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Original Contribution

# Lesions of anogenital mammary-like glands: Four cases including novel pathologic and immunohistochemical observations

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ABSTRACT

Anogenital mammary-like glands, formerly described as ectopic breast tissue, are currently considered to be normal histologic components of the anogenital region. Anogenital mammary-like glands can give rise to many lesions identical to counterparts in the native female breast. We describe four cases of such lesions, including fibroadenoma, gynecomastia-like hyperplasia, and ectopic mammary-type tissue with a spectrum of usual ductal hyperplasia, apocrine metaplasia, adenosis, and pseudolactational change. All four cases occurred in young women (ages 29–38) who presented with vulvar or perianal masses. Similar to previously reported cases, these lesions shared histologic and immunohistochemical characteristics identical to native female breast lesions. Novel findings in our cases included (1) the first case of gynecomastia-like change to be reported in the perianal area of a female, (2) Immunohistochemical staining identifying a 3-layered epithelium characterized by a population of CK14 and CK5/6 positive and hormone receptor negative superficial luminal cells, and (3) diffuse, strong positivity for GATA3 in all cases. Our study adds to the literature on these rare lesions and highlights findings which may be useful in understanding the pathogenesis and improving the diagnosis of anogenital mammary-like gland lesions.

## 1. Introduction

Ectopic breast tissue in the vulvar area was first reported by Hartnug et al. in 1872, as cited from Kazakov et al.'s paper [1]. This mammary-type tissue in the vulvar region was thought to arise early in life from the embryonic milk line. The milk lines develop from the embryonic ectoderm and are described as extending from the axilla to the medial aspect of the groin. This mammary tissue would then undergo spontaneous regression during embryogenesis, except in the chest area, giving rise to the breasts. Ectopic breast tissue has conventionally been thought to arise from a failure of regression of the milk line remnants outside the pectoral region [2]. It occurs more frequently in women and can involve any body site along the milk line, with the axilla as the most common site. Vulvar and anogenital area involvement is relatively rare.

A more recent theory explaining the presence of breast tissue in the vulvar and anogenital area proposes that these structures derive from normal eccrine-type glands present in the anogenital area [1-4]. van der Putte et al. demonstrated the presence of these structures and claimed these glands have the capacity to branch into lobules and form acini similar to mammary glands [4]. They are referred to as anogenital mammary-like glands (AGMLG). The histology of these mammary-like glands varies from simple glandular structures with round lumina surrounded by fibrous stroma to complex lobular units mimicking normal breast tissue [1-4]. These glands are located mostly between the labia minora and majora, as well as posteriorly in the paramedian zone of the perineum and around the anus. The occurrence of AGMLG lesions arising in regions that are not part of the caudal milk line, such as the perianal region, further supports the more recent theory proponed by van der Putte [3,4].

AGMLG can give rise to lesions that show a striking similarity to lesions originating in the mammary gland proper. These lesions often become symptomatic at puberty, pregnancy, or during lactation when they are influenced by female sex hormones. A variety of benign and malignant lesions arising from AGMLGs have been described, including sclerosing adenosis, columnar cell lesions, ductal lesions, various metaplastic changes affecting epithelium and myoepithelium, lactating adenoma, hidradenoma papilliferum, hidradenocarcinoma papilliferum, fibroadenoma, phyllodes tumor, pseudoangiomatous stromal hyperplasia, extramammary Paget disease, and carcinoma. We have

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**Table 1**
Specifications for antibodies used for immunohistochemical staining.

| Antibody | Clone | Dilution | Control | Vendor | Platform |
|---|---|---|---|---|---|
| ER | SP1 | Ready to use | Breast | Ventana[a] | Ventana Ultra |
| PR | 1E2 | Ready to use | Breast | Ventana | Ventana Ultra |
| AR | SP107 | Ready to use | Prostate | Ventana | Leica Bond III |
| CK14 | LL002 | Ready to use | Skin | Ventana | Ventana Ultra |
| CK5/6 | D5/16B4 | Ready to use | Skin | Leica[b] | Leica Bond III |
| GATA-3 | L50-823 | Ready to use | Breast | Leica | Leica Bond III |

[a] Ventana Medical Systems, Inc. 1910 E Innovation Park Drive. Tucson, Arizona 85,755. United States.
[b] Leica Biosystems Inc. 1700 Leider Lane. Buffalo Grove, IL 60089. United States.

encountered four cases of vulvar/perianal lesions arising from AGMLGs. Herein, we describe fibroadenoma, gynecomastia-like change, and two cases of ectopic breast tissue with a variety of mammary-type proliferative changes. To the best of our knowledge, gynecomastia-like change has not been described in the anogenital region in a female. Additionally, we describe a three-layered immunohistochemical profile and GATA3 positivity of epithelial cells occurring in these lesions.

