

## Polymorphic CAG/CAA Repeat Length in the *AIB1/SRC-3* Gene and Prostate Cancer Risk: A Population-based Case-Control Study

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### Abstract

**In an earlier report, we showed that a shorter CAG repeat length in the androgen receptor (AR) gene is associated with an increased risk of prostate cancer in China, the population with the lowest reported prostate cancer incidence in the world. Because AR coactivators enhance transactivation of AR, in this report we evaluated the relationship of a CAG/CAA repeat length polymorphism in the *AIB1/SRC-3* gene (amplified in breast cancer gene 1, a steroid receptor coactivator and an AR coactivator) with prostate cancer risk in a population-based case-control study in China. Genomic DNA from 189 prostate cancer patients and 301 healthy controls was used for the PCR-based assay. The *AIB1/SRC-3* CAG/CAA repeat length ranged from 24 to 32, with the most common repeat length being 29. Homozygous 29/29 and heterozygous 28/29 were the most common genotypes, with 44 and 30% of the controls harboring these genotypes, respectively. Relative to subjects homozygous for 29 CAG/CAA repeats (29/29 genotype), individuals with the <29/29 genotype had a nonsignificant 31% increased risk [odds ratio (OR), 1.31; 95% confidence interval (CI), 0.87–1.97], whereas those homozygous for the <29 allele had a significant 81% excess risk (OR, 1.81; 95% CI, 1.00–3.28). The combined effect of CAG repeat lengths in the *AR* and *AIB1/SRC-3* genes was also evaluated. Relative to men with both the 29/29 genotype of the *AIB1/SRC-3* gene and a long CAG repeat length ( $\geq 23$ ) in the *AR* gene, those with both the <29/<29 *AIB1/SRC-3* genotype and a short CAG repeat length in the *AR* gene (<23) had a 2.8-fold risk (OR,**

**2.78; 95% CI, 1.24–6.26). Together, our data indicate that the CAG/CAA repeat length in the *AIB1/SRC-3* gene may be associated with prostate cancer risk in Chinese men and that the combination of CAG/CAA repeat lengths in both the *AIB1/SRC-3* and *AR* genes may provide a useful marker for clinically significant prostate cancer. Expanded studies in other populations are needed to confirm this association and the combined effect of *AIB1/SRC-3* and other hormone-related genes in prostate cancer etiology.**

### Introduction

There exists large racial/ethnic variation in incidence rates of clinical prostate cancer, with African Americans having rates that are 10–30 times higher than those for Asians (1–3). The reasons for the substantial racial/ethnic differences in prostate cancer risk are unclear. Because androgens regulate the growth and division of prostatic cells, it has been suggested that population differences in androgen biosynthesis, metabolism, and transport may contribute to some of the marked racial/ethnic differences in the incidence of clinical prostate cancer (4–6).

*AR*<sup>2</sup> plays a key role in intraprostatic androgenic action. Within the prostate gland, testosterone is converted into DHT, a more potent androgen. DHT then binds to the *AR* to form an intracellular DHT-*AR* complex, which in turn modulates prostatic target genes to induce proliferation. It has been shown in healthy subjects that CAG repeat length in several genes is polymorphic and that a longer CAG repeat length in the *AR* gene, which encodes the polyglutamine region in the *AR* protein, interferes with *AR* transcriptional activation (and thereby, *AR* function). It has been suggested that in both Western and Asian men, variation in CAG repeat length in the *AR* gene is related to prostate cancer risk and may help explain part of the substantial differences in prostate cancer risk across populations (7–9). In an earlier report, we confirmed that a shorter CAG repeat length in the *AR* gene conferred a higher prostate cancer risk in Chinese men, a low-risk population (9).

Transactivation of *AR* is related not only to *AR* gene CAG repeat length but also to other factors, including the ligand and coactivators. *In vitro* studies have shown that several *AR* coactivators, including *ARA55*, *ARA70*, *Rb*, *BRCA1*, and *AIB1*, enhance *AR*-mediated transcription 2–10-fold (10–13), suggesting that *AR* coactivators may affect the risk of prostate cancer through their influence on intraprostatic androgenic action. The *AIB1* protein (also known as *SRC-3*), encoded by the *AIB1/SRC-3* gene located on chromosome 20 (20q12), is an *AR* coactivator and a member of the steroid receptor coactivator family, which interacts with members of the nuclear hormone

