

Androgen receptor and 5 $\alpha$ -reductase  
immunohistochemical profiles in extramammary  
Paget disease

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**Title page**

**Androgen receptor and 5  $\alpha$  -reductase immunohistochemical profiles in  
extramammary Paget's disease**

**Running head:** Androgenic microenvironment of extramammary Paget's disease

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**Abbreviation:** AR, androgen receptor; 5 $\alpha$ R, 5 $\alpha$ -reductase

**ABSTRACT**

**Aim:** Extramammary Paget's disease is an uncommon skin tumor occurring mostly in the genitoperineal region. Previous reports have shown that frequent expression of androgen receptor suggests tumor proliferative effects of androgens on Paget cells. Androgen-converting enzymes, such as 5 $\alpha$ -reductase, which locally produces a bioactive androgen, have recently gained attention in studies of the intratumoral actions of androgens. We investigated correlations between the androgenic microenvironment and invasiveness in extramammary Paget's disease, particularly in terms of sex differences. **Methods and Results:** We examined 58 cases of extramammary Paget's disease (32 men, 26 women; 42 non-invasive, 16 invasive) using immunohistochemistry for androgen receptor and 5 $\alpha$ -reductase. In all 58 cases, expression rates were 57% for androgen receptor and 55% for 5 $\alpha$ -reductase, with 38% double-positivity for androgen receptor and 5 $\alpha$ -reductase. Only 5 $\alpha$ -reductase expression rate was significantly higher in invasive cases (81%) than in non-invasive cases (45%; p=0.042). For invasive cases, numbers of double-positive results for androgen receptor and 5 $\alpha$ -reductase were significantly higher in men (90%) than in women (17%, p=0.039). **Conclusion:** Double-positivity for androgen receptor and 5 $\alpha$ -reductase in Paget cells suggests autocrine synthesis of androgen in extramammary Paget's disease. The different hormonal microenvironments in male and female cases and intratumoral androgen levels affect the invasiveness of extramammary Paget's disease.

## INTRODUCTION

Extramammary Paget's disease is a relatively rare cutaneous malignant neoplasm arising in apocrine gland-rich areas, such as the genitoperineal region and axilla<sup>1-3</sup>, and is principally classified as an apocrine gland tumor<sup>1</sup>. Normal apocrine glands of the skin consistently express androgen receptor (AR), but no detectable estrogen receptor and progesterone receptor<sup>4,5</sup>. Similarly, several previous reports have found that Paget cells frequently expresses only AR, but not estrogen receptor or progesterone receptor<sup>6-8</sup>. These findings have suggested the tumor proliferative effects of androgens on Paget cells<sup>6-8</sup>.

In peripheral tissues, a large population of steroid hormones are locally synthesized by steroid-producing enzymes from circulating precursor steroids<sup>9</sup>. Bioactive sex-hormones are produced in the target cells themselves (autocrine) or neighbouring cells (paracrine)<sup>9</sup>. Additionally, the importance of the local synthesis of bioactive sex-hormones has recently been noticed in hormone receptor-positive cancers<sup>9,10</sup>. As for androgen synthesis, 5 $\alpha$ -reductase (5 $\alpha$ R) mainly catalyzes the conversion of testosterone to a bioactive androgen<sup>10,11</sup>. Two isoforms of 5 $\alpha$ R have been detected: 5 $\alpha$ R type 1, expressed in the liver and skin; and 5 $\alpha$ R type 2, localized in the liver, prostate, seminal vesicle, and epididymis<sup>10,12</sup>. Androgens are synthesized primarily by 5 $\alpha$ R type1. Clarification of 5 $\alpha$ R expression and relationships between AR and 5 $\alpha$ R of Paget cells would thus reveal the androgenic microenvironment of extramammary Paget's disease.