## 2. Materials and methods

Four cases of mass lesions arising from AGMLGs were identified in the files of Houston Methodist Hospital Department of Pathology and Genomic Medicine. Immunohistochemical stains for estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), cytokeratin 14 (CK14), cytokeratin 5/6 (CK5/6), and GATA-3 were performed on 4 μm paraffin embedded sections in all cases. The slides were de-paraffinized in xylene, then rehydrated through graded alcohol series. Endogenous peroxidase was blocked using 3% hydrogen peroxide. Slides were immunostained with the primary antibodies listed above, and counterstained with hematoxylin and eosin (H&E). Clones, dilutions, controls, vendors and platforms used for each antibody are shown in Table 1.

This study was approved by the Institutional Review Board of Houston Methodist Hospital.

## 3. Case descriptions

### 3.1. Case 1

A 38-year-old female presented with menometrorrhagia and was found on pelvic examination to have a vulvar cyst. She underwent endometrial curettage along with excision of the vulvar cyst. Macroscopically, the resected lesion was a 3.5 cm well-circumscribed nodule with a tan pink whorled cut surface. Microscopically, the lesion had features of a mammary-type fibroadenoma with ducts and loosely cellular to focally myxoid stroma (Fig. 1). The compressed epithelial elements had a pericanalicular pattern and were lined by cuboidal to columnar cells with bland cytologic features. The distribution of epithelial and stromal elements was homogenous and there was no increased stromal cellularity, increased stromal mitotic activity, nuclear pleomorphism, heterologous element, or other atypical feature. The concurrently performed endometrial curettage showed a disordered proliferative endometrium with no hyperplasia or malignancy. Immunohistochemical stains on the fibroadenoma (Fig. 2) demonstrated estrogen and progesterone receptor (ER and PR) positivity in the majority of luminal epithelial cells, and positivity for androgen receptor (AR) in about half of the luminal cells. Myoepithelial cells expressed CK14 and CK5/6. A subset of superficial luminal epithelial cells also showed positivity for CK14 and CK5/6 and were negative for ER, PR, and AR. GATA3 was diffusely and strongly positive in all luminal epithelial cells.

### 3.2. Case 2

A 29-year-old female presented with a tender and inflamed perianal mass. The excised lesion was a 2.0 × 1.5 × 1.0 cm pedunculated polyp. Microscopically, the lesion had several mammary-type ducts with mild hyperplasia, focal tufting or micropapillae, and periductal edema and fibrosis (Fig. 3). No well-formed terminal duct-lobular units were present, but rather ducts were present throughout. A mild inflammatory cell infiltrate was present in the stroma and consisted predominantly of lymphocytes with scattered neutrophils, eosinophils and plasma cells. The overall findings were consistent with gynecomastia-like changes. No atypia or malignancy was identified. Immunohistochemical staining demonstrated focal positivity for ER in ductal luminal cells, with PR and AR positivity in the majority of luminal cells (Fig. 4). Myoepithelial cells were immunoreactive for CK14 and focally for CK5/6. A third distinct superficial luminal cell population was observed which expressed CK14 and CK5/6; and was ER-, PR-, and AR-negative. GATA3 was diffusely and strongly positive in all luminal cells.

### 3.3. Case 3

A 29-year-old female, G2P1 with gestational diabetes underwent excision of a right labial mass following a vaginal delivery at 39 weeks. The patient reported that the mass had been present since childhood and was not associated with pain or discharge. The clinical impression was a probable fibroma. The resected 0.7 × 0.5 × 0.4 cm tan-pink firm, irregular mass had no associated hemorrhage, cystic degeneration, or necrosis. Microscopically, there was typical mammary-type tissue with well-defined lobular architecture and usual ductal hyperplasia (Fig. 5). No atypical hyperplasia or malignancy was identified. Immunohistochemical stains demonstrated focal positivity for PR and AR in luminal cells, as well as weak patchy positivity for ER (Fig. 6). CK14 was diffusely positive in myoepithelial cells, but not in luminal cells. CK5/6 showed focal staining of superficial luminal cells and weak staining of myoepithelial cells. The CK 5/6 positive cells were ER-, PR-, and AR- negative. GATA3 was diffusely and strongly positive in all luminal cells.

### 3.4. Case 4

A 35-year-old female underwent excision of a labial cyst. The resected lesion consisted of a 2.7 cm irregular tan pink portion of tissue. Histologic examination demonstrated mammary-like tissue with well-formed ducts and lobules. A variety of benign mammary-type lesions were present (Fig. 7). There was focal usual ductal hyperplasia (UDH) consisting of ducts having a haphazard proliferation of bland cells forming slit-like lumina. Other areas showed cystically dilated glands with apocrine metaplasia, having focally tufted or cribriform lumina lined by cells with abundant eosinophilic cytoplasm, bland round nuclei, and apical snouts. Elsewhere, there was focal adenosis. The lesion also had focal pseudolactational changes consisting of glands with intraluminal secretions lined by cells with abundant clear secretory cytoplasmic vacuoles and hyperchromatic nuclei. Immunohistochemical stains of the background mammary-like tissue was positive for PR and AR and patchy weakly positive for ER (Fig. 8). Apocrine metaplastic cells were strongly AR positive, focally PR positive, and ER negative. The other lesions (UDH, pseudolactational change, and adenosis) were focal and were no longer present in the deeper sections prepared for immunohistochemical studies. Myoepithelial cells were immunoreactive for CK5/6 and CK14. A distinct population of superficial luminal cells was positive for CK14, CK5/6, and negative for ER, PR, and AR. GATA3 was diffusely and strongly positive in all luminal cells.

