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Physiology

Androgen Receptor Trinucleotide Polymorphism in Leiomyoma

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***Objective*:** Androgen receptor (AR) was detected in leiomyoma. AR gene has a polymorphic microsatellite encoding cytosine, adenine, and guanine (CAG) repeats. We aimed to investigate the association between the AR gene CAG repeats and leiomyoma.
***Methods*:** Women were divided into two groups: (1) leiomyoma ($n = 159$); (2) non- leiomyoma groups ($n = 129$). Their CAG repeats were detected by polymerase chain reaction. The CAG repeats ranged in length from 168 bp (9 CAG repeats, genotype A) to 234 bp (31 CAG repeats, genotype W). Distributions of CAG repeats in both groups were compared.
***Results*:** Genotype proportions of different CAG repeats for AR gene in both groups were significantly different. The genotype S (27 CAG repeats) is associated with higher susceptibility of leiomyoma. Distribution of CAG repeats in both groups appeared mono-peak distributions. Percentages of genotypes K-S (19–27 CAG repeats) in leiomyoma and non-leiomyoma groups were: (1) 5, 11, 19.5, 10.4, 12.9, 8.8, 7.5, 5.7, 4.4%; (2) 5.4, 14.3, 16.7, 12.8, 12.4, 5.8, 9.3, 7, 1.2%.
***Conclusions*:** AR trinucleotide polymorphism is associated with leiomyoma susceptibility. The 27 CAG repeats is related with higher risk of leiomyoma.

KEY WORDS: Androgen receptor; leiomyoma; multiallele polymorphism; trinucleotide repeat polymorphism.

INTRODUCTION

Leiomyoma, the most common benign uterine neoplasma, is occurred in around one forth of the women during their lifetimes (1). Leiomyoma growth may be derived from growth and proliferation of a single smooth muscle cell (2). Leiomyoma is a complex disease, which is caused by an interaction between multiple genes, hormone, growth factor, cytokines, and the environment. Steroid hormones secreted by the ovaries are necessary for the growth of leiomyoma. Leiomyoma is related with the auto-and paracrine interaction of sex-steroid hormone (3,4).

The identification of the related genes is essential for genetic diagnosis and therapy for genetic-associated disease. Genetic studies of the multifactorial disease such as leiomyoma are difficulty to approach due to the uncertainty of a polygenic trait. Polymorphisms are not directly linked to a certain disease. However, they are useful tools in the study of multifactorial disorders.

Androgen receptor (AR) is involved in various biological processes such as sexual differentiation, maturation and spermatogenesis. The AR gene has a polymorphic cytosine, adenine, and guanine (CAG) microsatellite in exon one that codes for variable-length glutamine repeats in the N-terminal domain

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of the AR protein (5). Mitsumori *et al.* (5) demonstrated that the shorter CAG alleles may be a genetic factor that promotes the growth of benign prostatic hyperplasia (BPH). Westberg *et al.* (6) demonstrated that the serum levels of androgens in premenopausal women may be influenced by CAG repeat polymorphism of the AR gene.

In our previous report, we observed that the AR gene polymorphism likely contributes to the pathogenesis of endometriosis (7). The 21 CAG repeats are related with higher risk of endometriosis. We also noted that the AR gene CAG repeat was associated with urolithiasis (8). However, the role of AR gene polymorphism in the development of the leiomyoma remains unclear. In this study, we aimed to evaluate the association between the leiomyoma and the AR gene CAG repeats. This is the first survey in this aspect.

PATIENTS AND METHODS

Pre-menopausal Taiwan Chinese women with surgically diagnosed leiomyoma and non-leiomyoma were included. All patients were divided into two groups: (1) leiomyoma ($n = 159$); (2) non-leiomyoma groups ($n = 118$). This study was approved by the Ethical Committee of the China Medical University Hospital. Informed consents were signed by all women who donated their blood. There were non-significant differences between both groups in age, weight, and height.

Deoxyribonucleic acid (DNA) was extracted from peripheral blood and subjected to analysis by PCR and gel electrophoresis of the PCR products. The CAG region of the AR gene was amplified by polymerase chain reaction (PCR). The primers were designed as follows: forward, 5′-TGCGCGAAGTGAT CCAGAAC-3′; reverse, 5′-CTTGGGGAGAACCA TCCTCA-3′ (534-513 % of coding region). The PCR reaction was carried out in a total volume of 25 μL containing genomic DNA; 2–6 pmole of each primer; 1XTaq polymerase buffer (1.5 mM $MgCl_2$); and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer Applied Biosystems, Foster City, U.S.A).

PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Perkin Elmer, Foster City, U.S.A.). Cycling condition was set as follows: on cycle at 94°C for 5 min, 35 cycles of 94°C for 10 s, 60°C for 45 s, and 72 °C for 1 min, and one final cycle of extension at 72°C for 40 min. Appropriate amount of PCR products (0.75 μL) were mixed with 1.75 μL of premixed solution (formamide: loading buffer (blue dextran, 50 mg/mL; EDTA, 25 mM):standard = 5:1:1). Genescan-350 TAMRA (6-carboxy-tetramethylrhodamine, red) (Perkin Elmer Applied Biosystems, Foster City, U.S.A) was used as the reference molecular size standard. Electrophoretic analysis was performed with the use of a 6% denaturing polyacrylamide gel and with ABI Prism 377 DNA Sequencer Perkin Elmer Applied Biosystems, Foster City, U.S.A). The data was analyzed with software GeneScan Analysis 2.1 (Perkin Elmer Applied Biosystems, Foster City, U.S.A.).

The PCR products ranged in length from 168 bp (containing 9 CAG repeats with the 141 bp of amplified flanking sequences) to 234 bp (31 CAG repeats). The genotype was classified into 'A' through 'T' according to the numbers of the CAG repeats from 9 to 31. The frequency and distribution of AR trinucleotide repeat polymorphisms were evaluated. Correlations of leiomyoma with the different AR genotypes were examined. The SAS system with χ^2 test and Fisher's exact test were used to examine the association between leiomyoma and genotype. The Fisher's exact test was performed when there exists the condition that more than 20% of cells have expected values less than 5, while χ^2 test was performed under no such condition. A P-value of <0.05 was considered statistically significant.

RESULTS

Genotype proportions of different AR gene polymorphisms in both groups were significantly different (Fig. 1). Their differences existed in the genotypes S (27 CAG repeats, $P = 0.04$). The genotype S (27 CAG repeats) is associated with higher susceptibility of leiomyoma. Women with genotypes S have higher risk of developing leiomyoma. The percentages of genotypes K-S (19–27 CAG repeats) in leiomyoma and non-leiomyoma groups were: (1) 5, 11, 19.5, 10.4, 12.9, 8.8, 7.5, 5.7, 4.4%; (2) 5.4, 14.3, 16.7, 12.8, 12.4, 5.8, 9.3, 7, 1.2%, respectively.

The distribution of CAG repeats for AR gene in both groups appeared mono-peak distributions. The main CAG repeats in patients with and without leiomyoma were 21 (genotype M). The χ^2 test was used in the analyses of genotype F and I-S. The Fisher's exact test was used in the analyses of genotype A-E, G, H, and T-W.

