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Association Between Confocal Morphologic Classification and Clinical Phenotypes of Multiple Primary and Familial Melanomas

Thaís Corsetti Grazziotin, MD, PhD; Ivette Alarcon, MD; Renan Rangel Bonamigo, MD, PhD; Cristina Carrera, MD, PhD; Miriam Potrony, PhD; Paula Aguilera, MD, PhD; Joan-Anton Puig-Butillé, PhD; Johanna Brito, MD; Celia Badenas, PhD; Llúcia Alós, MD; Josep Malvehy, MD, PhD; Susana Puig, MD, PhD

IMPORTANCE The improved knowledge of clinical, morphologic, and epidemiologic heterogeneity of melanoma in the context of multiple primary and familial melanomas may improve prevention, diagnosis, and prognosis of melanoma.

OBJECTIVE To characterize reflectance confocal microscopy (RCM) morphologic patterns of melanomas in multiple primary and familial melanomas.

DESIGN, SETTING, AND PARTICIPANTS In this cross-sectional, retrospective study, patients in a hospital-based referral center were recruited from March 1, 2010, through August 31, 2013; data analysis was conducted from September 1, 2013, through May 31, 2014. Consecutive primary melanomas, documented by dermoscopic and confocal examination, from multiple primary and familial melanomas with known *CDKN2A* mutational status were studied.

MAIN OUTCOMES AND MEASURES Epidemiologic, genetic, dermoscopic, and histologic data were evaluated according to an RCM morphologic classification: dendritic cell, round cell, dermal nest, combined, and nonclassifiable types.

RESULTS Fifty-seven melanomas from 50 patients (28 women [56%] and 49 white patients [98%]) were included: 23 dendritic cell (40%), 21 round cell (37%), 2 dermal nests (4%), 2 combined (4%), and 9 nonclassifiable (16%). The median (SD) age of the participants was 53.0 (16.9) years (interquartile range, 41.8-71.2 years), and the median (SD) age at the first melanoma was 46.0 (17.1) years (interquartile range, 35.8-61.5 years). Dendritic cell melanoma was characterized by older age at diagnosis, phototypes 2 and 3, more intense solar exposure, and moderate to severe solar lentigines; it was the most prevalent confocal type in facial lesions and was associated with the lentigo maligna histologic subtype. Round cell melanomas were identified more often in the familial context and in individuals with phototype 1 skin types; RCM features, such as junctional thickening, dense dermal nests, and nucleated cells within papillary dermis, were more frequently found in this subtype. Dermal nest and combined melanoma were associated with the absence of pigmented network on dermoscopy and thicker tumors on histologic analysis. Nonclassifiable type was associated, by RCM, with the absence of pagetoid cells on confocal examination and lower frequency of marked atypia on melanocytes in the basal cell layer; it presented with lower ABCD Total Dermoscopy Scores and RCM scores compared with the other types. *CDKN2A* mutation carriers may develop any RCM type of melanoma.

CONCLUSIONS AND RELEVANCE Different routes to develop melanoma can be identified according to RCM morphologic classification, with dendritic cell melanomas being associated with chronic sun damage and round cell melanoma with early age at onset and phototype 1 in the context of multiple primary and familial melanomas. The morphologic expression of melanomas via dermoscopy and confocal examination varies according to differences in tumor stage and biological behavior.

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Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Thaís Corsetti Grazziotin, MD, PhD, Department of Dermatology, Universidade Federal de Ciências da Saúde de Porto Alegre, Rua Sarmento Leite, 245 CEP 90050-170, Porto Alegre, Rio Grande do Sul, Brazil (thais.grazziotin@pucrs.br).

Currently, morphologic analysis–based melanoma classification is insufficient to provide enough information about causative factors or to select patients for specific treatments.[1] In fact, refining classification of melanomas into morphologic subtypes that correlate with phenotypical characteristics or prognostic factors may enhance knowledge about the disease and create a model for future investigations with molecular analysis and/or therapy stratification.

In vivo reflectance confocal microscopy (RCM) is a noninvasive imaging technique that allows for the en face visualization of microscopic structures and cellular detail in the epidermis and superficial dermis at histopathologic resolution.[2,3] Furthermore, integrating dermoscopy and confocal techniques is useful for distinguishing early-stage melanoma that lacks frankly malignant features, especially in high-risk patients during follow-up,[4,5] adding to morphologic information about the biology of the lesions. The use of in vivo RCM added a new dimension in melanoma knowledge because the entire surface of the melanoma can be analyzed at histologic resolution. Not only the tumor but also patient characteristics may influence the morphologic findings. In fact, *CDKN2A* germline mutations and genetic variants in *MC1R* may influence dermoscopic features. Among Spanish carriers of *CDKN2A* mutations, the presence of 2 *MC1R* red hair color variants was associated with melanomas with a less suspicious clinical and dermoscopic appearance.[2,6-10] Recently, 4 distinct melanoma phenotypes on RCM were described as follows: dendritic cell, round cell, dermal nest, and combined melanomas.[11] The authors found that melanomas with a predominant population of dendritic cells were thinner by Breslow index, and melanomas typified by roundish melanocytes or dermal nests were smaller but thicker by Breslow index.[11] The purpose of this study was to correlate morphologic RCM patterns with clinical data, genetic variants, dermoscopic features, and histologic criteria in the context of patients with multiple primary and familial melanomas.

