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Role of a *CYP17* Promoter Polymorphism for Familial Prostate Cancer Risk in Germany

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Abstract. *Background: A thymidine to cytosine transition (designated A2 variant) in the promoter region of CYP17 has previously been associated with a familial history of prostate cancer in North American families. The purpose of the present study was to determine whether this correlation could be replicated in a European population. Materials and Methods: Case-control comparisons were performed by modelling a dominant (A1/A2 + A2/A2 vs. A1/A1) and a recessive (A2/A2 vs. A1/A2 + A1/A1) effect of the promoter modification. Results: An insignificant overrepresentation of homozygous carriers of the A2 allele (recessive effect) was found in sporadic cases, as compared to controls. However, the A2 variant was not related to familial disease. Conclusion: Our results do not suggest a role of CYP17 as a high-risk susceptibility gene for familial prostate cancer, nor as a modifier for the disease risk in the European population.*

Prostate cancer is the most frequently diagnosed malignancy among men in many developed countries and its incidence is rising rapidly in most countries. It is assumed that prostate cancer is a multifactorial disease with genetic and environmental components involved in its etiology. High-risk susceptibility genes are believed to cause familial aggregation of the disease, that is seen in about 9% of all cases (1), while low penetrance genes are believed to contribute a moderate disease risk, probably having a considerable impact on sporadic prostate cancer. Predisposing variants have often been suggested within genes of the androgen metabolism pathway for several reasons. First, androgens are crucial for the normal development of the prostate gland and in maintaining its functional state in the adult. Second, it has been proven that prostate carcinoma is a highly hormone-dependent tumor (2). Third, studies have reported an elevated risk of prostate cancer with increasing levels of plasma testosterone (3).

One candidate gene related to androgen metabolism is the *CYP17* gene encoding the cytochrome P450c17α protein. This enzyme mediates two key reactions in steroid hormone biosynthesis: 17α-hydroxylation of pregnenolone and progesterone, as well as 17,20-lysis of 17α-hydroxypregnenolone and 17α-hydroxyprogesterone. *CYP17* is located on chromosome 10q24.3 and contains 8 exons (4). A single nucleotide polymorphism (SNP) has been described in the 5' untranslated region, 27 bp downstream from the transcription start site and 34 bp upstream from the initiation of translation. The thymidine (T) to cytosine (C) transition creates an additional recognition site (CCACC) of the transcription factor Sp-1, and is therefore suspected to alter the expression level of *CYP17*.

Association studies have been conducted to investigate a possible effect of the *CYP17* polymorphism on the risk of sporadic prostate cancer. However, the results were inconclusive concerning the question of whether the wild-type allele (referred to as A1 allele) or the altered allele (referred to as A2 allele) can be considered as a risk factor. A recent meta-analysis found no effect of the *CYP17* polymorphism on sporadic prostate cancer (5). The question still remains unresolved for familial disease, since only two investigators included prostate cancer families (6, 7). One North American study reported evidence that a familial history of the disease together with the *CYP17* A2 alteration may strongly increase prostate cancer risk (7).

In order to further investigate a possible role of *CYP17* in a familial disease aggregation, we conducted an association study in Germany, including 82 unrelated familial prostate cancer patients, each representing a pedigree of multiple affected men, along with 92 prostate

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Key Words: CYP17, familial prostate cancer, polymorphism, association.

Table I. *Disease history of families represented by 82 unrelated familial prostate cancer probands.*

Family characteristic	No. of families
All	82 (100%)
Hereditary prostate cancer[a]	
Yes	26 (32%)
No	56 (68%)
No. of affected members	
2	32 (39%)
3	33 (40%)
≥4	17 (21%)

[a]According to the Hopkins criteria of hereditary prostate cancer (14).

