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Nipple angiofibromas with loss of \textit{TSC2} are associated with tuberous sclerosis complex

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\section*{TO THE EDITOR}

Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous syndrome that leads to hamartoma formation in multiple organs, including the skin (Curatolo \textit{et al.}, 2008). Cutaneous hamartoma formation occurs secondary to loss of function of either the \textit{TSC1} or \textit{TSC2} gene in fibroblast-like cells and subsequent dysregulation of the mechanistic target of rapamycin complex 1, or mTORC1 (Li \textit{et al.}, 2008; Tyburczy \textit{et al.}, 2014). Angiofibromas are among the most well recognized TSC-related skin hamartomas and consist of multiple pink papules on the central face (Little, 1909). Here we describe angiofibromas of the nipple-areolar complex.

Between October 2012 and January 2015, 53 TSC patients (50 women, 3 men) participated in studies of lymphangioleiomyomatosis (LAM), a TSC-associated lung disease with female predominance, at the National Institutes of Health in Bethesda, Maryland. Written informed consent was obtained according to IRB-approved protocols 00-H-0051, 95-H-0186 and/or 82-H-0032.

Eleven TSC patients (10 women, 1 man; median age 41 years [range 21–71 years]) with papules on the nipple and/or areola were identified (Table), eight of whom had germline mutations in \textit{TSC2}. Clinical examination revealed 1 to 25, 1–3 millimeter, pink to red dome-shaped papules on the nipple and/or areola, affecting one or both breasts (Figure 1a). }
patient recollection, the median age of onset was 33 years (n=7 women; range 16–38 years). One patient reported painless bleeding from her nipple papules during breastfeeding while the rest remained asymptomatic.

Histopathological examination of ten biopsied nipple or areolar papules from seven patients revealed increased number of dilated capillaries in the papillary dermis and increased number of stellate and spindle-shaped fibroblasts interspersed in the dermal collagen, consistent with the diagnosis of an angiofibroma (Figure 1b).

Increased numbers of factor XIIIa positive spindle to stellate shaped cells were observed in the dermis of the nipple-areolar complex papules (Figure 1c), as characteristically observed in TSC-related facial angiofibromas (Li et al., 2005). To detect activation of mTORC1 in nipple-areolar complex papules, immunohistochemical staining against phosphorylated ribosomal protein S6 (pS6) (Ser235/236) was performed on ten samples, revealing avid positive dermal fibroblast-like cells as seen in other TSC-related skin hamartomas (Figure 1d) (Li et al., 2008).

Western blot analysis of fibroblast-like cells grown from two out of four nipple angiofibromas demonstrated loss or near complete loss of TSC2. All four showed increased pS6 under serum starvation, compared to dermal fibroblasts from normal-appearing skin (Supplementary Figure 1), consistent with prior molecular analysis of facial angiofibromas (Tyburczy et al., 2014). Mutational analysis of DNA extracted from papule-derived fibroblast-like cells or whole tissue biopsies was performed as described previously using targeted next generation sequencing (NGS) of TSC1 and TSC2, with validation by Sanger sequencing or SNaPshot analysis (Tyburczy et al., 2014). Seven angiofibromas from five patients were analyzed and biallelic mutations in TSC2 were identified in four samples (Table, Supplementary Figure 2). A nonsense mutation in TSC2 (p.Arg1459*) was present in one nipple angiofibroma fibroblast culture at 99% allele frequency, consistent with both a germline p.Arg1459* mutation and second hit loss of the wild-type allele (Table, P27, Supplementary Figure 2a). Three samples (2 whole tissue, 1 cultured cells; Table, P36 and P37) had second hit point mutations in TSC2 that were seen at low allele frequency (3% to 5%), albeit undetectable in patient controls, consistent with the low prevalence of neoplastic fibroblast cells in these biopsies. All three of the low frequency second hit mutations seen in these samples were validated by an independent method (Supplementary Figure 2e–j). The remaining samples in which neither second hit point mutations nor loss of heterozygosity (LOH) was seen in TSC2 may be due to the occurrence of LOH, which is very difficult to detect in a sample in which the tumor cells are at low frequency. This microscopic and molecular evidence of mTORC1 activation with inactivating mutations in TSC2 provides strong support for the concept that these lesions arise through two hit inactivation of TSC1/TSC2, as we have shown previously for facial angiofibromas (Tyburczy et al., 2014).