Cases 1–4 with immunohistochemical findings are summarized in Table 2.

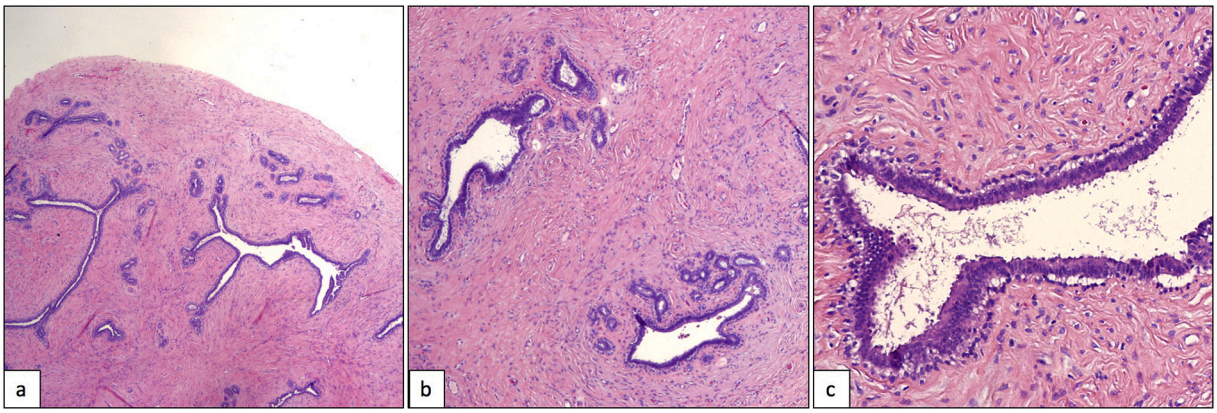


**Fig. 1.** Vulvar fibroadenoma consisting of a well circumscribed mass with epithelial and stromal components (a) (H&E stained section, 20× magnification). Open glands (pericanalicular pattern) are situated among bland mildly cellular stroma (b) (H&E stained section, 40× magnification). Epithelial lining consisted of cuboidal to columnar cells. Distinct myoepithelial cells with cytoplasmic clearing are visible beneath the luminal epithelial cells in this gland (c) (H&E stained section, 100× magnification).

## 4. Discussion

Our study describes four mammary-type lesions arising in the anogenital area, all of which showed a similar distinctive immunohistochemical staining pattern. Kornegoor et al. described a distinctive 3-layered ductal epithelium in male gynecomastia [5]. They observed a distinct layer of CK14 and CK5/6 positive luminal cells which unlike the intermediate luminal layer, was negative for ER, PR, and AR. Normal native breast epithelium is typically described as bilayered, consisting of basally situated myoepithelial cells and a single layer of luminal epithelial cells [6]. A CK14 and CK5/6 positive cell population is also described in the normal female breast, as a population "dispersed irregularly throughout the ductal-lobular system" [6]. However, besides Kornegoor et al.'s description of a superficial layer of this population in male gynecomastia [5], no mention is given to the precise localization of this population in normal breast or other lesions of breast origin. In all of our cases of AGMLG lesions, we observed these CK14 and CK5/6 positive cells (with the exception of case 3, in which these cells were CK5/6 positive but not CK14 positive) to be present predominantly superficially above other intermediately located luminal cells. Thus, in addition to gynecomastia as described by Kornegoor et al. [5], we show that this arrangement is similarly found in a fibroadenoma and in ectopic breast tissue with a variety of proliferative changes arising from the AGMLG. This suggests that the superficial epithelial cell population is also a characteristic of the AGMLG and lesions arising thereof. Future studies to more closely examine the localization of CK14 and CK5/6 positive superficial cells in the native female breast are needed to determine whether or not this arrangement