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<sup>2</sup> The abbreviations used are: *AR*, androgen receptor; *DHT*, dihydrotestosterone; *AIB1*, amplified in breast cancer 1; *SRC-3*, steroid receptor coactivator-3; *OR*, odds ratio; *CI*, confidence interval.

receptor family (14, 15). Like the AR protein, the AIB1/SRC-3 coactivator contains a stretch of glutamine residues encoded by a variable-length track of CAG/CAA repeats in the *AIB1/SRC-3* gene. The effect of different CAG/CAA repeat lengths on the activity of the final AIB1/SRC-3 coactivator is as yet unknown. However, because the CAG repeat length in the *AR* gene directly affects AR function, it is possible that the length of the polyglutamine stretch in the AIB1/SRC-3 protein alters protein stability and/or potency to enhance hormone action through nuclear receptors. Therefore, variation in CAG/CAA repeat length in the *AIB1/SRC-3* gene may affect not only sensitivity to hormones, but also susceptibility to prostate cancer.

As part of a multidisciplinary population-based case-control study, in this report we examine the relationship of CAG/CAA repeat length in the *AIB1/SRC-3* gene, both independently and in conjunction with each of two polymorphisms in the *AR* gene, with prostate cancer risk in Chinese men to elucidate further the role of genetic factors in prostate cancer.

## Materials and Methods

### Study Subjects

Details of the study have been described previously (9, 16–18). Briefly, cases of primary prostate cancer (ICD9 185) newly diagnosed between 1993 and 1995 were identified through a rapid reporting system established between the Shanghai Cancer Institute and 28 collaborating hospitals in urban Shanghai. Cases were permanent residents in 10 urban districts of Shanghai (henceforth referred to as Shanghai) who had no history of other cancer. Contrary to many Western countries, prostate cancer screening is not widespread in China; therefore, cases in this study were clinically significant prostate cancers who presented with symptoms.

On the basis of the personal registry cards of all adults over age 18 residing in urban Shanghai (maintained at the Shanghai Resident Registry), male population controls were selected randomly from the 6.5 million permanent residents of Shanghai and frequency-matched to the expected age distribution (5-year category) of prostate cancer cases. Included controls were negative for prostate cancer based on digital rectal exam and transrectal ultrasound.

Information on potential risk factors was elicited through an in-person interview by trained interviewers using a structured questionnaire. The interview included information on demographic characteristics, dietary and smoking history, consumption of alcohol and other beverages, medical history, family history of cancer, physical activity, body size, and sexual behavior. Of the 268 eligible cases (95% of the cases diagnosed in Shanghai during the study period), 243 (91%) were interviewed. After a consensus review by both the Chinese and American pathologists, four cases were classified as having benign prostatic hyperplasia and excluded from the study. Of the 495 eligible controls, 472 (95%) were interviewed. Most nonresponses were attributable to refusal.

### Blood Collection and DNA Extraction

Two hundred cases (82% of those interviewed) and 330 controls (70%) provided 20 ml of fasting blood for the study. The blood samples were processed at a central laboratory in Shanghai. The buffy coat samples were first stored at  $-70^{\circ}\text{C}$  and then shipped to the United States in dry ice for DNA extraction at the American Type Culture Collection (Manassas, VA), by a standard DNA extraction protocol. Quality-control procedures showed no evidence of contamination, and DNA purity and

length were satisfactory. After DNA extraction, 190 cases and 305 controls had sufficient DNA for genotyping. DNA samples were arranged in case-control pairs/triplets to minimize day-to-day laboratory variation, and laboratory personnel were masked to case-control status.

### Genotyping

**AIB1/SRC-3 Gene.** The polyglutamine region of the AIB1/SRC-3 protein is encoded by two glutamine codons in the *AIB1/SRC-3* gene on chromosome 20 (GenBank accession no. AF012108): CAG and CAA. The usual sense codon sequence of the polyglutamine stretch is  $(\text{CAG})_n \text{CAA}$   $(\text{CAG})_n (\text{CAA})_4 \text{CAG CAA}$   $(\text{CAG})_2 \text{CAA}$ . The two variable-length tracks of CAG repeats  $[(\text{CAG})_n]$  usually contain six repeats between nucleotides 3930 and 3947 and nine repeats between nucleotides 3951 and 3977, for a total repeat length of 29 (19). This polymorphism has previously been described by Shirazi *et al.* (19). However, whereas Shirazi *et al.* scored genotypes of this marker using only the two variable  $(\text{CAG})_n$  stretches, we scored the total number of continuous CAG and CAA triplets in the entire polyglutamine region of the *AIB1/SRC-3* gene, as has been done more recently (20, 21).

