The role of *TERT* promoter mutations in differentiating recurrent nevi from recurrent melanomas: A retrospective, case-control study

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Background: Repigmentation at previous biopsy sites pose a significant diagnostic dilemma given clinical and histologic similarities between recurrent nevi and locally recurrent melanoma. Though common in melanoma, the role of *TERT* promoter mutations (TPMs) in recurrent nevi is unknown.

Objective: We investigated the role of TPMs in recurrent nevi and whether the presence of hotspot TPM distinguishes recurrent nevi from locally recurrent melanoma. We also characterized clinical and histologic features differentiating these lesions.

Methods: We analyzed 11 locally recurrent melanomas, 17 recurrent nevi, and melanoma and nevus controls to determine TPM status. We also assessed clinical and histologic features of the recurrent groups.

Results: Hotspot TPMs were more common in recurrent melanomas than recurrent nevi (P = .008). Recurrent melanomas were more likely to have solar elastosis (P = .0047), multilayering of melanocytes in the epidermis (P = .0221), adnexal involvement (P = .0069), and epidermal consumption (P = .0204). Recurrent nevi had intra-epidermal atypia limited to the area above the scar ($P < .0001$) and occurred earlier after the original biopsy ($P < .0008$). Solar elastosis, months to recurrence, and hotspot TPMs were independently associated with recurrent melanoma in multivariate analysis.

Limitations: This was a retrospective study.

Conclusion: Hotspot TPMs are significantly more frequent in recurrent melanomas and could serve as a diagnostic clue in histologically ambiguous cases. (J Am Acad Dermatol 2019;80:685-93.)

Key words: borderline lesions; locally recurrent melanoma; melanoma in scar; recurrent nevi; regenerating nevus; repigmentation within a scar; *TERT*; *TERT* promoter mutation; TPM.

Melanocytic neoplasms that recur at the site of the scar after incisional biopsy or incomplete excision can be diagnostically problematic from both a clinical and histologic perspective.[1,2] Due to distortion from the prior biopsy scar, recurrent nevi might not conform to a typical dermoscopic pattern of benign nevi.[3-6] Conversely, slowly recurring melanomas might have only subtle dermoscopic clues suggestive of melanoma, such as shiny white streaks, which might be overlooked or incorrectly attributed to the prior procedure. Histologic distinction is even more

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complex, as recurrent nevi might have striking histologic similarities to melanoma to the extent that the term pseudomelanoma is often used.[2] This includes marked lentiginous single-cell growth, pagetosis, and adnexal involvement as seen in the early radial growth phase of melanoma.[2,7-9]

A number of studies have identified telomerase reverse transcriptase (*TERT*) promoter mutations (TPMs) as one of the most common mutations in melanoma.[10-13] *TERT* encodes the catalytic subunit of telomerase, an enzyme that adds nucleotide repeats to the ends of telomeres to prevent cellular senescence, which can occur from telomere attrition due to cell division.[14,15] Increased telomerase activity has been reported in up to 90% of cancers.[14-16] A recent study showed that these mutations tend to occur relatively early in the progression of melanoma.[12] In an assessment of melanomas with benign nevus precursors, TPMs were identified in areas of the tumor thought to be intermediate between nevus and melanoma.[12]

CAPSULE SUMMARY

- Telomerase reverse transcriptase (*TERT*) promoter mutations are significantly more common in both primary and recurrent melanomas compared with recurrent nevi.
- Repigmentation within a scar can be diagnostically challenging. *TERT* promoter mutation status can help distinguish recurrent nevi from recurrent melanomas in cases that cannot be diagnosed with confidence on histopathology alone.

In this study, we sought to answer 2 questions. Are TPMs frequent in recurrent nevi and a possible explanation of their clinical behavior? If TPMs are not frequent in recurrent nevi, can their presence be used to discriminate locally recurrent melanomas from recurrent nevi? We also compared the clinical and histologic features of locally recurrent melanoma and recurrent nevi.

METHODS

Case selection

After obtaining institutional review board approval, we searched our dermatopathology database for cases that occurred during 2008-2017 in which the histologic differential diagnosis was recurrent junctional or compound nevus or recurrent melanoma with an in situ component. We accepted all cases with a final diagnosis of melanoma occurring at a prior incisional biopsy site. We also accepted 17 cases of recurrent nevi that met these criteria, for which sufficient tissue was available for DNA extraction and Sanger sequencing for TPMs. We further required that all hematoxylin-eosin–stained slides were available for review and that a consensus diagnosis of recurrent nevus or melanoma was made by 3 board-certified dermatopathologists. Lesions with a region of ≥ 4 mm^2, which could be microdissected with a tumor concentration of $\geq$20% yielded sufficient DNA concentrations for sequencing. Among the melanomas, sufficient tissue was available for Sanger sequencing in 9 of 11 cases. Control nevi were randomly selected from dysplastic and compound nevi. Melanoma controls were randomly selected from a cohort of stage T1 superficial spreading melanomas. Relevant clinical data obtained included patient age and sex, lesion time to recurrence, and site.

