on susceptibility to PCa was observed under the additive model (OR = 2.15, 95%CI = 1.04-4.44, P = 0.04; Figure 1) and recessive model (OR = 2.07, 95%CI = 1.07-4.03, P = 0.03; Figure 2). There was no significant association between rs689470 polymorphism and PCa susceptibility when examining dominant contrast and allelic contrast (OR = 0.97, 95%CI = 0.71-1.33, P = 0.86; OR = 1.02, 95%CI = 0.72-1.46, P = 0.90). In subgroup analysis, no significant association with PCa risk was found in Caucasians. The detailed data are presented in Table 2.

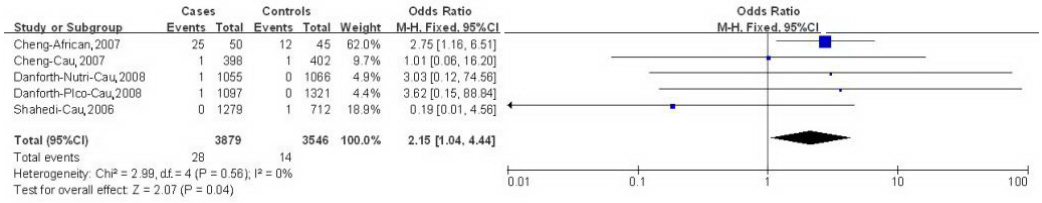

Figure 1. Forest plots of odds ratio with 95%CI (confidence interval) for the PTGS2 rs689470 polymorphism and risk of prostate cancer in overall population (TT *vs* CC).

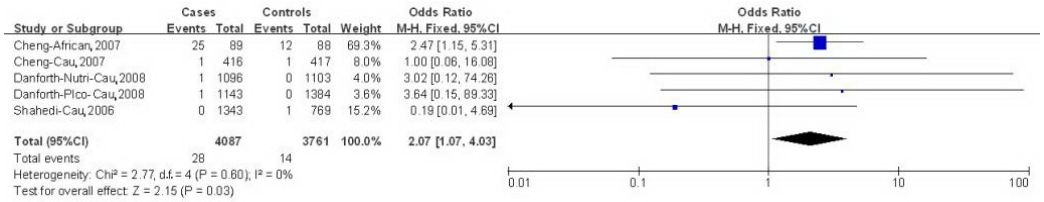

Figure 2. Forest plots of odds ratio with 95%CI (confidence interval) for the PTGS2 rs689470 polymorphism and risk of prostate cancer in overall population (TT *vs* CC + CT).

Publication bias

Begg's funnel plot and the Egger test were performed to assess publication bias. The shape of the funnel plots did not reveal any evidence of obvious asymmetry in all comparison models, and the Egger test was used to provide statistical evidence of funnel plot symmetry. The results did not show any evidence of publication bias. The detailed data are shown in Table 2.

DISCUSSION

Previous results suggested that single nucleotide polymorphisms are the most common sources of human genetic variation, and that they may contribute to an individual's susceptibility to cancer (Wu et al., 2009). In recent years, interest in the genetic susceptibility to cancers has led to a growing attention to the study of polymorphisms of genes involved in tumorigenesis. Since the identification of PTGS2 rs20417 and rs689470 polymorphisms, a growing number of studies suggested that the polymorphisms in the PTGS2 gene play important roles in the development of PCa. Epidemiological studies of the two polymorphisms in PTGS2, if large and unbiased, can provide insight into the *in vivo* relationship between the gene and PCa risk. However, these studies have appeared in the literature either supporting or refuting a significant association. Some reviewed studies are limited by their sample size and consequently suffer from having a power too low to detect effects that may exist. However, the pool ORs generated from a much larger population can increase the statistical power. Combining data from many studies have the advantage of reducing random error (Ioannidis et al., 2008). In order to provide a comprehensive and reliable conclusion, we performed the present meta-analysis of 11 independent case-control studies, including 14,282 cases and 16,277 controls.