Paget cells are generally located in the epidermis (so-called carcinoma in situ) for a long period in the early stage. Then, with disease progression, Paget cells invade the dermis. Once invasive growth occurs, Paget cells often metastasize to lymph nodes and/or other organs, and the prognosis worsens<sup>2,14</sup>. In hormone receptor-positive cancers, cell growth,

invasion and metastasis of cancers often vary with the hormonal microenvironment. For example, in prostate carcinoma, 5 $\alpha$ R type1 reportedly correlates with immunoreactivity for AR and tumor aggressiveness<sup>13</sup>. In extramammary Paget's disease, the association between tumor proliferation and androgenic microenvironment has yet to be examined. Thus, differences between extramammary and mammary Paget's disease have been discussed<sup>8</sup>. However, sex differences in extramammary Paget's disease remain unclear, while this tumor often occurs in not only female but also male<sup>1,3</sup>.

The present study immunohistochemically investigated expressions of AR and 5 $\alpha$ R, and estimated correlations between these expressions and clinicopathological factors including sex, invasiveness, and local recurrence and metastasis.

## **Materials and methods**

The surgical pathology files of Kanazawa University Hospital and Kanazawa Medical Center from 1990 to 2008 were reviewed for extramammary Paget's disease, as determined by intraepidermal spread of large cells with pale to granular cytoplasm and occasional nuclear displacement by cytoplasmic vacuoles. Diagnosis of Paget cells was confirmed by positive staining for mucin<sup>1-3</sup>. We selected 58 patients (mean age, 71.5 $\pm$ 9.4 years; range, 48-91 years), including 32 men (mean age, 71.4 years; range, 48-90 years) and 26 women (mean age, 72.1 years; range, 52-91 years). No significant difference in age was noted between sexes. Extramammary Paget's disease cases were further divided into groups according to histological findings of invasion: non-invasive cases (i.e., usual intraepithelial extramammary Paget's disease, n=42, 22 men, 20 women); and invasive cases (n=16, 10 men, 6 women). Invasion manifested as small irregular nests and single cells surrounded by

desmoplastic stromal reaction<sup>14</sup>. According to Goldblum et al., we determined minimal invasive cases as infiltration of Paget cells <1 mm in depth from the basement membrane of the epidermis, and massive invasive cases as infiltration of Paget cells >1 mm<sup>3</sup>.

Formalin-fixed, paraffin-embedded, serial 4- $\mu$ m-thick sections mounted on precoated slides were immunohistochemically stained using the Avidin-Biotin Complex method. Primary antibodies were for AR (clone AR411, 1:100; DAKO, Glostrup, Denmark) and 5 $\alpha$ R (5 $\alpha$ -reductase type 1, rabbit polyclonal, 1:1000; provided by Dr. D. W. Russell; University of Texas Southwestern Medical Center, Dallas, TX). Antigen retrieval for AR was undertaken by pressure cooking in ethylenediaminetetraacetic acid buffer (pH 6.0) for 5 min. Staining was performed using a Ventana standardized automated system (Ventana Medical Systems, Tucson, AZ) according to the manufacturer's protocols. Immunopositive labeling of  $\geq 10\%$  among all cells was considered as a positive result, according to the standardized criteria for hormone receptors and hormone-producing enzymes<sup>11,15</sup>. The entire analysis of immunohistochemical findings was performed by two pathologists (S.K and Z.Y) who were blinded to the clinical features of the sample.

Statistical analyses were performed using Fisher's test or the  $\chi^2$  test, as appropriate, with the Yates correction and the Mann-Whitney rank-sum test for unpaired nonparametric data, to compare each group using the parameters of clinicopathological factors and immunohistochemical data. Values of  $p < 0.05$  were considered significant.

## RESULTS

### *Clinical findings*

For the 58 cases of extramammary Paget's disease, mean tumor size was  $7.3 \pm 3.5$  cm

(range, 2-22 cm), with no significant difference noted between non-invasive cases (mean, 7.15±3.8 cm) and invasive cases (mean, 7.87±2.35 cm; p=0.4834). Tumor size was significantly larger in women (mean, 8.72±4.23 cm) than in men (mean, 6.17±2.01 cm; p=0.0049). Inguinal lymph node biopsy/dissection was performed in 18 cases (non-invasive cases, n=11; invasive cases, n=7). Lymph-node metastasis was observed in five cases (all male invasive cases) with significant correlations only seen for invasiveness of extramammary Paget's disease (p=0.001), and not with tumor size, sex or age. Lymph-node metastasis was equally seen in minimal invasive cases (2/3, 66%) and massive invasive cases (3/6, 50%; p=0.235). Using follow up data on all cases with a mean interval of 55 months. Local recurrence occurred in six cases (10%; non-invasive cases, n=4; invasive cases, n=2); no significant correlations were detected between local recurrence and other clinical parameters. No patient suffered distant metastasis or died of disease.

