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PATHOBIOLOGY IN FOCUS

Unexpected UVR and non-UVR mutation burden in some acral and cutaneous melanomas

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Ultraviolet radiation (UVR) mutagenesis causes nearly all cutaneous melanomas, however, since UVR signatures are largely absent in acral melanoma, as well as melanoma in sun-protected sites, the cause of these melanomas is unknown. Whole-genome sequencing data generated as part of the Australian Melanoma Genome Project was supplemented with a detailed histopathological assessment with the melanomas then classified as UVR or non-UVR related, based on their mutation signatures. The clinicopathological characteristics of melanomas with mutation signatures for their subtype were compared. Three (of 35 = 8.6%) acral melanomas, all clinically and pathologically verified as arising from acral or subungual locations, had predominant UVR mutation burden, whereas four (of 140 = 2.9%) cutaneous melanomas showed predominant non-UVR mutations. Among the acral melanomas, the few that were UVR dominant occurred in younger patients, had a higher mutation load and a proportion of mutation burden due to UVR, which was similar to that in melanomas from intermittently UVR-exposed skin. Acral melanomas with a UVR signature occurred most frequently in subungual sites and included tumors harboring BRAF or NF1 mutations. Cutaneous melanomas dominated by non-UVR signatures had lower mutation burdens counts and their primary tumors were thicker and had more mitoses than in other cutaneous melanomas. No histopathological features predicted UVR dominance in acral melanomas or non-UVR dominance in cutaneous melanomas. Our finding of acral/subungual melanomas with predominant UVR mutagenesis suggests that the nail plate and acral skin do not provide complete protection from UVR. Our data also confirm that cutaneous melanomas not caused by UVR are infrequent. Identifying where mutation burden is discordant with primary tumor anatomical site is likely to be clinically significant when determining treatment options for metastatic acral and cutaneous melanoma patients.

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Malignant tumors arise as a result of the accumulation of multiple genetic abnormalities and melanoma has the highest mutation load of any malignancy.[1] It has been established that melanomas arising in different anatomical locations with varying degrees of exposure to ultraviolet radiation (UVR) are caused by different genetic alterations.[2]

For many decades it has been recognized that cutaneous melanoma incidence is associated with fair skin[3] and UVR exposure,[4,5] however, this relationship is not simple.[6] Furthermore, melanomas arising in areas of intermittent UVR exposure, such as the trunk, back and abdomen, and those arising in sites of chronic UVR exposure, such as head and neck, have different associations with risk factors, histological appearances, and mutation profiles.[7,8] Melanomas from intermittently UVR-exposed sites tend to occur in younger patients, more commonly arise in pre-existing benign nevi, more often have superficial spreading histology and more often BRAF mutated, compared with melanomas arising in chronically exposed skin.[7] In contrast, the latter tend to occur in older people, are more commonly of lentigo maligna histological subtype and are less likely to carry BRAF mutation.[2]

Cutaneous melanomas are defined as those arising non-glabrous skin, thereby excluding the plantar or palmar and the nail apparatus. They are predominantly caused by exposure to UVR, which acts as a mutagen by forming

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covalent links between two adjacent pyrimidines.[9] The subsequent conformation change in the DNA is detected and normally repaired by DNA damage response mechanisms. However, inefficiencies in DNA damage repair can lead to mutations, most frequently C>T or CC>TT transitions, which are the dominant point mutations of UVR.[1,10,11]

The incidence of acral melanoma, defined as those arising on glabrous skin of the palms and soles and in the nail apparatus, is similar across different populations.[12] Furthermore, acral melanomas are thought to be protected from UVR by a thick strata corneum[13] or nail plate.[14] Therefore, it has been hypothesized that acral and sun-shielded mucosal melanomas have different genetic causal pathways unrelated to UVR exposure. This is typically reflected in low mutation burden, which is offset by numerous focused gene amplifications and deletions or structural abnormalities.[2,13,15] They also have characteristic histological features, including a predominantly lentiginous growth pattern.[8,16]

We recently performed the first high coverage whole-genome sequencing study of a large cohort of cutaneous, acral, and mucosal melanomas, which enable a high-resolution assessment of mutation signatures in melanomas arising in these different anatomical sites (currently submitted for review). Here we focus comprehensive clinical, pathological, and molecular analysis on the features of melanomas with mutation signatures that are discordant with their anatomical site, namely cutaneous melanomas dominated by non-UVR mutation signatures and acral melanomas dominated by UVR mutation signatures. Since a tumor's molecular profile, rather than the primary tumor anatomical site, will determine the most appropriate treatment options for individual melanoma patients, these features are likely to be clinically significant.

MATERIALS AND METHODS

Patients and Specimens

The study was undertaken with Human Ethics Review Committee approval and patient informed consent. One hundred and eighty-three melanomas were whole-genome sequenced and data processed as part of the Australian Melanoma Genome Project[17] (currently submitted for review). These cases included melanomas arising from cutaneous, acral, and mucosal primary anatomical sites and also melanomas with unknown primary sites. Fresh surgical specimens were macro-dissected and tumor tissues were procured (with as little contaminating normal tissue as possible) and snap frozen in liquid nitrogen within 1 h of surgery. All samples were pathologically assessed before inclusion into the study, with samples requiring greater than 80% tumor content and less than 30% necrosis to be included.[17] All samples were independently reviewed by a pathologist to confirm the presence of melanoma and fulfillment of the above criteria. Samples requiring tumor enrichment underwent macrodissection or frozen tissue coring (Cryoxtract, Woburn, MA, USA) using a marked H&E slide as a reference.

The histopathology of all mucosal and acral samples was reviewed by RVR and RAS to confirm the diagnosis and anatomical site of the primary tumor. Acral melanomas were classified as occurring within acral skin of the palm of the hand, sole of the foot, and in subungual locations under nail beds. Clinical site, lack of hair follicles, and thickened stratum corneum/nail apparatus was confirmed for all acral cases with reference to clinical photos and notes whenever possible. Primary mucosal melanomas were defined as occurring in the mucosal membranes lining oral, respiratory, gastrointestinal, and urogenital tracts. The H&E slides of the primary melanomas were reviewed for all mucosal and acral tumors and their anatomical location confirmed histologically.

