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Improving classification of melanocytic nevi: BRAF V600E expression associated with distinct histomorphologic features

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Abstract

Background: A subset of melanomas carrying a BRAF V600E mutation, the most common targetable mutation in melanoma, arises in association with a melanocytic nevus also harboring a BRAF V600E mutation. The detailed histomorphologic characteristics of BRAF V600E-positive nevi are not systematically documented.

Objective: To identify histomorphologic features correlating with BRAF V600E status in nevi.

Methods: We retrospectively identified melanocytic nevi from our laboratory reporting system. We performed a histomorphologic analysis and BRAF V600E expression analysis by immunohistochemistry.

Results: Thirteen (14.8%) nevi were wild type (WT) and 76 (86.4%) positive for BRAF V600E. BRAF V600E nevi were predominantly dermal (BRAF V600E 55.3% vs. BRAF WT 15.4%, p=0.01) and showed congenital growth pattern (BRAF V600E 51.3% vs. BRAF WT 15.4%, p=0.02). BRAF V600E nevi often exhibited predominantly nested intraepidermal melanocytes, larger junctional nests, abrupt lateral circumscription, and larger cell size. Architectural disorder

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and inflammatory infiltrates were more often seen in BRAF WT nevi. *BRAF* sequencing of a subset of nevi confirmed immunohistochemical results.

Limitations: Limitations include retrospective design and a small sample size of BRAF WT nevi.

Conclusions: BRAF V600E is associated with distinct histomorphologic features in nevi. This may contribute to improving the accuracy of classification and diagnosis of melanocytic neoplasms.

Keywords

melanocytic nevus; melanoma; dermatopathology; histomorphology; gene; BRAF; mutation; immunohistochemistry

Introduction

Melanocytic nevi are exceedingly common and commonly biopsied benign melanocytic neoplasms. They are mimickers, risk factors, and precursors of melanoma, the deadliest of the common forms of skin cancer. The most common genetic driver and therapeutically targetable mutation in melanoma is the V600E mutation of the v-raf murine sarcoma viral oncogene homolog B gene (BRAF), a gene encoding a serine/threonine kinase in the RAS-mitogen activated protein kinase (MAPK) pathway [1–5]. Notably, *BRAF* mutations are found in >80% of melanocytic nevi [2, 6, 7], including a subset of melanocytic nevi that act as precursors for melanoma [8, 9]. It is therefore paramount to better define the clinical, histomorphologic, and genetic features of melanocytic nevi that may predict progression to, or association with, melanoma.

In melanoma, the presence of the BRAF V600E mutation correlates with certain clinicopathologic findings, including age <55 years and intermittent sun-exposure, as well as histopathologic features of intraepidermal upward scatter of melanocytes, nest formation of intraepidermal melanocytes, thickening of the epidermis, and larger tumor cells [10]. Similarly, melanocytic nevi with a BRAF V600E mutation are of earlier onset [11]. Additionally, these nevi are associated with a globular dermoscopic pattern and a predominantly dermal histological growth pattern[1, 6, 12–14]. While these results are based on relatively small sample sizes [6], they suggest that the BRAF V600E mutation may impact the characteristics and behavior of the nevus.

The diagnosis of melanoma is based on histologic examination. In a subset of cases, the histological distinction between a nevus and a melanoma is challenging. Therefore, there is a growing interest in the development of novel molecular diagnostic tests and classification systems based on the combination of histomorphologic and molecular features. To expand our knowledge of the morphological-genetic correlation in melanocytic nevi, we retrospectively identified 150 melanocytic nevi and performed a detailed histomorphologic analysis as well as BRAF V600E immunohistochemistry to identify morphological features correlating with BRAF V600E status.

Materials and methods

Cases

This study was approved by the institutional review board of University of California Davis (no. 756049). Pathology archives were searched from January 2014 to March 2014 for histologically proven melanocytic nevi and 150 were included in this study. To ensure that the cohort consisted of both common nevi, dysplastic nevi, and nevi with congenital pattern, we included 30 consecutive cases using the search terms “junctional melanocytic nevus”, 30 consecutive cases using the search terms “compound melanocytic nevus”, “predominantly intradermal melanocytic nevus”, and “intradermal melanocytic nevus”, 30 consecutive cases using the search term “nevus, congenital pattern”, 30 consecutive cases using the search term “junctional melanocytic nevus, dysplastic type”, and 30 consecutive cases using the search term “compound melanocytic nevus, dysplastic type”. Exclusion criteria included the diagnosis of a blue nevus, a Spitz nevus, or a nevus with unusual or atypical growth.