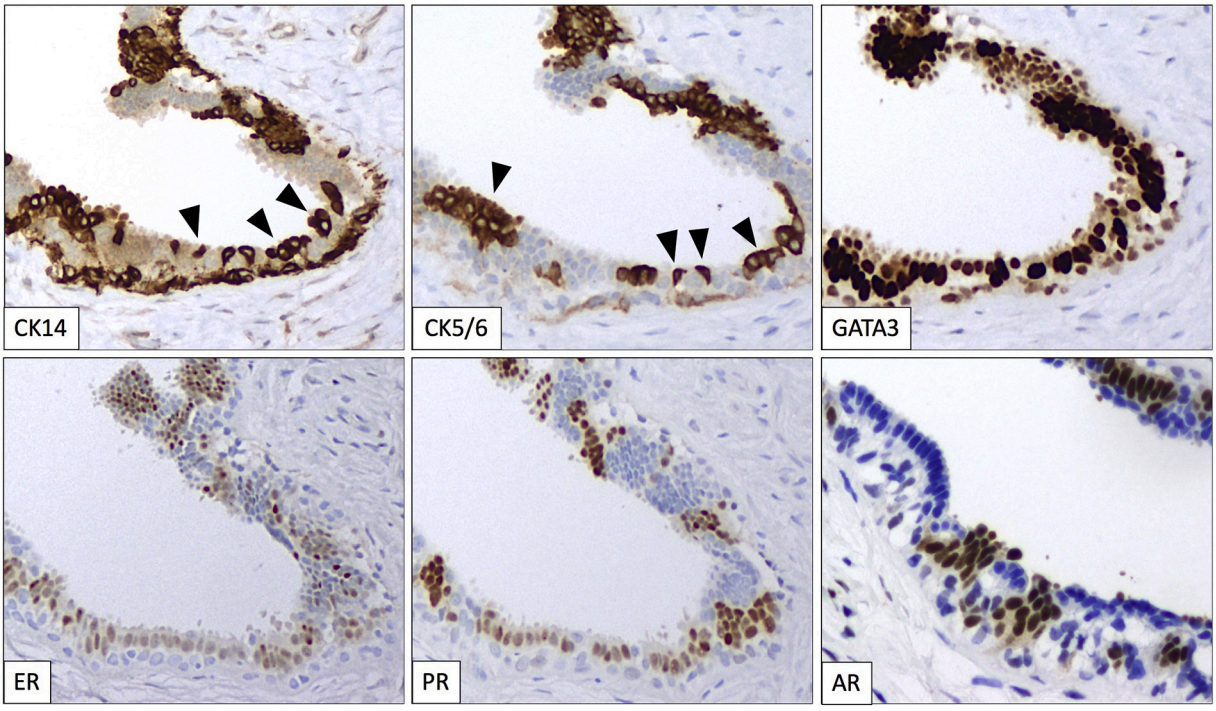


**Fig. 2.** Immunohistochemical staining of vulvar fibroadenoma shows 3 distinct cell populations: a luminal cell population positive for CK14 and CK5/6 and predominantly localized to the superficial epithelium (arrowheads), an intermediate luminal cell population positive for ER, PR, and focally AR, but negative for CK14 and CK5/6, and a myoepithelial cell population located basally and showing positivity for CK14 and CK5/6 and negative for ER, PR, and AR. GATA 3 shows diffuse strong positivity for all three glandular layers. Note pictures are all of the same area, however AR slide was cut deeper than the others, resulting in staining of an area slightly deeper than the area of the other slides. (IHC stained slides, 200× magnification for AR, 100× magnification for all other photomicrographs. Cropped).

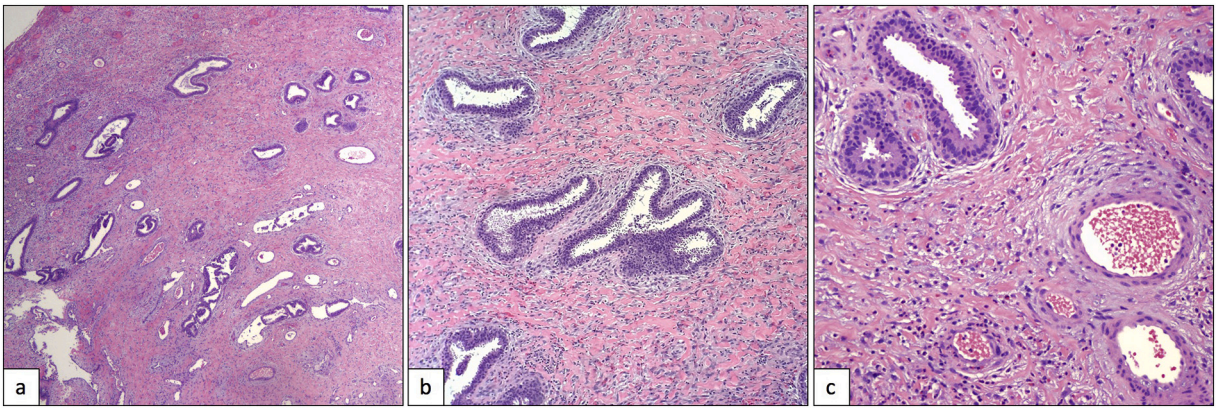


**Fig. 3.** Gynecomastia-like change consisting of ductal elements without lobule formation (a) (H&E stained section, 20× magnification). Periductal edema and fibrosis is apparent (b) (H&E stained section, 100× magnification). The stroma around ducts contains a mild inflammatory cell infiltrate consisting predominantly of lymphocytes (c) (H&E stained section, 100× magnification).