Histologic analysis

Hematoxylin-eosin–stained slides from recurrent melanomas and recurrent nevi were assessed for the following histologic features: presence of solar elastosis, mitoses, confluent junctional growth, adnexal involvement, consumption, dyscohesion of the dermal-epidermal junction (DEJ), epidermal effacement, limitation of the atypical component to the area above the scar, and multilayering of melanocytic cells in the epidermis, degree of pagetoid spread (extensive vs focal or none), nuclear atypia (mild or moderate vs severe), and whether nuclear atypia was confined to epidermal melanocytes or included dermal melanocytes. Solar elastosis was defined as the presence of thickened, basophilic elastic fibers in the dermis. Multilayering was defined as ≥ 2 stacked rows of prominent lentiginous growth of melanocytes in the basal layer. Consumption was defined as thinning of the epidermis as a direct result of extensive proliferation of melanocytes in the region. Hematoxylin-eosin–stained slides of melanoma controls and nevus controls were reviewed to confirm diagnoses. Hematoxylin-eosin–stained slides of the primary melanoma were available and reviewed for 9 of 11 cases.

DNA extraction and Sanger sequencing

DNA was extracted from formalin-fixed paraffin-embedded tissues using GeneRead DNA FFPE Extraction kit (QIAGEN, Hilden, Germany) and quantified by Qubit (Life Technologies, Carlsbad, CA). The core promoter region of *TERT* (−260 to +60) was amplified by using PCR primers (forward 5′-ATTCGCGGGCACAGACGC-3’; reverse 5′-TCGC GGTAGTGGCTGCGC-3′) (Life Technologies). A range

Abbreviations used:

DEJ: dermal-epidermal junction
TERT: telomerase reverse transcriptase
TPM: telomerase reverse transcriptase promoter mutation

of 30-150 ng of DNA were used in a 40 μL-reaction with 0.5 μM primers, 1x Invitrogen Platinum Hot Start PCR Master Mix and 8 μL Invitrogen Platinum GC Enhancer (Life Technologies). The thermal cycling process was initiated at 95°C for 4 min, followed by 40 cycles of 94°C for 1 min, 69°C for 1 min, and 72°C for 1 min and 1 cycle of 72°C for 5 min. PCR products were run on a 2% agarose gel and purified using QIAquick Gel Extraction Kit (QIAGEN). Sanger sequencing was performed at ACGT Inc (Wheeling, IL). The sequencing chromatograms were viewed with FinchTV 1.4.0.

Mutation analysis

The following 4 mutations were defined as hotspot mutations on the basis of their frequency reported in the COSMIC database and the published literature to date: c.1-146C>T, c.1-139_1-138CC>TT, c.1-124C>T, and c.1-125_1-124CC>TT.[10,11]

Statistical analysis

Statistical analysis was performed using RStudio, version 1.1.383. Fisher's exact tests were used to compare frequencies of histomorphologic features and hotspot TPMs. A Mann-Whitney *U* test was used to evaluate age and time to recurrence. A *P* value of $<.05$ was considered statistically significant.

Univariate and multivariate analyses were performed by using logistic regressions with Firth correction to account for variables with complete and quasiseparation. Significantly associated variables were identified in univariate analyses. We then used forward selection to determine features most predictive of recurrent melanoma when combined with hotspot TPMs.

Table I. Summary of clinical characteristics of recurrent nevus and recurrent melanoma groups

Characteristic	Recurrent nevus	Recurrent melanoma	*P* value
Median age, y	30	49	.0024
Sex, n (%)			1
Male	3/17 (17.6)	2/11 (18.2)	
Female	14/17 (82.4)	9/11 (81.8)	
Location, n (%)			.0104
Extremities	2/17 (11.7)	7/11 (63.6)	
Trunk	15/17 (88.2)	4/11 (36.3)	
Median time to recurrence, mon	3.0	63.5	<.0008

Table II. The frequency of various histologic features in recurrent melanomas and recurrent nevi