Histomorphologic analysis

Hematoxylin and eosin (H&E) stained slides and tissue blocks were available for 135 cases. H&E slides were reviewed by one to two board-certified dermatopathologists (MK, MF) prior to the availability of BRAF V600E staining results. The following parameters were recorded: dermal growth pattern, congenital pattern, nesting of intraepidermal melanocytes, size of junctional nests, epidermal contour, lateral circumscription, pigmentation, cell size, cytologic atypia, pagetoid melanocytes, architectural disorder, inflammatory infiltrate and solar elastosis. The detailed criteria for each category are described in Table I.

BRAF V600E immunohistochemistry

BRAF V600E expression was analyzed by immunohistochemistry using previously described conditions with minor modifications [15, 16]. Monoclonal antibody clone VE1 to BRAF V600E (Spring Bioscience) was used at a dilution of 1:200. For chromogenic detection, EnVision FLEX+DAB detection kit (Dako) was used. Presence, absence or indeterminate staining of BRAF V600E was identified with 100% consensus agreement by three board-certified dermatopathologists (MK, MF, TK).

Whole exome sequencing

Tumor tissue was manually microdissected from formalin-fixed paraffin-embedded tissue sections. DNA was isolated using standard protocols. Whole exome sequencing analysis, including the *BRAF* gene, was performed by next-generation sequencing (Novogene, Corp.) to an average read depth of >100-fold using the HiSeq 4000 sequencing system (Illumina). Raw sequence reads were aligned to the reference human genome (GRCh37) and variants identified using a DRAGEN hardware/software server platform utilizing a field-programmable gate array (FPGA) pipeline (for details of the DRAGEN server and methods see [17]).

Statistical analysis

Descriptive statistics were obtained stratified by BRAF V600E status, with mean and standard deviation (SD) for continuous variables, and count and percent for categorical variables. Two sample t tests were used to compare means between BRAF V600E groups. Chi-square tests were used to examine associations between categorical variables and Fisher's exact tests if any cell size was below 5. For those with all cell sizes >= 5, logistic regression models were used to study the association between a binary histomorphologic feature and BRAF V600E status while multinomial logistic regression models were used for associations with a categorical histomorphologic feature of more than 2 levels.

Results

Patient characteristics and the results of histomorphologic and immunohistochemical staining are shown in Table II. BRAF V600E immunohistochemistry was performed on 137 specimens. The staining was interpretable in 89 cases, while 48 cases were indeterminate. The reasons for classification as indeterminate included prominent melanin in melanocytes or keratinocytes, a small number of melanocytes, or weak staining. Most of the cases were considered indeterminate due to prominent melanin in melanocytes or keratinocytes (30/48 or 62.5%) or prominent melanin in melanocytes or keratinocytes and a small number of melanocytes (15/48 or 31.3%). Three cases were considered "indeterminate" due to weak staining (3/48 or 6.3%). Of the 89 interpretable cases, 13 (14.8%) were wild type (WT) and 76 (86.4%) positive for BRAF V600E by immunohistochemistry. This frequency of BRAF V600E is similar to prior reports [2, 6, 7].

The mean age was 53.5 years (SD = 11.3) for BRAF WT and 48.0 (SD = 16.7) for BRAF V600E (p=0.26). The ratio of males to females was 1:1.6 for BRAF WT and 1:2.3 for BRAF V600E (p=0.56). The anatomic location of head/neck, trunk, and extremity including hand or foot was similar in both groups, 7.7%, 53.9%, and 38.5%, respectively, for BRAF WT and 13.3%, 52.0%, and 34.7%, respectively, for BRAF V600E.

