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Association of a CYP17 polymorphism with the overall survival in Caucasian patients with androgen independent prostate cancer

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Abstract

Objectives—Prostate cancer risk may be increased in association with serum concentrations of testosterone, and polymorphisms have been identified in the 17-hydroxylase cytochrome P450 gene (CYP17) that is involved in the biosynthesis and metabolism of androgens. While a polymorphism in CYP17 has been associated with increased prostate cancer risk, the relationship of the CYP17 polymorphism and clinical outcome remains unclear. The purpose of this study was to investigate the association between a CYP17 polymorphism and survival in Caucasian patients with androgen independent prostate cancer (AIPC).

Methods—The study utilized 222 samples acquired from Caucasian patients with AIPC. The CYP17 polymorphism (-34 T>C) was analyzed using PCR amplification followed by RFLP detection.

Results—No significant differences were observed in the frequencies of the CYP17 genotype in relation to categorized Gleason scores, age at diagnosis, or hormone therapy. The median survival was significantly longer in 126 patients with the CYP17 A2 allele (8.9 years) genotype than 96 patients with the A1 allele (6.7 years) genotype ($p=0.040$ by logrank test). Similarly, the estimated survival probability at ten years (24% in A1 allele vs. 43% in A2 allele) is observed to have a statistically significant difference between the two groups ($p=0.002$ by the permutation test).

Conclusion—These results suggest that the CYP17 polymorphism is associated with overall survival in patients with AIPC.

Keywords

CYP17; Polymorphism; androgen independent prostate cancer; survival

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INTRODUCTION

Steroid hormones have been implicated in playing a fundamental role in the pathogenesis of prostate cancer. Polymorphisms in the genes that code for enzymes or hormones involved in the androgen regulatory pathway are proposed to influence an individual's risk for developing prostate cancer. Thus, assessments of these genetic variations would make reasonable candidates to evaluate as potential biomarkers for prostate cancer susceptibility. Such polymorphic genes that exist within the androgen biosynthesis and/or metabolism pathway and which have been suggested to be associated with prostate cancer risk include the cytochrome P450 17 α -hydroxylase (CYP17)¹⁻³.

CYP17 encodes an enzyme with both 17 α -hydroxylase and 17,20-lyase activities, the rate-limiting steps in androgen biosynthesis. 17 α -hydroxylase is responsible for hydroxylating pregnenolone and progesterone (Figure 1) to their 17 α -OH derivatives, which are then converted by 17, 20-lyase to dihydroepiandrosterone and androstenedione and subsequently to testosterone and estrogens⁴. A common single nucleotide polymorphism (SNP) in the CYP17 gene consists of a single base pair substitution (-34T>C) located in the 5'-untranslated region upstream from the translation initiation site and creates an Sp-1 type promoter site. Because the number of promoter elements may correlate with transcriptional activity, it has been postulated that the variant genotype (-34C, A2 allele) may result in increased transcription and therefore higher androgen levels, which may potentially alter susceptibility to prostate cancer. However, evidence that the level of testosterone increases the development of prostate cancer is inconclusive^{5,6}. Moreover, results from several studies examining the association of the CYP17 polymorphism to the incidence of prostate cancer have also been inconsistent^{3,7,8}. Some studies have found the CYP17 polymorphism to increase the risk of prostate cancer, whereas others failed to confirm this observation. While the association of the CYP17 polymorphism to the risk of prostate cancer development has been inconclusive, its association to prostate cancer progression and hence overall survival remains to be determined. To our knowledge, the association between the duration of overall survival and the CYP17 polymorphism in patients with androgen independent prostate cancer (AIPC) has not been previously reported. In the present study, we investigated the relationship between the CYP17 polymorphism and overall survival in Caucasian patients with AIPC.

MATERIALS AND METHODS

Study subjects

Two hundred twenty two Caucasian patients with AIPC were enrolled in this study. Patients underwent surgical castration, or leuteinizing hormone-releasing hormone (LHRH) analogs with or without anti-androgens as the initial hormone therapy and required resistance to hormone therapy. All patients were enrolled in an IRB approved clinical trial within the intramural program of the National Cancer Institute and were arbitrarily assigned a number in our database. Informed consents were obtained from all subjects prior to trial participation.