Feature	Recurrent melanoma, n	Recurrent nevi, n	*P* values
Solar elastosis present	5/11	0/17	.0047*
Atypical melanocytic proliferation limited to area above scar	0/11	17/17	<.0001*
Dermal component present	6/11	10/17	.5646
Extensive pagetoid spread	10/11	0/17	<.0001*
Epidermal effacement	7/11	15/17	.1741
Nuclear atypia	11/11	6/17	.0001*
Confluent junctional growth	10/11	10/17	.0987
Adnexal involvement	6/11	1/17	.0069*
Atypia confined to epidermis	5/11	14/17	.0946
Multilayering of melanocytes in epidermis	5/11	1/17	.0221*
Mitoses	6/11	1/17	.0069*
Epidermal consumption	7/11	3/17	.0204*
Dyscohesion of DEJ	7/11	5/17	.1212

DEJ, Dermal-epidermal junction.
*Statistically significant *P* value.

RESULTS

There were 20 melanoma control and 21 nevi control cases. All melanoma controls were superficial spreading melanomas; 18 were stage T1a and 2 were stage T1b, with an overall average Breslow depth of 0.38 mm. Among 21 nevi controls, there were 16 moderately or mildly dysplastic nevi and 5 conventional compound nevi. Among the control groups, 13 of 20 melanoma controls and 2 of 21 nevi controls were found to have hotspot TPMs ($P = .0001$). The 2 nevi controls with hotspot mutations were dysplastic nevi with moderate atypia.

There were 11 recurrent melanoma and 17 recurrent nevus cases. Histopathologic sections from the primary melanoma were available for review in 9 of 11 melanoma cases. Upon review of the original biopsies of the melanomas, 7 were thought to be melanoma from the onset, while 2 were felt to be morphologically most consistent with dysplastic nevi that transformed to melanoma. Review of the original biopsies of the recurrent