Patient demographics, primary tumor site, primary tumor characteristics (Breslow thickness, Clark level, lymphatic invasion, vascular invasion, neurotropism, mitotic rate, regression status, satellite status, T stage, sentinel node status, ulceration, and time to first recurrence) and follow-up data were retrieved from the MIA Research Database. In patients with multiple primary melanomas, the culprit melanoma was assigned using a previously described algorithm.[18]

Whole-Genome Sequencing

Whole-genome sequencing (WGS) was performed as previously described.[17] All the WGS data mentioned in this manuscript has been made publically available on the ICGC Data Portal.

Mutation Signatures

Mutation signatures were identified from WGS data as described.[1] Tumors were classified as 'UVR'-dominant when UVR signature accounted for more than 60% of the total mutation burden. Tumors were defined as 'non-UVR' dominant when the mutation burden did not fulfill the above definition.

Histopathological Features

For all cases, corresponding formalin-fixed, paraffin-embedded (FFPE) tumor specimens of the primary tumor were sought from the laboratories where the sequenced tissue was sourced and when identified, H&E-stained sections were obtained and reviewed ($n=74$) and a semi-quantative analysis was performed on a range of histopathological features as follows:

Tumor-Infiltrating Lymphocyte (TIL) Grade

TIL-grade was assessed by a seven-tier semi-quantitative grading scheme, which was been adapted by us from a previous published study[19] for pathological evaluation of cases in The Cancer Genome Atlas Melanoma Project (TCGA).[20] The scheme is based on a sum of the assessment of TIL density from 0 to 3 (negative, mild, moderate, or marked) and TIL distribution from 0 to 3 (negative, focal,

multifocal, or diffuse) across the entire extent of the invasive tumor (no TILs to marked-diffuse TILs, 0–6, respectively).

Pigmentation

Pigmentation was assessed by a seven-tier semi-quantitative grading scheme based on the sum of an assessment of pigment distribution from 0 to 3 (0, 0–25, 25–50, or >50% of cells pigmented) and average pigment density assessed from 0 to 3 (absent, mild, moderate, or severe) across the extent of the invasive tumor (no pigmentation to diffuse severely pigmented, 0–6, respectively), as previously described.[21] Melanophages were not considered in interpretation.

Regression

Regression was assessed as to the percentage of tumor affected from 0 to 3 (0, 0–25, 25–50, or >50%).

Size and Shape of Melanoma Cells

The size and shape of the melanoma cells were assessed utilizing a ×20 objective and based on a semi-quantitative grading scheme adapted from a previously published study.[21,22] The size of the lesional cells was compared with that of a lymphocyte and graded from 0 to 2 (<2×, 2×, and >2× the diameter of a lymphocyte, respectively). The shape of the cells was determined by the length of the long and short axis of the cells and graded from 0 to 2 (epithelioid with long and short axis approximately equal, mixed epithelioid and spindled, spindled with the long axis at least double the length of the short axis).

Tumor Border

The tumor border was assessed at ×20 power as to the predominant pattern at the advancing edge of the melanoma (pushing, infiltrative).

Solar Elastosis

The severity of solar elastosis was assessed in a semi-quantitative grading system as judged at a ×20 objective from 0 to 3 (absent, mild, moderate, or severe/homogeneous).

Upward Scatter

The amount of upward scatter of melanocytes within the epidermis was assessed in a semi-quantitative grading system based on a grading system previously reported,[22] which in short assess the amount of epidermal melanocytes located within the basal layer of the epidermis and graded 0 to 3 (100, 75–100, 50–75, or <50%)

Lateral Circumscription

The amount of lateral circumscription within the epidermal component was assessed in a semi-quantitative grading system previously reported,[22] which in short assess the abruptness of the transition from the intraepidermal component of the lesion to adjacent uninvolved epidermis. This is graded from 0 to 2 (areas of apparently uninvolved tumor interspersed with normal, gradual decrease in melanocytes with a transition that is difficult to pin point, abrupt transition from involved epidermis to normal appearing skin over a distance of 1 or 2 rete ridges or 0.1 mm).

Statistical Methods

Descriptive statistics were provided for UVR/non-UVR signature and acral/cutaneous melanomas. Continuous variables were summarized by their mean (s.d.), median, first and third quartiles. For categorical variables frequency and percentage are reported. No inference was performed given the limited sample size, however, difference in means and their 95% confidence interval are reported to assess the magnitude of the difference between the continuous variables

RESULTS

Proportion with UVR Dominance

Of the 183 melanomas, 35 were from acral sites including 10 from subungual locations, eight were mucosal melanomas, and the remaining 140 were from other cutaneous, non-acral skin, or had arisen from an unknown primary site. Melanomas from unknown primary sites were arbitrarily considered to be cutaneous melanomas as they have been shown to have similar mutation profiles to cutaneous melanoma in prior studies.[23,24] Cases were then classified as UVR or non-UVR based on the proportion of UVR signatures in the overall mutation burden, as defined in 'Methods' section. As expected, no mucosal melanomas were found to be UVR dominant. Seven tumors had mutation burdens that were discordant with their origin: three of the 35 (9%) acral melanomas (Figure 1) were found to be UVR dominant while four of the 140 (3%) (Figure 2) cutaneous melanomas lacked UVR signatures (Table 1 and Figure 3). All non-acral melanomas arising from the hands and feet had dominant UVR signatures (Table 4).

UVR Dominant Acral Melanoma

The three acral melanomas dominated by UVR signatures are described in Table 2. Two had arisen as primaries located on a subungual location involving the thumb, while the third occurred on the sole of the foot. All were males, aged 58–64 years (median 61 years) at the time of diagnosis of the primary. At the time of diagnosis, metastatic disease was present within regional lymph nodes in all cases with no distant metastatic disease, ie, American Joint Committee on Cancer (AJCC) 7th edition stage IIIA–IIIC.[25] All cases were of acral lentiginous melanoma subtype with Breslow thickness 1.3–4.0mm (median 3.2), tumor mitotic rate 2–3 mm/2 (median 3) and two of the cases were ulcerated.