We determined the number of CAG/CAA repeats in the polyglutamine stretch of the *AIB1/SRC-3* gene by amplifying the gene's COOH-terminal polyglutamine region in each sample, using custom flanking primers ( $5'$ -TCATCACCTCCGA-CAACAGAGG- $3'$  and  $5'$ -TATGGAACTGTTGCGGAG-GAG- $3'$ ) and the Advantage 2 Polymerase System (Clontech). The number of CAG/CAA repeats was determined by electrophoresis of the PCR products on an acrylamide gel and comparison with molecular weight standards. For confirmation, PCR products from selected samples were subsequently purified with the PCR Product Purification Kit (Qiagen) and sequenced directly with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems).

**AR Gene.** Genotypes of both the CAG (polyglutamine) and GGN (polyglycine) repeat length polymorphisms in exon 1 of the *AR* gene (located on the X chromosome) were determined as described previously (22). Briefly, we designed sets of oligonucleotide primers that flanked each of the two polymorphic regions for use in DNA amplification and direct sequencing. For the polyglutamine stretch, the number of continuous CAG or CAA triplets was counted directly, whereas for the polyglycine stretch, the number of continuous GGN repeats (where N represents T, G, or C) was counted directly.

**Quality Control.** Because the PCR procedure is prone to contamination, a negative, water-blank control was always included in each batch of the PCR reactions (usually 9–18 DNA samples plus one negative control). If the negative control was shown to be positive, the assay was repeated for the entire batch. Twenty-four split samples from a single individual were spaced at intervals among the study samples to assess the reproducibility of genotyping. For the *AIB1/SRC-3* gene, all of the 24 split samples had a CAG/CAA repeat length of 29 in both alleles. Of the 21 split samples with *AR* CAG results, 19 (90%) had the same repeat number (repeat length, 23); 1 had one more, and 1 had one less repeat. Of the 20 samples with *AR* GGN results, 19 (95%) had the same repeat number (repeat length, 23), and 1 had one less repeat.

### Statistical Analysis

To estimate the prostate cancer risks associated with *AIB1/SRC-3* genotypes, we used unconditional logistic regression

**Table 1** Frequencies of *AIB1/SRC-3* CAG/CAA repeat length alleles and genotypes in prostate cancer cases and controls in China

	Cases		Controls	
	n	%	n	%
<i>AIB1/SRC-3</i> allele				
24	0	0.0	1	0.2
26	22	5.8	39	6.5
27	6	1.6	7	1.2
28	114	30.0	140	23.5
29	224	58.9	391	65.6
30	11	2.9	13	2.2
31	1	0.3	5	0.8
32	2	0.5	0	0.0
Total	380		596	
<i>AIB1/SRC-3</i> genotype				
24/29	0	0.0	1	0.3
26/26	0	0.0	1	0.3
26/27	0	0.0	1	0.3
26/28	5	2.6	6	2.0
26/29	17	8.9	26	8.7
26/30	0	0.0	2	0.7
26/31	0	0.0	2	0.7
27/28	0	0.0	1	0.3
27/29	5	2.6	5	1.7
27/30	1	0.5	0	0.0
28/28	23	12.1	21	7.0
28/29	59	31.1	88	29.5
28/30	3	1.6	1	0.3
28/31	1	0.5	2	0.7
29/29	67	35.3	130	43.6
29/30	7	3.7	10	3.4
29/31	0	0.0	1	0.3
29/32	2	1.1	0	0.0
Total	190		298	

models to derive ORs and corresponding 95% CIs (23). Because of the lack of functional data regarding the alleles of the *AIB1/SRC-3* CAG/CAA repeat length marker, optimal categorization of the genotypes is unknown. In this investigation, the distribution of the number of the CAG/CAA repeat lengths among controls was used to derive the median cutoffs used to calculate the ORs. Because the *AIB1/SRC-3* gene is located on chromosome 20, each individual carries two alleles. In contrast, because the *AR* gene is located on the X chromosome, there is only one allele for each individual. We previously showed that 23 is the median repeat length for both the *AR* CAG and the *AR* GGN polymorphisms in this Chinese population (9); therefore, subjects in the present *AIB1/SRC-3* analysis were grouped by <23 versus  $\geq$ 23 repeats in analyses stratified by each of the two *AR* gene polymorphisms. The level of significance for all results reported herein is 0.05.