CYP17 Genotype Analysis

Genomic DNA was extracted from serum or white blood cell buffy coat layers of whole blood of patients using the either the QiAamp Ultrasens Viral DNA kit (serum) or the QIAamp DNA Blood Kit (buffy coat) as described by the manufacturer (Qiagen, Valencia, CA). The control DNA from healthy Caucasian males was purchased (Valley Biomedical Inc., Winchester VA). A 541-bp fragment of genomic DNA containing the -34T>C SNP in the CYP17 gene was amplified by PCR. Primer sequences were as follows: F1, 5'-ttcgactctggagtcattca-3' and R1, 5'-agtcttggtgcccatagaac-3'. The PCR was carried out in a 50- μ l reaction mixture containing approximately 200 ng genomic DNA, PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.8 pmol/

μl of each primer, and 1.25 U Platinum® Taq DNA polymerase (Invitrogen, Carlsbad, CA). After an initial denaturation at 94°C for 5 min, 40 cycles of amplification with denaturation at 94°C for 30 sec, annealing at 64°C for 30 sec, and extension at 72°C for 30 sec was performed, followed by a final extension step of 7 min at 72°C. The CYP17 polymorphism was analyzed using the previously described PCR-RFLP assay⁹. To validate genotypes, identification by sequencing was completed for 20% of the samples.

Statistical analysis

Associations between CYP17 genetic variation and patient characteristics were evaluated by the exact Kruskal-Wallis. The probability of survival as a function of time was determined by the Kaplan-Meier method. The statistical significance of the differences in median survival among the genotypes was determined by the logrank test and the permutation test.

RESULTS

We analyzed 222 Caucasian patients with AIPC for the presence of the -34T>C variant of CYP17. The median age of diagnosis was 60 years. The frequencies of the incidence of the CYP17 polymorphism in patients and healthy volunteers (controls) are shown in Table I. Statistical analyses of the genotype prevalence did not show significant differences between two groups. Next, analyses of the association of the CYP17 genotypes with age at diagnosis, Gleason score, and treated hormone therapy in patients were also evaluated (Table 2). No significant differences were observed in the frequencies of the CYP17 genotypes in relation to categorized Gleason scores, age at diagnosis or hormone therapy. These results indicated that the CYP17 polymorphism did not correlate the risk of prostate cancer and had no significant association with disease status of prostate cancer because no significant differences in patients treated hormone therapy was found in CYP17 genotypes.

We next determined whether the CYP17 polymorphism was associated with survival time from diagnosis. One hundred eighty seven of the 222 patients had expired prior to analysis. The probability of survival over time was determined by the Kaplan-Meier method according to the genotype expressions as shown in Figure 2. In the intermediate range of follow-up, the estimated median survival time of all patients was 7.7 years (95% confidence interval [CI] 6.7 to 8.6), but the median survival for the 96 patients with the A1A1 genotype was 6.7 years (95% CI 5.5 to 7.6), compared to 8.9 years (95% CI 7.7 to 10.4) for the combined 126 patients with either the A1A2 or A2A2 genotype. The statistically difference in overall survival between the two groups was observed ($p=0.040$ by logrank test, Hazard ratio was 1.36; 95% CI 1.36 to 1.82). The estimated survival probability at ten years was 35% (95%CI 28% to 41%) in all patients, 24% (95%CI 16% to 33%) in A1A1 patients, and 43% (95%CI 35% to 52%) in A1A2 + A2A2 patients. The difference between A1A1 and A1A2 + A2A2 patients was statistically significant ($p=0.007$ by the permutation test). These results suggest that patient survival was significantly prolonged for those with the CYP17 variant allele.