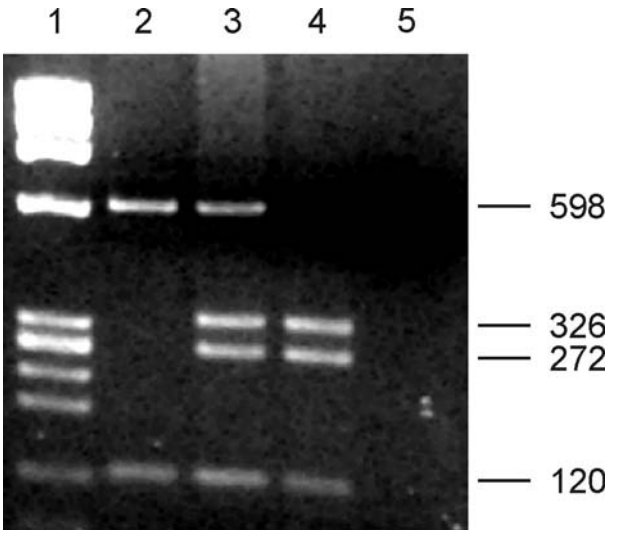

Figure 1. *MspAI 1 restriction fragments of CYP17 alleles, separated by agarose gel electrophoresis (2% agarose gel in TBE). Lane 1, DNA molecular weight marker; lane 2, genotype A1/A1 (wild-type); lane 3, genotype A1/A2; lane 4, genotype A2/A2; lane 5, negative control.*

cancer probands without affected relatives (sporadic cases) and 89 controls.

Materials and Methods

Study population. Prostate cancer cases and families were recruited as described elsewhere (8). For the present study, we genotyped 82 unrelated familial prostate cancer probands, each selected as the youngest case within a pedigree of multiple affected relatives. The characteristics of the represented families are shown in Table I. Ninety-two patients, who did not report any affected relatives, were genotyped and included as a sporadic case group. For a control group, we used 89 healthy, elderly men who were not diagnosed with prostate cancer before and who had a negative family history of the disease. For 17 control patients, the PSA level was known and verified to be normal at the time of sampling. The mean age at diagnosis was 60.4 years (range: 47 - 80) for familial prostate cancer probands, 63.6 (range 43 - 79) for sporadic probands and 56.7 (range 34 - 79) for the control sample.

Genotyping. Genomic DNA was obtained from blood according to standard protocols. The T to C transition in the promoter region of *CYP17* was amplified by polymerase chain reaction (PCR) and analyzed by restriction digestion analysis. PCR amplification of the 718 bp DNA fragment was performed using the following primer sequences: *CYP17* forward primer 5'-GTTCCAAGCCTTGACTCTG-3' and the reverse primer 5'-TGAAGACCTGAACCAATCCC-3'. The PCR products were incubated with the restriction enzyme *Msp*AI (New England Biolabs GmbH, Frankfurt am Main, Germany) for 3 hours at 37˚C and separated on an ethidium bromide-stained 2% agarose gel. The PCR product of the A1 allele is cut into two fragments at a constitutive *Msp*AI site, while the additional polymorphic *Msp*AI site of the A2 allele results in three restriction fragments (Figure 1).

Statistical analysis. For each study group, a χ^2 test was preformed to check if the distribution of the three possible *CYP17* genotypes matched the Hardy-Weinberg equilibrium. A putative influence of the A2 allele on disease risk was investigated by setting up a dominant and a recessive case control design. For the dominant model, we compared individuals being heterozygous (A1/A2) and homozygous (A2/A2) for the promoter modification against the wild-type genotype (A1/A1). In the recessive model, the homozygous state of A2 represented the risk genotype group and was compared to all others (A1/A1 and A1/A2) used as the reference group. The corresponding 2 x 2 cell contingency tables were analyzed by unconditional logistic regression. We used the software StatView (Madison SAS) to compute odds ratios, 95% confidence intervals and *p*-values.

Results

The *CYP17* promoter polymorphism was investigated in 89 controls, 92 non-familial (sporadic) and 82 unrelated familial prostate cancer probands. For each study group, genotype distributions were verified to be in accordance with the Hardy-Weinberg equilibrium ($p \geq 0.47$) and the observed allele frequencies (A1: 55 - 62%, A2: 38 - 45%) did not differ between the three groups investigated ($p > 0.30$).