## Results

Age at diagnosis ranged from 50 to 94 years (median, 73 years) for cancer cases. Because there is no widespread prostate cancer screening in China, cases in this study were mostly men with clinically significant prostate cancer. Accordingly, approximately two-thirds of the cases were diagnosed as having advanced (regional/remote stage) cancer, and most tumors were moderately or poorly differentiated. Most cases were symptomatic at diagnosis, and 77% had serum prostate-specific antigen levels >10 ng/ml (median, 87 ng/ml). Compared with population controls, cases had significantly higher caloric intake, had significantly larger waist-to-hip ratios, and were

**Table 2** Age-adjusted ORs for prostate cancer in relation to CAG/CAA repeat lengths in the *AIB1/SRC-3* gene in Chinese men

Allele 1	Allele 2	Cases	Control	OR	95% CI
29	29	67	130	1.00	
29	30, 31, 32	9	11	1.58	0.62–4.01
29	28	59	88	1.30	0.83–2.03
29	24, 26, 27	22	32	1.33	0.72–2.47
30, 31	26, 27, 28	5	7	1.38	0.42–4.52
28	28	23	21	2.12	1.09–4.12
26, 27	26, 27, 28	5	9	1.08	0.35–3.35
29	29	67	130	1.00	
<29	29	81	120	1.31	0.87–1.97
<29	<29	28	30	1.81	1.00–3.28
>29	29	9	11	1.59	0.63–4.03
>29	<29	5	7	1.39	0.43–4.54

somewhat less likely to be married, have attended college, or be smokers or drinkers, although not significantly so (data reported in Ref. 9).

The distributions of the alleles and genotypes of the *AIB1/SRC-3* gene CAG/CAA repeat length marker by case-control status are shown in Table 1. Among controls, the CAG/CAA repeat length ranged from 24 to 32, with 29, 28, and 26 being the most common repeat lengths (65.6, 23.5, and 6.5%, respectively). Eighty-eight percent of the controls had at least one 29 allele. Homozygous 29/29 and heterozygous 28/29 were the most common genotypes, with 44 and 30% of the controls harboring these genotypes, respectively.

Relative to men homozygous for 29 CAG/CAA repeats in the *AIB1/SRC-3* gene (29/29 genotype), subjects homozygous for the 28 allele had a significant risk increase (OR, 2.12; 95% CI, 1.09–4.12; Table 2). Subjects with the 28/29 genotype had a nonsignificantly increased risk relative to the 29/29 genotype (OR, 1.30; 95% CI, 0.83–2.03), as did men with one 29 allele and one 24, 26, or 27 allele (OR, 1.33; 95% CI, 0.72–2.47) and men with one 29 allele and one 30, 31, or 32 allele (OR, 1.58; 95% CI, 0.62–4.01).

On the basis of the median CAG/CAA repeat length of 29, we categorized the various *AIB1/SRC-3* alleles as <29, 29, and >29 (Table 2). Relative to men with the 29/29 genotype, men with one <29 and one 29 allele had a moderately but nonsignificantly increased risk (OR, 1.31; 95% CI, 0.87–1.97). Those with two <29 alleles had a marginally significant 81% increased risk (OR, 1.81, 95% CI, 1.00–3.28) relative to men with the 29/29 genotype. Finally, men with the >29 allele (>29/29 and >29/<29 genotypes) had a somewhat, although not significantly, increased risk.

The risks of prostate cancer associated with various repeat lengths in both the *AIB1/SRC-3* gene CAG/CAA polymorphisms as well as the two polymorphisms of the *AR* gene are shown in Table 3. Relative to men both homozygous for the 29 CAG/CAA allele (29/29 genotype) of the *AIB1/SRC-3* gene and having a long *AR* CAG repeat length ( $\geq$ 23 repeats), men with both the (<29/29) *AIB1/SRC-3* CAG/CAA genotype and a short *AR* CAG repeat length (<23) had a significant 2-fold risk (OR, 2.00; 95% CI, 1.12–3.59), whereas men both homozygous for the <29 *AIB1/SRC-3* CAG/CAA allele and having a short CAG repeat length (<23) in the *AR* gene had a significant 2.8-fold risk (OR, 2.78; 95% CI, 1.24–6.26). On the other hand, men with the *AIB1/SRC-3* >29/29 genotype and <23 *AR* CAG repeats had a nonsignificantly increased risk (OR, 2.50; 95% CI, 0.83–7.60) relative to men with *AIB1/SRC-3* 29/29 genotype and  $\geq$ 23 *AR* CAG repeats. Similarly, men both ho-