COMMENT

Since the -34T>C substitution (A2 allele) has been proposed to affect CYP17 promoter activity, the enhancement of CYP17 activity may increase the bioavailability of testosterone and its metabolite dihydrotestosterone. These androgen hormones play an essential role in regulating the growth of prostate cancer cells and are therefore important in the etiology of prostate cancer, however, our results indicated that patients with the A2 allele genotype had a significantly longer survival advantage compared with patients expressing the A1 allele. It can be suspected that genetically determined differences in androgen biosynthesis may explain some of the observed differences in the risk of prostate cancer, however, many studies have not shown a consistent relationship between serum testosterone levels and prostate cancer

development^{3,5,6}. Whether the A2 allele affects prostate cancer risk or survival, the functional consequence of this CYP17 polymorphism remains controversial^{1-3,5-8,10-14}. No association between the CYP17 variant and prostate cancer risk was observed in the present results as well as meta-analysis², whereas, Lunn et al.¹⁰ reported that the CYP17 A2 allele occurred at a higher frequency in Caucasian patients with prostate cancer than in a control population. Contrary to this report, Wadelius et al.¹¹ found that more Caucasian men were homozygous for the CYP17 A1 allele variant among prostate cancer patients compared with controls. Additionally, Oefelin et al.¹² reported higher pretreatment testosterone levels were associated with longer survival in androgen independent patients. Solaway et al.¹⁵ reported that a low level of testosterone has been negatively associated with survival in hormone therapy refractory prostate cancer. AIPC may not require androgens to grow the tumor cell, however, the mechanism by which androgen affect clinical outcome remains to be elucidated. Our approach to evaluate CYP17 genotypes versus survival relies upon data suggesting that prostate cancer can progress even with sub-castration levels of androgens for several reasons including: androgen receptor gene amplifications, altered mRNA expression, activating mutations, structure/function relationships, etc. Thus, we did not distinguish between treatments because CYP17, in its capacity to make androgens, is most likely involved in disease progression at some points between diagnosis and death despite the treatment(s) received during that period. Therefore, our results should be interpreted with caution, especially considering that there is no clear biological mechanism through which this CYP17 polymorphism associates with survival in patients with AIPC.

CONCLUSION

This study suggest that the CYP17 polymorphism can be a potential prognosis predictor for survival in patients with androgen independent disease because patients who carry the CYP17 variant A2 allele have a longer survival than patients who do not carry this variant. Future studies are warranted to determine the role of the CYP17 polymorphism as a prognostic biomarker, confirm its association with survival.

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Abbreviations

CYP17	cytochrome P450 17 α -hydroxylase
RFLP	restriction fragment length polymorphism
SNP	single nucleotide polymorphism
AIPC	androgen independent prostate cancer

Figure 1.
Biosynthesis and metabolism of androgens

Figure 2.

Kaplan-Meier over all survival curves in Caucasian patients with AIPC according to CYP17 genotypes (A1A1 vs A1A2 + A2A2). The duration of survival was computed from the date of prostate cancer diagnosis until the date of death or last follow-up.

Dotted line, survival for patients with CYP17 A1A1 genotype

Solid line, survival for patients with CYP17 either A1A2 or A2A2 genotype

Table 1
Distribution of CYP17 polymorphism among Caucasian prostate cancer patients and healthy volunteers

	Patients (n=222)	Volunteer (n=83)	OR	95% CI	p
A1A1	96 (43.2%)	33 (39.8%)	1	-	0.78
A1A2	97 (43.7%)	36 (43.4%)	1.08	0.62-1.87	0.37
A2A2	29 (13.1%)	14 (16.8%)	1.40	0.67-2.97	0.58
A1A2+A2A2	126 (56.8%)	50 (60.2%)	1.15	0.69-1.92	

Genotyping data was categorized into A1A1, A1A2, and A2A2.
OR compared with A1A1 genotype

Table 2

Associations of CYP17 genotypes with age at diagnosis, Gleason score, and treated hormone therapy

	A1A1	A1A2	A2A2	<i>p</i>
Age at Diagnosis				0.94
< 49	10	7	5	
50 – 59	32	35	6	
60 – 69	40	42	16	
> 70	14	13	2	
Gleason score				0.68
2 – 6	12	15	5	
7	28	24	4	
8 – 10	49	50	19	
unknown	7	8	1	
Hormone therapy				0.56
Surgical castration	1	4	1	
LHRH analogue	4	1	2	
CAB	91	92	26	

Gleason scores were categorized as mildly aggressive (<7), moderately aggressive (7) or highly aggressive (8-10).

Hormone therapy was categorized as surgical castration, LHRH (leuprolide or goserelin), and combined androgen blockade (CAB).

Associations between CYP17 genetic variation and Age at diagnosis, Gleason score, or hormone therapy were evaluated by the exact Kruskal-Wallis test.