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Apolipoprotein E genotype is not linked to locally recurrent hormone-refractory prostate cancer

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Mechanisms of prostate cancer recurrence during androgen deprivation are poorly understood. Recently, the putative role of apolipoprotein E ε4 allele in the aetiology of prostate cancer was raised. To investigate the hypothesis that ε4 allele of apolipoprotein E gene predisposes to prostate cancer and is involved in the relapse of hormonal therapy response, 38 hormone-refractory locally recurrent carcinoma samples from 38 prostate cancer patients were screened for apolipoprotein E genotype. The frequency distribution of apolipoprotein E genotypes among tumours did not differ significantly from that among controls. The allele frequency of ε4 was 19.7% and 19.3% in tumours and controls, respectively. The results suggest that clinical progression of prostate cancer during androgen withdrawal therapy is not associated with apolipoprotein E genotype. *Prostate Cancer and Prostatic Diseases* (2000) **3,** 107–109.

Keywords: apolipoprotein E; ε4 allele; aetiology; hormone refractory; hormonal therapy

Introduction

Despite the high prevalence of prostate cancer, its aetiology has remained poorly known. International and interethnic differences[1] in the incidence of prostate cancer suggest a predominantly environmental[2] aetiology for the disease. Most investigators agree that prostate cancer results from an interplay between genetic factors,[3] endogenous hormones[4,5] and environmental influences that may include, for example, dietary fat and heavy consumption of alcohol.[6,7] One of the strongest risk factors for prostate cancer is, however, positive family history. Male relatives of prostate cancer patients have a 2–5-fold risk for prostate cancer as compared with the general population.[8–11] Several genetic factors, such as androgen receptor, 5-α reductase and vitamin D receptor gene alleles, have been reported to be associated with an increased risk for prostate cancer.[12–14] Recently, Lehrer[15] has suggested that apolipoprotein E (APOE) ε4 allele has a possible relationship with prostate cancer.

APOE is a plasma protein that serves as a ligand of LDL lipoprotein receptors. It participates in cholesterol and lipid metabolism and transport to the cells. In addition, it is involved in repair responses to tissue injury and participates immunoregulation, as well as cell growth and differentation.[16]

APOE has three common isoforms, E2, E3 and E4, which are coded by the codominant alleles designated ε2, ε3 and ε4 by one gene located on chromosome 19. The most common APOE3 isoform represents the parental type, and isoforms E2 and E4 are suggested to be derived by mutations from this parent form. There are differences in APOE allele frequencies between different ethnic groups; the ε4 allele is associated with high cholesterol levels especially in Finnish and German populations.[17–19]

To investigate whether APOE is associated with the relapse of hormonal therapy in prostate cancer, in the present study 38 locally recurrent prostate cancers were screened for APOE genotype.

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Material and methods

Samples

The subjects were 38 prostate cancer patients treated by conventional androgen deprivation in the Tampere University Hospital between 1977 and 1991. All patients experienced a local tumour recurrence, as evidenced by symptoms of urethral obstruction and an increase of serum prostatic acid phosphatase. Non-malignant tissue from formalin-fixed, paraffin-embedded blocks was selected by histopathological examination of haematoxylin and eosin-stained slides.

Controls

The control subjects were 163 healthy Finnish males, of whom 88 were 70 y old and the rest 40 y old.[20]

Methods

Genomic DNA was extracted from tissue samples with the QIAamp tissue Kit (Qiagen Inc., USA). APOE genotypes were analysed using polymerase chain reaction (PCR), as described previously,[21] with slight modifications[22] by using the primers described by Emi *et al*.[23] Positive (APOE4/2) and negative controls were included in each amplification experiment. To determine the APOE genotype, 244 base pair PCR amplified-fragments were digested with *Hha*I (Promega) and banding formed from fragments of 91, 83, 72 to 48 base pairs were separated through 5% MetaPhor agarose gel. Resulting APOE genotype banding was visualized using ethidium bromide staining.