Table 3 ORs<sup>a</sup> for prostate cancer in relation to *AIB1/SRC-3* CAG/CAA and *AR* CAG or GGN repeat lengths in Chinese men

AR polymorphisms	CAG/CAA repeat length genotypes of the <i>AIB1/SRC-3</i> gene														
	29/29			<29/29			<29/<29			>29/29			>29/<29		
	<i>n</i> <sub>1</sub> / <i>n</i> <sub>2</sub> <sup>b</sup>	OR	95% CI	<i>n</i> <sub>1</sub> / <i>n</i> <sub>2</sub>	OR	95% CI	<i>n</i> <sub>1</sub> / <i>n</i> <sub>2</sub>	OR	95% CI	<i>n</i> <sub>1</sub> / <i>n</i> <sub>2</sub>	OR	95% CI	<i>n</i> <sub>1</sub> / <i>n</i> <sub>2</sub>	OR	95% CI
CAG repeat length <sup>c</sup>															
≥23	29/64	1.00		31/65	1.05	0.57–1.94	9/14	1.42	0.55–3.66	1/4	0.55	0.06–5.20	4/4	2.21	0.51–9.48
<23	37/64	1.27	0.70–2.32	50/55	2.00	1.12–3.59	19/15	2.78	1.24–6.26	8/7	2.50	0.83–7.60	1/3	0.72	0.07–7.34
GGN repeat length <sup>c</sup>															
≥23	49/104	1.00		69/93	1.57	0.99–2.49	19/23	1.75	0.87–3.51	8/8	2.12	0.75–5.99	3/6	1.06	0.25–4.42
<23	16/23	1.47	0.71–3.04	12/24	1.07	0.49–2.31	7/6	2.47	0.79–7.77	1/3	0.70	0.07–6.94	2/0		

<sup>a</sup> Adjusted for age.<sup>b</sup> *n*<sub>1</sub> number of cases; *n*<sub>2</sub>, number of controls.<sup>c</sup> Median number of repeats among controls was used for the cutoff.

mozygous for the <29 *AIB1/SRC-3* CAG/CAA allele and having a short GGN repeat length (<23) in the *AR* gene had a nonsignificant 2.5-fold risk (OR, 2.47; 95% CI, 0.79–7.77) relative to men both homozygous for the 29 *AIB1/SRC-3* CAG/CAA allele and having a long *AR* GGN repeat length (≥23 repeats).

There was no correlation between the repeat lengths in the *AR* and *AIB1/SRC-3* genes. In addition, the number of CAG/CAA repeats in the *AIB1/SRC-3* gene did not correlate with education, body mass index, waist-to-hip ratio, total caloric intake, serum levels of sex hormones (testosterone; DHT; 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol glucuronide; and estradiol), or sex hormone-binding globulin. These variables therefore were not included in the logistic model for adjustment. In addition, ORs were materially unchanged when the analysis was stratified by clinical stage (localized *versus* advanced stage disease, data not shown).

## Discussion

Results from this population-based case-control study in China suggest that the *AIB1/SRC-3* CAG/CAA repeat length marker is associated with prostate cancer risk and that men with one or more alleles other than the 29 CAG/CAA repeat allele may have an increased risk of clinically significant prostate cancer. Furthermore, our results suggest that this effect, although independent of *AR* genotypes, is more pronounced among men with a smaller number of *AR* CAG repeats.

The observed association with CAG/CAA repeat length in the *AIB1/SRC-3* gene is biologically plausible. Data from transient transfection studies show that the *AIB1/SRC-3* coactivator enhances *AR* transcriptional activity in the presence of DHT (12), suggesting that the *AIB1/SRC-3* coactivator, in conjunction with *AR*, may increase androgenic activity within the prostate gland. Amplification of the *AIB1/SRC-3* gene has been implicated in the etiology of several other hormone-dependent cancers as well, including breast and ovarian cancers (24). Furthermore, recent clinical data suggest that overexpression of *AR* in prostate tumors may contribute to hormone sensitivity and tumor progression (25). One possible explanation for this could be a change in the ratio of *AR* to *AR* coactivators. The relative distribution of *AR* coactivators in prostate tumors may therefore play an important role in prostate tumor progression.