Key Points

Question Is reflectance confocal microscopy a reliable tool to classify melanomas into subtypes and correlate them with phenotypic and genetic background?

Findings This cross-sectional, retrospective study evaluated 57 melanomas from high-risk patients. Dendritic cell melanoma was characterized by older age at diagnosis, phototypes 2 and 3, more intense solar exposure, and solar lentigines; round cell melanomas were identified more often in familial context and in individuals with phototype 1 skin type; and patients with *CDKN2A* mutational status may develop any type of reflectance confocal microscopy melanoma.

Meaning Specific phenotypic features of high-risk patients were associated with some types of melanomas on confocal microscopy classification.

Methods

Patient and Melanoma Selection

A cross-sectional, retrospective, hospital-based study was performed that included patients treated and followed up at the Melanoma Unit of the Hospital Clinic, Barcelona, Spain, from March 1, 2010, through August 31, 2013; data analysis was conducted from September 1, 2013, through May 31, 2014.

The inclusion criteria were patients undergoing periodic follow-up at the Melanoma Unit with a history of at least 2 melanomas (multiple primary melanoma) or hereditary familial melanoma (≥2 patients affected by melanoma in first- or second-degree relatives or at least 1 primary melanoma and at least 1 first- or second-degree relative with pancreatic cancer). Primary melanomas proven by histopathologic examination (any histologic subtype, Breslow Index ≤3 mm) and documented by photographic dermoscopic and confocal examination and known *CDKN2A* status (wild type or mutated) were included. Tumors thicker than 3 mm were excluded to avoid outliers that could modify the interpretation of results. Because this study is focused on morphologic classification based on confocal microscopy of primary melanomas, we wanted to avoid the more advanced tumors because losing primary morphologic features and acquiring more heterogeneity could obscure the results of our study. Patients with genetic conditions, such as Li-Fraumeni syndrome, xeroderma pigmentosum, and albinism, and patients undergoing systemic treatment for advanced melanoma were excluded. Clinical data were collected for each patient, and tumor characteristics were also registered.

The study was approved by the ethical committee of Hospital Clinic of Barcelona. Written informed consent was obtained from each patient, and data were deidentified.

Dermoscopic Analysis

Dermoscopic images were captured using digital cameras (Olympus Camedia [Olympus America Inc], Canon G7 [Canon Inc], and/or Nikon Coolpix 4500 [Nikon Corp]) equipped with a polarized dermatoscope (DermlitePhoto; 3 GEN LLC) and with a epiluminescence microscope system (Mole Max II; Derma Medical Systems). Dermoscopic evaluation was based on the pattern analysis and ABCD Total Dermoscopy Score (TDS) as follows: (A) asymmetry, (B) borders, (C) colors, and (D) different structural components (structureless areas, pigment network, branched streaks, dots, and globules).[12,13] A TDS less than 4.75 is indicative of a benign melanocytic lesion, a TDS of 4.75 through 5.45 is suggestive but not diagnostic of melanoma, and a TDS greater than 5.45 should be considered melanoma.

Confocal Analysis

Confocal examinations were performed by Vivascope 1500 (Lucid Inc). Distinct melanoma types based on RCM were classified as recently proposed by Pellacani et al[11]: (1) dendritic cell melanomas, with a predominantly dendritic cell population and rings and/or thin meshwork pattern; (2) round cell melanomas, with predominantly large, roundish cells with a tendency to aggregate into nests forming a meshwork pattern; (3) dermal nest melanomas, with predominantly large aggregates of cells in the papillary dermis; (4) combined melanomas, with a combination of the 3 previous confocal patterns; and (5) nonclassifiable melanoma, without any of the previous patterns.