The 32 acral melanomas lacking UVR signatures are described in Table 3. In brief, 21 of the cases were female and 11 male, aged 34–90 years (median 72 years) at the time of primary diagnosis. Breslow thickness ranged from 0.8 to

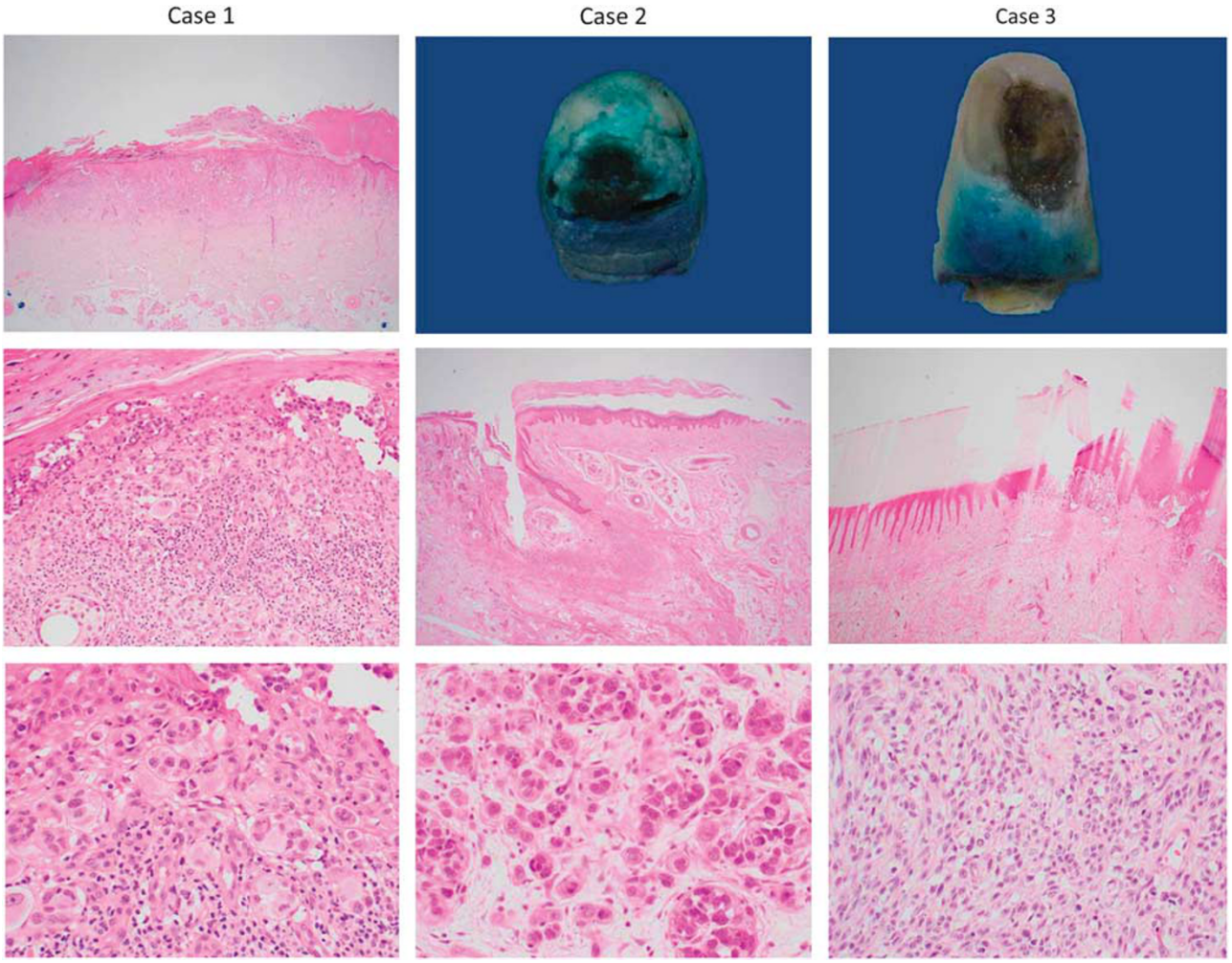

Figure 1 Acral melanoma with a UVR signature. Panel 1: Scanning power of case 1, H&E x20, macroscopic photo of case 2, macroscopic photo of case 3. Panel 2: Medium view of case 1, H&E x100. Scanning power of case 2 and 3, H&E x20. Panel 3: High-power magnification of case 1, 2, and 3, H&E x400.

14.5 mm (median 4.3 mm), tumor mitotic rate 0 to 15 mm/2 (median 4.0 mm/2), 14 (44%) cases were ulcerated, and 13 (41%) of cases were AJCC stage either III or IV at time of diagnosis.

Even though no *P*-value was calculated, due to the small sample size, the 95% confidence intervals (CI) have shown non-UVR acral melanomas are more likely in older patients and have more mitoses than acral melanoma with a UVR signature (Table 3).

Cutaneous Melanoma Cases without Dominant UVR Signatures

The four cases of cutaneous melanoma without UVR signatures, two male and two females aged 49–68 years (median 60 years), are described in Table 2. In two cases, the primary melanoma was located on the thorax, one was on the abdomen, and one was on the lower ankle/lateral aspect of the foot. At the time of diagnosis, three patients had metastatic disease within regional lymph nodes with no distant metastatic disease, AJCC 7th edition stage IIIB–IIIC[25] while the other case was stage IB at the time of diagnosis. Breslow thickness ranged from 0.5 to 20 mm (median 8.65 mm), tumor mitotic rate 5 to 30 mm/2 (median 10.5 mm/2) with two cases ulcerated.

The 136 cutaneous melanomas with a UVR signature are described in Table 3. In brief, 46 of the cases were female and 89 male aged 17–92 years (median 60 years). The Breslow thicknesses ranged from 0.3 to 20 mm (median 2.7 mm), tumor mitotic rate ranged from 0 to 40 mm/2 (median 6.0 mm/2), 42 (31%) cases were ulcerated, and 41 (30%) of cases were AJCC stage III or IV at time of diagnosis.

Although the Breslow thickness and tumor mitotic rate were higher in the non-UVR group than the UVR group there was no significant difference as the 95% CI includes zero (Table 3).

