CLINICAL STUDY

Impact of constitutional genetic variation in androgen/oestrogen-regulating genes on age-related changes in human prostate

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Abstract

Objective: Benign prostatic hyperplasia (BPH) is the most common benign tumour in ageing men. While the etiopathology remains unsolved, a disruption in the endocrine/autocrine–paracrine prostatic homeostasis, involving steroid hormones, contributes to the pathogenesis of BPH. DNA polymorphisms in genes involved in hormone synthesis, signalling and metabolism may, therefore, be responsible for these changes. We have evaluated the correlation between specific genotypes in androgen- and oestrogen-regulating genes (AR, SRD5A2, CYP17 and CYP19), and age-related prostatic changes.

Methods: We have tested genetic susceptibility to morphological and pathological criteria in 195 French Caucasians, using allelic variants for candidate genes involved in androgen/oestrogen prostatic activity: androgen receptor (CAG repeats), 5 α -reductase type 2 (TA repeats, V89L and A49T mutations), A2 variant of the 17 α -hydroxylase (CYP17) and the simple tandem repeat polymorphism (STRP) aromatase (CYP19) polymorphisms.

Results: The A2 variant of 17 α -hydroxylase (CYP17) and allele 191 of STRP aromatase (CYP19) showed an opposite effect on age-related prostate hyperplasia: CYP17 being associated with increased risk of prostate enlargement and CYP19 with reduced risk. The 5 α -reductase type II variants studied did not show links with prostate hyperplasia. The androgen receptor gene CAG repeat length showed a low correlation with the increase of prostate weight, suggesting some effect on age-related prostate growth.

Conclusion: These results suggested that common variants of the CYP17 gene are associated with prostate enlargement and therefore may increase the risk of development of BPH in this population, while infrequent variants of the aromatase gene (CYP19) could be of a protective nature.

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Introduction

Benign prostatic hyperplasia (BPH) is the most common benign tumour in ageing men (1). The microscopic prevalence of BPH is fairly consistent across national and ethnic groups, increasing with age in all male populations (2). However, while age is the main determinant of these morphological changes in the prostate gland, the etiopathology of benign prostate enlargement remains unclear and needs further study. A better understanding of risk factors is thus expected to be determined, particularly from the development of molecular epidemiological studies of these complex

diseases. Furthermore, identification of the genetic determinants in age-related changes of the prostate could provide insights for risk assessment, prevention and indicate new targets for BPH management.

BPH is a multifactorial and probably heterogeneous disease, involving environmental, endocrine and genetic factors. The study of the interactions between genes and multifactorial disease has expanded in recent years.

Highly penetrating genes involved in BPH are likely to account for only a relatively small proportion of the disease. However, the majority of cases of BPH cannot be explained by a single gene model. Other

predisposing mutations with low penetrancy could confer a moderate increase in the risk of specific diseases but they are widespread in the general population. This is particularly true for polymorphisms in genes encoding enzymes or receptors involved in the endocrine control of prostate homeostasis during ageing (3, 4).

Previous contributions, using case control design approaches, have identified genetic polymorphisms in genes involved in steroid metabolic pathways as risk markers in BPH (4–6).

Androgen receptor CAG polymorphisms

One of the critical functions of the products of the androgen receptor gene is to activate the expression of target genes. This transactivation activity resides in the N-terminal domain of the protein, which is encoded in exon 1 and contains polymorphic CAG repeats. A shorter CAG repeat is associated with a higher level of receptor transactivation function, thereby possibly resulting in a higher risk of BPH. These data were interpreted to suggest a possible association between the polymorphisms of the androgen receptor gene and the development of BPH (6–9).

SRD5A2 gene variants (TA repeats, A49T and V89L)

Conversion of testosterone in prostatic target cells to its metabolite and active androgen, dihydrotestosterone (DHT), is catalysed by the enzyme 5 α -reductase. Two genes encode the two 5 α -reductase isoforms. They are located on chromosomes 5p15 and 2p23 for isoforms type I and type II respectively.