Table 1. Description of Tumor Characteristics and Genetic Findings According to Reflectance Confocal Microscopy Pattern[a]

Tumor Feature	Dendritic Cell	Round Cell	Dermal Nest	Combined	Nonclassifiable
Location					
Trunk	10/23 (43.5)	13/21 (61.9)	0/2 (0)	1/2 (50.0)	7/9 (77.8)
Extremity	7/23 (41.2)	7/21 (33.3)	1/2 (50.0)	1/2 (50.0)	1/9 (11.1)
Acral	1/23 (4.3)	1/21 (4.8)	0/2 (0)	0/2 (0)	1/9 (11.1)
Facial	5/23 (21.7)[b]	0/21 (0)	1/2 (50.0)	0/2 (0)	0/9 (0)
Tumor size, mm					
<5	6/23 (26.1)	4/21 (19.0)	1/2 (50.0)	1/2 (50.0)	3/9 (33.3)
5-10	13/23 (56.5)	13/21 (61.9)	0/2 (0)	1/2 (50.0)	5/9 (55.6)
>10	4/23 (17.4)	4/21 (19.0)	1/2 (50.0)	0/2 (0)	1/9 (11.1)
Tumor thickness, mean (SD), mm	0.5 (0.1)	0.6 (0.5)	2.0 (1.3)[b]	2.1 (1.2)[b]	0.3 (0.2)[b]
Subtype					
SSM	18/23 (78.3)	15/17 (88.2)	0/2 (0)	1/2 (50.0)	9/9 (100)
NM	0/23 (0)	1/17 (5.9)	1/2 (50.0)	1/2 (50.0)	0/9 (0)
LM	5/23 (21.7)[b]	1/17 (5.9)	0/2 (0)	0/2 (0)	0/9 (0)
Nevoid melanoma	0/23 (0)	0/17 (0)	1/2 (50.0)	0/2 (0)	0/9 (0)
CDKN2A mutation	5/23 (21.7)	3/21 (14.3)	1/2 (50.0)	2/2 (100)	2/9 (22.2)
Any *MC1R* variant	16/23 (69.6)	16/20 (80.0)	1/2 (50.0)	2/2 (100)	8/9 (88.9)
RHC variant[c]	5/23 (21.7)	7/20 (35.0)	0/2 (0)	2/2 (100)	4/9 (44.4)
ABCD TDS, mean (SD)	6.3 (0.8)	6.4 (0.6)	6.3 (0.6)	6.1 (0.7)	5.6 (0.5)[b]

Abbreviations: LM, lentigo maligna; NM, nodular melanoma; RHC, red hair variant; SSM, superficial spreading melanoma; TDS, Total Dermoscopy Score.

[a] Data are presented as number/total number (percentage) of patients unless otherwise indicated.

[b] $P < .05$ compared with other types.

[c] The RHC variants in patients are R151C, R160W, D294H, and I155T.

On the basis of the presence of major or minor criteria of malignancy, the lesion was classified according to the Pellacani and Barcelona confocal microscopy total score.[14,15] According to the Pellacani score, the cutoff for malignancy was a score equal to or greater than 3. According to the Barcelona algorithm, the cutoff for malignancy was a score equal to or greater than −1.

Genetic Study

The study only included patients for whom *CDKN2A* and *MC1R* sequencing results were available. DNA samples were obtained from peripheral blood lymphocytes of all patients by standard methods, and molecular analysis was conducted at the Molecular Genetics Department of the Hospital Clinic, Barcelona, Spain. Specific mutations in *CDKN2A* and polymorphisms in *MC1R* were studied as previously described.[16-20] *MC1R* polymorphisms were classified as red hair variants or non-red hair variants. Variants that produce no change in amino acid sequence (synonymous changes) were considered as wild type for statistical analysis.

Statistical Analysis

Continuous variables were analyzed and reported as mean (SD) and median (interquartile range). The analysis of the association between categorical variables and outcomes used the Pearson χ^2 test. The Fisher exact test was applied when the expected frequency in the 2 × 2 table is below 5 and the *t* test was used in the comparison of quantitative variables. A κ value for the evaluation of the interobserver reproducibility was calculated for the percentage of concordant ratings. Interobserver reproducibility was calculated based on the independent evaluation of 2 masked readers (T.C.G., I.A.) who analyzed dermoscopy and confocal images of 10 preselected cases. The differences are considered significant at $P < .05$. The calculations were made using Haploview (Broad Institute of MIT and Harvard) and SPSS statistical software, version 16.0 (SPSS Inc).

