



Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma (PPAR- γ) gene and risk of prostate cancer among men in a large cancer prevention study

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Abstract

The nuclear hormone receptor peroxisome proliferator-activated receptor-gamma (PPAR- γ) may play a role in prostate carcinogenesis. We examined the association between the PPAR- γ *Pro12Ala* polymorphism and prostate cancer risk in a cohort of Finnish male smokers. In a nested case-control analysis that included 193 prostate cancer cases and 188 matched controls, we found no significant association between this polymorphism and prostate cancer risk (odds ratio, OR = 1.27, 95% confidence interval, CI: 0.83–1.94), or significant trend or association with tumor stage (OR = 1.28, 95% CI: 0.54–3.04 for metastatic disease) or grade (OR = 1.57, 95% CI: 0.63–3.91 for poorly differentiated disease). The *Pro12Ala* polymorphism does not appear to play a significant role in prostate cancer risk in this cohort of men.

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1. Introduction

The peroxisome proliferator-activated receptor (PPAR) is a nuclear hormone receptor that belongs to the family of ligand-activated transcription factors [1]. Three distinct isoforms, PPAR- α , PPAR- β , and

PPAR- γ , have been identified, each with a specific pattern of expression. These receptors are activated by dietary factors such as fatty acids and their metabolites [2], and may also be involved in controlling genes relevant to the process of carcinogenesis [3]. PPAR- γ specifically plays an important role in adipocyte differentiation and is involved in the uptake, metabolism and storage of fatty acids [2]. Physiologically, activation of PPAR- γ decreases the production of glucose in the liver, increases muscle utilization of glucose, and increases the efflux of cholesterol that is mediated by macrophages [2], and controls cell proliferation and differentiation [4]. PPAR- γ has

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been implicated to play a role in the progression of prostate cancer. Both normal and malignant prostatic tissues have been shown to express PPAR- γ [5–7]. Heterozygous deletions in the 3p25 region containing PPAR- γ have been found in up to 30% of patients with prostate cancer [4]. Further, data have indicated that ligand activation of PPAR- γ inhibits growth, induces apoptosis, and modulates prostate specific antigen (PSA) levels in prostate cancer cell lines, and stabilizes PSA levels in prostate cancer patients [8].

Pro12Ala is a missense mutation in the PPAR- γ_2 specific exon whereby proline (Pro) has been substituted for alanine (Ala) in codon 12. The frequency of the PPAR- γ_2 Ala allele in Caucasian populations, including Finnish populations, has been shown to range from 0.11 to 0.22 [9–12]. In humans, the Ala12 allele has been associated with physiologic changes such as weight gain over time and alterations in body mass index (BMI) [7, 11,13], increased insulin sensitivity [10,14], and altered lipid profiles [15], thus possibly implicating this polymorphism in cardiovascular disease risk. These findings, however, are not consistent across the literature.

The fact that PPAR- γ is expressed in malignant prostatic tissue and that its ligands have been shown to inhibit prostate cancer cell growth and stabilize serum PSA levels over time in patients [8] led us to examine the *Pro12Ala* polymorphism and its association with prostate cancer. In this investigation, we assess the relationship of the *Pro12Ala* germline polymorphism in the PPAR- γ_2 gene with prostate cancer risk in participants of the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study, a large, randomized intervention trial conducted in Finland. As a secondary analysis, we also examined the association between this polymorphism and the clinical aggressiveness of prostate cancer, as measured by stage and grade of disease.

2. Materials and methods

2.1. Study subjects

This study was conducted in a cohort of the 29, 133 white male smokers who participated in the ATBC Study conducted in Finland (1985–1993). The ATBC

Study was a randomized, placebo-controlled primary prevention trial that assessed whether supplementation with α -tocopherol (50 mg/day) or β -carotene (20 mg/day), or both, reduced the incidence of lung and other cancers among men that smoked at least five cigarettes per day [16]. This study received institutional review board approval from both the National Cancer Institute (USA) and the National Public Health Institute of Finland. At baseline, a fasting blood sample was collected from all subjects as well as medical, demographic and other information. Prior to randomization, study participants were administered a food-use questionnaire with a portion size booklet to obtain dietary information, and a serum sample was collected. The overall design, rationale, objectives and main findings of the ATBC Study have been published [16]. The results of the trial indicated that incidence of lung cancer among study subjects increased 16% for those in the β -carotene supplement group, and that the incidence of prostate cancer was reduced by 32% for those in the α -tocopherol supplement group [17,18].