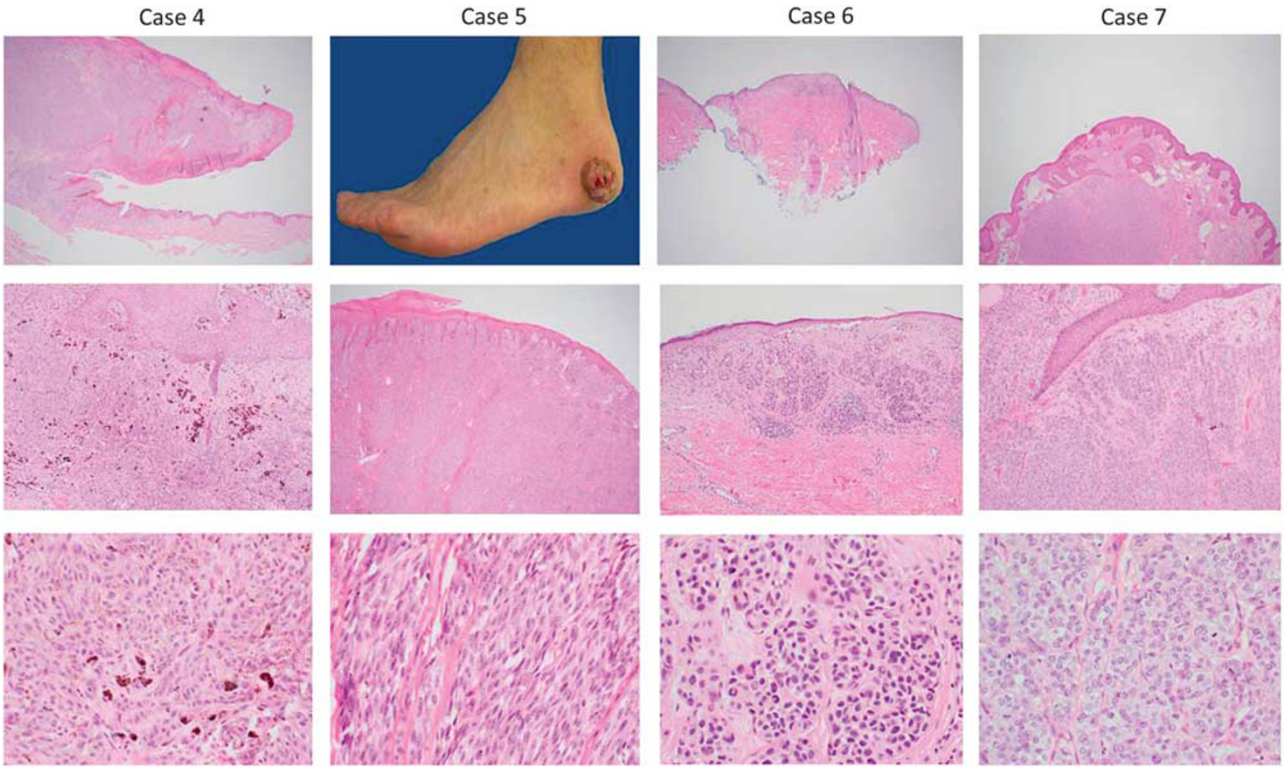

Figure 2 Cutaneous melanoma with a non-UVR signature. Panel 1: Scanning power of case 4, 6, and 7, H&E x20, macroscopic photo of case 5. Panel 2: Medium view of case 4, 6, and 7, H&E x100. Scanning power of case 5, H&E x20. Panel 3: High-power magnification of case 4, 5, 6, and 7, H&E x400.

Table 1 Summary of UVR/non-UVR signatures following high coverage whole-genome sequencing of a cohort of 183 melanomas

Site of primary	Total cases	UVR signature cases *n* (%)	Non-UVR signature cases *n* (%)
Cutaneous	140	136 (97)	4 (3)
Acral	35	3 (9)	32 (91)
Subungual	10	2 (20)	8 (80)
Non-subungual	25	1 (4)	24 (96)
Mucosal	8	0 (0)	8 (100)

Histopathological Features

H&E-stained sections of the primary tumors were available for review in three (100%) and 23 (72%) of the acral melanomas with a UVR signature and non-UVR signature, respectively, and the findings are outlined in Table 4. Forty-four (32%) and four (100%) of the cutaneous melanomas with a UVR signature and non-UVR signature, respectively, had H&E-stained sections available for review and the findings are outlined in Table 4.

All cutaneous melanomas with a non-UVR signature and all non-cutaneous melanoma with a UVR signature, where the sections of the primary were available, were reviewed to confirm anatomical location of the primary tumor. For example, in case 5 the primary site was described as the lateral ankle, however, acral skin was noted at one end of the histological specimen but not overlying the primary tumor. Clinical photos confirmed this to be correctly classified as a cutaneous melanoma, which occurred in close proximity to the transition to acral skin (Figure 2).

No histopathological feature predicted a discordant classification by UVR status. Of note, there was no solar elastosis present in the three UVR-dominant acral melanomas or the four cutaneous melanomas without UVR dominance.

Tumor Mutation Burden and UVR Dominance

The total burden of point mutations, comprising of single nucleotide variations (SNV) and indels, detected in the three cases of acral melanoma with a UVR signature and the four cases of cutaneous melanoma with a non-UVR signature are detailed in Table 4 and compared with those of the comparison groups of acral and cutaneous melanomas. Figure 3 relates total count of SNV and structural variants (SV) to the relative burden of UVR-related point mutation signatures.

For three acral melanomas with dominant UVR signatures, the total mutation burden per case was 7175 to 45 751 (median 26 271), including 4405 to 36 139 (median 16 717) C>T transitions, 41 to 310 (median 244) CC>TT transitions, and 71 to 234 (median 118) SV. One case was found to harbor a BRAF mutation, one a NF1 mutation, and the third