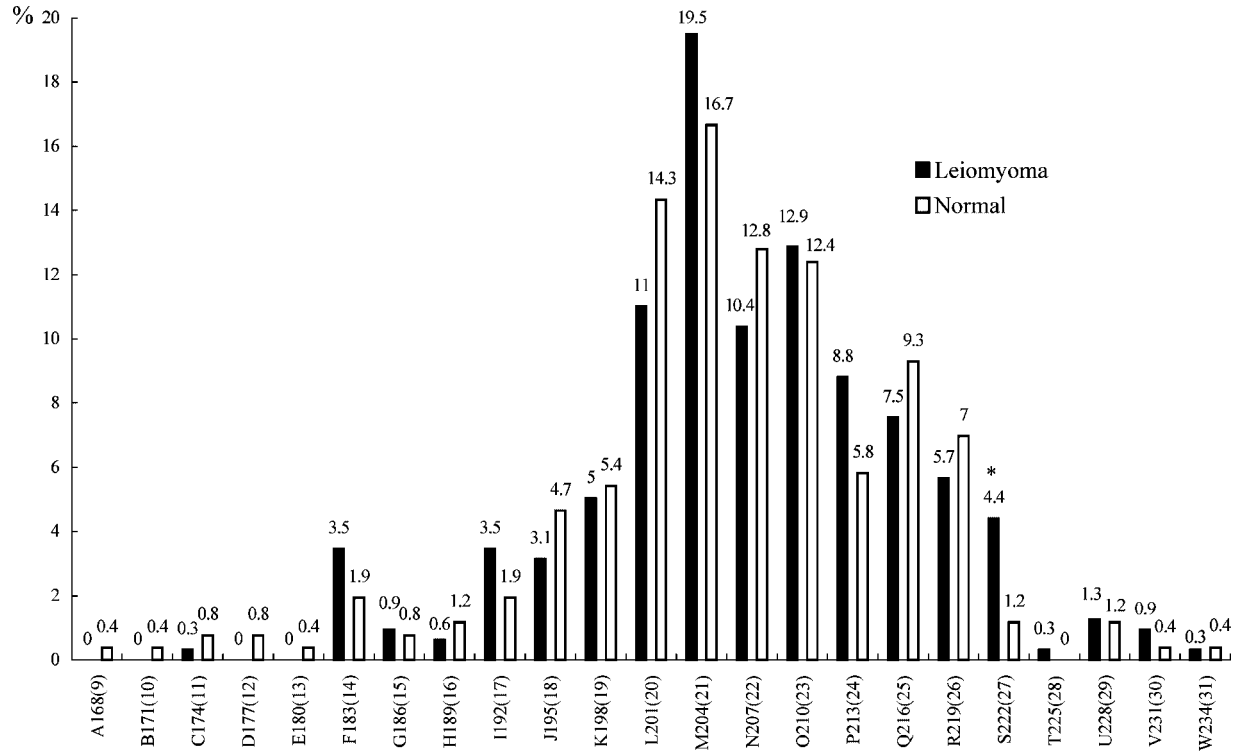

Fig. 1. Frequency distributions of androgen receptor CAG repeat length polymorphism in patients with leiomyoma ($n = 159$) and without leiomyoma ($n = 129$). The PCR products ranged in length from 168 bp (9 CAG repeats, genotype A) to 234 bp (31 CAG repeats, genotype W). The number demonstrates the percentage of individual genotype in each group (*difference existed in the genotypes between both groups).

DISCUSSION

Leiomyoma is the most common tumor in women, but its etiology is still unclear. Leiomyoma is thought to be monoclonal neoplasm (9). It has been demonstrated biochemical and immunocytochemical evidence about the hormone receptors in leiomyoma cells (10). Volume decrease of leiomyoma in GnRHa-treated patients is partly dependent on the concentrations of unbound sex-hormone receptors in the leiomyoma (11). Development of leiomyoma is mediated mainly by estrogen receptor. Estrogen may exert its mitogenic effects on leiomyoma through estrogen-dependent growth factors (12). Leiomyoma contain significantly more estrogen-binding estrogen receptor than the myometrium (13). However, AR also plays some role in development of leiomyoma. The AR was detected in leiomyoma, adenomyosis, and endometrial carcinoma (14).

Numerous disorders, such as leiomyoma, endometriosis, osteoporosis, hypertension, diabetes, and asthma, have been attributed to genetic susceptibility. Susceptibility genes are considered to interact with other genes and environment to produce the corresponding disorder (15). Unlike mutations, polymorphisms are not directly linked to a certain disease. However, they are useful tools in the study of multifactorial disorders (16,17). Polymorphism is related with the leiomyoma development, including AR (18), estrogen receptor (19), and insulin-like growth factor II gene polymorphisms (20). In our previous surveys, we observed the relationships of leiomyoma with estrogen receptor and IGF2 gene polymorphism (Hsieh *et al.*, unpublished data). We observed that the ER gene polymorphism likely contributes to the pathogenesis of leiomyoma (Hsieh *et al.*, unpublished data).

Numerous diseases are related with AR gene, including prostate carcinoma (21), BPH (5), uterine endometrial carcinoma (22), polycystic ovarian disease (23), breast carcinoma (24), androgen insensitivity (25), hirsutism (26), oligozoospermia (27), X-linked spinal and bulbar muscular atrophy (28), ankylosing spondylitis (29), amyotrophic lateral sclerosis (30), hypertrophic cardiomyopathy (30), Huntington's

disease (31). Levine and Boyd (32) demonstrated that the ovarian cancer patients who carried a short AR allele were diagnosed an average of 7.2 yr earlier than patients who did not carry a short allele. Mifsud *et al.* (33) observed that the increased CAG length was associated with a increased risk of azoospermia.

In contrast, some investigator demonstrated the non-association between AR gene polymorphism and the individual diseases, including isolated familial breast and ovarian cancers (34), familial prostate cancer (35), cryptorchidism (36), and impaired spermatogenesis of Klinefelter's syndrome (37). These controversies may be due to the multiple enzymatic processes and interactions, different illness classification, racial, environmental and disease variation.

In this study, we observed that the distributions of CAG repeats for AR gene were different between the individuals with leiomyoma and normal populations. We noted that the women with genotypes S (27 CAG repeats) have higher risk of developing leiomyoma. The higher prevalence of genotype S in women with leiomyoma suggested its genetic contribution upon the leiomyoma formation. Although the other CAG repeat between both groups appeared non-significantly different, their difference may exist after a larger series survey.

In conclusion, AR trinucleotide repeat polymorphism is associated with leiomyoma susceptibility. AR gene polymorphism likely contributes to the pathogenesis of leiomyoma. The 27 CAG repeats are related with higher risk of leiomyoma. The CAG repeats frequencies of AR gene polymorphism may become the candidate genetic marker for the prediction of leiomyoma susceptibility. This could provide the database for the further survey of the AR gene polymorphism. However, the real role of the AR trinucleotide polymorphism upon the leiomyoma remains to be clarified. Larger series are warranted to confirm these observations and to further examination interaction between AR gene polymorphism and leiomyoma development. Furthermore, other hormone gene polymorphisms upon leiomyoma development merit further surveys.

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