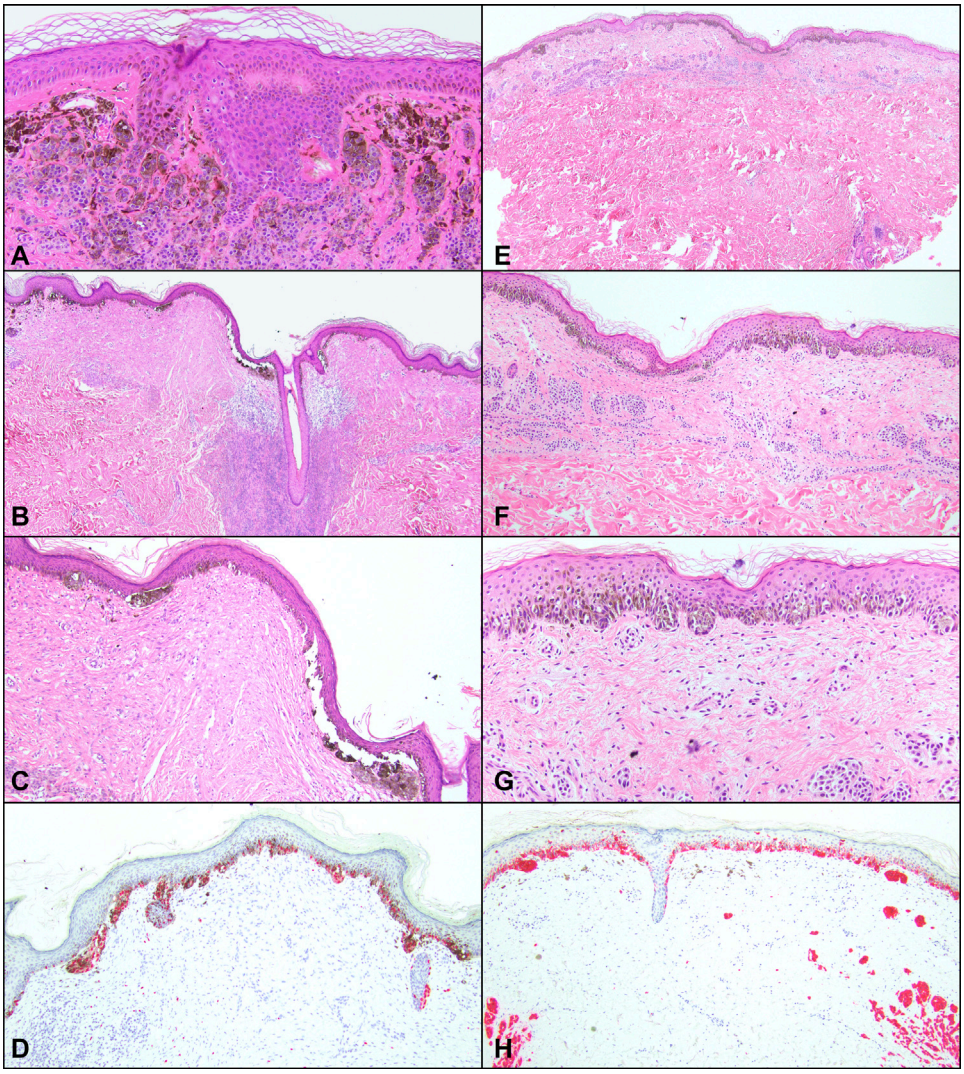

Fig 1. Two cases of recurrent nevi with concerning histologic features. **A**-**D**, Case 1. **A**, The original nevus shows no atypical features with minimal intraepidermal involvement. **B**, Low-power image of the recurrence of the nevus (**A**) shows confluent lentiginous growth of melanocytes in the epidermis, resulting in dyscohesion of the dermal-epidermal junction, as well as some horizontal streaming of irregular nests. **C**, Higher power imaging highlights the dyscohesion and lentiginous growth. **D**, A SOX-10 staining highlights the lentiginous and suprabasal growth of melanocytes in the epidermis. **E**-**H**, Case 2. **E**, A low-power view shows that atypical intra-epidermal growth of melanocytes is limited to the area above the scar. **F** and **G**, Higher power views show broad lentiginous growth and pagetoid cells in the epidermis above a dermal scar. **H**, MART-1 staining shows confluence of junctional nests and focal pagetosis.

nevi showed 9 dysplastic nevi and 8 conventional nevi. The median age for patients with recurrent nevi was 30 (range 23-61) years and recurrent melanoma was 49 (range 29-71) years (P = .0024). The median time to recurrence within a scar for recurrent nevi was 3.0 (range 1-90) months and for melanoma was 63.5 (range 23-240) months ($P < .0008$). Two of 17 recurrent nevi and 7 of 11 recurrent melanomas were on the extremities; 15 of 17 nevi and 4 of 11 melanomas were on the trunk (P = .0104). There was no significant difference by sex. Table I summarizes the clinical data.

Histologic features are summarized in Table II. Of note, there was a statistically significant difference between recurrent melanoma and recurrent nevi for 8 distinct histologic features. The recurrent melanoma group was significantly more likely to have solar elastosis (P = .0047), extensive pagetoid

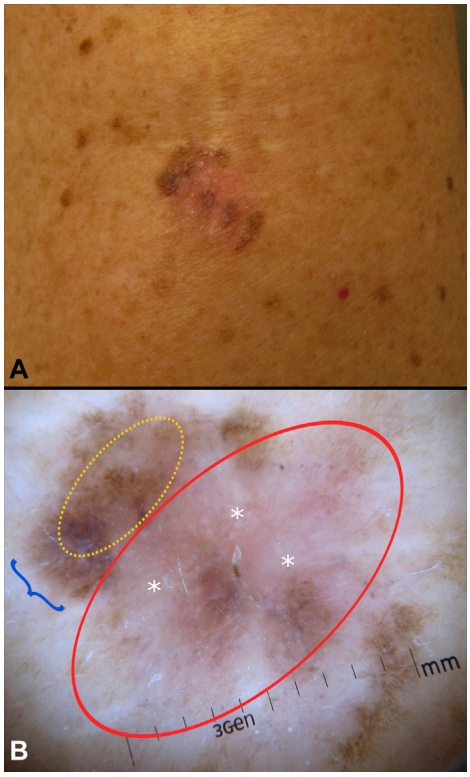

Fig 2. An incompletely excised locally recurrent melanoma in a 52-year-old woman. **A**, A clinical photograph in 2014 of repigmentation within a scar on the left upper arm. The patient had 2 prior biopsies at this site in 2005 and 2007. **B**, The dermoscopic image shows shiny white streaks (*white asterisks*), vascular blush (*red solid circle*), radial streaming (*blue brace*), and an atypical network (*yellow dotted circle*).

spread ($P < .0001$), severe nuclear atypia ($P = .0001$), multilayering of cells ($P = .0221$), dermal or junctional mitoses ($P = .0069$), extensive adnexal involvement ($P = .0069$), and consumption ($P = .02034$). The recurrent nevus group was statistically more likely to have the atypical component confined to the area above the scar ($P < .0001$); 0 of 17 recurrent nevi had extensive pagetoid spread, and 10 of 17 recurrent nevi had focal suprabasal movement of melanocytes confined to the area above the scar. Fig 1 illustrates some histologic features of recurrent nevi that might be mistaken for melanoma. Figs 2 and 3 show a locally recurrent melanoma that was originally diagnosed as a recurrent nevus in a middle-aged woman.