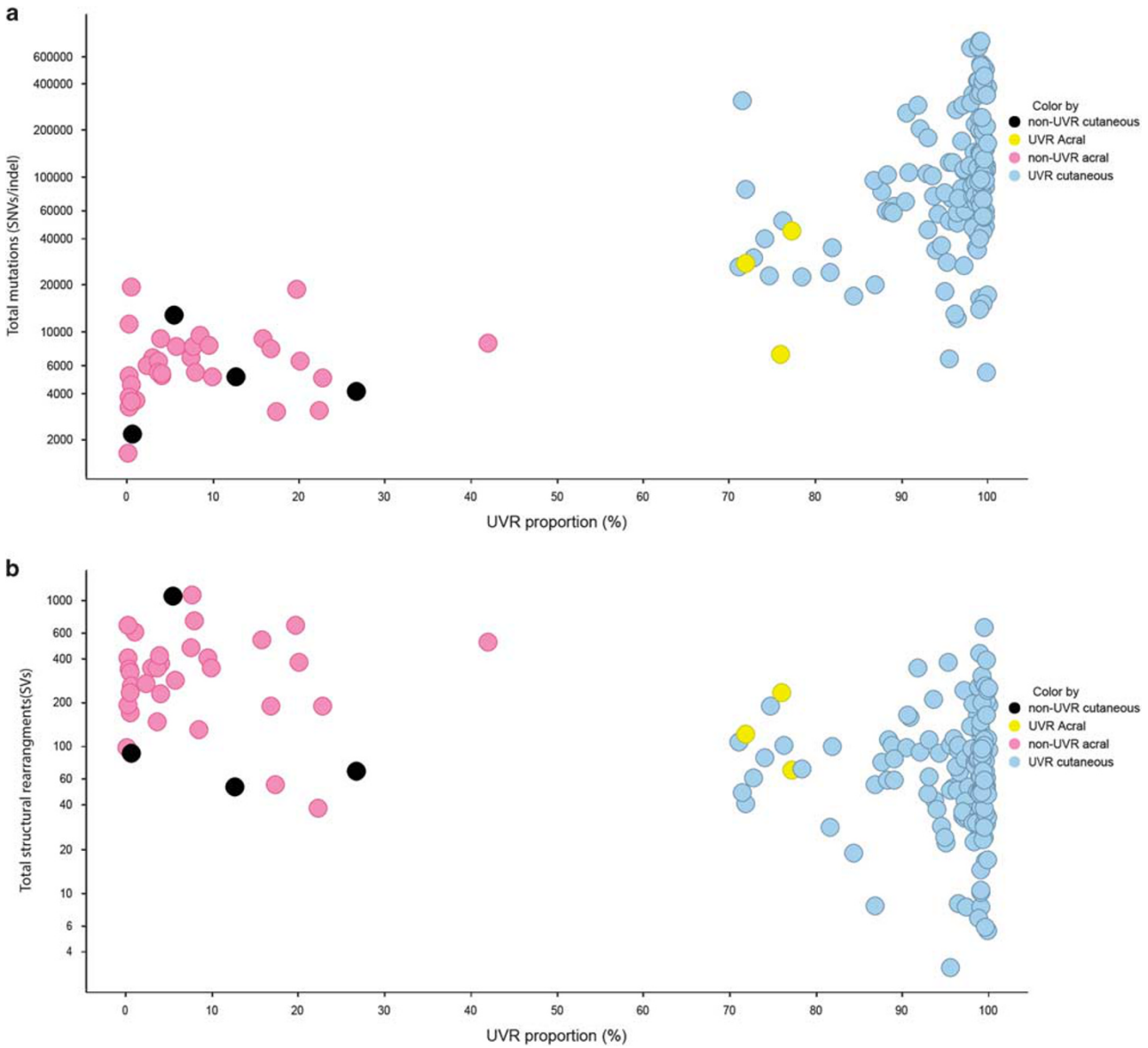

Figure 3 Cohort of 175 melanomas (140 cutaneous, 35 acral) illustrating total mutations of each melanoma against proportion of UVR signature in total mutation burden. (**a**) Depicts the total number of mutations (SNVs/indels) per sample, patients are colored based on the melanoma subtype/mutation signature. (**b**) Depicts the relationship between number of structural variants and the proportion of UVR-related mutations.

had no mutation detected in BRAF, RAS, or NF1. The proportion of the mutation burden due to UVR was 72.5 to 76.9%. In contrast, the acral melanomas without a dominant UVR signature had a total mutation burden from 1610 to 19 128 per case (median 5779), including 202 to 8416 (median 1709) C>T transitions, 1 to 431 (median 4) CC>TT transitions, 41 to 1132 (median 335) SV; the proportion of the mutation burden due to UVR signatures was much lower in these cases, ranging from 0 to 41.5% (median 4.2%).

For the four cutaneous melanomas with no dominant UVR signature, the total mutation burden per case was 2122 to 11 583, including 138 to 3469 (median 1870) C>T transitions, 2 to 203 (median 6.5) CC>TT transitions, and 52 to 1123 (median 79) SV. Two of the four cases harbored a BRAF mutation while the other two cases harbored no mutation in BRAF, RAS, or NF1. The proportion of the mutation burden due to UVR was 0 to 26.3%. In contrast, the cutaneous melanomas with dominant UVR signatures had a total mutation burden from 13 111 to 775 848 (median 92 605), including 12 258 to 645 517 (median 76 817) C>T transitions, 124 to 6189 (median 932) CC>TT transitions, and 3 to 657 (median 63.5) SV; the proportion of the mutation burden due to UVR signature was much higher in these cases ranging from 70.3 to 100% (median 98.6%).

Table 2 Demographics and pathological information of acral melanoma with a UVR signature and cutaneous melanoma with a non-UVR signature

	Site of primary tumor	Tissue sequenced	Gender	Age at time of primary diagnosis	Overall stage at primary diagnosis[a]	T stage at primary diagnosis[a]	N stage at primary diagnosis[a]	M stage at primary diagnosis[a]	Subtype	Breslow thickness (mm)	Ulceration	Clark level	Tumor mitotic rate (/mm^2)	Vascular invasion	Lymphatic invasion	Neurotropism
Cases of acral melanoma with a UVR signature																
Case 1	Sole of right foot	Metastasis in right inguinal lymph node	Male	61	IIIA	T2a	N1a	M0	AL	1.3	No	IV	2	Yes	No	No
Case 2	Subungual left thumb	Primary	Male	58	IIIC	T3b	N2b	M0	AL	4.0	Yes	IV - V	3	No	No	No
Case 3	Subungual right thumb	Metastasis in right axillary lymph node	Male	64	IIIB	T3b	N1a	M0	AL	3.2	Yes	V	3	No	No	No
Cases of cutaneous melanoma with a non-UVR signature																
Case 4	Medial lower abdomen	Primary	Female	68	IIIB	T4b	N2a	M0	SSM	8.5	Yes	IV	16	Yes	Yes	No
Case 5	Lateral ankle	Primary	Male	59	IIIC	T4b	N3	M0	SSM	8.8	Yes	V	30	Yes	Yes	No
Case 6	Right pectoral region	Metastasis in brain	Male	49	IB	T1b	N0	M0	SSM	0.5	No	III	5	No	No	No
Case 7	Right axilla	Primary	Female	61	IIIC	T4b	N2b	M0	NM	20.0	Yes	V	5	No	No	No

Abbreviations: AL, acral lentiginous; SSM, superficial spreading melanoma; NM, nodular melanoma.
[a]American Joint Committee on Cancer, 7th edition 2010.

