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Biochemical and Biophysical Research Communications 329 (2005) 100–104
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GGC and *Stu*I polymorphism on the androgen receptor gene in endometrial cancer patients

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Received 11 January 2005

Abstract

Androgens have an anti-proliferative effect on endometrial cells. Human androgen receptor (AR) gene contains two polymorphic short tandem repeats of GGC and CAG, and a single-nucleotide polymorphism on exon 1 that is recognized by the restriction enzyme, *Stu*I. Prior studies have shown that the lengths of the CAG repeat are inversely and linearly related to AR activity and associated with endometrial cancer. However, little is known about the GGC repeat and the *Stu*I polymorphism of the AR gene. Thus, we investigated whether these AR polymorphisms are risk factors for endometrial cancer. To test this hypothesis, the genetic distributions of these polymorphisms were investigated in blood samples from endometrial cancer patients and healthy controls. The allelic and genotyping profiles were analyzed by polymerase chain reaction (PCR), PCR–restriction fragment length polymorphism (PCR–RFLP), and direct DNA sequencing, and analyzed statistically. The GGC repeat was significantly longer in endometrial cancer patients as compared to normal healthy controls. In general, an increased risk of endometrial cancer was found with increasing GGC repeat. The relative risk for the 17 GGC repeat was greater than 4, as compared to controls. However, the *Stu*I polymorphism was not significantly different between patients and controls. The findings suggest that increased numbers of GGC repeat on the AR gene may be a risk factor for endometrial cancer.

Keywords: Androgen receptor gene; Endometrial cancer; GGC repeat; *Stu*I polymorphism

Endometrial carcinoma is the most common malignancy of the female genital tract and its incidence has recently increased [1]. However, the genetic basis of this disease is not well understood. The origin and growth of this tumor is thought to be influenced by a variety of steroid hormones [1,2]. Androgens influence endometrial cancer risk by binding to the androgen receptor (AR) and inhibit endometrial cell growth [2–5]. The AR gene is located on chromosome Xq11-12 and consists of eight exons [6,7]. The AR has two polymorphic repeats on exon 1, characterized by different numbers of GGC and CAG repeats resulting in variable lengths of polyglycine and polyglutamine regions [6,7]. A single-nucleotide polymorphism (SNP) at codon 211 (G1733A) has also been reported between the GGC and CAG repeats on exon 1 that is recognized by the restriction enzyme, *Stu*I [8]. Prior studies have shown that the length of the CAG repeat is inversely and linearly related to AR activity and has significant correlation with several cancers, including endometrial cancer [9–12]. However, little is known about the GGC repeats and *Stu*I polymorphism. Only one paper has been published indicating that the *Stu*I polymorphism was

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doi:10.1016/j.bbrc.2005.01.104

associated with prostate cancer [13]. Since androgens act directly on endometrium via the AR to inhibit endometrial cancer, women with decreased AR transactivation (carriers of longer repeat alleles) may have an elevated risk of endometrial cancer. Therefore, we hypothesized that these polymorphisms on the AR gene may be associated with increased risk for endometrial cancers. To test this hypothesis, the allelic and genotypic distributions of the GGC repeat and *Stu*I polymorphisms on the AR gene were investigated in endometrial cancer patients and compared to those of healthy controls by polymerase chain reaction (PCR), PCR–restriction fragment length polymorphism (PCR–RFLP), and direct DNA sequencing.

Materials and methods

Samples. A total of 113 sporadic endometrial cancer blood samples were obtained from the Hokkaido University Hospital (Hokkaido, Japan) between 1992 and 2000. Only Japanese patients, who were diagnosed with histologically confirmed endometrial cancer, were enrolled in the study. The age range among the endometrial cancer patients was 39–87 years, with a median of 61.7 years. Cancer-free control blood samples were obtained from 202 unrelated Japanese healthy volunteers in the same prefecture (100 women and 102 men) at the same time period as the cancer patient samples. No patients or healthy controls of other ethnic groups were recruited, therefore this study was limited to a Japanese population. There were no differences between patients and control groups in regard to race, family history of cancer, indices of body size (height, weight, and body mass index), or income. Appropriate informed consent was obtained from the patients in accordance with Local Ethical Committee guidelines of the hospital.

GGC repeat. The protocol for DNA extraction and PCR methods was previously outlined [2,12]. The primers used in this study were as follows: GGC-f, TCCTGGCACACTCTCTCTTCAC; GGC-r, GCCA GGGTACCACACATCAGGT. PCR amplifications were performed with 10 ng DNA solution containing 1.5 mM $MgCl_2$, 0.8 mM dNTP, and 0.5 U *Taq* polymerase (Cetus) using each primer pair in a total volume of 50 μl. The PCR for GGC consisted of 30 cycles (98 °C for 60 s and 70 °C for 300 s). PCR amplification of CAG consisted of 30 cycles (95 °C for 45 s, 60 °C for 30 s, and 72 °C for 30 s). Aliquots of PCR products (5 μl) were mixed with 1 μl of 10× loading dye and were placed on a denaturing 8% polyacrylamide gel containing 7 M urea and 1× Tris–borate–EDTA buffer. Electrophoresis was carried out at 400 V and ambient temperature. The bands on the gels were visualized by silver staining. Allele designations were performed according to an allelic ladder produced in our laboratory [2,12].