Hotspot TPMs were more common in the recurrent melanoma group than the recurrent nevi group ($P = .0008$). Nine of 11 recurrent melanoma cases were successfully sequenced, and 4 had hotspot TPMs (1 had mutations c.1-146C>T and c.1-139_1-138CC>TT). In 3 of these cases, sequencing of the original biopsy showed the presence of the same TPM. None of the 17 recurrent nevus cases had a hotspot TPM. Fig 4 shows the Sanger sequencing chromatogram for hotspot mutations compared with a negative control. Table III summarizes the frequency of hotspot TPMs among the different lesional groups.

Univariate logistic regression is displayed in Table IV. Clinical features associated with recurrent melanoma were older age, truncal location, and longer time to recurrence. Histologic features included solar elastosis, lack of sharp demarcation, extensive pagetosis, severe atypia, adnexal involvement, atypia confined to the epidermis, multilayering of melanocytes in the epidermis, mitoses, epidermal consumption, and hotspot TPM. In the multivariate analysis (Table V), hotspot TPM in combination with solar elastosis and months to recurrence were most significantly associated with a diagnosis of recurrent melanoma.

DISCUSSION

The distinction of a recurrent nevus from locally recurring melanoma at the site of an incisional biopsy or incomplete excision can be challenging from both a clinical and histologic perspective. On histology, the term pseudomelanoma has been coined to describe these lesions because they might have many of the morphologic attributes of melanoma in situ, including an effaced epidermis, lentiginous single cell growth along the DEJ with some junctional confluence, suprabasal movement of melanocytes, and nuclear atypia.[2,7-9] Recurrent nevi typically recur in <1 year and the recurring pigment is often confined to the scar clinically.[3,7-9,17]

The clinical distinction between recurrent nevi and melanoma occurring within a scar is complicated because recurrent nevi can have dermoscopic features of melanoma. Blum et al reported radial lines in 29.6% of recurrent nevi and pseudopods in 7.1% of benign lesions.[3] Although pigment extending beyond the scar was significantly associated with a diagnosis of recurrent melanoma, the group found that 42.9% of benign recurrent nevi also displayed this feature.[3]

In this study, we compared the clinical and histologic features of 17 recurrent nevi and 11 melanomas recurring at the site of a prior incomplete surgical procedure. Recurrent lesions occurring in younger patients after <1 year in less heavily sun-damaged skin were more likely to be recurrent nevi. In contrast, locally recurrent melanomas tended to occur in older patients with a median time to recurrence of 63.5 months.

Multiple histologic factors were statistically significantly different between the recurrent nevus