Table 3 Details of cutaneous and acral melanoma by UVR and non-UVR signature

	Cutaneous melanoma			Acral melanoma		
	UVR signature *N* (%)	Non-UVR signature *N* (%)	Difference in means (95% CI)	UVR signature *N* (%)	Non-UVR signature *N* (%)	Difference in means (95% CI)
Cases	136	4		3	32	
Gender						
Male	86	2		3	11	
Female	49	2		0	21	
Age at time of primary (years)						
Mean (s.d.)	57.3 (18.6)	59.1 (7.9)	1.8 (−10.1, 13.6)	60.9 (3.2)	70.5 (13.6)	9.6 (3.0, 16.2)
Q1, Median, Q3	46, 59.0, 71	56, 59.8, 63		59, 61.2, 62	63, 71.8, 80	
Breslow thickness (mm)						
Mean (s.d.)	4.1 (4.0)	9.5 (8.0)	5.3 (−7.3, 18.0)	2.8 (1.4)	4.9 (3.2)	2.1 (−0.5, 4.7)
Q1, Median, Q3	1.4, 2.7, 5.2	6.5, 8.6, 11.6		2.2, 3.2 3.6	2.6, 4.3, 6.3	
Mitoses (/mm^2)						
Mean (s.d.)	7.9 (8.1)	14.0 (11.9)	6.1 (−12.6, 24.7)	2.7 (0.6)	4.5 (3.6)	1.8 (0.4, 3.3)
Q1, Median, Q3	3, 6.0, 10	5, 10.5, 19.5		2.5, 3, 3	2, 4.0, 6 .5	
Ulceration present	42 (31)	3 (75)		2 (67)	14 (44)	
Cases at least stage III at diagnosis	41 (30)	3 (75)		3 (100)	13 (41)	
WHO melanoma subtype						
NM	59 (43)	1(25)		0 (0)	3 (9)	
SSM	48 (35)	3 (75)		0 (0)	0 (0)	
DM	9 (7)	0 (0)		0 (0)	2 (6)	
AL	0 (0)	0 (0)		3 (100)	30 (85)	
LM	3 (2)	0 (0)		0 (0)	0 (0)	
Not classified/unknown primary	17 (13)	0 (0)		0 (0)	0 (0)	
Vascular invasion present	8 (6)	2 (50)		1 (33)	5 (16)	
Lymphatic invasion present	8 (6)	2(50)		0 (0)	6 (19)	

Table 3 Continued

	Cutaneous melanoma			Acral melanoma		
	UVR signature *N* (%)	Non-UVR signature *N* (%)	Difference in means (95% CI)	UVR signature *N* (%)	Non-UVR signature *N* (%)	Difference in means (95% CI)
Neurotropism present	3 (2)	0 (0)		0 (0)	4 (13)	
Satellites present	5 (4)	0 (0)		0 (0)	3 (9)	
Primary tumor H&E slides reviewed	44 (32)	4 (100)		3 (100)	23 (72)	
TIL distribution						
Negative	9 (20)	3 (75)		0 (0)	6 (26)	
Focal	25 (57)	1 (25)		3 (100)	10 (43)	
Multifocal	8 (18)	0 (0)		0 (0)	7 (30)	
Diffuse	2 (5)	0 (0)		0 (0)	0 (0)	
TIL density						
Negative	9 (20)	3 (75)		0 (0)	6(26)	
Mild	27 (61)	1 (25)		3 (100)	13 (57)	
Moderate	8 (18)	0 (0)		0 (0)	4 (17)	
Marked	0 (0)	0 (0)		0 (0)	0 (0)	
Pigment distribution						
0%	23(52)	2 (50)		1 (33)	11 (48)	
0–25%	14 (32)	1 (25)		2 (67)	9 (39)	
25–50%	5 (11)	1 (25)		0 (0)	2 (9)	
>50%	2 (5)	0 (0)		0 (0)	1 (4)	
Pigment density						
Negative	23 (52)	2 (50)		1 (33)	11 (48)	
Mild	8 (18)	0 (0)		0 (0)	4 (17)	
Moderate	7 (16)	2 (50)		2 (67)	7 (30)	
Marked	6 (14)	0 (0)		0 (0)	1 (4)	
Regression in melanoma						
0%	34 (77)	4 (100)		1(33)	11 (48)	
0–25%	7 (16)	0 (0)		2 (67)	10 (43)	
25–50%	3 (7)	0 (0)		0 (0)	1 (4)	

Table 3 Continued

	Cutaneous melanoma			Acral melanoma		
	UVR signature *N* (%)	Non-UVR signature *N* (%)	Difference in means (95% CI)	UVR signature *N* (%)	Non-UVR signature *N* (%)	Difference in means (95% CI)
>50%	0 (0)	0 (0)		0 (0)	1 (4)	
Cell size						
< ×2 lymphocyte	0 (0)	0 (0)		0 (0)	1 (4)	
= ×2 lymphocyte	2 (5)	0 (0)		0 (0)	1 (4)	
> ×2 lymphocyte	42 (95)	4 (100)		3 (100)	21 (91)	
Cell shape						
Epithelioid	25 (57)	3 (75)		1 (33)	10 (43)	
Mixed	13 (30)	1 (25)		2 (67)	9 (39)	
Spindled	6 (14)	0 (0)		0 (0)	4 (17)	
Tumor border						
Pushing	30 (68)	4 (100)		1 (33)	14 (61)	
Infiltrative	13 (30)	0 (0)		2 (67)	8 (35)	
Unable to assess	1 (2)	0 (0)		0 (0)	1 (4)	
Solar elastosis						
Absent	7 (16)	4 (100)		3 (100)	23 (100)	
Mild	13 (30)	0 (0)		0 (0)	0 (0)	
Moderate	15 (34)	0 (0)		0 (0)	0 (0)	
Severe	8 (18)	0 (0)		0 (0)	0 (0)	
Unable to assess	1 (2)	0 (0)		0 (0)	0 (0)	
Lateral circumscription						
Discontinuous	0 (0)	0 (0)		0 (0)	0 (0)	
Gradual	16 (36)	1 (25)		3 (100)	11 (48)	
Abrupt	19 (43)	2 (50		0 (0)	9 (39)	
Unable to assess	9 (20)	1 (25)		0 (0)	3 (13)	
Upward spread						
Absent	13 (30)	0 (0)		1 (33)	15 (65)	

Table 3 Continued

	Cutaneous melanoma		Difference in means (95% CI)	Acral melanoma		Difference in means (95% CI)
	UVR signature *N* (%)	Non-UVR signature *N* (%)		UVR signature *N* (%)	Non-UVR signature *N* (%)	
Occasional	20 (45)	3 (75)		1 (33)	2 (9)	
Moderate	2 (5)	0 (0)		1 (33)	4 (17)	
Marked	0 (0)	0 (0)		0 (0)	1 (4)	
Unable to assess	9 (20)	1 (25)		0 (0)	1 (4)	

Abbreviations: AL, acral lentiginous; SSM, superficial spreading melanoma; NM, nodular melanoma; DM, desmoplastic melanoma; LM, lentigo maligna; TILS, tumor-infiltrating lymphocytes.
Values are expressed as *n* (%) unless otherwise noted. Total percentages that do not add up to 100% have missing values.