A nested case-control sample group was selected from ATBC study participants who provided a whole blood sample between April 1992 and March 1993 ($n = 20\,305$). Incident prostate cancer cases ($n = 208$) diagnosed up to December 31, 1994 were identified through the Finnish Cancer Registry and the Register of Causes of Death (ICD9-185). Cases were detected by abnormal prostate findings on clinical examination or by symptoms; approximately 73% of the cases were identified by symptoms due to local tumor effects. PSA was not used to screen for prostate cancer in this population of men. Study oncologists centrally reviewed the medical records of the incident cases, and pathologists reviewed histopathologic specimens. Information of Tumour node (TNM) staging and Gleason grade based on the American Joint Committee on Cancer (1992) were available for 181 (94%) and 163 (86%) of the prostate cancer cases, respectively. Controls ($n = 208$) were frequency matched to cases on age (± 5 years), intervention group, and study clinic.

2.2. DNA isolation and PPAR- γ genotyping analysis

DNA was isolated from whole blood samples and matrix assisted laser desorption/ionization-time-of-

flight mass spectrometry (MALDI-TOF) (Sequenom, San Diego, California) was used to genotype for the PPAR- γ polymorphism. This method generates allele-specific products with mass differences that can be determined using mass spectrometry [19,20]. The initial step of MALDI-TOF involves polymerase chain reaction (PCR) amplification of the PPAR- γ gene region containing the polymorphism followed by allele-specific primer extension using extension primers that create two products differing by 1–3 nucleotides 3' to the single nucleotide polymorphism. The allele-specific extension products are then distinguished by mass spectrometry. PCR of the PPAR- γ gene containing the *Pro12Ala* polymorphic regions was performed using 5'-ACGTTGGATGCAAACCCCTATTCCATGCTG as the sense primer and 3'-ACGTTGGATGTGTATCAGTGAAGGAATCGC as the antisense primer. All PCR reactions were performed in 5 μ l volume using 2.5 ng of genomic DNA, 1 \times PCR buffer (Qiagen), 2.5 mM MgCl₂, 200 μ M of each dNTP, 0.1 unit of Taq Polymerase (Qiagen) and 200 nM each primer. Thermal conditions were 95°C for 15 min, 45 cycles of 95°C for 20 s, 56°C for 30 s and 72°C for 2 min with a final extension of 72°C for 3 min. Purified PCR products were used for the primer extension reaction after addition of extension mix (9 l), containing 600 nM extension primer (5'-TCTGGGAGATTCTCC-TATTGAC), 50 μ M each of ddATP, ddCTP, ddTTP, and dGTP, 1 \times buffer, and 0.064 unit of Thermo-Sequenase (Sequenom, San Diego, CA). Primer extension reactions were performed at 94°C for 2 min followed by 40 cycles of 94°C for 5 s, 52°C for 5 s and 72°C for 5 s. Primer extension products were purified using SpectroCLEAN resin (Sequenom). Approximately 14 nanoliters of each extension product was transferred onto a silicon microchip forming a crystalline matrix that is then pulsed with ultraviolet light, thus ionizing the analyte molecules. Raw spectral data was transferred from the Spectro-READER Biflex detector (Sequenom) into a pre-programmed template using SpectroTYPER software (Sequenom) in order to assign genotype probabilities based on programmed assay definitions and calculated mass differences. Batches containing equal number of cases and controls, and negative controls (PCR reagents without DNA), were genotyped. For quality control purposes, genotyping for a random sample of

study samples ($n = 121$) was repeated and showed 90% concordance. Only six samples were not concordant and misclassification was not differential by case-control status, and thus did not bias the genotyping results. The study was conducted in a population unscreened for prostate cancer, and there is no reason to believe that genotyping is correlated with PSA levels, thus limiting any bias by genotype for undetected prostate cancers. Clinical symptoms used in the identification of prostate cancer cases did not differ between wildtype and variant genotypes. Genotyping was successful for 193 cases and 189 controls, and this group comprises the study population used in the data analyzes.