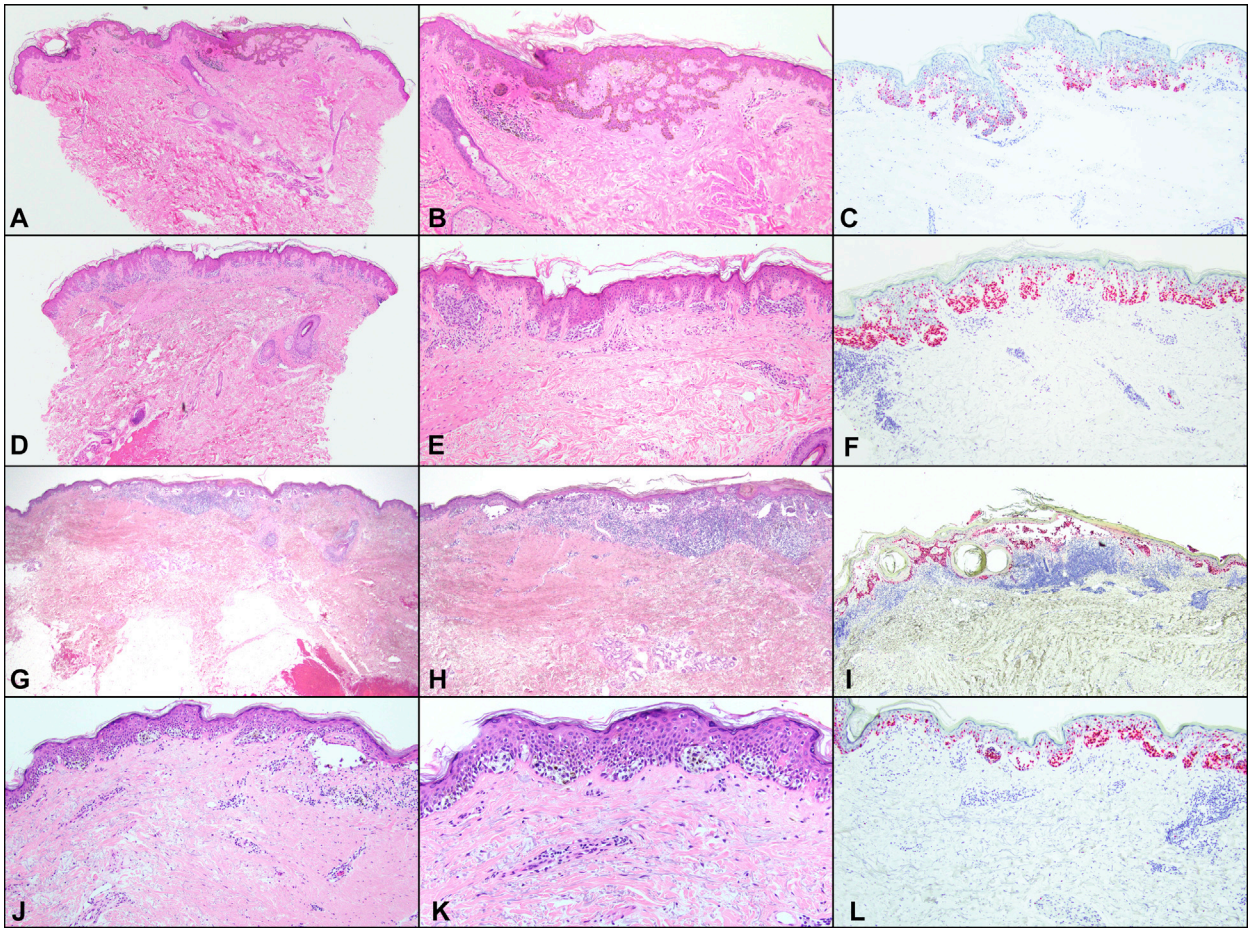

Fig 3. Original biopsy (**A**-**C**) and subsequent biopsies (**D**-**L**) of 52-year-old woman with incompletely excised locally recurrent melanoma (patient described in Fig 2). **A**, Histologic section from the original biopsy performed in 2005 showing a junctional melanocytic proliferation with melanocytes clustered around rete ridges without confluence or pagetosis, most consistent with a diagnosis of a junctional nevus. **B**, Higher magnification showing the melanocytic proliferation mostly clustered around the rete ridges. **C**, SOX-10 staining highlights the melanocytes along the rete ridges and shows a lack of melanocytic confluence or pagetosis. **D**-**F**, A second biopsy was performed in October 2007 due to recurrence at the scar. The 4x and 20x views (**D** and **E**) show a broad intra-epidermal proliferation of single and nested melanocytes along the dermal-epidermal junction, with prominent lentiginous and pagetoid changes. **F**, SOX-10 staining shows extensive pagetosis. **G**-**L**, A third biopsy in February 2014 after another recurrence at the site of the scar. The diagnosis rendered was superficial spreading melanoma, Breslow 0.24 mm, without ulceration and with positive margins. The low-power and high-power views (**G** and **H**) show an irregular confluent lentiginous proliferation of atypical melanocytes in the epidermis with focal involvement of the papillary dermis. **I**, The SOX-10 staining shows prominent pagetoid spread of melanocytes. Views from the lateral edge beyond the scar (**J**-**L**) show that the atypical component extends well beyond the scar. A higher power view (**K**) and SOX-10 (**L**) show prominent pagetosis in the area beyond the scar.

and recurrent melanoma groups. This included the tendency of lentiginous growth to extend beyond the scar, multilayering of melanocytes along the DEJ, extensive pagetosis, accumulation of solar elastosis, severe nuclear atypia, dermal or junctional mitoses, extensive adnexal involvement, and consumption. A limitation of these findings is that only cases in which a conclusive consensus diagnosis could be made were included. Hence, although these histologic parameters will discriminate most recurrent nevi from melanoma, some cases might not be solved by histopathology alone.