In our study, the frequency of nipple-areolar complex angiofibromas in adult women was 20%. Median age of onset was 33 years (range 16–38), nearly three decades after onset of facial angiofibromas in the same female patients (median age 4 years; range 3–13). This indicates that nipple-areolar complex angiofibromas are an adult manifestation of TSC, occurring later than any cutaneous feature currently described. In addition, the presence of

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nipple and areolar angiofibromas in a man from our cohort suggests that unlike LAM, these hamartomas are not limited to women. The differential diagnosis of nipple-areolar complex papules in a TSC patient should include several benign tumors, such as nipple adenomas/florid papillomatosis, leiomyomas, fibromas, and folliculosebaceous cystic hamartomas (Badr and Lakshmiah, 2009, Spyropoulou et al., 2014). For patients without known TSC, consideration should also be given to nipple papules and nodules related to other tumor syndromes, including myxomas and neurofibromas (Bongiorno et al., 2010, Carney et al., 1986). Biopsy and pathologic examination are valuable to confirm the diagnosis of angiofibroma, both for diagnostic purposes and for reassurance in regards to natural history. Treatment may not be necessary; unlike facial angiofibromas, angiofibromas of the nipple and areola do not bleed spontaneously.

Nipple-areolar complex angiofibromas were less common than facial angiofibromas in our cohort, affecting 20% versus 100% of female patients, respectively. Less frequent occurrence of angiofibromas on the nipple or areola may be explained by protection from sun exposure as our group previously demonstrated that signature second hit UV mutations promote facial angiofibroma formation (Tyburczy et al., 2014). We propose that repetitive trauma from breastfeeding may stimulate hamartoma development in female patients, just as shoe wear compression may stimulate ungual fibroma pathogenesis (Aldrich et al., 2010). One patient reported onset of a nipple papule in the weeks following initiation of breastfeeding and two additional patients reported onset of papules after discontinuing breastfeeding. Thus, both somatic mutations and trauma may drive TSC-related skin tumor pathogenesis.

In summary, we report nipple-areolar complex angiofibromas, a cutaneous manifestation of TSC. Similar to facial angiofibromas, they occur following second hit loss of the wild type allele of TSC2, but manifest much later in life. They have a benign clinical course, with symptomatic manifestations only associated with local trauma. When verified by histologic analysis, they may provide additional information in favor of TSC diagnosis.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>TSC</td>
<td>tuberous sclerosis complex</td>
</tr>
<tr>
<td>LAM</td>
<td>lymphangioleiomyomatosis</td>
</tr>
<tr>
<td>pS6</td>
<td>phosphorylated ribosomal protein S6</td>
</tr>
<tr>
<td>mTORC1</td>
<td>mechanistic target of rapamycin complex 1</td>
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</table>
NGS  

next generation sequencing

LOH  

loss of heterozygosity

References


Figure 1. Gross and microscopic features of nipple-areolar complex angiofibromas
(a) Pink papules on the nipple and areola. (b) Hematoxylin and eosin stain shows increased number of dilated vessels and fibroblasts consistent with the diagnosis of an angiofibroma. (c) Factor XIIIa staining demonstrates positive cells in the dermis as seen in facial angiofibromas. (d) Immunoreactivity to phosphorylated ribosomal protein S6 (pS6) in scattered dermal fibroblast-like cells suggests hyperactivation of the mechanistic target of rapamycin.
Clinical, microscopic and mutational analysis of nipple-areolar complex angiofibromas.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>TSC cutaneous major features</th>
<th>History of breastfeeding</th>
<th>Lesions on nipple (biopsied)</th>
<th>Lesions on areola (biopsied)</th>
<th>Histopathology</th>
<th>Tissue for mutational analysis</th>
<th>TSC2 mutation (af)</th>
<th>TSC2 protein change</th>
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<tr>
<td>P25 F</td>
<td>AF, HM</td>
<td>no</td>
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<td>13 (1)</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>P27 F</td>
<td>AF, FCP</td>
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<td>3 (1)</td>
<td>3</td>
<td>angiofibroma, associated hidrocystoma</td>
<td>cultured fibroblasts</td>
<td>c.4375C&gt;T (.99)</td>
<td>p(Arg1459*)</td>
<td>–</td>
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<td>P28 F</td>
<td>AF, SP, FCP</td>
<td>yes</td>
<td>3 (1)</td>
<td>0</td>
<td>angiofibroma, prominent vascular component</td>
<td>cultured fibroblasts</td>
<td>c.4868_4875delCC CTGATG (.54)</td>
<td>Out-of-frame deletion</td>
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<td>AF, HM, UF</td>
<td>no</td>
<td>1</td>
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<td>–</td>
<td>–</td>
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<td>no</td>
<td>1</td>
<td>0</td>
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<td>–</td>
<td>–</td>
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<td>5 (1)</td>
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<td>cultured fibroblasts</td>
<td>c.2098-1G&gt;A (.5)</td>
<td>splice</td>
<td>p.(His1620Tyr)</td>
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<td>11 (2)</td>
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<td>splice</td>
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<td>3</td>
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<tr>
<td>P47 F</td>
<td>AF, HM, UF</td>
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<td>–</td>
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</table>

Abbreviations: AF, angiofibromas; af, allelic frequency; F, female; FCP, fibrous cephalic plaque; HM, hypomelanotic macules; L, left; M, male; R, right; SP, shagreen patch; TSC, tuberous sclerosis complex; UF, ungual fibromas.

Bold denotes germline mutation when documented in blood or control skin.