#### *Immunohistochemical findings for all cases of extramammary Paget's disease*

Immunohistochemical findings of extramammary Paget's disease are summarized in Table 1 and Figure 1. Among 58 cases, Paget cells were positive for AR in 33 cases (57%), 5 $\alpha$ R in 32 cases (55%), and double-positive for AR and 5 $\alpha$ R in 22 cases (38%). AR and 5 $\alpha$ R were often detected in the same cells (Fig. 1), but the association between AR and 5 $\alpha$ R in tumor cells was not significant (p=0.0739). Male cases showed relatively higher immunoreactivity for AR (men 66%, women 46%) and 5 $\alpha$ R (men 63%, women 46%), but these differences did not reach the level of statistical significance (p=0.1346, p=0.1252, respectively). No association was observed between clinical factors such as tumor size, age, lymph-node metastasis and local recurrence, and the presence of AR and 5 $\alpha$ R.

*Differences between extramammary Paget's disease with and without invasion*

Compared with non-invasive cases, the expression rate of 5 $\alpha$ R was significantly higher in invasive cases of extramammary Paget's disease. Indeed, for 5 $\alpha$ R expression, invasive cases was 81% (13/16) and non-invasive cases was 45% (19/42) (p=0.0423) (Table 2, Fig. 2). In particular, in men, 5 $\alpha$ R expression was more frequent in invasive cases (9/10, 90%) than in non-invasive cases (11/22, 50%; p=0.0303). In comparison with intraepithelial Paget cells, relatively strong both AR and 5 $\alpha$ R immunostaining was seen in peripheral nests of the invasion area (Fig.1c,d). In addition, in invasive cases, the number of double-positives for AR and 5 $\alpha$ R was significantly higher in men (7/10, 70%) than in women (1/6, 17%; p=0.0389)(Fig.2). There were no differences in any parameters of immunophenotype between minimal and massive invasive cases.

**DISCUSSION**

Frequent expression (approximately 60%) of 5 $\alpha$ R was seen in Paget cells, and a new finding of about 40% double-positivity for AR and 5 $\alpha$ R in Paget cells was identified. In addition, the close association between AR and 5 $\alpha$ R in Paget cells suggests probable autocrine function of androgen.

Previous reports examining hormone receptors in extramammary Paget's disease have mainly investigated women, with all women in the 23 cases described by Liegl et al.<sup>8</sup>, and 23 women and only 5 men examined by Diaz de Leon et al.<sup>7</sup>. Similar frequencies of AR expression in both sexes (men, 3/5; women, 12/23) were described in the report by Diaz de

Leon et al.<sup>7</sup>. Sex differences in extramammary Paget's disease have not previously been noticed. However, in this report, examination of more male cases revealed that extramammary Paget's disease in men shows higher positivity for AR and 5 $\alpha$ R, along with higher double-positivity for AR and 5 $\alpha$ R. Sex differences in the androgenic microenvironment would thus appear to be characteristic for extramammary Paget's disease.

The invasiveness of extramammary Paget's disease has been described in a few previous reports concerned with p53 overexpression<sup>14</sup>, high expression of MIB-1 and cyclin D1<sup>16</sup>, and loss of MUC5AC<sup>17</sup>. For the first time, we have clarified androgenic differences correlated with the progression of extramammary Paget's disease. Compared to non-invasive cases, invasive cases showed significantly higher expression of 5 $\alpha$ R and double-positivity for both AR and 5 $\alpha$ R. These findings indicate that local androgen production is associated with progressive proliferation of Paget cells, with a tendency toward stromal invasion. In addition, an androgenic role in the invasive growth of extramammary Paget's disease was emphasized in male cases. Only male patients showed lymph-node metastasis, instead of smaller tumor size, in association with intratumoral androgenic action in male cases. In prostate carcinoma, biological aggressiveness and expression of AR and androgen-converting enzymes have been linked<sup>13</sup>. In contrast, some reports of breast carcinoma have shown that AR status relates to growth inhibition, particularly in cases positive for both AR and 5 $\alpha$ R<sup>11</sup>. In brief, Paget cell proliferation was accelerated under an androgenic microenvironment, particularly in male cases, similar to prostate carcinoma, but not breast carcinoma.