Racial/ethnic variation in the *AIB1/SRC-3* CAG/CAA repeat length mirrors the risk patterns of prostate cancer in high- and low-risk populations (19, 26), thus indirectly supporting a role of *AIB1/SRC-3* in prostate cancer etiology. In a small survey of 112 African Americans, 19 Chinese, and 18 Caucasians, the allele frequency of 29 CAG/CAA repeats was 61, 76, and 58%, respectively. Given the genetic variation in *AIB1/*

*SRC-3* and its role as an *AR* coactivator in androgenic signaling pathways, it is possible that polymorphisms of *AIB1/SRC-3*, and perhaps other *AR* coactivators, may play a role in the development of prostate cancer.

Our observed association between the *AIB1/SRC-3* gene and risk of clinically significant prostate cancer in Chinese men is inconsistent with the results of a previous study conducted among Caucasian men in the United States, which found no such association (20). The reasons for this inconsistency are unclear. Because there is no widespread prostate cancer screening in China, a larger proportion of the cases in the present study were at a clinically advanced stage compared with those of the previous study (20). However, even when limited to men with high-grade tumors, the previous study found no excess risk associated with *AIB1/SRC-3* CAG/CAA repeat lengths shorter than 29 (20). Differences between the populations may also partly explain the inconsistency of the findings. For example, Chinese men appear to have a higher prevalence of the 29 allele than do Caucasians in the United States (65.6 *versus* 47.8%; Ref. 20). Regardless, further investigation is needed to confirm our results.

The molecular mechanism by which steroid-ligand and coactivators interact with *AR* to enhance gene transcription is unclear. The coactivator domains most likely to be involved in altered receptor-coactivator-mediated transcription are the interaction domains between the receptor and the cofactor, as well as the cofactor domain that interacts with the basal transcriptional machinery (14, 27). Many steroid receptor coactivators use LxxLL motifs (or NR boxes) to interact with the ligand-binding domain of the receptor (28). Thus, it is possible that germ-line or somatic mutations in this region of the coactivator gene may be involved in prostate cancer development.

In an earlier report (9), we showed that Chinese men have a longer mean CAG repeat length in the *AR* gene than do Western men and that a shorter *AR* CAG repeat length was associated with an increased risk of prostate cancer in this low-risk population (OR for <23 *versus* >23 repeats, 1.65; 95% CI, 1.14–2.39). The observed association with CAG/CAA repeat length in the *AIB1/SRC-3* gene is independent of the *AR* gene: regardless of CAG repeat length in the *AR* gene, men homozygous for the <29 *AIB1/SRC-3* allele had a higher risk than those homozygous for the 29 allele. However, the risk associated with the <29/<29 *AIB1/SRC-3* genotype was more pronounced among those with the short *AR* CAG repeat length, suggesting that genetic variations in *AR* and its coactivator may affect *AR* transactivation and alter the risk of prostate cancer.

It is possible that the *AIB1/SRC-3* CAG/CAA repeat length polymorphism is related to other polymorphic regions within the *AR* and *AIB1/SRC-3* genes, or even to a nearby gene

in linkage disequilibrium with this locus that may be the actual susceptibility locus and confound the results. Future and expanded studies are needed to evaluate the individual and combined effects of *AIB1/SRC-3* with other hormone-related genes to further clarify the underlying mechanism of androgenic pathways in prostate carcinogenesis.

Rare genetic factors with high penetrance that confer a much higher relative risk to the few individuals who carry them (e.g., *HPC1* on chromosome 1 has been estimated to explain 10% of the prostate cancer cases in the United States; Ref. (29)) are unlikely to explain the large racial difference in prostate cancer risk. In contrast, the common polymorphism of the *AIB1/SRC-3* gene has the potential to confer a more variable risk on all individuals, which in turn may result in a much larger proportion of prostate cancer cases attributable to certain genotypes.

Survival and selection biases in our study should be minimal because well over 90% of the eligible cases participated in the study and most cases were interviewed within 30 days after diagnosis. Seventy to 80 percent of the study subjects gave blood for the study, so it is unlikely that response status among cases and controls was related to the observed allele frequencies.