Significant associations were found between the histomorphologic features of nevi and BRAF V600E expression. A predominantly junctional growth pattern was associated with BRAF WT nevi (11 of 13, or 84.6%, Figure 1A and 1B), while BRAF V600E nevi showed predominantly dermal growth pattern (42 of 76 or 55.3%, p=0.01, Figure 1C and 1D). Furthermore, most BRAF WT nevi did not display congenital features (2 of 13 or 15.4%), defined as adnexal and/or periadnexal growth, perivascular growth, and/or splaying of melanocytes among collagen fibers, in contrast to BRAF V600E nevi (39 of 76, or 51.3%, p= 0.02, Figure 1E and 1F). Additionally, differences in many other histomorphologic variables were noted, though not reaching statistical significance in this data set. These included nesting of intraepidermal melanocytes (predominantly nested intraepidermal melanocytes in 16.7% of BRAF WT versus 39.3% of BRAF V600E) and size of junctional nests (medium to large nests in 16.7% of BRAF WT versus 43.6% of BRAF V600E), and lateral circumscription (abrupt borders in 15.4% of BRAF WT versus 40% of BRAF V600E). Additionally, architectural disorder (present in 61.5% of BRAF WT versus 44.7% of BRAF V600E) and inflammatory infiltrates (present in 76.9% of BRAF WT versus 50% of BRAF V600E) were more frequently observed in BRAF WT nevi. Finally, although no

differences were observed in the presence of cytologic atypia, cell size appeared larger in BRAF V600E nevi (large cell size in 23.1% of BRAF WT versus 47.4% of BRAF V600E).

BRAF gene sequencing was performed in 4 cases in a blinded fashion, *i.e.* without knowledge of the results of the immunohistochemistry prior to analyzing sequencing data. Two nevi with negative BRAF V600E staining by immunohistochemistry were negative for *BRAF* mutations, while two nevi with positive BRAF V600E staining showed *BRAF* chr7:140453136A>T (BRAF V600E) mutations. No other recurrent somatic mutations were identified.

Discussion

Our study shows that histomorphologic features of a melanocytic nevus can yield meaningful information on the mutation status of *BRAF,* the most common genetic driver of melanocytic tumors. We demonstrate that melanocytic nevi with BRAF V600E expression are more likely to show a predominantly dermal growth pattern and congenital features. Additionally, BRAF V600E nevi often exhibit predominantly nested intraepidermal melanocytes, medium to large junctional nests, abrupt lateral circumscription, and larger cell size. By contrast, architectural disorder and the presence of an inflammatory infiltrate are more often seen in a BRAF WT nevus. These results validate prior studies showing a correlation between dermal growth and BRAF V600E status[1, 6, 12–14]. Furthermore, the findings of this study expands the knowledge of genotype-phenotype correlation in melanocytic tumors potentially facilitating a more accurate and comprehensive classification of melanocytic tumors [10].

Our findings are consistent with prior results on the frequency of BRAF V600E in melanocytic nevi [2, 6, 7], the predominance of compound or dermal growth pattern in these tumors [1, 6, 7, 12–14, 18], as well as the association with large junctional nests [6]. In our data set, BRAF V600E was expressed in 86% of melanocytic nevi. Compound or predominantly dermal growth pattern was seen in 55.3% of BRAF V600E nevi, while only 15.4% of BRAF WT nevi were compound or predominantly dermal. In prior studies, 63–84.6% of BRAF V600E-positive nevi were intradermal, 51–82% compound, and 2–35% junctional [1, 7, 12]. Based on prior studies, BRAF V600E is frequent not only in common acquired and dysplastic nevi, but also in congenital nevi [7, 13]. We further demonstrated that congenital features can in fact serve as another distinguishing finding between BRAF V600E and BRAF WT nevi, in our series present in 51.3% of BRAF V600E and 15.4% of BRAF WT nevi. Of note, similar to prior studies, the cases with BRAF V600E expression and congenital features in our study were typically small nevi and not clinically confirmed large congenital nevi, which typically show an *NRAS* mutation instead of BRAF V600E (reviewed in [19]).

As pointed out by Viros *et al.* [10], who studied the associations between morphological and genetic features in melanoma, genotype-phenotype correlation may allow generation of hypotheses on the function or role of the gene in the biology of the tumor type. Notably, the benign melanocytic tumors with BRAF V600E examined in this study share many features with their malignant counterparts studied by Viros *et al.* [10], namely nest formation of

intraepidermal melanocytes, sharper demarcation to the surrounding skin, and larger tumor cells. This suggests an overlap of phenotypic effects shared by benign and malignant melanocytic tumors associated with a mutant BRAF.

BRAF V600E nevi can serve as precursors for melanoma[20]. Borderline lesions and possibly also dysplastic nevi, appear to contain other drivers such as the NRAS proto-oncogene, GTPase, gene (*NRAS*) [20]. In our series, architectural disorder and inflammation, some of the hallmarks of a dysplastic nevus, were less common in BRAF V600E nevi than BRAF WT nevi. Further work is needed to establish whether a characteristic genotype exists for dysplastic nevi and to identify genetic drivers of *BRAF* and NRAS-negative nevi.