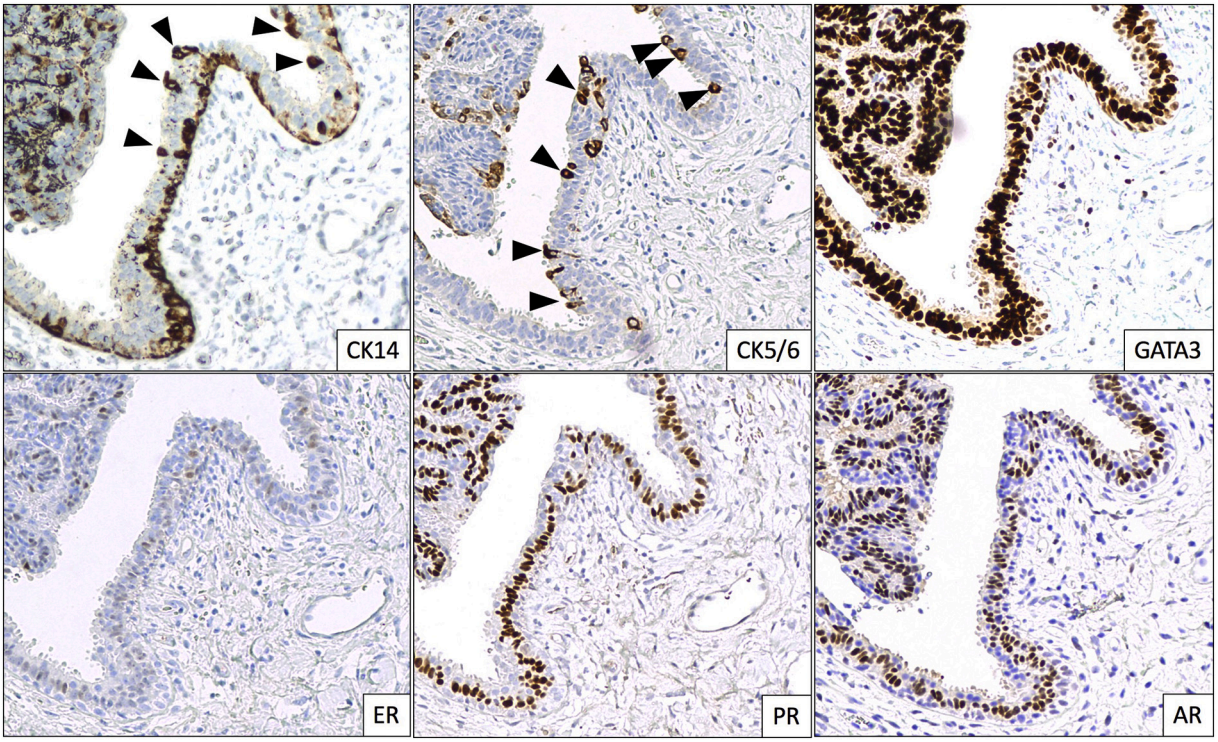


**Fig. 4.** Immunohistochemical staining of a case of gynecomastia-like change showing three distinct cell populations, similar to that seen in the vulvar fibro adenoma (Fig. 2). The myoepithelial cells are positive for CK14 and CK5/6, in addition to a population of luminal cells predominantly present superficially (arrowheads). Intermediate cells between the myoepithelial cells and superficial cells are positive for ER (focally), PR, and AR, but negative for CK14 and CK5/6. GATA 3 shows diffuse strong positivity for all three glandular layers. (IHC stained slides, 100× magnification. Cropped).

can be seen there in addition to gynecomastia and AGMLG lesions. Kornegoor et al. [5] postulated that the unique 3-layered pattern of staining in male gynecomastia militates against gynecomastia as an origin for male breast carcinoma because of the multiclonality of the luminal cell population. Whether this is analogous to the pathogenesis of carcinomas arising in AGMLG is not known.

Another novel finding in our study is the first reported observation of gynecomastia-like change arising from AGMLG in a female patient. Gynecomastia-like change in the female breast is uncommon, having been described in only a few case reports and small series. In the two relatively largest series, gynecomastia-like changes occurred as clinically detected breast masses or as incidental findings within other breast lesions [7,8]. Outside of the native breast, cases of gynecomastia-like change have only been reported at the periphery of a phyllodes tumor in the perianal region of a male patient [9] and occurring as a mass lesion within the axilla of a female [10], but not in the anogenital area of a female. All reported cases of gynecomastia-like hyperplasia, whether in the native breast or in ectopic locations, shared similar features, including a propensity to occur in younger patients and unique histologic findings of sole ductal structures, edematous or fibrotic stroma surrounding the ducts, and mild stromal lymphocytic infiltration.

