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Association of the polymorphisms of genes involved in androgen metabolism and signaling pathways with familial prostate cancer risk in a Japanese population

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Abstract

Background: Androgen plays a central role in the normal and malignant development of prostate glands. Genetic polymorphisms of genes involved in androgen metabolism and signaling might be associated with the risk of prostate cancer. Methods: One hundred and two patients with prostate cancer with a family history and 117 healthy age- and residence-matched male controls were enrolled. Genotypes of the CAG repeat length of androgen receptor (AR), CYP17, 5α-reductase type II (SRD5A2), UDG-glucuronosyltransferase (UGT) 2B15, PSA promoter genes were analyzed. Results: For single polymorphisms, the presence of Y alleles showed a significantly lower risk of prostate cancer in comparison with the D/D genotype in UGT2B15 (odds ratio [OR] = 0.41, 95% confidence interval [CI] = 1.40–4.28, $p = 0.0015$), and the presence of A2 alleles showed a weak tendency to decrease prostate cancer risk in comparison with the A1/A1 genotype in CYP17 (OR = 0.69, 95% CI = 0.39–1.23, $p = 0.21$). The stratification of cases according to clinical stage and pathological grade showed that the A2/A2 genotype was significantly associated with localized stage cancer in comparison with metastatic stage cancer (OR = 5.18, 95% CI = 1.49–17.95, $p = 0.007$). The combination of UGT2B15 and CYP17 genotypes could identify higher risk subjects even in subjects with low-risk UGT2B15 genotypes, i.e., Y/Y + D/Y genotypes (OR = 1.97, 95% CI = 0.92–4.22, $p = 0.079$). Conclusion: Genetic polymorphisms of the genes involved in androgen metabolism and signaling were significantly associated with familial prostate cancer risk. Single nucleotide polymorphisms of low-penetrance genes could be targets to understand genetic susceptibility to familial prostate cancer.

Keywords: AR CAG repeat; Single nucleotide polymorphisms; Genetic susceptibility; Risk factors; Genotyping; Genomic DNA; SRD5A2; UGT2B15; CYP17; PSA promoter; 5α-Reductase; Genetic polymorphism; Prostate cancer; Family history

1. Introduction

The incidence of prostate cancer is lower in Japan than in western countries [1]; however, it has been increasing every year in the prostate-specific antigen (PSA) era [2]. Understanding the etiopathophysiology of prostate cancer would provide important information for a screening program and the development of new treatment modalities. Androgen plays a central role in the normal and malignant growth and function of prostate glands, and differences in androgen metabolism and signaling pathways might influence the carcinogenesis of prostate cancer [3]. Genetic polymorphisms of the genes related with these pathways have attracted the attention of researchers in relation to genetic susceptibility to prostate cancer.

In androgen metabolism, key enzymes for androgen synthesis, activation and deactivation were elucidated. Cytochrome P-450 (CYP) 17 catalyzes steroid 17α-hydroxylase and 17–20 lyase activities in testosterone biosynthesis in both testes and adrenal glands [4]. A polymorphic T to C substitution in the 5′-untranslated region of the CYP17 gene creates a recognition site for the MspAI restriction enzyme. This polymorphic substitution also creates an SP-1 (CCACC box) promoter site, and this might increase transcriptional activities [5]. Steroid 5α-reductase type II (SRD5A2)

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catalyzes the conversion of testosterone to dihydrotestosterone (DHT). SRD5A2 contains two well-characterized polymorphic sites: an alanine to threonine substitution at codon 49 (A49T) and a valine to leucine substitution at codon 89 (V89L) [6,7]. The mutated enzyme resulting from T49 showed about 3.6-fold higher activity than the wild-type A49 enzyme, and the association of T49 with prostate cancer risk was reported [6]. Although the association of V89L genotypes in SRD5A2 with prostate cancer risk is controversial, a recent study from a Japanese group showed that the presence of the V allele significantly increased prostate cancer risk [8]. UDP-glucuronosyltransferases (UGTs) are phase II detoxification enzymes catalyzing the inactivation of endogenous and exogenous compounds by transfer of the glucuronyl group from 5′-diphosphoglucuronic acid [9]. UGT2B enzymes glucuronidate the primary steroid and UGT2B15 catalyzes the biotransformation of DHT in prostate glands [10]. Differences of the UGT2B15 enzyme could alter the intraprostatic DHT concentration.