The gold standard for the diagnosis of melanoma is histological examination. In a subset of melanocytic tumors, typically in up to 10% but based on some reports in as many as 25% of cases[21], the histological distinction between melanoma and nevus is problematic, if not impossible [21–23]. As early diagnosis significantly increases survival rates of melanoma, improving diagnostic accuracy is imperative and may require ancillary molecular testing. Understanding the genetic pathway of early melanomagenesis, such as but likely not limited to telomerase reverse transcriptase (*TERT*) promoter mutations and p16 loss [20], is required to identify clinically useful markers and predictors of malignant transformation.

Conclusions

Our study demonstrates that histomorphologic features of a melanocytic nevus can provide relevant information on its molecular background, including the presence of BRAF V600E, the most common driver of melanocytic tumors and the most common therapeutically targetable alteration in melanoma. A greater understanding on the genotype-phenotype correlation in melanocytic nevi may facilitate the development of a more accurate classification system for melanocytic tumors that ultimately leads to improved diagnostic accuracy and management of these exceedingly common human tumors as well as a better understanding of markers and predictors of malignant transformation.

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Clinicaltrials.gov (or equivalent) listing (if applicable): Not applicable.

Abbreviations used:

BRAF	v-raf murine sarcoma viral oncogene homolog B gene
WT	wild type
MAPK	mitogen activated protein kinase
H&E	hematoxylin and eosin
TERT	telomerase reverse transcriptase
NRAS	NRAS proto-oncogene GTPase gene

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Capsule summary

- *BRAF* V600E mutation is a common genetic driver of melanocytic nevi and melanoma.
- BRAF V600E is associated with distinct histomorphologic features in melanocytic nevi, including dermal and congenital growth patterns.
- Understanding the genetic-morphologic correlates in melanocytic nevi may facilitate a more accurate classification and improved diagnosis of melanocytic neoplasms.