The StuI polymorphism. The PCR–RFLP method was used to detect the *Stu*I polymorphism by a previously published protocol [8,13]. A 416 bp fragment of exon 1 of the AR gene was amplified using primers (5′-CAC AGG CTA CCT GGT CCT GG-3′ and 5′-CTG CCT TAC ACA ACT CCT TGG C-3′) with negative and positive controls [8]. The PCR consisted of 30 cycles of denaturation (94 °C for 60 s), annealing (60 °C for 60 s), and extension (72 °C for 60 s), and was followed by a final incubation at 72 °C for 8 min. DNA fragments were separated on an ethidium bromide-stained agarose gel (4%) and visualized under UV light. Laboratory personnel were blinded to case–control status [2,12].

Sequence analysis. The PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA) [3,4]. Next, double-strand sequence analysis of the PCR products was performed using the first PCR primer, and a ABI 377 Sequencer and Dye Terminator Cycle sequencing kit (Applied Biosystems, Foster City, CA) [2,12].

Statistical analysis. Since the AR gene is located on the X chromosome, females have two alleles on their two X chromosomes and males have only one allele on their one X chromosome [12,14]. Homogeneity was tested as described previously [12,14]. χ^2 and t tests that were two-sided for statistical significance were used to test the deviation of allelic and genotype distributions [11,12,14]. Case subjects and control subjects were compared for GGC repeats initially as a continuum. Then the relative risks were estimated using cutoff points, comparing the odds of cancer patients and controls in the category below and above the cutoff point. We used a cut-point of 16 and 17 for GGC repeat, because this divided the distribution of these repeats approximately in half.

Results

GGC repeat

The frequencies of genetic distribution of the GGC repeats on the AR gene in endometrial cancer patients and healthy controls are shown in Table 1. There were no differences in genetic distribution of the GGC repeats between male and female healthy controls (Table 1A). Genotyping of endometrial cancer patients and female controls revealed 11 and 8 distinct alleles, respectively (Table 1B). The mean GGC repeat was 15.9 and 15.4 repeats for cancer patients and female controls, respectively. The length of GGC repeats of the endometrial cancer patients was significantly longer compared to that of female controls ($P < 0.05$). The relative risks for endometrial cancer were estimated using a cutoff point of 16 and 17 repeats, and then comparing the endometrial cancer patients and controls in the category below and above the cutoff point (Table 1C). No significant association was observed for the 16 GGC repeat between endometrial cancer patients and female controls (the relative risks: 1.10). However, the 17 GGC repeat was significantly overrepresented in endometrial cancer patients compared to female controls ($P < 0.001$). Thus, 14.6% of endometrial cancer patients (33 of 226) had the 17 allele while 3.5% of female controls (7 of 226) had it (both: $P < 0.001$) (Table 1B). The relative risks for the 17 repeat of GGC were 4.17 as compared to female controls. Moreover, 5.7% of endometrial cancer patients (13 of 226) had the 18 allele although none of the female controls (0 of 226) did ($P < 0.001$) (Table 1C).

StuI polymorphism

The frequency of distribution of the *Stu*I polymorphism of the AR gene in endometrial cancer patients and the control groups is shown in Table 2. The genotypic distributions of this polymorphism were consistent with the Hardy–Weinberg equilibrium in cancer patients and control groups. No difference was observed for the allelic distribution of this polymorphism between men and women in control groups ($P > 0.75$) (Table 2A).

Table 1
The GGC repeat of androgen receptor gene in endometrial cancer patients and healthy controls

(A) Repeat	Female controls	Male controls[a]	Total controls
10	3/200 (1.5%)	1/102 (1.0%)	4/302 (1.3%)
11	4/200 (1.5%)	2/102 (2.0%)	6/302 (2.0%)
12	10/200 (5.0%)	4/102 (4.0%)	14/302 (4.6%)
13	4/200 (2.0%)	2/102 (2.0%)	6/302 (2.0%)
14	5/200 (2.5%)	3/102 (3.0%)	8/302 (2.6%)
15	21/200 (10.5%)	12/102 (12.0%)	33/302 (10.9%)
16	146/200 (73.0%)	74/102 (74.0%)	220/302 (72.8%)
17	7/200 (3.5%)	3/102 (3.0%)	10/302 (3.3%)
18	0/200 (0.0%)	1/102 (1.0%)	1/302 (0.3%)
19	0/200 (0.0%)	0/102 (0.0%)	0/302 (0.0%)
20	0/200 (0.0%)	0/102 (0.0%)	0/302 (0.0%)
21	0/200 (0.0%)	0/102 (0.0%)	0/302 (0.0%)