The roles of the two isoforms in the adult tissue are still subject to controversy and it is believed that isoform type II predominates in the prostate. The possibility that different alleles of the 5 α -reductase type II (SRD5A2) gene may be associated with different levels of 5 α -reductase activity (4) and consequently different levels of prostate enlargement risk has motivated studies of the ethnic variation in allelic distribution of this gene. The first DNA polymorphism described for the SRD5A2 gene as a susceptibility determinant in prostate cancer is a TA dinucleotide repeat polymorphism in the 3' untranslated region of the gene. Three alleles with 0, 9 or 18 TA repeats have been considered but the 18 TA repeats are common only in Afro-American men (10).

The V89L mutation reduces steroid 5 α -reductase activity. This substitution is particularly common among Asians. The *Leu* allele has been associated with lower serum levels of 3 α -androstane diol glucuronide which is another product of 5 α -reductase activity (11).

CYP17 variants

CYP17 encodes the cytochrome P450c 17 α -enzyme which catalyses steroid 17 α -hydroxylase/17,20 desmolase activities at key points in testosterone and oestrogen biosynthesis in both gonads and adrenals (12). The CYP17 A2 allele contains a T to C transition polymorphism in the 5' promoter region that creates an additional SP1 (CCACC box) promoter site. The polymorphism is correlated with increased promoter activity in the CYP17 gene (13) and circulating testosterone levels in men and oestrogen levels in women. This A2 allele has been associated with increased risk of baldness, polycystic ovarian cancer and recently with prostate tumours (14, 15).

CYP19 simple tandem repeat variants

Constitutional genetic variation at the human aromatase gene, so-called CYP19, mapping to chromosome 15q21 has been related to aromatase activity and breast cancer risk. The aromatase gene (CYP19) encodes the enzyme which converts testosterone to oestrogen. In the prostate, oestrogens interact potentially with oestrogen receptor- α in fibromuscular cells and with oestrogen receptor- β in prostatic epithelial cells. A common high heterozygosity tetranucleotide simple tandem repeat polymorphism named STRP in intron 4 had previously been described suggesting that, on the one hand, the 171 bp allele was associated with high breast cancer risk and, on the other hand, that the 191 bp allele could be linked with a lower risk (16, 17). The role of oestrogens and aromatase activity in the development of BPH has been suggested and aromatase inhibitors have been proposed to target BPH.

We have performed an association study on a homogeneous French Caucasian population using pathological prostatic phenotypes strictly defined by morphological features from surgical and autopsy prostate dissection in elderly men. The genetic variants of androgen- and oestrogen-regulating genes (AR, SRD5A2, CYP17 and CYP19) have been analysed in a multiparametric statistical process with age-related prostatic changes. We hypothesise that specific genotypes in androgen- and oestrogen-regulating genes are correlated with prostate hyperplasia, and could provide information relevant to the detection and prevention of PBH.

Subjects and methods

Subjects and data collection

Included, prospectively, in this study were 195 subjects identified through the Department of Urology and Anatomy at the University of Paris VII and Paris V between December 1997 and March 2000. To be eligible for the study the subjects had to be of French

Caucasian ethno-geographic origin and informed consent had to be provided.

All subjects included in the study provided constitutional DNA and complete histopathological analysis of the prostate gland. Prostate glands were obtained by radical prostatectomy ($n = 109$) and from cadavers during autopsy ($n = 86$) using the same surgical technique. Measurement of the weight of the autopsy and the operation specimens was carried out with a standard weighing machine (Teraillon, Paris, France) immediately after removal. Prostate glands were characterised for their macroscopic and microscopic aspects by systematic sections from apex to prostate base. We divided the prostates into categories using a combination of the age-related morphologic changes (prostate weight).