The additional lesions described in our study, albeit rare, have been reported as associated with AGMLG. These lesions, namely fibroadenoma [1,11-19], pseudolactational change [20], usual ductal hyperplasia [21,22], apocrine metaplasia [23-25], and adenosis [26], are reported as histologically and/or immunohistochemically identical to their mammary counterparts. The histology and

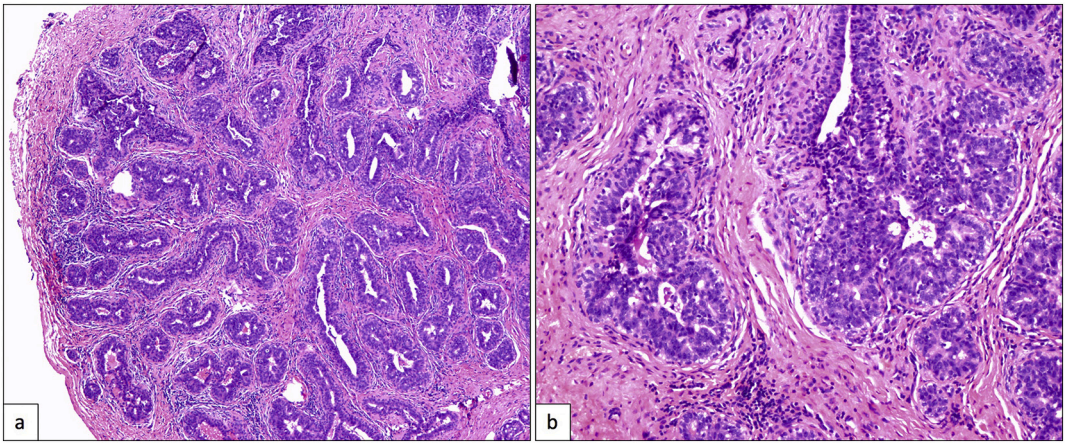


**Fig. 5.** Benign mammary-type tissue in a labial mass. The lesion is composed of mammary type lobular units (a) (H&E stained section, 40× magnification). Usual ductal hyperplasia is also present (b) (H&E stained section, 100× magnification).

immunohistochemistry of the lesions we describe were identical to those found in the native breast, with the only exception being the novel CK14 and CK5/6 superficial cell population we observed.

GATA3 was diffusely positive in all luminal cells in all four lesions we studied Expression of GATA3 in AGMLG has been documented [27] and so it does not seem unusual that lesions arising from the AGMLG should also show GATA3 positivity. Indeed, GATA3 was positive in a case of periclitoral accessory breast tissue with secretory change [28], an AGMLG tumor with lactational features [29], in two cases of fibroadenoma-like lesions of the vagina [30], and in three separate reports of primary vulvar mammary-type carcinoma [31-33]. Given that all of our cases, in addition to those previously reported in the literature, showed strong diffuse positivity for GATA3, this suggests that GATA3 may be useful in identifying the origin of a lesion from the AGMLG, similar to how GATA3 can be used to help identify lesions of native breast origin. As the number of cases to date that were stained for GATA3 is limited, sensitivity of GATA3 in identifying lesions arising in AGMLG awaits confirmation in a larger number of cases. GATA3 is also expressed in urothelial lesions, and thus urothelial lesions extending to the anogenital area may enter the differential diagnosis of such cases.

In summary we describe four cases of lesions arising from the AGMLG with novel findings of: (1) a three-layered epithelium having a superficial cell population with a specific immunohistochemical staining profile similar to that described in male gynecomastia, (2) the first case of gynecomastia-like hyperplasia in the perianal region of a female, and (3) diffuse GATA3 positivity in all four cases of AGMLG lesions. These findings contribute to our understanding of these rare