Recently, pre/post-operative hormonal therapeutic strategies have been established

in hormone receptor-positive cancers. However, for the patients of extramammary Paget's disease, surgical treatment remains the only treatment, whereas local recurrence occurred in about half of cases, due to the difficulty of determining adequate surgical margins in extramammary Paget's disease and the resulting presence of residual tumor cells<sup>18</sup>. For such cases, topical chemotherapy with fluorouracil and radiotherapy has been used, but effective chemoradiation regimens for extramammary Paget's disease have yet to be established<sup>2,18</sup>. Regarding high expression of AR in extramammary Paget's disease, recently, androgen-deprivation therapy has been reported in the successful treatment of a patient of extramammary Paget's disease with multiple metastases<sup>19</sup>. Due to local regulation of androgenic synthesis, 5 $\alpha$ R inhibitors allowed decreased side-effects and longer period of hormonal response for prostate hormonal therapy<sup>20</sup>. Our findings indicate that regulation of 5 $\alpha$ R is one potential therapeutic tool for extramammary Paget's disease, particularly for local recurrence and/or invasive state.

In conclusion, we have demonstrated the characteristic androgenic microenvironment of extramammary Paget's disease. Higher expressions of 5 $\alpha$ R and co-expressions 5 $\alpha$ R and AR in invasive cases compared to non-invasive cases suggest that androgen might accelerate the invasive state of Paget cells. Interestingly, androgenic involvement in the invasiveness of Paget cells was emphasized in male. These novel findings of the androgenic microenvironment of extramammary Paget's disease help to clarify the tumorigenesis, and seem likely to lead to the use of adjuvant endocrine therapy for extramammary Paget's disease.