2.3. Statistical analysis

Differences in demographic and medical characteristics by case/control status were tested using the chi-square test for heterogeneity for categorical variables, and the Wilcoxon rank sum test for continuous variables. Logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between genotype and prostate cancer incidence, adjusting for confounders. The homozygous wildtype genotype was used as the reference for total prostate cancer. When the association between PPAR- γ and prostate cancer was assessed by stage and grade of disease (among the cases only), localized and well-differentiated prostate cancer were used as the reference categories, respectively. Baseline covariates were identified as potential confounders by examining their distribution by case-control status. They were included in the model if they changed the OR by more than 20% or significantly changed the likelihood ratio statistic ($P < 0.05$). Effect modification of the association between the *Pro12Ala* polymorphism and prostate cancer was tested by including a cross-product interaction term of the PPAR- γ genotype and selected covariates (e.g. age, BMI, and dietary fats) in the regression model, and examining any change in the likelihood ratio statistic. Trends were calculated across three categories for stage and grade, and the significance level was set at $P < 0.05$. All statistical analyzes were performed with SAS software (SAS Institute, Cary, NC).

Table 1

Demographic, dietary and medical characteristics of study participants by prostate cancer case status

	Cases (<i>n</i> = 193)	Control (<i>n</i> = 188)	<i>P</i> -value ^a
Age at randomization (years) ^b	60.5 (5.2)	60.6 (4.9)	0.78
α-tocopherol supplementation group (%)	42.0	42.5	0.92
Body mass index (kg/m ²) ^b	26.3 (8.3)	25.8 (8.3)	0.11
Total cigarettes/day ^b	18.6 (8.3)	18.6 (8.7)	0.96
Energy (kcal/day) ^b	2724.8 (724)	2812.3 (825)	0.40
History of diabetes mellitus (%)	5.2	2.1	0.17
History of coronary heart disease (%)	6.7	11.2	0.13
Total fat (g/day) ^b	119.6 (37.8)	123.3 (43.1)	0.62
Total fatty acids (g/day) ^b	98.7 (31.9)	101.6 (36.2)	0.60
Polyunsaturated N-3 fatty acids (g/day) ^b	2.1 (0.9)	2.2 (0.9)	0.15
Polyunsaturated N-6 fatty acids (g/day) ^b	9.7 (6.3)	10.2 (6.7)	0.55
Triglycerides (g/day) ^b	103.2 (33.4)	106.2 (37.9)	0.60
Cholesterol (mg/day) ^b	564.0 (198)	564.1 (240)	0.46

^a Determined from Wilcoxon Rank Sum Test or χ^2 Test for heterogeneity.^b Values presented as mean (standard deviation).

3. Results

Incident prostate cancer cases and their matched controls did not differ statistically by various demographic, dietary or medical characteristics (Table 1). For the prostate cancer cases, the mean age at prostate cancer diagnosis was 66.1 ± 5.4 years, with an average time to diagnosis of 5.5 ± 2.1 year from baseline.

We observed no significant differences in the distribution of the PPAR- γ genotype among the cases and controls (Table 2). The distribution among our control population is similar to that observed in other populations of Caucasian men [12] and is in Hardy–Weinberg equilibrium. Due to the small proportion of subjects who were homozygous variant (4%), we combined the heterozygous and homozygous variant genotypes to represent the variant genotype for the

Table 2

Frequencies of PPAR- γ genotype among prostate cancer cases and controls (A), and association between PPAR- γ polymorphism and prostate cancer risk (B)