In summary, this population-based study conducted in a low-risk population suggests that the CAG/CAA repeat length polymorphism in the *AIB1/SRC-3* gene may be associated with risk of clinically significant prostate cancer. Although we are unable to generalize our results directly to Western populations, similar underlying biological mechanisms may exist for other racial/ethnic groups. Laboratory studies of *AIB1/SRC-3* CAG/CAA repeat length polymorphism functionality and epidemiological studies in other ethnic groups are needed to confirm the observed association and to clarify whether *AIB1/SRC-3* together with *AR* or other hormone-related genes can serve as useful molecular markers for identification of men at higher risk of developing clinically significant prostate cancer.

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### References

- Hsing, A. W., Devesa, S. S., Jin, F., and Gao, Y. T. Rising incidence of prostate cancer in Shanghai, China. *Cancer Epidemiol. Biomark. Prev.*, 7: 83–84, 1998.
- Hsing, A. W., Tsao, L., and Devesa, S. S. International trends and patterns of prostate cancer incidence and mortality. *Int. J. Cancer*, 85: 60–67, 2000.
- Hsing, A. W., and Devesa, S. S. Trends and patterns of prostate cancer: what do they suggest? *Epidemiol. Rev.*, 23: 3–13, 2001.
- Ross, R. K., Pike, M. C., Coetzee, G. A., Reichardt, J. K., Yu, M. C., Feigelson, H., Stanczyk, F. Z., Kolonel, L. N., and Henderson, B. E. Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. *Cancer Res.*, 58: 4497–4504, 1998.
- Ross, R. K., Bernstein, L., Lobo, R. A., Shimizu, H., Stanczyk, F. Z., Pike, M. C., and Henderson, B. E. 5- $\alpha$ -Reductase activity and risk of prostate cancer among Japanese and US white and black males. *Lancet*, 339: 887–889, 1992.
- Shibata, A., and Whittemore, A. S. Genetic predisposition to prostate cancer: possible explanations for ethnic differences in risk. *Prostate*, 32: 65–72, 1997.
- Stanford, J. L., Just, J. J., Gibbs, M., Wicklund, K. G., Neal, C. L., Blumenstein, B. A., and Ostrander, E. A. Polymorphic repeats in the androgen receptor gene: molecular markers of prostate cancer risk. *Cancer Res.*, 57: 1194–1198, 1997.
- Giovannucci, E., Stampfer, M. J., Krithivas, K., Brown, M., Dahl, D., Brufsky, A., Talcott, J., Hennekens, C. H., and Kantoff, P. W. The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. *Proc. Natl. Acad. Sci. USA*, 94: 3320–3323, 1997.