Consequently, the odds ratios comparing familial cases with controls or sporadic cases *versus* controls were small and did not differ significantly from 1.0 (Table II). If the A2 allele confers a risk, this may correlate either to the presence of one or two A2 alleles in the genotype depending on the mode of action. These possibilities can be accounted for by combining the corresponding genotypes in the analysis, which is equivalent to the use of dominant and recessive models. Neither of these models yielded significant results (Table II).

Table II. *Correlation of CYP17 alleles with prostate cancer risk in the groups of familial prostate cancer cases (A) and sporadic disease (B) compared to controls.*

A) Families Genotype	Controls	Cases	OR	95%CI	*P*-value
Dominant model (A_{12}+A_{22} *vs* A_{11})					
A_1A_1	29 / 89 (33%)	33 / 82 (40%)		Reference	
A_1A_2+A_2A_2	60 / 89 (67%)	49 / 82 (60%)	0.72	0.38 - 1.34	0.30
Recessive model (A_{22} *vs* A_{12}+A_{11})					
A_1A_1+A_1A_2	79 / 89 (89%)	69 / 82 (84%)		Reference	
A_2A_2	10 / 89 (11%)	13 / 82 (16%)	1.48	0.60 – 3.60	0.38
B) Sporadic cases					
Genotype	Controls	Cases	OR	95%CI	p-value
Dominant model (A_{12}+A_{22} *vs* A_{11})					
A_1A_1	29 / 89 (33%)	29 / 92 (32%)		Reference	
A_1A_2+A_2A_2	60 / 89 (67%)	63 / 92 (68%)	1.05	0.56 - 1.96	0.90
Recessive model (A_{22} *vs* A_{12}+A_{11})					
A_1A_1+A_1A_2	79 / 89 (89%)	72 / 92 (78%)		Reference	
A_2A_2	10 / 89 (11%)	20 / 92 (22%)	2.20	0.96 – 5.00	0.06

Table III. *Correlation of CYP17 with prostate cancer risk in general. All prostate cancer probands, regardless of family history, were compared to controls.*

Genotype	Controls	Cases	OR	95%CI	*P*-value
Dominant model (A_{12}+A_{22} *vs* A_{11})					
A_1A_1	29 / 89 (33%)	62 / 174 (36%)		Reference	
A_1A_2+A_2A_2	60 / 89 (67%)	112 / 174 (64%)	0.88	0.50 - 1.50	0.60
Recessive model (A_{22} *vs* A_{12}+A_{11})					
A_1A_1+A_1A_2	79 / 89 (89%)	141 / 174 (81%)		Reference	
A_2A_2	10 / 89 (11%)	33 / 174 (19%)	1.85	0.87 – 3.95	0.11

In a further step, we examined a possible association between *CYP17* genotypes and the risk of prostate cancer in general. For this purpose, all prostate cancer probands with and without a familial history of the disease were compared with the group of healthy men (Table III). Under the dominant model, the frequency of risk genotypes (A1/A2 + A2/A2) was equal between all cases and controls. The corresponding odds ratio was 0.88 (95% CI, 0.50 – 1.50). Applying the recessive model, we found a slight but not significant elevation of risk genotypes (A2/A2) in the total group of cases as compared to controls (OR=1.85; 95% CI, 0.87 – 3.95). The excess of A2/A2 genotypes was mainly due to a high frequency of A2 homozygous carriers in the sporadic prostate cancer sample. When the comparison was restricted to only sporadic cases *versus* controls, the corresponding odds ratio was elevated to OR=2.20, but still had an insignificant interval of confidence (CI=0.96 – 5.00; p=0.06 Table II).