(A)	Cases, No. (%)	Controls, No. (%)
<i>Pro12Pro</i> (wildtype)	121 (62.7)	128 (68.1)
<i>Pro12Ala</i> (heterozygous)	64 (33.2)	54 (28.7)
<i>Ala12Ala</i> (homozygous variant)	8 (4.2)	6 (3.2)
(B)	Cases, No. (%)	OR (95% CI) ^a
Wildtype	121 (63.0)	1.0 (reference)
Variant ^b	72 (37.5)	
Univariate		1.27 (0.83–1.94)
Multivariate ^c		1.24 (0.80–1.92)

^a Estimated from a logistic regression model.^b Represents both the heterozygous and homozygous variant.^c Estimated from a logistic regression model after adjusting for age at randomization, BMI, total fatty acids, carbohydrates, total cholesterol, and total fat.

Table 3
Association between PPAR- γ polymorphism and stage and grade of prostate cancer

	Variant allele cases, No. (%)	OR (95% CI) ^a
Stage ^b	(<i>n</i> = 72)	
Localized (0–II)	47 (65.3)	1.00 (reference)
Regional (III)	5 (6.9)	0.55 (0.17–1.80)
Metastatic (IV)	20 (27.8)	1.28 (0.54–3.04)
		<i>P</i> = 0.71 (trend)
Grade ^c	(<i>n</i> = 62)	
Well differentiated	21 (33.9)	1.00 (reference)
Moderately differentiated	29 (46.8)	1.48 (0.73–3.00)
Poorly differentiated	12 (19.3)	1.57 (0.63–3.91)
		<i>P</i> = 0.33 (trend)

^a Estimated from an unadjusted logistic regression model; adjusting for potential confounders did not alter the associations.

^b Based on information of TNM stage obtained from 181 cases: localized (stage T0–T2), regional (stage T3), metastatic (stage T4).

^c Based on Gleason grade obtained from 163 cases: Well differentiated (Gleason 2–4), Moderately differentiated (Gleason 5–7), Poorly differentiated (Gleason 8–10).

risk analysis. All associations reported are in reference to the homozygous wild-type genotype.

The unadjusted risk of prostate cancer associated with the variant alleles was 27% greater than that for the wild type as shown in Table 2, however, this association was not significant (OR = 1.27, 95% CI: 0.83–1.94). Baseline anthropometric and dietary intake factors (e.g. age, BMI, dietary fats) did not confound the association between the variant genotype and prostate cancer. Further, among the baseline covariates examined, we observed no significant gene-environment interaction (*P* > 0.05). The study population is relatively genetically homogeneous and therefore study site was not included as a potential confounder. Further, risk of prostate cancer among those with the variant allele did not differ between men who were in (OR = 1.18, 95% CI: 0.60–2.22) or not in (OR = 1.36, 95% CI: 0.78–2.37) the α -tocopherol supplementation treatment group. Given the sample size of our study and the prevalence of the PPAR- γ genotypes in our control population, we had 80% power (two-sided test of significance, α = 0.05) to detect an odds ratio as low as 1.8 for carriers of at least one variant allele (versus no variant alleles) for the PPAR- γ *Pro12Ala* polymorphism.

In a secondary analysis among prostate cancer cases, we assessed the association of the variant allele with clinical stage and tumor grade of disease (Table 3). Our findings indicate that having the variant allele diminished the risk of regional disease by 45%, but

that this relationship was not statistically significant (OR = 0.55, 95% CI: 0.17–1.80) and only five men with the variant allele were diagnosed with regional disease. No apparent association existed between the variant allele and the risk of metastatic disease (OR = 1.28, 95% CI: 0.54–3.04). In examining tumor grade, the risk of more poorly differentiated cancer increased for those men with the variant allele, although the risk was not statistically significant (OR = 1.57, 95% CI: 0.63–3.91). Tests of the linear trend for risk across both tumor grade and stage were not significant (*P* > 0.05, Table 3).

4. Discussion

In the present investigation, we assessed the association between the *Pro12Ala* polymorphism in the PPAR- γ_2 gene and prostate cancer among participants in the ATBC Cancer Prevention Study. We did not observe a significant association between the variant genotype and risk of prostate cancer overall or between the genotype and disease stage or grade.