(B) Repeat	The patients[b,c]	Female controls
10	1/226 (0.4%)	3/200 (1.5%)
11	1/226 (0.4%)	4/200 (1.5%)
12	6/226 (2.7%)	10/200 (5.0%)
13	4/226 (1.8%)	4/200 (2.0%)
14	4/226 (1.8%)	5/200 (2.5%)
15	17/226 (7.5%)	21/200 (10.5%)
16	160/226 (70.7%)	146/200 (73.0%)
17	20/226 (8.8%)	7/200 (3.5%)
18	11/226 (4.9%)	0/200 (0.0%)
19	1/226 (0.4%)	0/200 (0.0%)
20	0/226 (0.0%)	0/200 (0.0%)
21	1/226 (0.4%)	0/200 (0.0%)

(C) Allele	The patients	Female controls	Relative risk for female controls	95% CI for female controls
≧16	193/226 (85.4%)	153/200 (76.5%)	1.10	1.00–1.21
≧17	33/226 (14.6%)	7/200 (3.5%)	4.17	1.89–9.22

[a] Male healthy controls vs. female healthy controls: $0.95 < P$.
[b] The patients vs. female healthy controls: $P < 0.05$.
[c] The patients vs. total healthy controls: $P < 0.001$.

Table 2
The *Stu*I polymorphism of androgen receptor gene in endometrial cancer patients and healthy controls

(A) Allele	Female controls	Male controls[a]	Total controls
G	185/200 (92.5%)	95/102 (93.1%)	280/302 (92.7%)
A	15/200 (7.5%)	7/102 (6.9%)	22/302 (7.3%)

(B) Allele	The patients[b,c]	Female controls	Relative risk for female controls	95% CI for female controls
G	207/226 (91.6%)	185/200 (92.5%)		
A	19/226 (8.4%)	15/200 (7.5%)	1.12	0.59–2.15

(C) Genotype	The patients[d]	Female controls	Relative risk for female controls	95% CI for female controls
G/G	95/113 (84.1%)	86/100 (86.0%)		
G/A	17/113 (15.0%)	13/100 (13.0%)	1.16	0.59–2.26
A/A	1/113 (0.9%)	1/100 (1.0%)	NT	NT

[a] Male healthy controls vs. female healthy controls: $0.75 < P$.
[b] The patients vs. female healthy controls: $0.75 < P$.
[c] The patients vs. total healthy controls: $0.75 < P$.
[d] The patients vs. female healthy controls: $0.75 < P$.

There was no significant difference in allelic distribution in endometrial cancer patients compared to that of female controls ($P > 0.75$) (Table 2B). The relative risk of allele A of this SNP was calculated as 1.1.2 compared to allele G for female controls. There was also no significant difference in genotypic distribution in endometrial cancer patients compared to that of female controls ($P > 0.75$) (Table 2C). The relative risk of the genotype

G/A of this SNP was calculated as 1.16 compared to allele G.

Discussion

Androgens have an anti-proliferative effect on endometrial cells. The GGC repeat encodes polyglycine region, which has a length that is inversely and linearly related to AR activity [9,10]. It has been observed that shorter repeats impose a higher transactivation activity on AR and have an increased binding affinity for androgens [15,16]. In contrast, longer repeats lead to lower androgen responsiveness, weaker androgen inhibition, and increased proliferative activity, and promote carcinogenesis of the uterine endometrial cells [2,12,17–19].

Our data show that the length of GGC CAG repeat in the endometrial cancer patients was significantly longer compared to that of total controls. Moreover, increased risks of endometrial cancer were found with increasing GGC repeat. Based on a previous report, it was confirmed that even small differences in these repeats cause a significant difference in transcriptional activation of the AR gene [20]. An appreciable decrease in the transcriptional activity of an AR with a long polyglycine tract could lead to a reproductive lifetime of functional hyperestrogenicity in the endometrium, and this might contribute to initiation and/or progression of endometrial cancer. We observed no significant differences in allelic or genotypic distribution of the *Stu*I polymorphism between endometrial cancer patients and control groups. These data may reflect the fact that the *Stu*I nucleotide change is silent and does not induce any amino acid change in the AR protein. In conclusion, our findings suggest that the GGC repeat in the AR gene is a risk factor for endometrial cancer. Therefore, these polymorphic tandem repeats of the AR gene can serve as biomarkers to identify a population with higher risk for endometrial cancer.

Acknowledgments

We thank Dr. Roger Erickson for his support and assistance with the preparation of the manuscript. This work was supported by NIH Grants RO1AG016870; RO1AG21418, and VA REAP award and Merit Review grants.

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