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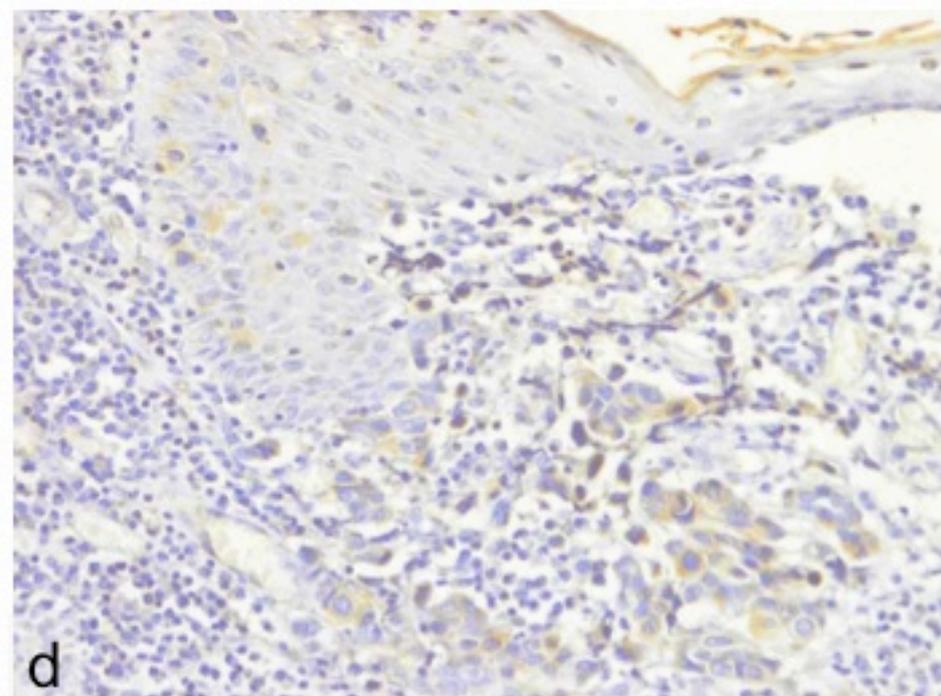
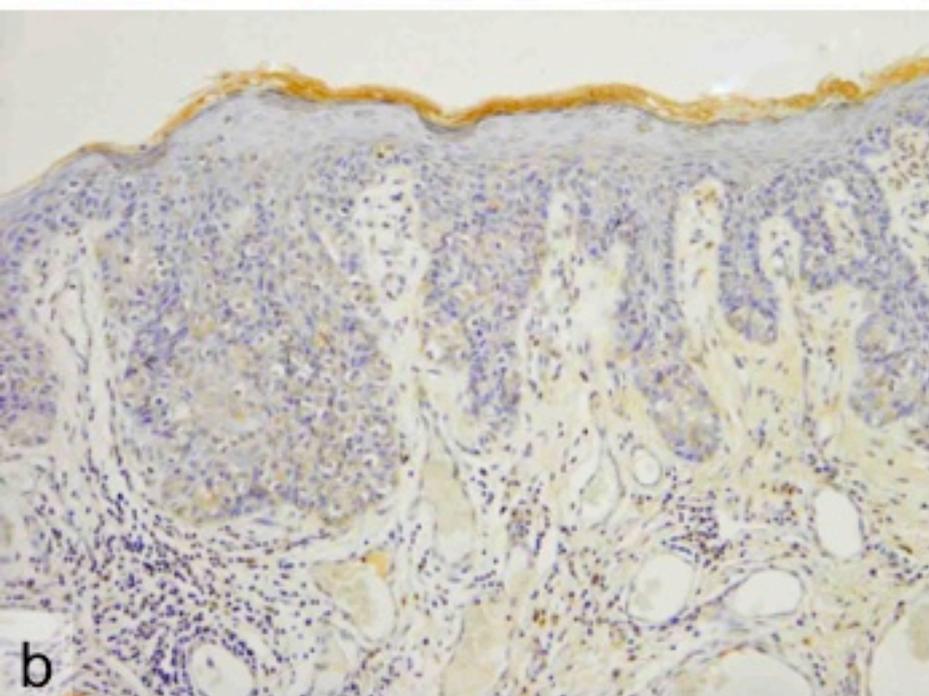