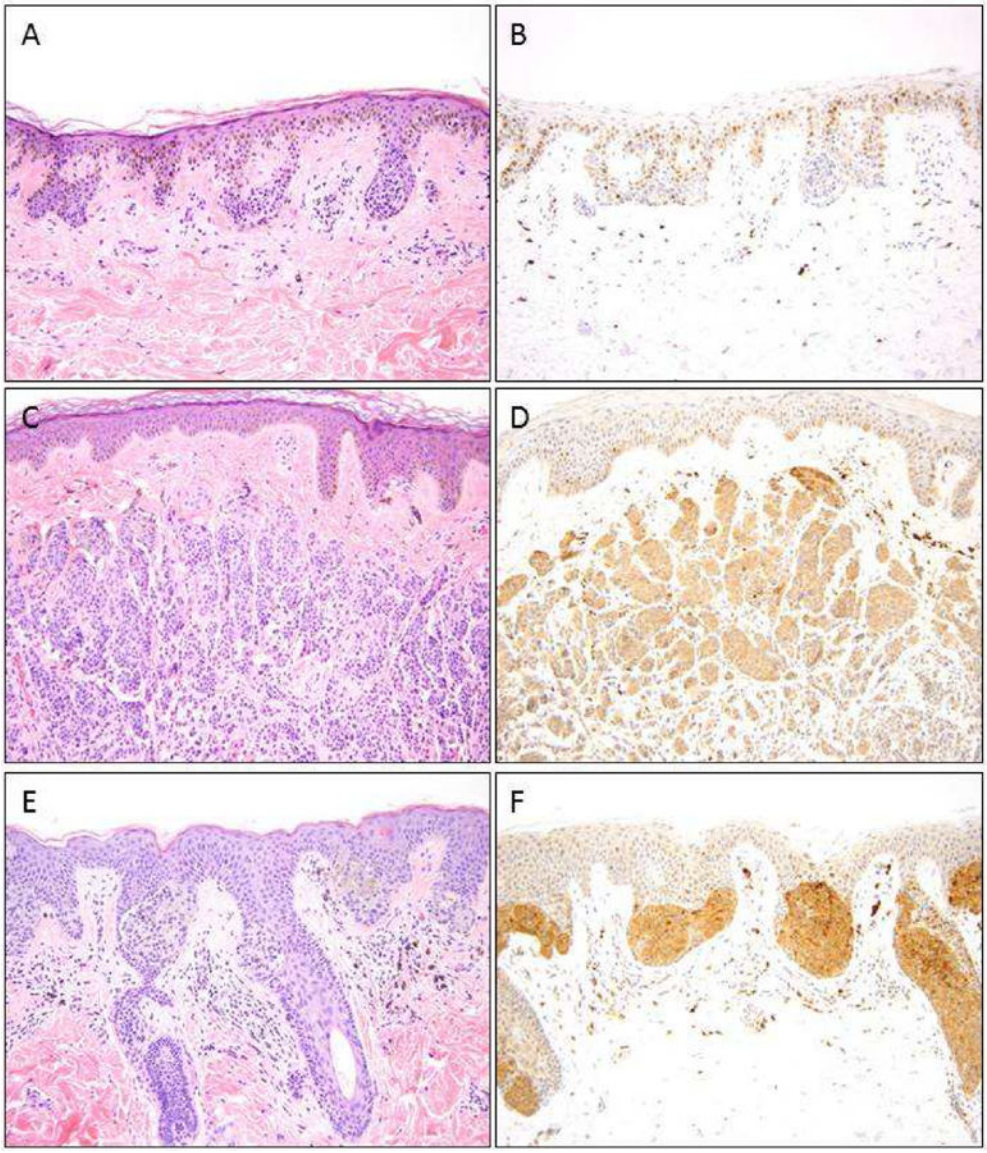

Figure 1. Histomorphologic features and BRAF V600E immunohistochemistry in melanocytic nevi.

Junctional growth pattern (A) in a nevus with negative BRAF V600E immunohistochemistry (B). Dermal growth pattern (C) in a nevus with positive BRAF V600E immunohistochemistry (D). Congenital growth pattern involving adnexal epithelium (E) in a nevus with positive BRAF V600E immunohistochemistry (F). A-F, magnification 200X. A,C,E, H&E stain. B,D,F, brown indicates positive staining, except for endogenous melanin in keratinocytes.

Table I.

Histological variables and criteria for interpretation.

Variable	Interpretation/definition	Additional remarks	Reference
Nesting of intraepidermal melanocytes	• Indeterminate: junctional component absent • Almost exclusively as single cells: <5% as nests • Predominantly as single cells: <25% as nests • Single cells and nests equal: 25%−50% as nests • Predominantly as nests: >50%as nests	A nest is defined as a cluster of 5 or more melanocytes at or above the junction	[10]
Epidermal contour	• Thinned: effacement or attenuation of rete ridges • Normal: epidermal silhouette similar to the adjacent uninvolved epidermis • Thickened: maximum 2-fold increase in epidermal thickness • Hyperplastic: greater than 2fold increase in epidermal thickness		[10]
Lateral circumscription	• Indeterminate: nevus extends to lateral margins or no junctional component • Discontinuous: areas of apparently uninvolved epidermis interspersed with tumor • Gradual: continuous decrease of the number of intraepidermal melanocytes making it difficult to pinpoint the transition to normal skin • Abrupt: transition from involved epidermis to the adjacent normal skin easily determined within one or two rete ridges		Modified from [10]
Pigmentation	• Absent: no pigment discernible even at high power • Faint: a faint diffuse melanin pigment or a few pigment granules at high power • Moderate: pigmentation visible at low power with translucent cytoplasm that is significantly lighter than the hematoxylin stained nuclei • High: pigmentation easily visible at low power with the cytoplasmic pigmentation reaching an intensity approximating that of the nucleus	Pigmentation assessed using the maximum pigmentation scored anywhere in the tumor	Modified from [10]
Cell size	• Small: the largest diameter <8 microns • Medium: the largest diameter 8–12 microns • Large: the largest diameter >12 micrometers	8 microns estimated as the size of two normal lymphocytes	Modified from [10]
Size of junctional nests	• Indeterminate: junctional/intraepidermal component absent	The depth of the epidermis measured from the granular	

