The Effect of Smoke Combined with the Multicancer Marker, Rs6983267, Located at Region 3 of Chromosome 8q24 in Prostate Cancer Patients

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Abstract

Introduction: Common variants on human chromosome 8q24 were found to be associated with prostate cancer risk with different frequency and incidence among the investigated populations. We examined the effect of smoke on this type of cancer and its relationship with the risk variant rs6983267, located at region 3 of chromosome 8q24, in a prostate cancer case-control study conducted in the Greek population in light, intermediate and heavy smokers.

Materials and methods: Samples of total blood from 74 patients with histologically confirmed prostate cancer and 24 healthy individuals were genotyped using real time polymerase chain reaction (PCR). Tumor-node-metastasis (TNM) stage, Gleason score and levels of prostate-specific antigen (PSA) at diagnosis were included in the analysis.

Results: Light (Packyears, PY<10) and heavy (PY>30) smokers are positive associated with prostate cancer, with an additive risk for the carriers of rs6983267 with positive smoking history (ORadj=21.36, C.I.=3.79-120.39) to develop the disease.

Conclusions: The SNP, rs6983267, has an independent risk for carriers to develop prostate cancer and in combination with smoke, it confers additive risk for the disease, similarly to others, well established risk factors such as age, family history and ethnicity.

Keywords: Prostate cancer; Smoke; 8q24; Rs6983267

Abbreviations: SNP: Simple Nucleotide Polymorphism; PSA: Prostate Specific Antigen; DRE-Digital Rectal Examination; PCR: Polymerase Chain Reaction; GWAS: Genome-Wide Association Studies; PAR: Population Attributable Risk; UADT: Upper Aero-Digestive Tract; FSH: Follicle Stimulating Hormone; IARC: International Agency for Research on Cancer; ROS: Reactive Oxygen Species; tPA: Tissue-type Plasminogen Activator; MMPs: Metalloproteinases

Introduction

The evidence for a genetic susceptibility to prostate cancer (PCA) has been well documented but the only firmly established risk factors for PCA are age, family history and ethnicity [1]. In an attempt to identify genetic variants underlying risk for PCA, genome-wide linkage and association studies (GWAS) have been performed and multiple chromosomal regions have been designated to harbour major susceptibility genes for PCA [2]. Three independent regions on chromosome 8q24 have been investigated thoroughly, at the beginning in small populations (Islander) and afterwards in bigger races like African-American, European-American, Japanese-American, Natives of Hawaii and Latinos, with different frequency and indecent for the PCA [3-6]. In men of European ancestry the single nucleotide polymorphism (SNP) rs6983267, at the region 3 of chromosome 8q24 has shown the highest odds ratio and population attributable risk (PAR) for PCA, compared to other SNPs at the same region [3]. Rs6983267 also has been associated with colorectal cancer, kidney and thyroid cancers and smoking-related cancers and thus was characterised as a multicancer marker with a genetic role yet to be clarified [7-9].

Smoke is a well established environmental carcinogen and it has been associated with many malignancies including the urinary track: bladder, prostate and kidney [10]. For PCAs there are conflicting data on the effect of cigarette smoking on serum levels of the various sex hormones and epidemiological studies have not compounded to a conclusive relationship. Smoke-mediated changes have been documented not only in endocrine pathways but also in enzymatic systems showing positive association between current smoking and fatal cancer of the prostate in many prospective cohort studies [11].

Methods

Study subjects

In total, 74 patients with PCAs and positive smoking history participated in the study. They were identified and recruited from the “Laiko” General University Hospital and from the “Gennimatas” General Hospital with an age distribution between 48-87 years. The inclusion criterion for case subjects was histological biopsy-confirmed adenocarcinoma of the PCa, diagnosed between October 2008 and January 2010. Tumor-node-metastasis (TNM) stage, Gleason score as...
determined by biopsy and levels of prostate-specific antigen (PSA) at diagnosis were available for all the patients.

Twenty-four control subjects with positive smoking history were recruited concurrently with case subjects, randomly selected from the «ΚΑΤ» General Hospital of Attika with an age distribution between 47-71 years. They were all healthy individuals, with PSA levels <4ng/ml, negative Digital Rectal Examination (DRE) and no family history.

Both case and control subjects provided written informed consent. The study received institutional approval from the scientific committee (Reference Number 697/17-10-2008).