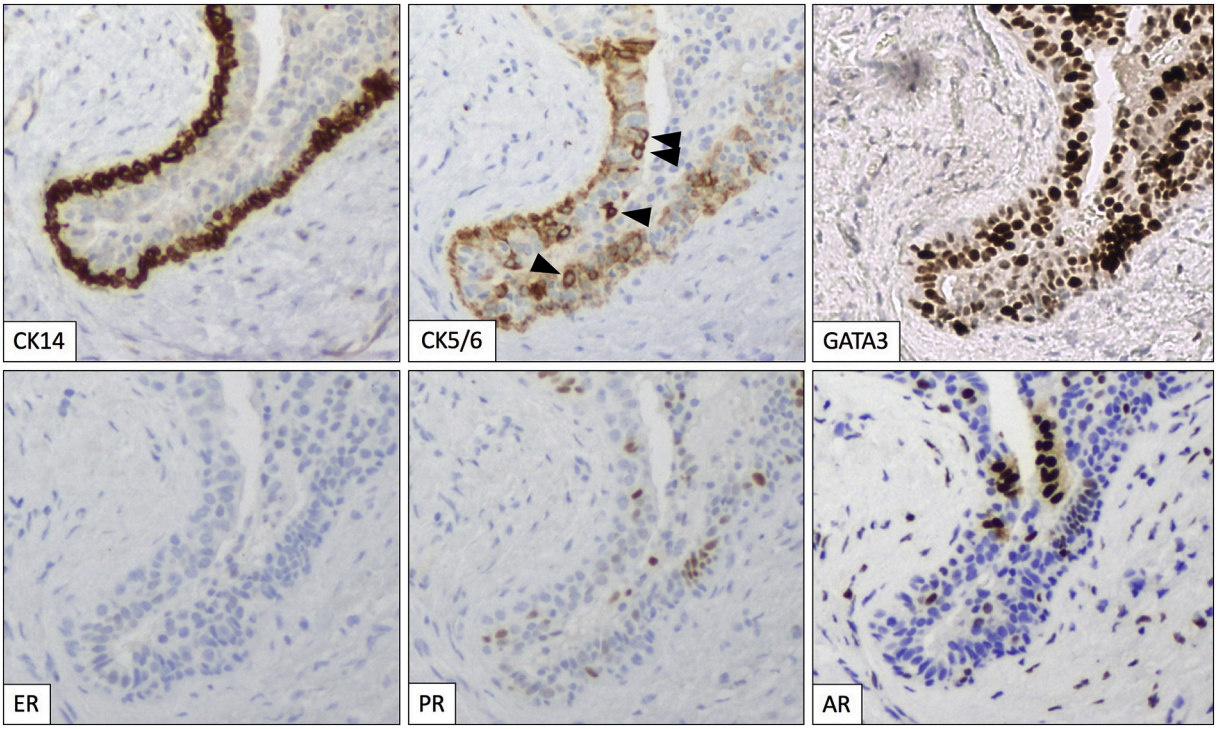


**Fig. 6.** Immunohistochemical staining of mammary type tissue from a labial mass. CK14 in this case stained only myoepithelial cells and not superficial luminal cells (unlike our other cases), whereas CK5/6 demonstrated staining of both myoepithelial cells and superficial luminal cells (arrowheads) as was seen in the other three cases. ER staining was negative to positive in only very rare luminal cells. PR and AR were patchy positive in luminal cells and GATA3 showed a similar staining pattern of all luminal cells similar to the other cases. (IHC stained slides, 100× magnification. Cropped).

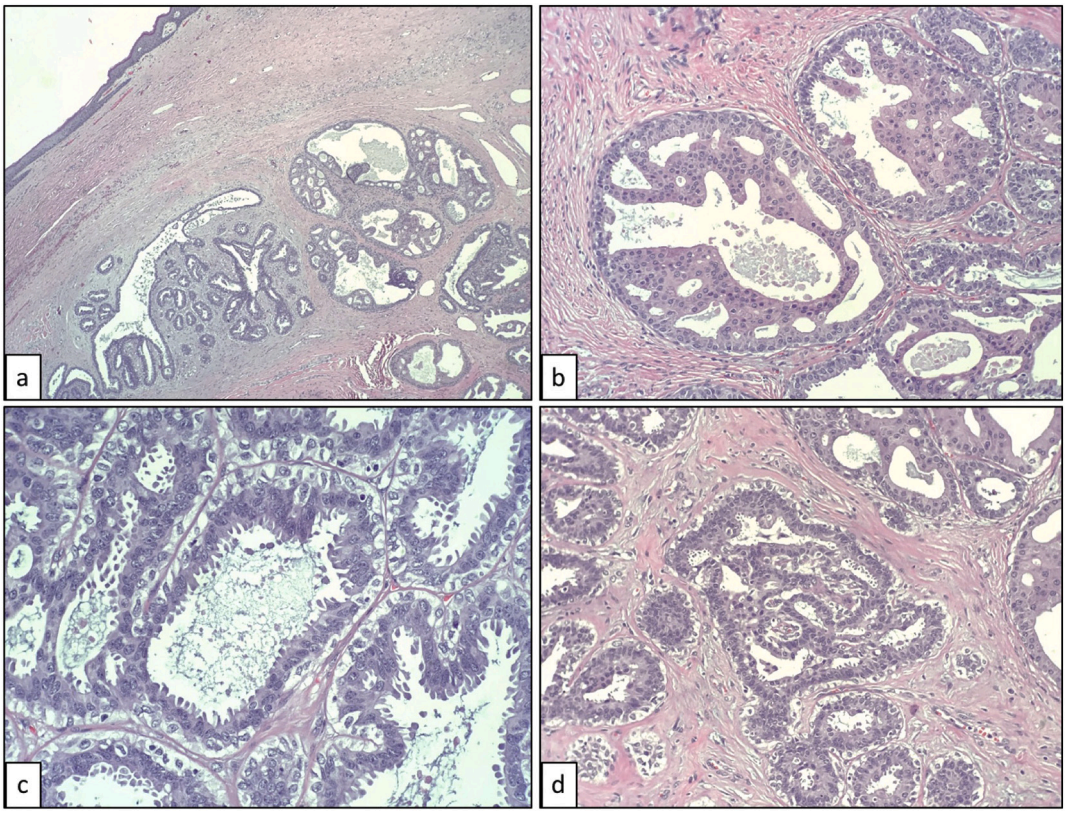


**Fig. 7.** Benign mammary-type glands (ectopic breast tissue) with overlying labial skin (a) (H&E stained section, 40× magnification). Apocrine metaplasia (b), pseudolactational changes (c), and usual ductal hyperplasia (d) present simultaneously in the labial lesion (H&E stained sections, 200× magnification).