Androgen signaling is mediated by AR, and the association of the genetic polymorphism of the CAG repeat length in exon 1 with prostate cancer risk was extensively examined. The transcriptional activity of AR was negatively correlated with the CAG repeat length of AR [11,12]. The shorter CAG repeat length could be associated with prostate cancer risk [13–17]. The PSA gene is the androgen-regulated gene and might influence the biology of prostate cancer [18,19]. The PSA promoter gene contains several genetic polymorphisms and the association of these polymorphisms with prostate cancer was examined [20,21].

We have extensively collected familial prostate cancer pedigrees in Japan [22,23]. Family history is one of the important risk factors for prostate cancer [24]. Several susceptibility loci or candidate genes have been reported to explain the genetic susceptibility of prostate cancer [25,26]; however, these genetic factors could explain the susceptibility of a very small percentage of familial/hereditary prostate cancer pedigrees [27,28]. Thus, we considered familial prostate cancer as a high-risk group for prostate cancer development, and reported the association of prostate cancer risk with the genetic polymorphism of high-penetrance genes including *HPC2/ELAC2* [29] and *RNASEL* [30] or low-penetrance genes encoding Vitamin D receptor gene [31], CYP1A1 [32], glutathione *S*-transferase [33] and p53 [34]. In this study, we examined the association of gene polymorphisms involved in androgen metabolism and signaling pathways with familial prostate cancer risk in a Japanese population.

2. Materials and methods

2.1. Patients

This case-controlled study included 102 prostate cancer patients with a family history of prostate cancer in first-degree relatives. The numbers of affected family members were two in 73 patients, three in 14 patients and four in 15 patients. Seventy-three patients with 2 affected family members were categorized into familial/not-hereditary prostate cancer, and 29 patients with 3 or more affected family members were categorized into hereditary prostate cancer according to Carter et al.'s definition [35]. All prostate cancer patients were confirmed histologically at Gunma University Hospital and its affiliated hospitals. Their ages ranged from 40 to 88 years old with a mean age of 69.9 years. Clinical stages were A in 2, B in 41, C in 32, D in 25 and unknown in 2 patients according to Jewett's staging system. Gleason scores were less than 7 in 25 and equal or more than 7 in 77 patients. One hundred seventeen non-cancer controls were recruited from clinics at Gunma University Hospital. Controls were excluded if they had an abnormal PSA level (i.e., $\geq$4.0 ng/ml), and abnormal digital rectal examination, and previous diagnosis of cancer. Their ages ranged from 51 to 88 years old with a mean of 71.0 years. No significant differences in age were observed between cases and controls. All cases and controls enrolled in this study under informed consent. The Ethical Committee of Gunma University approved this study.

2.2. Genotyping

Genomic DNA was isolated from whole blood using a GENOMIX kit (Talent srl. Trieste, Italy). Samples were diluted to 10 μg/μl and stored at −20 °C. To analyze the CAG repeat length of the AR gene, CAG repeat containing regions were amplified according to the method described by Yoshida et al. [36], and CAG repeat length was measured by direct sequencing of PCR products. Sequencing analysis was performed by the method described previously [37]. For the detection of UGT2B15 variants, allele-specific PCR was performed according to the method described by MacLeod et al. [38]. The G allele and T allele corresponded to aspartate (D) and tyrosine (Y) as encoded amino acids, respectively. Genotypes of CYP17, SRD5A2 and PSA genes were determined by the PCR-based restriction fragment length polymorphism method. For T/C polymorphism of the CYP17 gene, the wild-type allele, A1, and mutated allele, A2, were determined by the method described by Gsur et al. [39]. For SRD5A2, V89L polymorphisms were determined by the modified method described by Yamada et al. [40] Genomic DNA (40 ng) was mixed with dNTP (300 μM), 1 μM forward and reverse primers and 2.5 units of ProofStart DNA polymerase (Qiagen, Valencia, CA, USA) in the presence of 1× ProofStart PCR buffer and 1× Q-Solution according to the manufacturer's protocol (Qiagen). Cycling conditions were: 95 °C for 5 min for 1 cycle; 94 °C for 30 s; 73 °C for 60 s for 35 cycles, followed by an elongation cycle of 72 °C for 7 min. Obtained PCR products were digested with *Rsa*I for detection of the V89L polymorphism of SRD5A2. For the PSA gene, the G/A polymorphism in the promoter region was determined by the method described by Xue