- Hsing, A. W., Gao, Y. T., Wu, G., Wang, X., Deng, J., Chen, Y. L., Sesterhenn, I. A., Mostofi, F. K., Benichou, J., and Chang, C. Polymorphic CAG and GGN repeat lengths in the androgen receptor gene and prostate cancer risk: a population-based case-control study in China. *Cancer Res.*, 60: 5111–5116, 2000.
- Yeh, S., Miyamoto, H., Shima, H., and Chang, C. From estrogen to androgen receptor: a new pathway for sex hormones in prostate. *Proc. Natl. Acad. Sci. USA*, 95: 5527–5532, 1998.
- Heinlein, C. A., Ting, H. J., Yeh, S., and Chang, C. Identification of ARA70 as a ligand-enhanced coactivator for the peroxisome proliferator-activated receptor gamma. *J. Biol. Chem.*, 274: 16147–16152, 1999.
- Yeh, S., Kang, H. Y., Miyamoto, H., Nishimura, K., Chang, H. C., Ting, H. J., Rahman, M., Lin, H. K., Fujimoto, N., Hu, Y. C., Mizokami, A., Huang, K. E., and Chang, C. Differential induction of androgen receptor transactivation by different androgen receptor coactivators in human prostate cancer DU145 cells. *Endocrine*, 11: 195–202, 1999.
- Yeh, S., and Chang, C. Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. *Proc. Natl. Acad. Sci. USA*, 93: 5517–5521, 1996.
- Anzick, S. L., Kononen, J., Walker, R. L., Azorsa, D. O., Tanner, M. M., Guan, X. Y., Sauter, G., Kallioniemi, O. P., Trent, J. M., and Meltzer, P. S. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science (Wash. DC)*, 277: 965–968, 1997.
- Bautista, S., Valles, H., Walker, R. L., Anzick, S., Zeillinger, R., Meltzer, P., and Theillet, C. In breast cancer, amplification of the steroid receptor coactivator gene *AIB1* is correlated with estrogen and progesterone receptor positivity. *Clin. Cancer Res.*, 4: 2925–2929, 1998.
- Hsing, A. W., Chen, C., Chokkalingam, A. P., Gao, Y. T., Dightman, D. A., Nguyen, H. T., Deng, J., Cheng, J., Sesterhenn, I. A., Mostofi, F. K., Stanczyk, F. Z., and Reichardt, J. K. V. Polymorphic markers in the *SRD5A2* gene and prostate cancer risk: a population-based case-control study. *Cancer Epidemiol. Biomark. Prev.*, 10: 1077–1082, 2001.
- Hsing, A. W., Deng, J., Sesterhenn, I. A., Mostofi, F. K., Stanczyk, F. Z., Benichou, J., Xie, T., and Gao, Y. T. Body size and prostate cancer: a population-based case-control study in China. *Cancer Epidemiol. Biomark. Prev.*, 9: 1335–1341, 2000.
- Hsing, A. W., Chua, S., Jr., Gao, Y. T., Gentschev, E., Chang, L., Deng, J., and Stanczyk, F. Z. Prostate cancer risk and serum levels of insulin and leptin: a population-based study. *J. Natl. Cancer Inst.* (Bethesda), 93: 783–789, 2001.
- Shirazi, S. K., Bober, M. A., and Coetzee, G. A. Polymorphic exonic CAG microsatellites in the gene amplified in breast cancer (*AIB1* gene). *Clin. Genet.*, 54: 102–103, 1998.
- Platz, E. A., Giovannucci, E., Brown, M., Cieluch, C., Shepard, T. F., Stampfer, M. J., and Kantoff, P. W. Amplified in breast cancer-1 glutamine repeat and prostate cancer risk. *Prostate J.*, 2: 27–32, 2000.
- Rebeck, T. R., Wang, Y., Kantoff, P. W., Krithivas, K., Neuhausen, S. L., Godwin, A. K., Daly, M. B., Narod, S. A., Brunet, J. S., Vesprini, D., Garber, J. E., Lynch, H. T., Weber, B. L., and Brown, M. Modification of *brca1*- and *brca2*-associated breast cancer risk by *aib1* genotype and reproductive history. *Cancer Res.*, 61: 5420–5424, 2001.
- Chang, C. S., Kokontis, J., and Liao, S. T. Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. *Proc. Natl. Acad. Sci. USA*, 85: 7211–7215, 1988.
- Breslow, N. E., and Day, N. E. *Statistical Methods in Cancer Research. I. The Analysis of Case-Control Studies.* IARC Scientific Publ. No. 32, pp. 5–338. Lyon, France: IARC, 1980.
- Thenot, S., Charpin, M., Bonnet, S., and Cavaillès, V. Estrogen receptor cofactors expression in breast and endometrial human cancer cells. *Mol. Cell. Endocrinol.*, 156: 85–93, 1999.
- Bubendorf, L., Kononen, J., Koivisto, P., Schraml, P., Moch, H., Gasser, T. C., Willi, N., Mihatsch, M. J., Sauter, G., and Kallioniemi, O. P. Survey of gene amplifications during prostate cancer progression by high-throughput fluorescence *in situ* hybridization on tissue microarrays. *Cancer Res.*, 59: 803–806, 1999.
- Hayashi, Y., Yamamoto, M., Ohmori, S., Kikumori, T., Imai, T., Funahashi, H., and Seo, H. Polymorphism of homopolymeric glutamines in coactivators for nuclear hormone receptors. *Endocr. J.*, 46: 279–284, 1999.
- McKenna, N. J., Xu, J., Nawaz, Z., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. Nuclear receptor coactivators: multiple enzymes, multiple complexes, multiple functions. *J. Steroid Biochem. Mol. Biol.*, 69: 3–12, 1999.
- Heery, D. M., Kalkhoven, E., Hoare, S., and Parker, M. G. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature (Lond.)*, 387: 733–736, 1997.
- Smith, J. R., Freije, D., Carpten, J. D., Gronberg, H., Xu, J., Isaacs, S. D., Brownstein, M. J., Bova, G. S., Guo, H., Bujnovszky, P., Nusskern, D. R., Damber, J. E., Bergh, A., Emanuelson, M., Kallioniemi, O. P., Walker-Daniels, J., Bailey-Wilson, J. E., Beaty, T. H., Meyers, D. A., Walsh, P. C., Collins, F. S., Trent, J. M., and Isaacs, W. B. Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. *Science (Wash. DC)*, 274: 1371–1374, 1996.

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