DISCUSSION

Melanoma has the highest rate of somatic mutation of any malignancy.[1] Exome and whole-genome sequencing studies have confirmed that this mutation burden is dominated (median 82.5% in one study) by cytosine to thymidine transition (C>T),[10,11,26,27] which results from UVR exposure. In contrast, UVR signatures account for a much lower proportion of the mutation burden in acral melanomas; 25–49% in one study. The total number of mutations correlates with UVR exposure, ranging from greater than 100 000 mutations for those in chronic sun-exposed locations to 30 000 mutations in skin of intermittently UVR-exposed locations to <1000 mutations for those in acral/mucosal sites. Conversely, acral and mucosal melanomas have been shown to harbor more chromosomal aberrations and other structural variations[2] than cutaneous melanomas.

Utilizing data generated in the first high coverage whole-genome sequencing study of a large cohort of melanomas (183 cases, currently under review), we have analyzed the characteristics of acral and cutaneous melanomas with mutation burdens that are discordant with these patterns: three acral melanomas with a dominant UVR signature and four cutaneous melanomas without a dominant UVR signature.

Acral and Subungual Melanomas with a Dominant UVR Signature

The acral melanomas with a dominant UVR signature had a high number of mutations when compared with the acral melanomas without a dominant UVR signature. In previous exome and whole-genome sequencing studies, mutations per Mb in acral melanomas have been reported to range from 1.02 to 3.68 (5 cases),[15] 1.95, [28] and 2.79 to 14 (two cases).[27] In contrast, the mean mutation rate of The Cancer Genome Atlas Network study,[20] which consisted entirely of cutaneous melanomas, was 16.8 mutations/Mb. Two of the cases we identified in the current study, cases 2 and 3, displayed a mutation burden of 8.76 and 15.25 mutation/Mb, respectively. Although mutation rates are not generally comparable from one study to the next due to different methodologies, it would appear that these cases have a mutation burden more in keeping with cutaneous melanomas. The only other reported similar rate of mutation burden in an acral melanoma was that in case ME032.[27]

There have been recent conflicting reports on UVR signatures in sequenced acral melanomas. Some studies report no dominant UVR signature within acral melanomas[10,15,26] while other studies report a dominant UVR signature in cases of acral melanoma: one case report of whole-genome sequencing;[28] two of six cases of melanoma cell lines examined;[29] and one of two cases of whole-genome sequencing.[27] In our view, these findings probably relate to the case selection; some cases designated as arising from acral sites may have involved the non-acral surfaces of the hands or feet. However, in one study,[10] the

Table 4 Total mutations, mutations detected, and UVR proportion of mutational burden

	Total mutations	Mutations/ Mb	Structural variations	Total C>T	Total CC>TT	UVR proportion (%)	Mutation detected (BRAF/RAS/NF)
Acral melanoma – UVR							
Average	26 399	8.80	141	19 087	198	75.3	
Case 1	7175	2.39	234	36 139	310	76.9	BRAF V600E
Case 2	45 751	15.25	71	16 717	244	76.6	NF1
Case 3	26 271	8.76	118	4405	41	72.5	None detected
Acral melanoma including subungual – non-UVR							
N = *32*							
Average	6722	2.24	361	2057	22	7.8	BRAF = 5 (16)
							HRAS = 1 (3)
							NRAS = 4 (13)
							NF1 = 8 (25)
							None detected = 14 (44)
Range	1610–19128	0.54–6.38	41–1132	202–8416	1–431	0–41.5	
Median	5779	1.92	335	1709	4	4.2	
Subungual melanoma – non-UVR							
N = *8*							
Average	9522	3.17	456	3188	24	14.4	NRAS = 1 (12.5)
							NF1 = 2 (25)
							None detected = 5 (62.5)
Range	5467–19128	1.82–6.38	131–1132	1430–8416	2–66	3.1–41.5	
Median	7875	2.63	377	2486	7	7.9	
Cutaneous melanoma – UVR of hands and feet (non-acral sites)							
N = *2*							
Average	59 707	19.90	26	49 292	536	99.4	NRAS = 1 (50)
							None detected = 1 (50)
Range	44 344–75 069	14.78–25.02	31–30	35 244–63 340	295–778	99.3–99.5	
Median	59 707	19.90	26	49 292	536	99.4	

Table 4 Continued

	Total mutations	Mutations/ Mb	Structural variations	Total C>T	Total CC>TT	UVR proportion (%)	Mutation detected (BRAF/RAS/NF)
Cutaneous melanoma – non-UVR							
Average	5799	1.93	333	1837	54.5	11.0	
Case 4	4995	1.67	52	2098	6	13	BRAF V600E
Case 5	4170	1.39	69	1642	7	26.3	BRAF V600E
Case 6	11 908	3.97	1123	3469	2	4.8	None detected
Case 7	2122	0.71	89	138	203	0	None detected
Cutaneous melanoma – UVR							
N = *136*							
Average	151 664	50.55	94	126 827	1319	95.7	BRAF = 62 (46)
							HRAS = 3 (2)
							NRAS = 44 (32)
							NF1 = 14 (10)
							None detected = 13 (10)
Range	5840–779 056	1.95–259.69	3–657	12 258–645 517	124–6189	70.3–100	
Median	92 605	30.87	63.5	76 817	932	98.6	

authors postulated that this discrepancy may be due to a low threshold used for C>T transitions used to constitute a UVR signature and the percent of total mutations at a dipyrimidine and the percent of C>T transitions that were at a dipyrimidine was not reported. In the three cases, we describe the proportion of UVR signature constituting the total mutation burden ranged from 72.5 to 76.9%. These levels, while at the lower end of the range for UVR signature (Figure 3), appear sufficient to provide confidence that they are correctly identified as primarily UVR driven. Further evidence of this is the elevated total mutation load within these cases and the generally lower level of structural variations when compared with acral melanomas with a non-UVR signature.