Several studies have examined the associations between PTGS2 polymorphisms and other cancers. Meta-analysis results showed that the rs20417 polymorphism is significantly associated with esophageal squamous cell carcinoma and adenocarcinoma susceptibility, especially in Asians (Liang et al., 2011). Similarly, the rs20417 polymorphism has been associated with an increased risk of colorectal cancer among Asian population (Cao et al., 2010). The meta-analysis by Liu et al. (2010) suggested that the rs20417 polymorphism may be associated with gastric cancer susceptibility. However, one meta-analysis suggested a lack of association between the rs20417 polymorphism and breast cancer risk (Yu et al., 2010). In the present meta-analysis, it was found that the rs20417 polymorphism is not a risk factor for PCa

on the basis of all eligible studies. When stratifying for race, a consistent result was found. For rs689470, our results indicated that the rs689470 is associated with PCa in the overall population. However, no significant associations were found in Caucasians for all genetic models, suggesting a possible role of ethnic differences in genetic backgrounds and the environment in which they lived. There are many factors influencing the result, such as differences in populations, selection factors and so on. Considering the limited studies and population numbers of Africans and Asians included in the meta-analysis, our results should be interpreted with caution.

Heterogeneity is a potential problem when interpreting the results of the present meta-analysis. In overall analysis, significant between-study heterogeneity existed in dominant model comparison and allelic contrast. After subgroup analyses by ethnicity, the heterogeneity was effectively removed in Caucasians. The reason may be that differences in genetic backgrounds and the environment existed among different ethnicities.

Some limitations of this meta-analysis should be acknowledged. First, because only published and English articles were included in the meta-analysis, publication and potential English language biases might have occurred, even though it was not determined by the use of statistical tests. Second, although most of the controls were selected mainly from healthy populations, the result should be cautiously interpreted because controls were not uniformly defined. Third, in the subgroup analyses, the number of Asians and Africans was relatively small, where there was not enough statistical power to explore the true association. It is quite important to have more studies and samples of Asians and Africans in the future, so that a more precise conclusion about the associations between rs20417 and rs689470 polymorphisms and PCa risk can be achieved. Fourth, our results were based on unadjusted estimates, while a more precise analysis should be conducted if individual data were available, which would allow for an adjustment estimate by confounding factors.

In conclusion, significant associations were not detected between rs20417 and the risk of PCa in the overall population, Caucasians and Africans. For rs689470 polymorphism, our meta-analysis suggests that there is an association between rs689470 polymorphism and increased PCa risk in the overall population, but not in Caucasians. Due to the limitations in this analysis noted above, it is critical that larger and well-designed multicenter studies be conducted to confirm our results.

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REFERENCES

Balistreri CR, Caruso C, Carruba G, Miceli V, et al. (2010). A pilot study on prostate cancer risk and pro-inflammatory genotypes: pathophysiology and therapeutic implications. *Curr. Pharm. Des.* 16: 718-724.

Cao H, Xu Z, Long H, Li XQ, et al. (2010). The -765C allele of the cyclooxygenase-2 gene as a potential risk factor of colorectal cancer: a meta-analysis. *Tohoku J. Exp. Med.* 222: 15-21.

Cheng I, Liu X, Plummer SJ, Krumroy LM, et al. (2007). COX2 genetic variation, NSAIDs, and advanced prostate cancer risk. *Br. J. Cancer* 97: 557-561.

Danforth KN, Hayes RB, Rodriguez C, Yu K, et al. (2008). Polymorphic variants in PTGS2 and prostate cancer risk: results from two large nested case-control studies. *Carcinogenesis* 29: 568-572.

