Nevertheless, our ability to identify meaningful differences between these two populations suggests that this is a reasonable cutpoint.

One hypothesis for the increasing incidence rates of early-onset BCC has been increased awareness and skin surveillance. However, lesion size has not decreased over time, as might be anticipated if earlier detection were the underlying cause of increased incidence (Christenson et al., 2005). Our results likewise suggest that early-onset BCC is associated with aggressive histologic characteristics, as opposed to a less aggressive phenotype that might be expected if surveillance bias were operating. Although additional studies are needed, these results suggest there may be underlying biological differences between early- and late-onset BCC.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2015.11.002.

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Nipple Angiofibromas with Loss of *TSC2* Are Associated with Tuberous Sclerosis Complex

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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 et al., 2008). Cutaneous hamartoma formation occurs secondary to loss of function of either the *TSC1* or *TSC2* gene in fibroblast-like cells and subsequent dysregulation of the mechanistic target of rapamycin complex 1 (Li et al., 2008; Tyburczy 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.

Abbreviation: TSC, tuberous sclerosis complex

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Table 1. Clinical, microscopic, and mutational analysis of nipple-areolar complex angiofibromas

Patient	Sex	TSC cutaneous major features	History of breastfeeding	Lesions on nipple (biopsied)	Lesions on areola (biopsied)	Histopathology	Tissue for mutational analysis	*TSC2* mutation (af)	TSC2 protein change
P25	F	AF, HM	No	1	13 (1)	Angiofibroma	–	–	–
P27	F	AF, FCP	Yes	3 (1)	3	Angiofibroma, associated hidrocystoma	Cultured fibroblasts	**c.4375C>T (.99)**	**p.(Arg1459*)**
P28	F	AF, SP, FCP	Yes	3 (1)	0	Angiofibroma, prominent vascular component	Cultured fibroblasts	c.4868_4875delCCCTGATG (.54)	Out-of-frame deletion
P34	F	AF, HM, UF	No	1	0	–	–	–	–
P35	F	AF, HM, UF, FCP	No	1	0	–	–	–	–
P36	F	AF, HM, UF	Yes	5 (1)	5 (1)	Angiofibroma	Cultured fibroblasts	**c.2098-1G > A (.5)** c.4858C>T (.05)	**Splice** p.(His1620Tyr)
P37	F	AF, HM, UF	Yes	11 (2)	14 (1)	Angiofibroma	Whole tissue – T1	**c.2098-1G > A (.5)** c.138+1G > A (.05)	**Splice** splice
							Whole tissue – T2	**c.2098-1G > A (.5)** c.3574C > T (.03)	**splice** p.(Gln1192*)
							Whole tissue – T3	**c.2098-1G > A (.5)**	**Splice**
P43	F	AF, UF	No	3 (1)	0	Angiofibroma	Cultured fibroblasts	**c.3999C > A (.5)**	**p.(Tyr1333*)**
P46	F	AF, HM, UF	No	1	3	–	–	–	–
P47	F	AF, HM, UF	Yes	1 (1)	0	Angiofibroma	–	–	–
P48	M	AF, HM, UF	–	11	0	–	–	–	–

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.

Between October 2012 and January 2015, 53 patients with TSC (50 women, 3 men) participated in studies of lymphangioleiomyomatosis, 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 patients with TSC (10 women, 1 man; median age 41 years [range 21–71 years]) with papules on the nipple and/or areola were identified (Table 1), eight of whom had germline mutations in *TSC2*. Clinical examination revealed 1 to 25, 1–3 mm, pink to red dome-shaped papules on the nipple and/or areola, affecting one or both breasts (Figure 1a). Per 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, whereas the rest remained asymptomatic.

Histopathological examination of 10 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 mechanistic target of rapamycin complex 1 in nipple-areolar complex papules, immunohistochemical staining against phosphorylated ribosomal protein S6 (Ser235/236) was performed on 10 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 of four nipple angiofibromas demonstrated loss or near complete loss of TSC2. All four showed increased phosphorylation of ribosomal protein S6 under serum starvation, compared with dermal fibroblasts from normal-appearing skin (Supplementary Figure S1 online), 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 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 1; Supplementary Figure S2 online). 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 1, P27; Supplementary Figure S2a). Three samples (two whole tissue, one cultured cells; Table 1, 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