Variable	Interpretation/definition	Additional remarks	Reference
	• Small: <33% of the depth of the epidermis • Medium: 33–66% of the depth of the epidermis • Large: >66% of the depth of the epidermis	layer to the tip of the rete ridge	
Growth pattern	• Junctional: 100% of the melanocytes junctional • Predominantly junctional: <75% of the melanocytes junctional • Compound: approximately 50% of melanocytes junctional and 50% dermal • Predominantly dermal: >75% of the melanocytes are dermal		
Congenital pattern	• Absent • Present	One or several of the following features: periadnexal growth, perivascular growth, growth along adnexal epithlium, splaying of melanocytes among collagen fibers	[24]
Architectural disorder	• Absent • Present: two major criteria and at least two minor criteria present:	Major criteria include 1) junctional proliferation of atypical melanocytes extending three rete ridges beyond the dermal component if present, 2) junctional melanocytic proliferation present Minor criteria include 1) bridging of rete ridges and/or nests along the sides of rete and above dermal papillae, 2) lamellar fibroplasia, 3) inflammatory infiltrate with melanophages	Modified from [25,26]
Cytologic atypia	• Absent • Mild: nuclear size approximately the size of keratinocyte nucleus and/or nucleolus absent/small and/or mild pleomorphism: • Moderate: nuclear size approximately 1–2 x keratinocyte nucleus and/or nucleolus absent/small and/or moderate pleomorphism • Severe: nuclear size approximately >2 x keratinocyte nucleus and/or prominent/ enlarged nucleolus and/or severe pleomorphism		Modified from [27]
Pagetoid melanocytes	• Absent • Present	Singly disposed melanocytes in the upper spinous layer or granular layer	Modified from [10]
Inflammatory infiltrate	• Absent • Present		
Solar elastosis	• Indeterminate: tissue sections too superficial to determine • Absent • Present: individual elastotic fibers separated by collagen visible on low		[28]

Variable	Interpretation/definition	Additional remarks	Reference
	power or solid/nodular aggregates of elastosis		
BRAF V600E	• Indeterminate: unable to determine due to technical artifact, weak staining, prominent melanin, or small number of melanocytes		
	• Absent		
	• Present		

Table II.

The results of histomorphologic analysis and BRAF V600E immunohistochemistry.

		BRAF WT	%	BRAF V600E	%	P-value
Number of nevi		13	14.8	76	86.4	
Age (years)	**Mean (SD)**	53.5 (11.3)		48.0 (16.7)		0.26
Gender						0.56
	Female	8	61.5	53	69.7	
	Male	5	38.5	23	30.3	
Anatomic location						1.00
	Head/neck	1	7.7	10	13.3	
	Trunk	7	53.9	39	52.0	
	Extremity including hand or foot	5	38.5	26	34.7	
Nesting of intraepidermal melanocytes[1]						0.19
	Almost exclusively or predominantly as single cells	10	83.3	34	60.7	
	Single cells and nests equal or predominantly nested	2	16.7	22	39.3	
Epidermal contour						0.36
	Normal	3	23.1	29	38.2	
	Thickened or hyperplastic	10	76.9	47	61.8	
Lateral circumscription[2]						0.12
	Gradual	11	84.6	42	60.0	
	Abrupt	2	15.4	28	40.0	
Pigmentation						0.69
	Absent or faint	8	61.5	35	46.1	
	Moderate	4	30.8	33	43.4	
	High	1	7.7	8	10.5	
Cell size						0.14
	Small	10	76.9	40	52.6	
	Medium or large	3	23.1	36	47.4	
Size of junctional nests[3]						0.14
	Small	10	83.3	31	56.4	
	Medium or large	2	16.7	24	43.6	
Growth pattern						0.01
	Junctional of predominantly junctional	11	84.6	34	43.7	
	Compound or predominantly dermal	2	15.4	42	55.3	
Congenital pattern						0.02
	Absent	11	84.6	37	48.7	
	Present	2	15.4	39	51.3	
Architectural disorder						0.26
	Absent	5	38.5	42	55.3	
	Present	8	61.5	34	44.7	

		BRAF WT	%	BRAF V600E	%	P-value
Cytologic atypia						0.54
	Absent	7	53.9	34	44.7	
	Mild, moderate or severe	6	46.2	42	55.3	
Pagetoid melanocytes						1.00
	Absent	12	92.3	66	86.8	
	Present	1	7.7	10	13.2	
Inflammatory infiltrate						0.13
	Absent	3	23.1	38	50.0	
	Present	10	76.9	38	50.0	
Solar elastosis[4]						0.69
	Absent	10	76.9	63	84.0	
	Present	3	23.1	12	16.0	

For statistical analyses, the following adjustments 353 were made:

1= Excluded “Indeterminate”

2= Excluded “Indeterminate”

3= Excluded “Indeterminate”

4= Excluded “Indeterminate”