Results

The allelic frequencies of ε2, ε3 and ε4 were 7.9%, 72.4%, 19.7% in prostate cancer patients and 3.7%, 77.0%, 19.3% in controls, respectively ($P=0.52$, χ^2-test). A statistically significant difference was found when patients and controls with ε2/ε4 genotype were compared to other genotypes ($P=0.013$, Fischer's exact test, two-tailed). Table 1 summarizes the distribution of APOE genotypes.

Table 1 Distribution of APOE genotypes and alleles in prostate cancer patients and controls

	Prostate cancer patients ($n=38$)	Controls ($n=163$)
Genotype[a]	*n* (%)	*n* (%)
ε2/ε2	0	0
ε2/ε3	2 (5.3)	10 (6.4)
ε2/ε4	4 (10.5)	2 (1.2)
ε3/ε3	22 (57.9)	96 (58.6)
ε3/ε4	9 (23.7)	49 (30.1)
ε4/ε4	1 (2.6)	6 (3.7)
Allele frequency[a]	%	%
ε2	7.9	3.7
ε3	72.4	77.0
ε4	19.7	19.3

[a]In the χ^2-test there were no statistically significant differences between APOE genotype or allele distributions between controls and patients.

Discussion

Despite of the increased use of PSA to screen for prostate cancer, almost half of the carcinomas are still diagnosed at advanced clinical stage,[24] when cure is no longer considered possible. For these patients androgen deprivation therapy remains the only effective palliative treatment. Initially, hormonal therapies are effective in about 70–80% of patients but later the disease commonly progresses. The prognosis of patients whose tumours do not respond to hormonal therapy or whose disease progresses during the therapy is poor with median survival of only 6 months. There is also no effective treatment for such hormone-refractory prostate cancer.[25]

Understanding the molecular events that underlie the development and progression of prostate cancer could help answer many clinical questions on the treatment of prostate cancer. For example, the possibility of predicting the response to hormonal therapy could greatly help in making treatment decisions. Thus, we investigated the possible involvement of APOE in prostate cancer recurring locally during hormonal therapy.

The frequency distribution of APOE alleles did not differ significantly between prostate cancers and controls. However, the ε2/ε4 genotype was significantly more common among prostate cancer patients than controls. The biological importance of this genotype is unclear since ε2 and ε4 alleles are supposed to have opposite effects. Although the negative correlation between APOE genotype and locally recurrent, hormone-refractory prostate cancer indicates that APOE is not of importance in the aetiology and progression of prostate cancer during hormonal therapy, it is still tempting to speculate that APOE might be contributing to the development of prostate cancer. Firstly, APOE has a special role in cholesterol and lipid metabolism. Cholesterol is a precursor of steroid hormones, which raises the question whether higher blood cholesterol levels could provide extra precursors to testosterone synthesis, and thus, raise blood testosterone levels. Secondly, the APOE alleles have antioxidant activity that is suggested to protect tissues from oxidative damage. This has been shown in cultured cells with the ε2 allele being most protective, ε3 less so and ε4 least protective.[26] In addition, the APOE3 isoform has been shown to inhibit proliferation of endothelial and tumour cells *in vitro*, suggesting that APOE may be effective in modulating angiogenesis, tumour cell growth and metastasis.[27] Finally, Lehrer[15] has found higher prevalence of ε4 allele in 35 prostate cancer cases than in controls, and correlation of ε4 allele with colon[28] and breast[29] cancers has also been established. For example, Kervinen *et al*[28] have reported that colonic neoplasias show low frequency of the ε4 allele of APOE in patients with proximal adenoma and carcinoma compared with the control subjects. Their data suggest that ε4 allele of APOE protects against development of tumours of the proximal colon, but not against distal tumours. In addition, Tanne[29] recently showed that women who have raised concentrations of triglycerides and one or two ε4

alleles have four times the risk of developing breast cancer when compared with women with low triglyceride concentrations. High triglyceride concentration as such elevated the risk slightly, but ε4 allele alone did not have such an effect.

In summary, we have shown here a negative link between hormone-refractory, locally recurrent prostate cancer and the APOE genotype. This result suggests that APOE is not a key factor in the aetiology and progression of prostate cancer. However, our limited material consisted only of hormone-refractory local recurrences, and thus further studies with more subjects are needed to evaluate the aetiologic role of APOE in prostate cancer on population level.

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