Genotyping

Primers are designated by the nucleotide position (relative to *AR*, GenBank no. M23263; *SRD5A2*, GenBank no. L03843; *CYP17*, GenBank no. M31146; *CYP19*, GenBank no. M30798) corresponding to the 5' position, followed by the letter U for upper (sense strand) or L for lower (antisense strand). Primer pairs were chosen with the assistance of the computer programs Oligo 4.0 (National Biosciences, Plymouth, MN, USA) and Primer Express (Perkin Elmer Applied Biosystems, Foster City, CA, USA). The nucleotide sequences of primers are shown in Table 1.

PCR amplification was carried out in a final volume of 50 μ l, containing 50 ng DNA, 20 pM of each primer, 100 μ M of each dNTP, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl and 1 unit Taq DNA polymerase.

DNA amplification was performed conventionally: samples were subjected to 35 cycles of amplification consisting of 40 s of denaturation, 30 s of annealing and 30 s of extension. The final extension step at 72 °C was for 10 min. The annealing temperature was optimised for each primer set.

The sensitive Applied Biosystems model 377 DNA sequencing system (Perkin Elmer) was used to identify CAG repeats of the *AR* gene, TA repeats of the *SRD5A2* gene and STRP of *CYP19*. Dilutions of PCR products were optimised for each target gene and 1 μ l aliquots of each amplified product were added to 3.5 μ l deionised formamide containing 0.3 μ l of a molecular size marker (Genescan 500 ROX, Perkin Elmer, Courtaboeuf, France). The mixtures were heat-denatured and 1.5 μ l aliquots of each were loaded on 4% polyacrylamide/8 M urea gel and run for 3 h at 1200 V in the sequencer. Primers 559U of *AR* and 2334U of *SRD5A2* were fluorescein-labelled at their 5' end by adding Fluorprime (MWG, Courtaboeuf, France) during oligonucleotide synthesis and the size of the different fragments was determined with Genescan 672 Fragment Analysis software (Perkin Elmer Applied Biosystems).

V89L missense substitution in the *SRD5A2* gene and the T to C polymorphism in *CYP17* detection were based upon restriction fragment length polymorphism analysis after PCR amplification.

The G to C substitution at codon 89 of the *SRD5A2* gene leads to the loss of an Rsa-I restriction site. The upper primer (974U) leads to a single nucleotide substitution in the amplified product (G mutated to T in position 987) in order to abolish a second Rsa-I site. Thus, the polymorphic allele of interest leads to the loss of a restriction site unique within the amplified fragment, and Rsa-I digestion readily identifies the genotype for each sample.

The T to C polymorphism in *CYP17* creates a recognition site for the Msp-A1 restriction enzyme and Msp-A1 digestion identified a second allele (A2).

PCR products were, therefore, digested for 3 h at 37 °C using either Msp-A1 or Rsa-I, separated by agarose gel electrophoresis and stained with ethidium bromide to identify the base pair change.

Sequencing of PCR products

Samples were screened for the A49T mutation of the *SRD5A2* gene by PCR amplification with the primer

Table 1 Oligonucleotide primer sequences used for genotyping.

Gene	Oligonucleotides	Sequence
<i>AR</i>	599U	5'-TCCAGAATCTGTTCCAGAGCGTGC-3'
	874L	5'-ACTGCGGCTGTGAAGGTTGCTGT-3'
<i>SRD5A2</i>		
	A49T	837U 943L 5'-GGCTACGGGAAGCACACGGA-3' 5'-ATCCCGCGGGCACC GCGAA-3'
V89L	974U	5'-GCCACCTGGGACGTTACTTCTG-3'
	1102L	5'-TCCTTGGCGTTCCTCGGTGC-3'
(TA) _n	2334U	5'-CTCCACAAATGCTGATGAA-3'
	2482L	5'-CAGACACCACTCAGAATCCC-3'
<i>CYP17</i>	230U	5'-CATTCGCACTCTGGAGTC-3'
	642L	5'-GGCTCTTGGGGTACTTG-3'
<i>CYP19</i>	594U	5'-TTATGAAAGGTAAGCAGGTACTTAG-3'
	766L	5'-GTCGTGAGCCAAGGTCACT-3'

Table 2 Association of androgen receptor CAG, SRD5A2, CYP17 genotypes and CYP19 alleles with prostate weight. Values in parentheses are S.D.