It has been shown that the mean age of diagnosis for acral melanoma is higher than that of cutaneous melanoma.[30] In our series of acral cases with a dominant UVR signature, we report the age of the patient at the time of diagnosis of the primary to be lower than those without a dominant UVR signature. This finding provides further evidence that these acral melanomas possibly behave more like cutaneous melanomas than acral melanomas due to the different mutation processes that are driving them.

Interestingly, two of the three cases of acral melanoma with a dominant UVR signature occurred in subungual locations on the thumb and these two cases had the more elevated mutation burden. Although the eight other subungual melanomas, which had no UVR signature, had a mutation burden more in keeping with an acral melanoma (Table 4). Examining of human cadaveric nails has previously showed that all of UVB and the vast majority of UVA light is blocked by the nail plate.[14] The fact that two of the cases we reported in the thumb and a previous case report in the toe[28] of subungual melanoma with a dominant UVR signature appears to refute these findings. It would therefore appear that in some patients sufficient UVR can penetrate the nail plate to cause mutation effects in the melanocytes in the underlying subungual tissue. The other acral melanoma with a dominant UVR signature was noted to arise on the sole of the foot. This would also seem to argue against the traditional theory that the thickened stratum corneum on the soles of the feet and the palms of the hand, in addition to the fact that in the majority of the population they represent anatomical locations rarely exposed to UVR, provides a protective environment from UVR.[13] However, it should be noted that in some people through various occupational or recreational activities, such as excessive sun bathing, these areas could be exposed to a significant amount of UVR. In case 1, which involved the sole of the foot, we could not confirm that the patient had been involved in any such activities.

This is the first study, as far as we are aware, to compare histopathological features of acral and cutaneous melanomas with and without a dominant UVR signature. Within all the parameters examined, there were no particular histopathological features which appeared predictive of an acral melanoma with a dominant UVR signature compared with a non-UVR dominant signature. Of note, no histopathological evidence of solar damage was noted in the three acral melanomas with a UVR signature.

In summary, the intermediate total mutation count, lower number of structural variations, and lack of solar damage histologically indicate that these acral melanomas with a dominant UVR signature have a similar pathogenesis, and therefore possibly have similar clinical behavior, to melanomas arising in intermittently exposed skin, such as the torso or abdomen. This novel hypothesis appears to broaden our understanding of the heterogeneous nature of mutagenesis in acral melanoma.

Cutaneous Melanomas Lacking a UVR Signature

Of the cutaneous melanomas with a non-UVR dominant signature, the mutation load, C>T, and CC>TT transitions are lower than those with a UVR dominant signature and in the range of acral melanomas with a non-UVR dominant signature (Figure 3 and Table 3). Interestingly, the number of structural variations in case 4, 5, and 7 are low when compared with acral melanomas with a non-UVR dominant signature with case 6 harboring an exceedingly large burden of structural variations. These findings lead us to believe that there is considerable heterogeneity in melanomas with a non-UVR dominant signature with multiple, at this stage unknown, causative factors.

Case 5 was anatomically located in the ankle and clinically not an acral melanoma. Histopathology revealed that the tumor was centered on non-acral skin, which at the inferior aspect of lesion merged with acral skin. The remainder of these cases were from sites which would generally only receive intermittent UVR exposure (torso and abdomen).

Interestingly, in cutaneous melanomas with a non-UVR dominant signature, the tumor mitotic rate and Breslow thickness appeared higher than in the comparison group of cutaneous melanomas with a dominant UVR signature, although this difference was found not to be significant. These two criteria along with tumor ulceration are known to represent the most important prognostic factors for primary melanomas.[25] Results from the TCGA melanoma project,[20] which looked only at cutaneous melanoma, were also reviewed for any differences in high-risk features between UVR and non-UVR dominant melanomas (Supplementary Figure 1). These findings support our results with a greater Breslow thickness ($P=0.0223$) noted in the non-UVR group. Also, although no statistical significance was found, the non-UVR group were more frequently ulcerated ($P=0.0691$) and had a greater mitotic rate than the UVR group. Previously, cutaneous melanomas have been found to be thinner than acral melanomas and also to have a better prognosis.[30] Extrapolating these findings, although the numbers we have reviewed are small and no statistical significance was found between the two groups, it could indicate that cutaneous melanomas with a non-UVR

dominant signature are more similar in pathogenesis and possible clinical behavior to acral melanoma than cutaneous melanoma with a UVR signature.

Also of interest was the finding that, two of these four cutaneous melanoma cases (case 4 and 5) harbored a BRAF V600E mutation and these same two cases had a larger proportion of UVR signature compared with the two BRAF wild-type cases. As BRAF V600E mutations occur early in mutagenesis and are associated with exposure to intermittent UVR exposure rather than chronic UVR exposure,[2,7,31] it is possible that early low-dose intermittent UVR exposure is sufficient to enable BRAF mutation after which other, at this point unknown, mutation drivers become the predominant mutagenic process in this subset of melanomas.

Within the histopathological parameters examined, there was no particular feature found that was predictive of cutaneous melanoma with a non-UVR dominant signature compared with a dominant UVR signature.

Following the first high coverage whole-genome sequencing study of a large cohort of melanomas, we identified interesting cases which appeared to have contradictory UVR signatures based on the anatomical site of the primary tumor. These findings seem to resolve an area of uncertainty in the literature that a proportion of acral melanomas, particularly from subungual locations, are driven by UVR mutagenic process. We hypothesize that, based on mutation load, number of structural variations, age of onset and at times BRAF mutation status, these types of acral melanoma might more appropriately be grouped and viewed with melanomas arising from sites of intermittent UVR exposure. Conversely, some melanomas from cutaneous sites are not caused by UVR-associated mutation processes and could be more similar to acral melanomas in terms of mutagenesis, epidemiological features, and clinical behavior. Interestingly, in comparison with acral melanoma with a non-UVR dominant signature, three of the four cases of non-UVR cutaneous melanoma had relatively low structural variations. This highlights the genetic heterogeneity in non-UVR signature melanoma. Further research to investigate the spectrum of potential causative mechanisms in these melanomas will be of great interest.

In addition, we are the first study, as far as we are aware, to perform in-depth histopathological correlation of these interesting cases with seeming mismatch of mutation signatures. Taken together, these findings add to an expanding knowledge of the heterogeneous nature of melanoma. In particular, the novel findings we have presented could lead to further investigation into the poorly understood non-UVR signature mutagenic processes in melanoma, which could help guide preventative measures, ensure appropriate management, and guide research into future treatment options for melanoma.

Supplementary Information accompanies the paper on the Laboratory Investigation website (http://www.laboratoryinvestigation.org)

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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