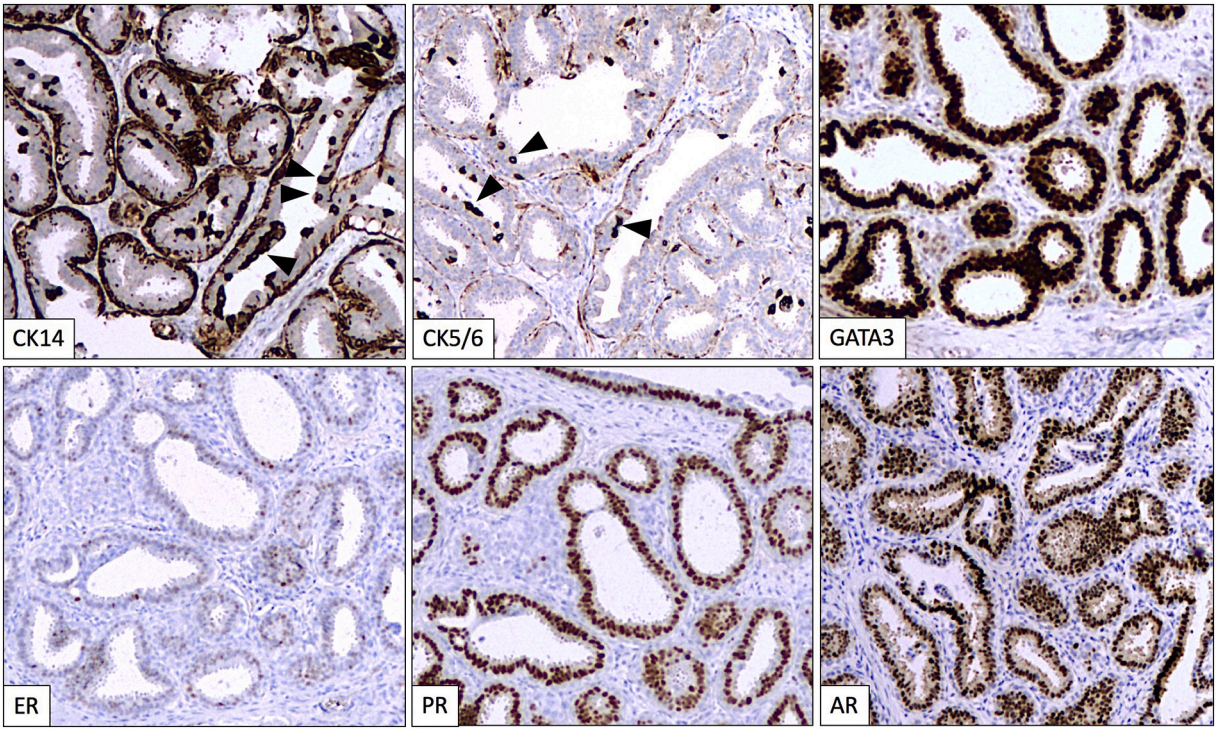


**Fig. 8.** Immunohistochemical staining of mammary type tissue from the labial mass in case 4. CK14 and CK5/6 stained superficial luminal cells (arrowheads) and myoepithelial cells. ER showed patchy positive staining, and PR and AR showed positivity in most luminal cells, but did not stain the superficial cell population. GATA3 was diffusely and strongly positive in all luminal epithelial cells. (IHC stained slides, 40× magnification).

**Table 2**
Summary of four cases of AGMLG lesions with immunohistochemical findings.

| Case # | Diagnosis | Age | Location | Superficial luminal cells IHC | Middle luminal cells IHC | Myoepithelial cells IHC |
|---|---|---|---|---|---|---|
| 1 | Fibroadenoma | 38 | Vulvar | CK14+ CK5/6+ ER- PR- AR- GATA3+ | CK14- CK5/6- ER+ PR+ AR+(p) GATA3+ | CK14+ CK5/6+ ER- PR- AR- GATA3- |
| 2 | Gynecomastia-like change | 29 | Perianal | CK14+ CK5/6+ ER- PR- AR- GATA3+ | CK14- CK5/6- ER+ PR+ AR+ GATA3 + | CK14+ CK5/6 + (f) ER- PR- AR- GATA3- |
| 3 | UDH | 29 | Labial | CK14- CK5/6+ ER- PR- AR- GATA3+ | CK14- CK5/6- ER+(w,p) PR+(f) AR+(f) GATA3+ | CK14+ CK5/6+ ER- PR- AR- GATA3- |
| 4 | UDH, AM, adenosis, PLC[a] | 35 | Labial | CK14+ CK5/6+ ER- PR- AR- GATA3+ | CK14- CK5/6- ER+ (w) PR+ AR+ GATA3+ | CK14+ CK5/6+ ER- PR- AR- GATA3- |

UDH = usual ductal hyperplasia; AM = apocrine metaplasia; PLC = pseudolactational changes; IHC = Immunohistochemistry; p = partial; w = weak; f = focal.
[a] IHC results shown are for background mammary-like tissue. Apocrine metaplasia was strongly AR+, PR+ and ER-. Other changes including UDH, PLC and adenosis were lost on deeper sections prepared for IHC.

lesions; including their clinicopathological and diagnostic characteristics.

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