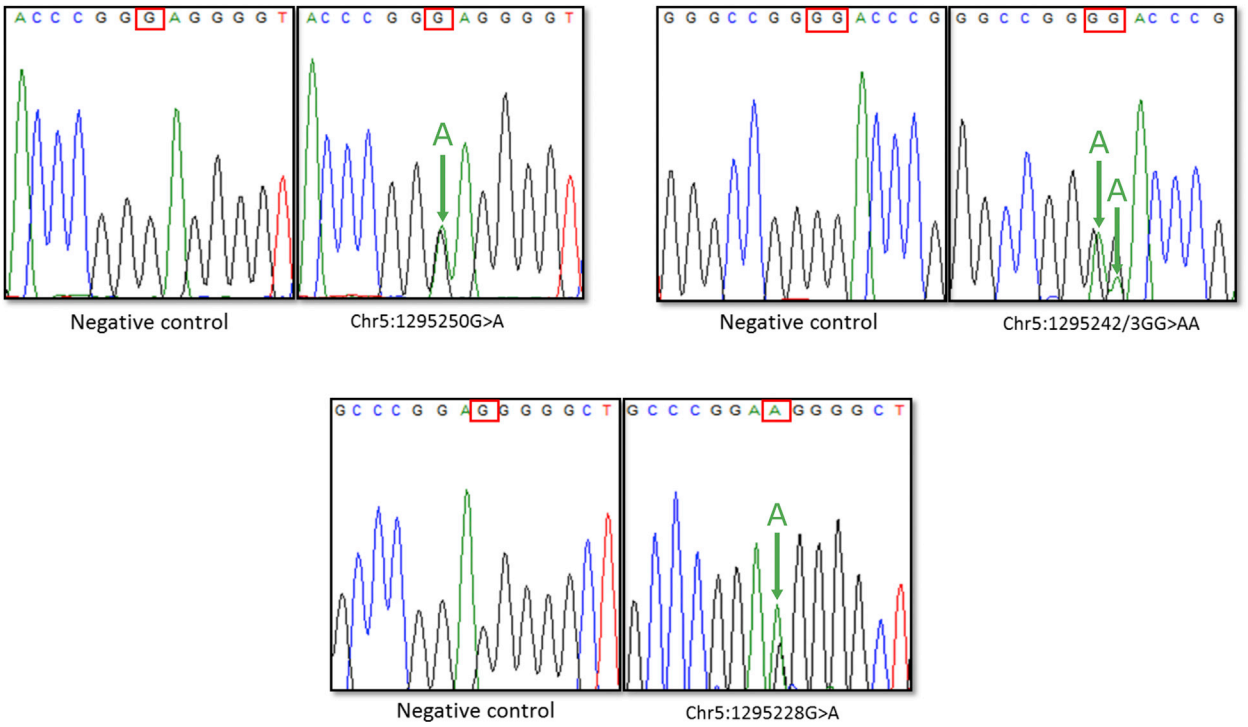

Fig 4. Sanger sequencing chromatogram of the telomerase reverse transcriptase (*TERT*) locus. The hotspot *TERT* promoter mutations Chr5:1295250 G>A, Chr5:1295242/3GG>AA, and Chr5:1295228G>A from 3 melanoma cases in our series are highlighted. *Arrows* show a nucleotide change to A at the indicated position.

Table III. Frequency of hotspot mutations in the *TERT* promoter by group

Category	No. with hotspot mutation	*P* value
Recurrent melanoma	4/9*	.008†
Recurrent nevus	0/17	
Melanoma control	13/20	.0001†
Nevus control	2/21	

TERT, Telomerase reverse transcriptase.
*One case had 2 hotspot mutations at both c.1-146C>T and c.1-139_1-138CC>TT.
†Statistically significant *P* value.

Our findings are consistent with the Blum study that showed a statistically significant association between longer time to recurrence and recurrent melanoma.[3] However, in our study, we did not include cases on the head and neck, which were the most common anatomic locations in the Blum study cohort and a sun-exposed site. Early paucicellular patterns of recurrent melanoma with insufficient tissue for sequencing were excluded. Analogous to their results though, we found solar elastosis on histology to be strongly associated with recurrent melanoma. In our multivariate model, TPMs, solar elastosis, and time to recurrence were independently associated with recurrent melanoma. Importantly, these clinical and molecular variables could all be considered when deciphering histologically ambiguous cases.

In many studies, the limitations of histology to unequivocally distinguish recurrent nevi from melanoma have been described.[8,9] In a study of 357 recurrent nevi, up to 15% of cases were difficult to distinguish from and at risk for misdiagnosis as melanoma.[8] A separate study of 175 recurrent nevi had a similar conclusion that the histologic distinction of a proportion of recurrent nevi from superficial spreading melanoma was challenging.[9] Also, investigators have described cases originally diagnosed as recurrent nevi that later showed clear changes consistent with invasive melanoma on re-excision.[8] Several groups have suggested that reviewing the original biopsy specimen is the most reliable way to confidently and accurately make a diagnosis in these difficult cases.[8,9] Although this is a highly useful and important process in our experience, this strategy might have some limitations. For example, in some cases the original biopsy might be highly incisional and not representative, have borderline features, or be unavailable. Last, we had 2 examples of unequivocal