	SRD5A2 alleles						CYP17 alleles					
	CAG repeats			V89L			TA repeats			CYP17 alleles		
	Under 19	20 to 23	Over 24	WV	VL	LL	TA0-0	TA0-9	TA9-9	A1A1	A1A2	A2A2
Number	30	91	57	87	74	18	127	48	3	60	94	25
Mean prostate weight (g)	47.2 (16.3)	44.4 (21.6)	42.1 (19.0)	42.8 (28.4)	45.9 (17.4)	44.3 (20.4)	44.7 (20.6)	42.7 (18.5)	47.9 (15.0)	39.9 (17.4)	46.2 (21.4)	47.3 (18.8)
Mean prostate weight related to age (weight (g) × 100/age (years))	67.1 (25.4)	64.5 (34.6)	60.8 (27.4)	61.3 (26.7)	66.4 (31.7)	65.2 (44.6)	64.6 (31.9)	61.5 (28.9)	66.1 (6.7)	56.4 (26.6)	66.8 (33.0)	70.4 (29.8)
CYP19 alleles												
Number	167	171	175	179	183	187	191	195				
Mean prostate weight (g)	128	61	25	2	5	126	10	1				
Mean prostate weight related to age (weight (g) × 100/age (years))	45.3 (20.5)	45.5 (19.9)	39.5 (20.5)	43.5 (0.7)	44.2 (25.1)	44.5 (19.3)	28.7 (8.4)	74.5 (ND)				
	65.0 (32.0)	66.8 (32.3)	58.8 (31.4)	63.6 (5.0)	60.3 (35.9)	64.0 (29.5)	40.4 (13.7)	82.8 (ND)				

V, Val; L, Leu; A1, Allele 1; A2, Allele 2.

set 837U/943L. The 107 PCR fragment was purified and sequenced using the Applied Biosystems (ABI) PRISM Big Dye Terminator Cycle Sequencing kit (Perkin Elmer) on the Applied Biosystems model 377 DNA sequencing system (Perkin Elmer). Potential A49T substitution was confirmed by performing sequence analysis both on the sense and antisense strand.

Statistical analysis

Prostate weight or prostate weight related to age (prostate weight (g) × 100/age (years)) was tested for correlation as a continuous variable by logistic regression with CGA repeat number and by the Kruskal–Wallis test and Student's *t*-test with CYP17 and SRD5A2 genotypes or CYP19 allele groups as nominal variables.

In addition, the alleles of CAG had to be grouped as discrete traits. The following categories were used: ≤ 19, 20–21–22–23, ≥ 24.

The alleles of the TA polymorphism were categorised as TA₀ and TA₉ according Reichardt *et al.* (10), since no TA₁₈ alleles were observed.

The statistical analyses were carried out with the PC program Stat View (SAS Institute, Cary, IN, USA).

Results

CAG repeats

Both weight and weight related to age showed correlation of CAG repeat number with coefficient of correlation $R^2 = 0.01$ ($P = 0.1823$) and $R^2 = 0.006$ ($P = 0.3025$) respectively (Table 2). An association between these parameters could be evoked but did not reach statistical significance.

CYP17

As shown in Table 2, the A2 variant was associated with weight and weight related to age ($P = 0.039$). The Student's test conducted on allele A1A1 ($n = 60$) versus A1A2 and A2A2 ($n = 119$) showed even better significance with $P = 0.0226$.

SRD5A2

No difference was observed between SRD5A2 genotypes analysed (A49T, V89L or TA repeat) and prostate weight or age-related prostate weight (Table 2).

CYP19

As shown in Table 2, the prostate weight and the age-related prostate weight were significantly lower in the 191 allele group ($P < 0.01$). It is noteworthy that the age of the subjects with allele 191 (72 years old,

s.d. = 9) was not statistically different from subjects with other *CYP19* alleles (71 years old, s.d. = 10).