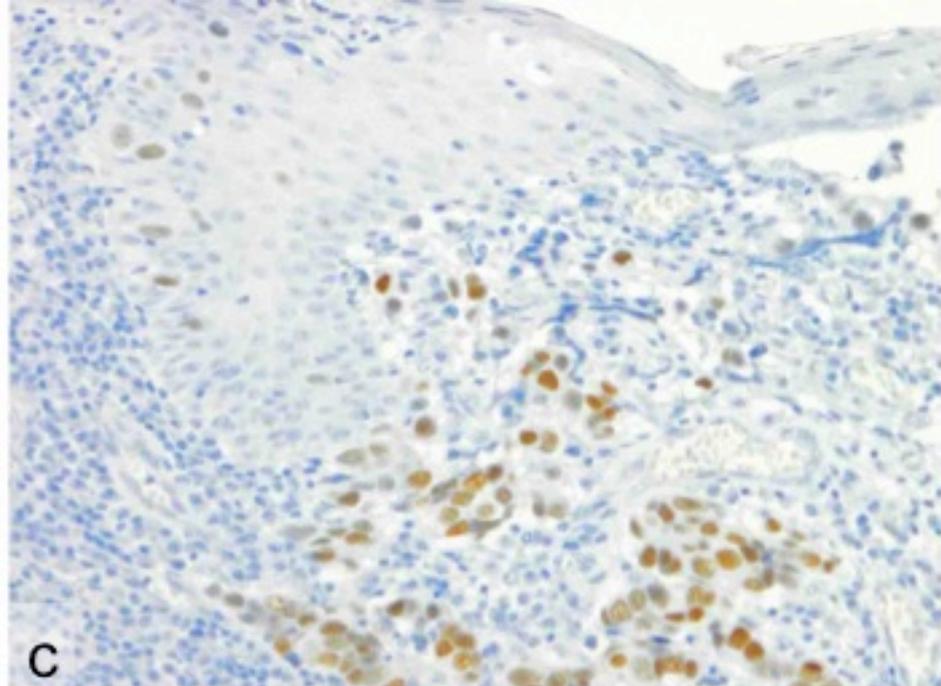
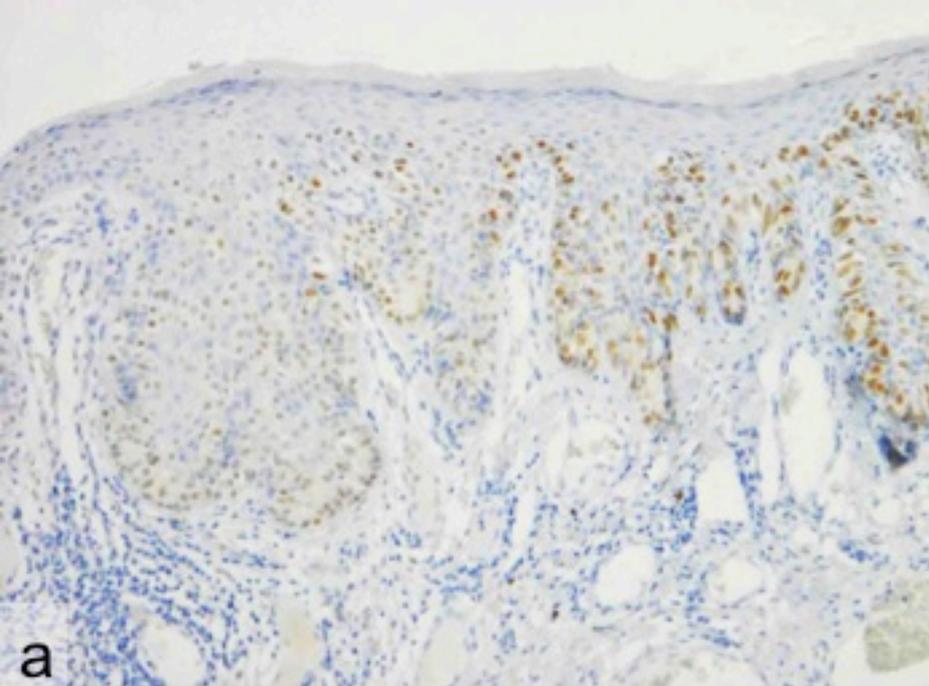
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**LEGENDS TO FIGURES**