Genotyping of SNP rs6983267

Whole EDTA blood samples (5ml) were drawn from all subjects and DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA). The yield was measured by spectrophotometry (NanoDrop, 1000, UK). Genotyping was performed by Real-time PCR using the Light Cycler 480 system (Roche Diagnostics, Germany) using the primers rs6983267 F: 5’-CTATCCATAAACAAGgggACgAAT and rs6983267 R: 5’-gggTTCTCtgCCCTTTgATT and the hybridization probes Sensor wt: 5’-TTTCTCAgTgCCCTTTCTgTgC—FL and anchor LC640- gCTCAAAggAgATgAgggTATTAACTCTg—PH, Fast Start hybridization probe master (Roche) running 45 cycles each 5 sec 95°C, 10 sec 60°C and 10 sec 72°C and a subsequent melting curve from 45°C to 85°C with a slope 0.2°C/sec and continuous fluorescence detection.

Statistical analysis

Qualitative variables are presented with absolute and relative frequencies, while quantitative variables are presented with mean (standard deviation). For the comparison of mean values between two groups, student’s t-test was used. For the comparison of proportions between the PCa and the control groups chi-square tests were computed. Logistic regression analysis was performed in order to evaluate the association of pack years with the presence of PCa. Adjusted odds ratios with 95% confidence intervals were performed from the results of logistic regression analysis. All reported p values are two-tailed. Statistical significance was set at p<0.05 and analyses were conducted using SPSS statistical software (version 17.0) (Figures 1-3).

**Results**

Data from 74 patients with PCa and 24 controls were analyzed. Sample characteristics of the PCa and control groups are presented in Table 1. Significantly greater mean age was found in the PCa group (67.5±6.6 vs. 56.6±9.4). The overall distribution of rs6983267 genotype TT (wild type), GT (heterozygotes), GG (homozygotes) was 18.9%, 54.1%, 27.0% in patients and 66.7%, 29.2%, 4.2% in controls, respectively. The proportion of homozygotes (GG) or heterozygotes (GT) was significantly greater for PCa group (p<0.001). Also, the proportion of subjects with pack years less than ten or more than thirty was significantly greater for PCa group (p<0.001). The proportion of homozygotes or heterozygotes for rs6983267 genotype for those with less than ten, ten to thirty and more than thirty pack years was 69.7%, 62.5% and 75.8% respectively (p=0.510).
Logistic regression analysis with dependent variable the presence of prostate cancer. Odds ratios (OR) and 95% confidence intervals (CI) derived from multiple linear regression analyses have indicated that rs6983267 may play a role in non-smoking related pathways for these cancers. The positive association with lung cancer among ever-smokers indicates that rs6983267 may play a role in non-smoking related pathways for these cancers. The positive association with lung cancer among ever-smokers indicates that rs6983267 may play a role in non-smoking related pathways for these cancers.

Table 1: Characteristics of the prostate cancer and control group.

| Characteristic     | Control | Cases | P     |
|-------------------|---------|-------|-------|
|                  | N(%)    | N(%)  | χ² test |
| Age (years), mean (SD) | 56.6(9.4) | 67.5(6.6) | <0.001* |
| Pack years        |         |       |       |
| <10               | 3(12.5) | 30(40.5) | <0.001* |
| 10-30             | 18(75)  | 14(18.9) |       |
| >30               | 3(12.5) | 30(40.5) |       |
| RT-PCR            |         |       |       |
| TT                | 16(66.7)| 14(18.9) | <0.001 |
| GT                | 7(28.2)| 40(54.1) |       |
| GG                | 1(4.2) | 20(27.0) |       |
| RT-PCR            |         |       |       |
| GT/GG             | 8(33.3)| 60(81.1) | <0.001 |

Table 2: Odds ratios (OR) and 95% confidence intervals (CI) derived from multiple logistic regression analysis with dependent variable the presence of prostate cancer.

| Characteristic | OR(95% CI) | P     |
|---------------|------------|-------|
| Age (years)   | 1.14(1.04-1.26) | 0.006 |
| Pack years    |            |       |
| 10-30         | 1.00‡      |       |
| <10           | 11.49(1.56-84.52) | 0.016 |
| >30           | 10.88(1.63-72.5) | 0.014 |
| RT-PCR        |            |       |
| TT            | 1.00       |       |
| GT/GG         | 21.21(3.75-119.92) | 0.001 |

*Student’s t-test

Discussion

In men with European ancestry, rs6983267 has shown the highest odds ratio and population attributable risk % (PAR%) for PCa, compared to other SNPs at the same region with little evidence of linkage disequilibrium with them and an overall population frequency in northern Europeans of 50% for the at risk allele [3]. When we examined rs6983267 in the Greek population, in a PCa case-control study, homozygotes or heterozygotes had 2.84 times greater likelihood for PCa (p=0.002) and the overall population frequency for the G allele was 61.85%. The carriers had almost two times greater odds for having the G allele (p=0.001) with a sensitivity for the disease of 81.40% [12].