Discussion

This paper is based on multiple correlations between allelic variants in genes involved in the androgen pathway and phenotypes in the age-related changes observed in the prostate. We performed a study on age-related prostatic enlargement, including an autopsy series in elderly men with their potential association with androgen/oestrogen-regulating genes.

The prostate is a hormonally regulated organ (18). Men with androgen metabolic pathway deficiency have been recognised as very low risk subjects for developing BPH. It is possible that a large proportion of familial aggregation is due not to segregation of mutations in a few major genes but rather to familial sharing of alleles at many loci, each in itself exerting only a small risk effect. The fact that genetic heritability explained 70% of the variance of plasma levels of androstane diols in male twins suggests that familial clustering may be due, at least in part, to familial correlation with hormonal status (19). These observations and previous reports on hormonal carcinogenesis suggest that functionally relevant sequence variants in genes involved in androgen metabolism and targeting receptors could define high-risk or low-risk profiles for age-related prostate hyperplasia.

Inherent difficulties in studies involving various ethnicities probably explain the discrepancies obtained in the different studies. Cases of BPH are frequently selected from patients undergoing surgical procedures consecutive to miscellaneous histopathological lesions associated with lower urinary tract symptoms.

Genetic susceptibility and phenotypic variations in age-related prostate hyperplasia

We report, on the one hand, that men who carry the A2 variant in *CYP17* are more likely to exhibit age-related prostate hyperplasia and, on the other hand, that men who carry the 191 allele in *CYP19* have a low risk of prostate hyperplasia (Table 2). The hypothesis that *CYP17* and *CYP19* may be involved in age-related prostate growth is based on our knowledge of testosterone synthesis and its metabolism to oestrogen. The product of *CYP17* is responsible for testosterone biosynthesis. Testosterone is either transformed to DHT by the 5 α -reductase gene to act on androgen receptors or into oestrogen by aromatase (*CYP19*), both of which have been suggested as targets for BPH treatment. In a recent report, the polymorphism of *CYP17* (T to C transition in the promoter region) had no association with prostate volume; however, the *CYP17* wild-type (A1/A1) genotype had a higher

stroma/epithelium ratio than those with A1/A2 and A2/A2 genotypes (20).

Although not confirmed by a case control collection of men from Northern Europe, previous studies support the role of the CAG repeat in the development of BPH. Giovannucci *et al.* (7) found an inverse correlation between androgen receptor gene CAG repeats and prevalence of surgery for BPH and enlarged prostate. Mitumori *et al.* (8) examined CAG repeats lengths in BPH patients who underwent prostatectomy. A statistically significant ($P < 0.02$) trend for large adenoma size with short CAG repeat length was found. Conversely, Bousema *et al.* (5) found a similar mean number of CAG repeats in the androgen receptor gene in patients (21.8) and controls (21.9). They concluded that even if an association could exist between BPH and CAG repeat polymorphism, it is probably weak. In our hands, no significant correlation between CAG repeat length and prostate hyperplasia was observed. However, some statistically non-significant magnitude effects showed a low but suggestive correlation between CAG repeat length and age-related increase in prostate weight.

We also report that the *SRD5A2* genotypes (V89L, A49T and TA repeats) had no significant impact on age-related prostate hyperplasia.

Conclusion

Interest in constitutional genetic variation is based on the possibility of identifying population subgroups that are at increased risk of developing BPH. In addition, knowledge of androgen metabolic enzymes or receptor gene genotypes can be used for chemopreventive strategies in BPH. Our study provides some evidence that genes comprising the steroid biogenesis pathway appear to be involved in age-related prostate gland growth, but their role on direct genesis still remains unclear. These observations are in agreement with the concept that androgens promote prostate growth. The association of no increased age-related prostate hyperplasia with *CYP19* variants supports the development of aromatase inhibitor trials for the prevention of prostate hyperplasia. These results confirm the need for molecular screening and a pharmacogenomic approach in order to discriminate good candidates for chemoprevention in the common age-related prostate diseases.

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