Figure 1. Immunohistochemical findings for androgen receptor and androgen-synthesizing enzymes in extramammary Paget's disease. Tumor cells are diffusely and strongly positive for androgen receptor in the nuclei of Paget cells (a,c) and 5 $\alpha$ -reductase in the cytoplasm of Paget cells (b,d) Peripheral nests in the invasive area show stronger immunoreactivity for both AR (c) and 5 $\alpha$ -reductase (d) than non-invasive area.

Figure 2. Numbers of cells showing double-positive and double-negative results for androgen receptor and 5 $\alpha$ -reductase in extramammary Paget's disease. Male cases with invasion are characterized by co-existence of androgen receptor and 5 $\alpha$ -reductase. AR, androgen receptor; 5 $\alpha$ R, 5 $\alpha$ -reductase.



cases

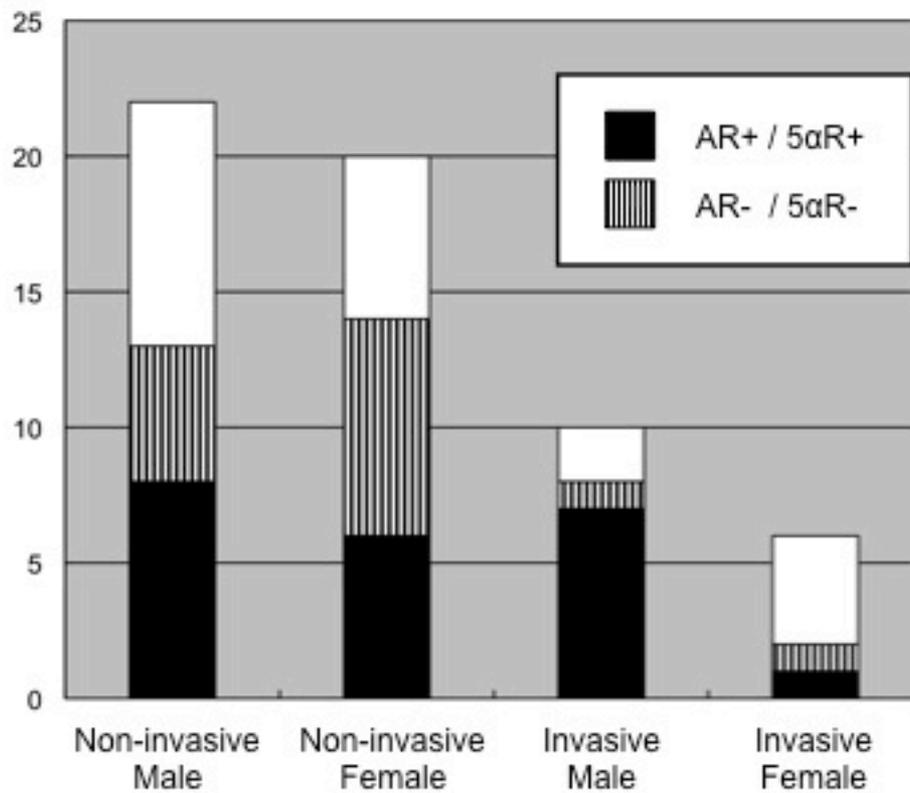


Table 1. Comparison of AR and 5  $\alpha$  R expression between male and female, and invasive and non-invasive cases of extramammary Paget's disease

	Total	<u>Male cases</u>	<u>Female cases</u>		<u>Non-invasive cases</u>	<u>Invasive cases</u>	
	(n=58)	(n=32)	(n=26)	<i>p value</i>	(n=42)	(n=16)	<i>p value</i>
AR	33 (56.8%)	21 (65.5%)	12 (46.2%)	0.134	24 (57.1%)	9 (56.3%)	0.951
5 $\alpha$ R	32 (55.2%)	20 (62.5%)	12 (46.2%)	0.122	19 (45.2%)	13 (81.3%)	<b>0.042</b>
AR and 5 $\alpha$ R double-positive	22 (37.9%)	15 (46.9%)	7 (26.9%)	0.116	14 (33.3%)	8 (50%)	0.242
AR and 5 $\alpha$ R double-negative	15 (25.8%)	6 (18.8%)	10 (38.5%)	0.121	13 (30.9%)	2 (10.5%)	0.263

AR, androgen receptor; 5  $\alpha$  R, 5  $\alpha$  -reductase

Table 2. Comparison of AR and 5  $\alpha$  R expression among subgroups of extramammary Paget's disease.

	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>		
	<u>non-invasive cases</u>	<u>non-invasion cases</u>	<u>Invasive cases</u>	<u>Invasive cases</u>	<i>P-value 1</i>	<i>P-value 2</i>
	(n=22)	(n=20)	(n=10)	(n=6)		
AR	14 (63.6%)	10 (50%)	7 (70%)	2 (33.3%)	0.725	0.152
5 $\alpha$ R	11 (50.0%)	8 (40%)	9 (90%)	4 (66.7%)	<b>0.030</b>	0.073
AR and 5 $\alpha$ R double positive	8 (36.4%)	6 (30%)	7 (70%)	1 (16.7%)	0.077	<b>0.039</b>
AR and 5 $\alpha$ R double negative	5 (22.7%)	8 (40%)	1 (10%)	1 (16.7%)	0.554	0.696

AR, androgen receptor; 5  $\alpha$  R, 5  $\alpha$  -reductase

p-value 1; Among male cases, compare with invasive and non-invasive cases of extramammary Paget's disease

p-value 2; Among invasive cases, compare with male and female cases of extramammary Paget's disease