DerSimonian R and Laird N (1986). Meta-analysis in clinical trials. *Control Clin. Trials* 7: 177-188.
Dossus L, Kaaks R, Canzian F, Albanes D, et al. (2010). PTGS2 and IL6 genetic variation and risk of breast and prostate cancer: results from the Breast and Prostate Cancer Cohort Consortium (BPC3). *Carcinogenesis* 31: 455-461.
Egger M, Davey SG, Schneider M and Minder C (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315: 629-634.
Gupta S, Srivastava M, Ahmad N, Bostwick DG, et al. (2000). Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma. *Prostate* 42: 73-78.
Hiatt RA, Armstrong MA, Klatsky AL and Sidney S (1994). Alcohol consumption, smoking, and other risk factors and prostate cancer in a large health plan cohort in California (United States). *Cancer Causes Control* 5: 66-72.
Hori S, Butler E and McLoughlin J (2011). Prostate cancer and diet: food for thought? *BJU Int.* 107: 1348-1359.
Ioannidis JP, Boffetta P, Little J, O'Brien TR, et al. (2008). Assessment of cumulative evidence on genetic associations: interim guidelines. *Int. J. Epidemiol.* 37: 120-132.
Kirschenbaum A, Klausner AP, Lee R, Unger P, et al. (2000). Expression of cyclooxygenase-1 and cyclooxygenase-2 in the human prostate. *Urology* 56: 671-676.
Kosaka T, Miyata A, Ihara H, Hara S, et al. (1994). Characterization of the human gene (PTGS2) encoding prostaglandin-endoperoxide synthase 2. *Eur. J. Biochem.* 221: 889-897.
Lee LM, Pan CC, Cheng CJ, Chi CW, et al. (2001). Expression of cyclooxygenase-2 in prostate adenocarcinoma and benign prostatic hyperplasia. *Anticancer Res.* 21: 1291-1294.
Liang Y, Liu JL, Wu Y, Zhang ZY, et al. (2011). Cyclooxygenase-2 polymorphisms and susceptibility to esophageal cancer: a meta-analysis. *Tohoku J. Exp. Med.* 223: 137-144.
Liu JL, Liang Y, Wang ZN, Zhou X, et al. (2010). Cyclooxygenase-2 polymorphisms and susceptibility to gastric carcinoma: a meta-analysis. *World J. Gastroenterol.* 16: 5510-5517.
Madaan S, Abel PD, Chaudhary KS, Hewitt R, et al. (2000). Cytoplasmic induction and over-expression of cyclooxygenase-2 in human prostate cancer: implications for prevention and treatment. *BJU Int.* 86: 736-741.
Mahmud S, Franco E and Aprikian A (2004). Prostate cancer and use of nonsteroidal anti-inflammatory drugs: systematic review and meta-analysis. *Br. J. Cancer* 90: 93-99.
Mantel N and Haenszel W (1959). Statistical aspects of the analysis of data from retrospective studies of disease. *J. Natl. Cancer Inst.* 22: 719-748.
Masferrer JL, Leahy KM, Koki AT, Zweifel BS, et al. (2000). Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer Res.* 60: 1306-1311.
McArdle PA, Mir K, Almushatat AS, Wallace AM, et al. (2006). Systemic inflammatory response, prostate-specific antigen and survival in patients with metastatic prostate cancer. *Urol. Int.* 77: 127-129.
Murad A, Lewis SJ, Smith GD, Collin SM, et al. (2009). PTGS2-899G>C and prostate cancer risk: a population-based nested case-control study (ProtecT) and a systematic review with meta-analysis. *Prostate Cancer Prostatic Dis.* 12: 296-300.
O'Byrne KJ and Dalgleish AG (2001). Chronic immune activation and inflammation as the cause of malignancy. *Br. J. Cancer* 85: 473-483.
Panguluri RC, Long LO, Chen W, Wang S, et al. (2004). COX-2 gene promoter haplotypes and prostate cancer risk. *Carcinogenesis* 25: 961-966.
Shacter E and Weitzman SA (2002). Chronic inflammation and cancer. *Oncology* 16: 217-26, 229.
Shahedi K, Lindstrom S, Zheng SL, Wiklund F, et al. (2006). Genetic variation in the COX-2 gene and the association with prostate cancer risk. *Int. J. Cancer* 119: 668-672.
Tsujii M and DuBois RN (1995). Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 83: 493-501.
Uotila P, Valve E, Martikainen P, Nevalainen M, et al. (2001). Increased expression of cyclooxygenase-2 and nitric oxide synthase-2 in human prostate cancer. *Urol. Res.* 29: 23-28.
Wu GY, Hasenberg T, Magdeburg R, Bonninghoff R, et al. (2009). Association between EGF, TGF-beta1, VEGF gene polymorphism and colorectal cancer. *World J. Surg.* 33: 124-129.
Wu HC, Chang CH, Ke HL, Chang WS, et al. (2011). Association of cyclooxygenase 2 polymorphic genotypes with prostate cancer in taiwan. *Anticancer Res.* 31: 221-225.
Yoshimura R, Sano H, Masuda C, Kawamura M, et al. (2000). Expression of cyclooxygenase-2 in prostate carcinoma. *Cancer* 89: 589-596.
Yu KD, Chen AX, Yang C, Qiu LX, et al. (2010). Current evidence on the relationship between polymorphisms in the COX-2 gene and breast cancer risk: a meta-analysis. *Breast Cancer Res. Treat.* 122: 251-257.