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Original article

Elevated serum triiodothyronine (t3) in Ashkenazi Jewish prostate cancer patients carrying the 11307k allele of the APC (adenopolyposis coli) gene

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Abstract

Purpose: The risk of developing any cancer in carriers of the 11307K mutation of the adenopolyposis coli (APC) gene is significantly increased (odds ratio 1.5, P = 0.01). One of the cancers associated with the 11307K mutation is prostate cancer (odds ratio 2.0, P = 0.14). Also, there is an association of APC mutations with thyroid cancer. In this study, we measured triiodothyronine (t3) levels in Ashkenazi Jewish prostate cancer patients, with and without the 11307K mutation of the APC gene. *Materials and Methods:* Participants in our study were found through urology and radiation oncology clinics in 1999 and 2000. All eligible patients were asked to take part. All patients had been initially diagnosed on the basis of rising PSA or abnormal physical examination. Histological confirmation of diagnosis was obtained for all subjects. Ethnic background was confirmed for all subjects by self-report or interview. The 11307K allele of the APC gene was detected by amplification of DNA isolated from peripheral blood according to standard polymerase chain reaction (PCR) and dot blot procedures. Serum t3 level was determined by fluorescent immunoassay with a standard, commercially available instrument. *Results:* We studied 77 patients. The youngest patient was 46, the oldest 88, average age 67 ± 7.2 (mean ± SD). Eleven males carrying the APC I 1307K allele had significantly higher serum t3 levels than 66 males carrying the wild type allele. There were no homozygotes for the 11307K allele. None of the males had a t3 level that was above the normal range for our laboratory (137 ng/dl). *Conclusions:* Our finding of increased serum t3 level with the APC 11307K allele in prostate cancer patients is not surprising, given the mitogenic potential of t3. Further studies may clarify whether t3 elevation is the mechanism whereby APC gene mutations increase the risk of prostate cancer; or whether other pathophysiologic abnormalities are involved.

Keywords: Ashkenazi Jews; A denopolyposis coli gene; Prostate cancer; Triiodothyronine (t3)

1. Introduction

Prostate cancer is the most common malignancy in American men [1]. Though dietary fat plays a role in the development of this cancer [2] and possibly also vasectomy [3], family history is one of the strongest risk factors [4].

Chromosome 5 abnormalities have been described in some studies of prostate cancer [5- 8]. Moreover, there have been multiple reports that the adenopolyposis coli gene (APC), located on chromosome 5g21-22, is associated with the development of prostate cancer [9-12].

APC is a tumor suppressor gene, and somatic loss occurs in tumors. A T-to-A germline mutation at APC nucleotide 3920, the 11307K mutation, is found in 6% of Ashkenazi Jews. Laken et al. [13] reported that the 11307K increased the risk of colorectal cancer, but Petrukhin et al. [14] reported no increased risk in 264 Ashkenazi Jews. In a study of 5081 Ashkenazi Jews, Woodage et al. [9] reported that the 11307K mutation confers an increased risk of colorectal cancer (odds ratio 1.9) though the increase was not statistically significant (P = 0.12).

However, Woodage et al. did find that the risk of devel-

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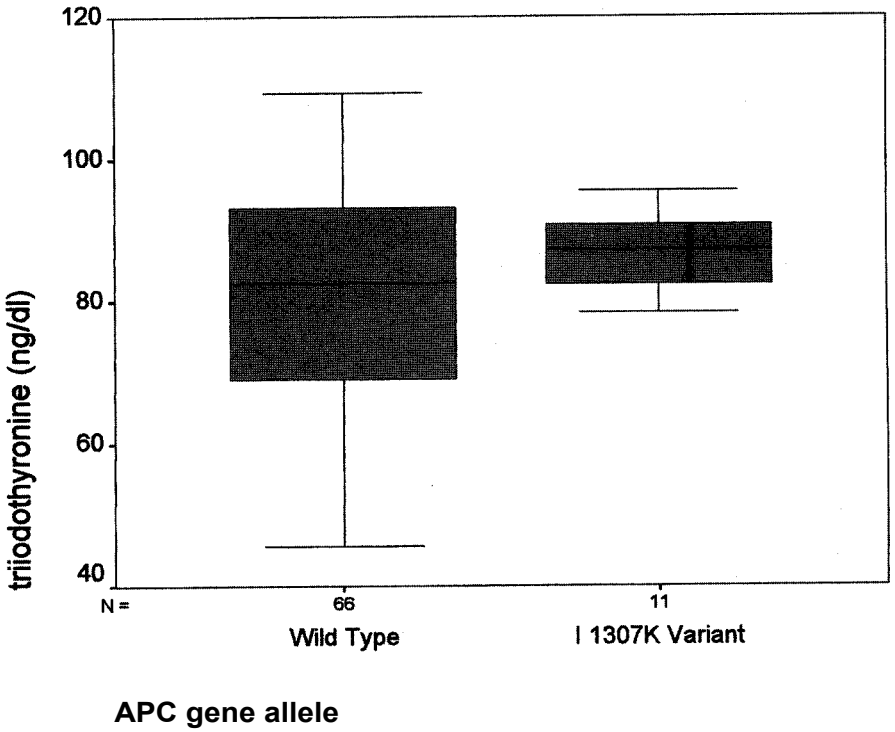