Table IV. Univariable logistic regression

Variable	OR	95% CI	*P* value
Age	1.09	1.03-1.19	.0021*
Site	10.3	1.95-74.1	.0052*
Months to recurrence	1.04	1.01-1.10	.0036*
Sex	0.92	0.15-6.43	.9259
Solar elastosis	29.6	2.74-4.1 $\times$ 10^{3}	.0028*
Sharp demarcation	0.0014	4.28 $\times$ 10^{-6}-0.03	4.6473 $\times$ 10^{-8}*
Dermal component	0.84	0.19-3.76	.8216
Pagetosis	245	18.1-3.85 $\times$ 10^{4}	3.7527 $\times$ 10^{-7}*
Epidermal effacement	0.11	7.56 $\times$ 10^{-4}-1.51	.1033
Nuclear atypia	40.7	4.09-5.54 $\times$ 10^{3}	.0004*
Confluent junctional growth	5.00	0.86-53.7	.0755
Adnexal involvement	13.0	2.08-147	.0049*
Atypia confined to epidermis	0.20	0.04-0.99	.0481*
Multilayering of melanocytes in epidermis	9.31	1.47-105	.0168*
Mitoses	13.0	2.08-147	.0049*
Epidermal consumption	6.90	1.42-41.0	.0159*
Dyscohesion of the DEJ	3.79	0.85-19.2	.0823
Hotspot TPM	35.0	2.93-5.0 $\times$ 10^{3}	.0030*

CI, Confidence interval; *DEJ*, dermal-epidermal junction; *OR*, odds ratio; *TPM*, telomerase reverse transcriptase promoter mutation.
*Statistically significant.

Table V. Multivariate logistic regression

Variable	OR	95% CI	*P* value
Hotspot TPM	51.5	2.12-7.8 $\times$ 10^{9}	.0119*
Site	8.60	0.37-1.07 $\times$ 10^{9}	.1892
Months to recurrence	1.02	1.00-1.11	.0221*
Solar elastosis	30.7	1.24-8.7 $\times$ 10^{8}	.0351*

CI, Confidence interval; *OR*, odds ratio; *TPM*, telomerase reverse transcriptase promoter mutation.
*Independently associated parameters.

melanomas in which the original biopsy was histopathologically most consistent with a dysplastic nevus.

TPMs have been extensively studied in cutaneous melanoma.[10-13,18,19] Four specific hotspot mutations have been well-characterized in the literature as pathogenic mutations, which create de novo ETS transcription factor binding sites that can result in a 2-4 fold increase in *TERT* transcription.[10,11,20,21] In 1 study, it was found that when c.1-124C>T and c.1-146C>T mutations were experimentally introduced, the cells continued to express *TERT*, even after differentiation.[22,23] Shain et al suggested that TPMs are an early event in the progression of melanoma from precursor lesions after reporting TPMs in a large percentage of intermediate lesions and early stage melanomas.[12]

In this study, we sought to determine whether the presence of these known pathogenic TPMs is involved in the pathogenesis of recurrent nevi, and whether any significant differences existed between recurrent nevi and recurrent melanomas in terms of TPM status. Interestingly, none of our 17 recurrent nevi had a hotspot TPM. Conversely, 4 of the 9 recurrent melanomas did (P = .008). In 3 of these 4 cases Sanger sequencing showed the same TPM was present in the original specimen. Hence, our findings suggest that in cases where there is histologic ambiguity in the distinction of recurrent nevus from locally recurrent melanoma, the presence of a TPM would strongly favor a diagnosis of melanoma. Furthermore, our data suggest that TPMs do not play a role in the clinical behavior of recurrent nevi, although we cannot exclude the possibility that alterations in telomerase expression as a result of methylation changes or other epigenetic alterations play a role.

The absence of a TPM does not exclude melanoma since in our series, only 44% of recurrent melanomas and 65% of melanoma control cases had hotspot TPMs. Although none of our recurrent nevi had a TPM, 2 of 21 (10%) of our nevi controls had hotspot TPMs. These 2 cases were diagnosed as moderately dysplastic nevi by a unanimous consensus of 3 experienced board-certified dermatopathologists. Hence, as is the case with most parameters used to distinguish melanoma from nevi, no single factor is absolute. However, we conclude that in addition to the clinical and histologic features that can be used to favor recurrent nevus or locally recurrent melanoma, the presence

of a TPM strongly favors a diagnosis of melanoma. In addition, TPMs are not responsible for the recurrent growth seen with recurrent nevi.

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