Epithelial tissues of several human cancers have been found to express the PPAR- γ subtype including colon cancer, breast cancer, and prostate cancer [4]. Studies have shown that PPAR- γ can act as a tumor suppressor by playing a role in cell cycle withdrawal and by promoting cell differentiation. Controversy

exists regarding PPAR- γ since it may also act as a tumor promoter [21–23] by exhibiting dual action on cell processes such as differentiation, cell cycle regulation, and apoptosis. Further, the tumor suppressor activity of PPAR- γ may be cell-type specific, perhaps due to the presence or absence of co-factors in different cell types [23]. Recently, Segawa and associates [5] reported that PPAR- γ is strongly expressed in tissue samples of prostate cancer and prostatic intraepithelial neoplasia, but only weakly expressed in benign prostatic hypertrophy and normal prostatic tissue. They speculated that the process of cell differentiation may be the mechanism by which PPAR- γ ligands mediate anti-proliferation in prostate cancer cells. Such data suggest that the PPAR- γ receptor may be well suited as a prostate cancer molecular target for therapeutic approaches utilizing differentiating agents [5].

Ligands of PPAR- γ , both natural and synthetic (e.g. the glitazones, polyunsaturated fatty acids, arachidonic acid metabolites, and endogenous prostaglandins), may bind to and/or activate the receptor, thus facilitating the regulation of fatty acid metabolism and transcription of apolipoproteins [24–26]. Synthetic PPAR- γ ligands have been used clinically to treat conditions associated with Type II diabetes mellitus [4,27]. A phase II clinical trial using troglitazone, a PPAR- γ ligand developed to treat insulin resistance, reported that men with histologically confirmed prostate cancer that took troglitazone maintained stable PSA levels for up to 18 weeks [8]. Experimentally, troglitazone has also been shown to decrease PSA production by 50% and induce differentiation in prostate cancer cell lines, and significantly inhibit prostate tumor growth in mice [6]. It has also been reported to produce necrosis in human prostate tumors but not normal adjacent tissue [6], possibly through the inhibition of androgen signaling [28]. Loss of the 15-lipoxygenase-2 pathway through the inactivation of PPAR- γ has also been implicated in prostate carcinoma [29]. Levels of polyunsaturated and essential fatty acid levels have been shown to be higher in less aggressive prostate cancerous tissue [30]. Further, consumption of fatty fish, which contains high levels of the polyunsaturated fatty acid docosahexanoic acid, a natural PPAR- γ agonist, has been suggested to decrease the risk of prostate cancer [31].

The substitution of proline for alanine in the PPAR- γ_2 gene (*Pro12Ala* polymorphism) has been associated with clinical outcomes and physiologic changes [7,10,11,13–15]. Stumvoll et al. [32] have hypothesized that the polymorphism may alter transcriptional activity and lead to suppression of lipolysis, thus increasing insulin sensitivity and decreasing the availability of free fatty acids. A single nucleotide polymorphism in the PPAR- γ gene may have implications in prostate carcinogenesis. Because the variant allele is less transcriptionally active than the wild-type [10], it may be less responsive to ligand activation, and less capable of inducing prostate cell differentiation and inhibiting prostate cancer growth.

We found no direct association between the variant allele of PPAR- γ and both prostate cancer risk and the risk of more clinically aggressive disease in this population. In addition, the association between the PPAR- γ polymorphism and prostate cancer risk was not significantly modified by measures of pre-diagnostic dietary intake of free fatty acids, potential natural ligands of the receptor. Although our study had limited power to detect more modest associations between this polymorphism and prostate cancer risk as well as trends across stage and grade of disease, we can conclude from this analysis that the *Pro12Ala* polymorphism is not strongly associated with risk of prostate cancer, nor is it associated with the aggressive phenotype among the cases in this population of Finnish men. Similar studies should be performed in other ethnically diverse populations with larger sample sizes to either confirm the present study or determine the role that this polymorphism may play in prostate cancer risk and progression.

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