Fig. 1. Serum t3 levels in 77 prostate cancer patients, stratified by APC allele. Number of cases in each group is indicated below corresponding box. The wild type carriers had t3 = 81.97 ng/dl ± 14.93 (mean ± SD). The 11307K variant carriers had t3 = 87.05 ± 5.35. There is a significant difference between the means of the two groups (P = 0.044, t-test for unequal variance). The boundaries of the box represent the 25th and 75th percentiles. The horizontal line inside the box represents the median. The "whiskers" extend from the boundaries of the box to the largest and smallest values that are not outliers (outliers are cases with values between 1.5 and 3 box lengths from the boundaries of the box).

oping any cancer with the 11307K mutation was significantly increased (odds ratio 1.5, P = 0.01). One of the cancers associated with the 11307K mutation was prostate cancer (odds ratio 2.0, P = 0.14). Woodage et al. [9] postulated that their findings are consistent with impaired function of the APC protein in cancer patients. In addition, Korinek et al. [15], Rubinfeld et al. [16], and Morin et al. [17] have shown that APC can interact with (3-catenin, a multi-functional cellular protein, and inappropriately activate the transcription factor Tcf4; this process is important in neoplasia.

The relationship of APC to prostate cancer is still controversial. Gao et al. [10] and Phillips et al. [11] reported that APC may be involved in the development of prostate cancer; whereas Suzuki et al. [18] and Watanabe et al. [19] found no such involvement.

In this study, we analyzed triiodothyronine (t3) levels of Ashkenazi Jewish prostate cancer patients, with and without the APC 11307K mutation, because triiodothyronine is necessary for the growth of prostate cancer cells. For example, a special serum-free defined medium that can support short-term, long-term, and clonal growth of the human prostatic carcinoma cell lines LNCaP, DU 145, PC-3, and ALVA-31 must contain t3 [20].

2. Methods

Participants in our study were found through urology and radiation oncology clinics in 1999 and 2000. All eligible patients were asked to take part. All patients had been initially diagnosed on the basis of rising PSA or abnormal physical examination. Histological confirmation of diagnosis was obtained for all subjects. All participants gave informed consent and the study had Institutional Review Board approval. All staging was clinical, because the patients were to receive 1-125 seed implant. We studied 77 males referred for treatment of localized prostate cancer.

Ethnic background was confirmed for all subjects by self report or interview. All participants gave informed consent for genetic studies and were not given the option to know their test results. Extensive genetic counseling, covering options for detection and prevention, was available. Although we used mostly sporadic cases of prostate cancer, some germ line mutations were still expected.

The 11307K allele of the APC gene was detected by amplification of DNA isolated from peripheral blood according to standard polymerase chain reaction (PCR) and dot blot procedures. The following primers for PCR were added to the reaction mixture:

2.1. APC primers

5'> G CAG ATT CTG CTA ATA CCC TGC <3' (forward)
5'> C TTC GCT CAC AGG ATC TTC AGC <3' (reverse)

Aliquots of amplified DNA were transferred to membranes (Hybond) using a standard protocol [21]. Hybridization was performed for 60 min at 57°. The following 32P labeled probes were used for dot blot analysis:

5'> GCA GAA ATA AAA GAA AAG 3'< (wild type)
5'> GCA GAA AAA GAA AAG 3'< (11307K mutant)

Positive and negative controls were included in all runs.

Serum t3 level was determined by a fluorescent immunoassay with a standard, commercially available instrument (Abbott AxSym, Abbott Laboratories, Abbott Park, Illinois).

3. **Results**

The youngest patient was 46, the oldest 88, average age 67 ± 7.2 (mean ± SD).

Males carrying the APC 11307K allele had significantly higher serum t3 levels than males carrying the wild type allele (Fig. 1). None of the males carrying the 11307K allele was homozygous for it. None of the males had a t3 level that was above the normal range for our laboratory (45-137 ng/dl).

Of the 66 males carrying the APC wild type allele, eight had a family history of prostate cancer, seven in a father or brother. Of the 11 males carrying the APC 11307K allele, three had a family history of prostate cancer, one in a father, one in a brother, and one in a maternal uncle.

Of the 66 males carrying the APC wild type allele, seven had a family history of colon cancer; four had a brother or mother with colon cancer, and two others had a father or sister with colon cancer. Of the 11 males carrying the APC 11307K allele, one had a family history of colon cancer in both parents, two had a family history of colon cancer in mother or father, and one had a brother with colon cancer.

4. **Discussion**

Epidemiologic studies have identified no definite relationship among t3, thyroid disease, and prostate cancer. One study of localized and metastatic prostate cancer revealed no abnormality of t3 [22]. Another study found a marginally decreased incidence of prostate cancer among males with myxedema [23].

But there is an association of the APC allele with thyroid cancer. A cribriform variant of papillary thyroid cancer is characteristic of patients with familial adenomatous polyposis and mutations of the APC gene [24]. In addition, thyroid carcinoma in patients with familial adenomatous polyposis requires damage to only a single allele of the APC gene, not biallelic activation [25].

Triiodothyronine plays an important role in the regulation of prostate cell growth and differentiation. Zhang et al. [26] have shown the interactive effects of t3 and androgens on the growth response and expression of the prostate-specific genes, PSA (prostate-specific antigen) and hK2 (human glandular kallikrein), in the human prostate cancer cell line, LNCaP. Triiodothyronine alone enhanced growth in a dose-dependent fashion. However, in the presence of androgens, higher concentrations of t3 were required to produce additional proliferative effects. Moreover, t3, androgens, or a combination of the two up-regulated PSA protein production in a dose-dependent fashion.

Therefore, our finding of increased serum t3 level with the APC 11307K alle in prostate cancer patients is not surprising, due to the growth-regulating potential of t3. Further studies may clarify whether t3 elevation is the route by which APC gene mutations increase the risk of prostate cancer; or whether other pathophysiologic abnormalities may also be involved.

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