Since the only striking effect of the A2 allele has previously been reported for affected men with one affected relative (6), we also compared our familial cases to all other individuals having no affected relatives. These comprised

Table IV. *Correlation of CYP17 with familial aggregation of prostate cancer. Familial prostate cancer probands were compared to individuals having no affected relatives (sporadic cases and controls).*

Genotype	Sporadic and controls	Families	OR	95%CI	*P*-value
Dominant model ($A_{12}+A_{22}$ *vs* A_{11})					
A_1A_1	58 / 181 (32%)	33 / 82 (40%)		Reference	
$A_1A_2+A_2A_2$	123 / 181 (68%)	49 / 82 (60%)	0.70	0.40 – 1.20	0.20
Recessive model (A_{22} *vs* $A_{12}+A_{11}$)					
$A_1A_1+A_1A_2$	151 / 181 (83%)	69 / 82 (84%)		Reference	
A_2A_2	30 / 181 (17%)	13 / 82 (16%)	0.95	0.50 – 1.93	0.88

the combined groups of sporadic probands and healthy men (Table IV). No evidence for an association was seen for the risk genotypes under the dominant (OR=0.70; 95% CI, 0.40 – 1.20) or under the recessive model (OR=0.95; 95% CI, 0.50 – 1.93). The same result was obtained when A2/A2 familial cases were compared to A1/A1 controls without affected relatives (OR=1.10; CI=0.40 – 2.90; p=0.80).

Discussion

The prostate gland is an hormonally-regulated organ, so androgens may play a major role in the etiology of prostate cancer. The *CYP17* gene is a likely candidate for prostate cancer because it is directly involved in the production of testosterone. A polymorphism identified in the promoter of *CYP17* was suggested as a low penetrance modifier and was therefore investigated in numerous case control studies. These approaches, which did not take into consideration a familial disease history, led to inconsistent reports on associations between the polymorphism and the development of prostate cancer. Some investigators observed elevated risk estimates in men homozygous for the frequent A1 allele (9, 10). Others, in contrast, noticed a borderline significance for A2 genotypes associated with prostate cancer (11-13), or no effect at all. The inconclusive situation has recently been resolved by a meta-analysis combining ten single studies which dealt predominantly with sporadic prostate cancer probands (5). The authors found no correlation between *CYP17* and disease risk, at least when they restricted the study populations to Caucasians. In our study, we identified an unequal distribution of *CYP17* genotypes among sporadic cases and controls. This small difference was not significant and, thus, is consistent with the conclusion that *CYP17* has no influence on prostate cancer risk in general. However, the power of our sample was limited by two factors. First, our sample might have been too small to detect moderate disease effects. Second, the disease-free status is usually not histologically confirmed, and thus a residual prevalence of prostate cancer among controls could bias the results in favor of the null hypothesis.

To our knowledge, only two studies have examined a putative role of the *CYP17* polymorphism in familial aggregation of prostate cancer. Recently, North American investigators (7) applied a family-based association test on pedigrees with at least three affected first-degree relatives. These thoroughly selected families, that come close to the defined entity of hereditary prostate cancer, did not support a role for *CYP17* as a high-risk factor. A previous study, also performed in North America (6), included men with an affected first-degree relative. These authors observed a strong correlation of the proposed risk genotype A2/A2 with familial disease history. The odds ratio for being homozygous for the A2 allele associated with having a family history of prostate cancer was 26.1 (95% CI, 3.41-199.6) relative to men without a family history of disease and the A1/ A1 genotype. In our study, we questioned whether the reported correlation could be verified in a European population. Our results showed no evidence that the *CYP17* genotype might predispose for a familial aggregation of prostate cancer. Our comparison was made between small samples and has, therefore, a limited power to detect moderate effects of the potential risk genotype. However, with respect to the obtained interval of confidence, that is 0.6 to 3.6, our results are not compatible with a disease impact of the strength reported by the previous American study (6).

Several reasons are under discussion to explain the divergent outcomes of association studies. The most plausible interpretation, that is compatible with the null hypothesis of no disease effect, simply is chance. Chance fluctuations in a series of individual studies could be eliminated by combining results in the course of a meta-analysis. Such an approach has already been applied to address the role of *CYP17* in sporadic

prostate risk, and may also be helpful to explore a putative influence on familial aggregation of the disease. However, there have been arguments that divergent outcomes would indicate true disease effects, especially if single studies represented different populations. The impact of a risk gene under study might be complicated by environmental factors, depending on culture, and by the genetic backgrounds specific for ethnicity.

Our study of German prostate cancer probands and controls does not provide evidence for a role of *CYP17* as a high-risk susceptibility gene in familial aggregation, nor as a modifier for the disease risk in general.

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