Rs6983267 has also been associated with colorectal cancer, kidney, thyroid cancers and smoking-related cancers and so was characterised as a multicancer marker [7-9] with an important carcinogenetic role yet to be clarified. In the study of Lani Park et al. [9] note worthy are the associations between rs6983267 and the upper aero-digestive tract (UADT) cancers (ORadj=1.69, 95% CI=1.28, 2.24), particularly in oropharynx (ORadj=1.80, 95% CI=1.30, 2.49) and larynx (ORadj=2.04, 95% CI=1.12, 3.72). When they stratified the analysis by smoking status, rs6983267 was positively associated with lung cancer among ever-smokers (ORadj=1.45, 95% CI=1.05, 2.00) and inversely associated with bladder cancer among ever-smokers (ORadj=0.35, 95% CI=0.14, 0.83). The positive association with UADT cancers, independent of smoking status, indicates that rs6983267 may play a role in non-smoking related pathways for these cancers. The positive association with lung cancer among ever-smokers indicates that rs6983267 may also play an important role in tobacco related carcinogenesis involving target specific carcinogens and perhaps metabolic, DNA repair and other related pathways. In our study, light (PY<10) and heavy (PY>30) smokers were positively associated with PCa, independently of rs6983267, indicating probably different biological pathways in the process of carcinogenesis for these two factors regarding this type of cancer.

Smoking has an anti-estrogene effect mainly caused by dose-dependent elevated hepatic clearance of estrogens [13] and may also alter the metabolism of other sex steroids such as testosterone and adrenal hormones. Thereby, tobacco smoke may influence negatively the incidence of benign and malignant growth of the prostate gland [14]. In adolescent male humans it also decreases the serum levels of follicle-stimulating hormone (FSH) [15] while in male rats it has a direct toxic effect on Leydig cells reducing the production of testosterone [16,17].

The intermediate smokers (PY=10-30) haven’t been associated with the disease probably because this amount of inhaled smoke could provoke an inhibitory effect to hormonal pathways acting beneficially on the status of PCa development, without severe interference in DNA repair mechanisms [13-17]. Very light smokers (PY<10) or non-smokers were positively associated with prostate cancer, probably because of the existing effect of hormones in conjunction with other factors such as family history and rs6983267.

Heavy smokers (PY>30) were also positively associated with PCa, probably because smoke has a multiple effect in enzymatic systems aggravating the carcinogenic process. In that case smoke is influencing positively PCa susceptibility in conjunction with all the other established factors. Tobacco smoke is one of the most common sources of cadmium (Cd) in the general population. At the cellular level, cadmium affects proliferation, differentiation and causes apoptosis. It has been classified as a carcinogenic by the International Agency for Research on Cancer (IARC). Indirect effects of cadmium provoke generation of reactive oxygen species (ROS) and DNA damage. Cadmium modulates also specific gene expression and signal transduction and reduces activities of proteins involved in antioxidant defences [18].

Also, genetic polymorphisms in genes expressing phase I and II metabolic enzymes, in conjunction with smoke have been reported to enhance the PCa susceptibility. Constitutive, hormonal and cancer specific factors like benzo (a) pyrene included in smoke, affect the expression and induction of the phase I metabolic enzymes, CYP1A1 and CYP1A2, in prostate cells resulting to an increased metabolism of nicotine and therefore to an increased intake by the smokers and to an increased carcinogenic process [19]. In addition, some polymorphisms of phase II glutathione S-transferase enzymes (GSTP1, GSTM3, GSTM1) decrease the ability to detoxify carcinogen compounds found in cigarette smoke and modify PCa susceptibility [20-22]. Finally, the rapid arylamine N-acetyltransferase 2 (NAT2) genotype which plays a major role in the metabolic activation of carcinogenic amines of smoke, has been correlated with the development of PCa risk [23,24].

Smoke also affects bone metabolism by different mechanisms. It induces proliferation and cytokine release in human osteoblasts and stimulates bone matrix turnover by increasing production of tissue-type plasminogen activator (tPA) and metalloproteinases (MMPs) [25,26]. These mechanisms tip the balance toward the resorption process and lead to bone loss, something that also characterise the PCa metastatic disease and partially explain the positive association between current smoking and fatal cancer of the prostate, in many prospective cohort studies [11]. All these observations converge in the smoking-linked higher risk of PCa development and morbidity of PCa patients who smoke.

In order to reach more safe and clear conclusions, further

*Student’s t-test

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investigations should be performed analyzing the effect of different exposure to smoke in the various stages of PCA and the carcinogenic pathways in which smoke is involved.

In conclusion, our findings support the established model for PCAs, of being a complex disease with genetic and environmental factors contributing to the carcinogenesis through different mechanisms.

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