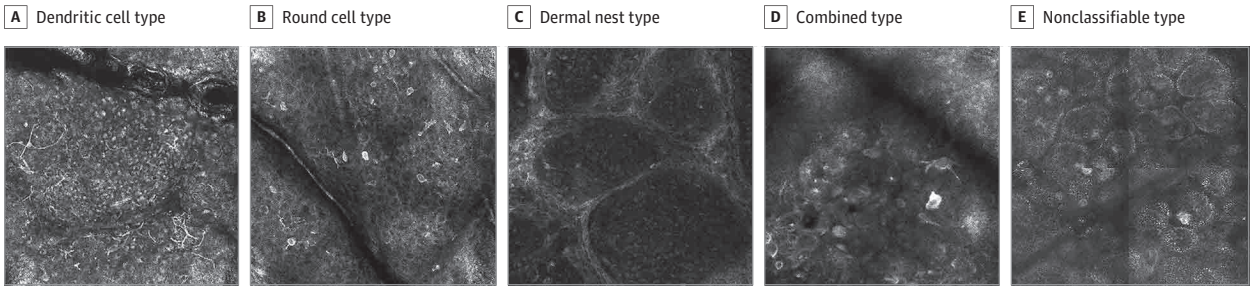
Results

Fifty-seven melanomas from 50 patients (28 women [56%] and 49 white patients [98%]) were included in the study. The median (SD) age of the participants was 53.0 (16.9) years (interquartile range, 41.8-71.2 years), and the median (SD) age at the first melanoma was 46.0 (17.1) years (interquartile range, 35.8-61.5 years). The most prevalent histologic subtype was superficial spreading melanoma (43 of 53 [81%]), followed by lentigo maligna melanoma (6 of 53 [11%]), nodular melanoma (3 of 53 [6%]), and nevoid melanoma (1 of 53 [2%]). According to the American Joint Committee on Cancer classification, 31 lesions (54%) were in situ melanomas, 23 (40%) were stage I, and 3 (5%) were stage II. Forty patients (80%) had multiple primary melanoma, and the 10 individuals (20%) with a single melanoma were members of families with hereditary cancer syndrome. Ten patients (20%) were *CDKN2A* mutation carriers.

The mean (SD) Breslow thickness was 0.79 (0.75) mm, and the median thickness was 0.52 mm. The mean (SD) general TDS was 6.2 (0.7), the mean (SD) Barcelona general RCM score was 1.0 (1.0), and the mean (SD) Pellacani general RCM score was 5.0 (1.7). Melanomas arising in patients with multiple primary melanoma had a significantly lower mean (SD) TDS than single melanomas developed in a familial context (6.0 [0.6] vs 7.0 [0.4], $P < .001$).

The classification of melanomas according to morphologic subtypes via confocal analysis resulted in 23 dendritic cell types (40%), 21 round cell types (37%), 2 dermal nest types (4%), 2 combined types (4%), and 9 nonclassifiable types (16%) (**Table 1** and **Figure 1**). The description of the data of the 3 less

Figure 1. Reflectance Confocal Microscopy Morphologic Presentation According to Confocal Microscopy Classification



A, Reflectance confocal microscopy reveals dendritic cells with large projections and nucleus within upper epidermis layers seen as an atypical cobblestone pattern; B, small round nucleated cells with refractive cytoplasm and dark nucleus in an atypical honeycomb epidermal pattern; C, large cerebriform nests formed by confluent aggregates of low reflecting cells, brainlike in appearance; D, pleomorphic cellular population with dendritic and round cells within a disarranged epidermis; and E, dermoepidermal junction with ringed pattern, edged and nonedged papillae with irregular junctional nests, and mild cytologic atypia in the basal layer.

Table 2. Association Between Reflectance Confocal Microscopy Dendritic Cell and Round Cell Melanoma Types and Clinical and Epidemiologic Findings[a]

Variable	Dendritic Cell	Round Cell	*P* Value
Female	12/23 (52.2)	15/21 (71.4)	.19
Age, mean (SD), y	60.4 (17.5)	49.9 (15.6)	.04[b]
Age at first CM, mean (SD), y	53.6 (17.5)	43.8 (14.8)	.053
Multiple CMs	19/23 (82.6)	16/21 (76.2)	.72
Familial CMs	8/23 (34.8)	13/21 (61.9)	.07
Phototype			
1	0/23 (0)	5/21 (23.8)	.02[b]
2	13/23 (56.5)	9/21 (42.9)	.36
3	10/23 (43.5)	6/21 (28.6)	.30
4	0/23 (0)	0/21 (0)	NA
5	0/23 (0)	1/21 (4.8)	.48
Nevus count >100	8/22 (36.4)	8/16 (50.0)	.40
Solar exposure, y			
<10	9/23 (39.1)	10/21 (47.6)	.57
10-18	11/23 (47.8)	7/21 (33.3)	.33
>18	10/23 (43.5)	2/20 (10.0)	.02[b]
Solar lentigo, moderate to severe	7/18 (38.9)	1/15 (6.7)	.046[b]

Abbreviations: CM, cutaneous melanoma; NA, not applicable.

[a] Data are presented as number/total number (percentage) of patients unless otherwise indicated.

[b] $P < .05$ (Pearson χ^2, Fisher exact, and *t* tests).

representative types (dermal nest, combined, and nonclassifiable) is given in Table 1 and eTable 2 in the Supplement.

The interobserver κ index was moderate to very good for single confocal features included in RCM melanoma scores, ranging from 0.458 to 1.000 (eTable 1 in the Supplement).

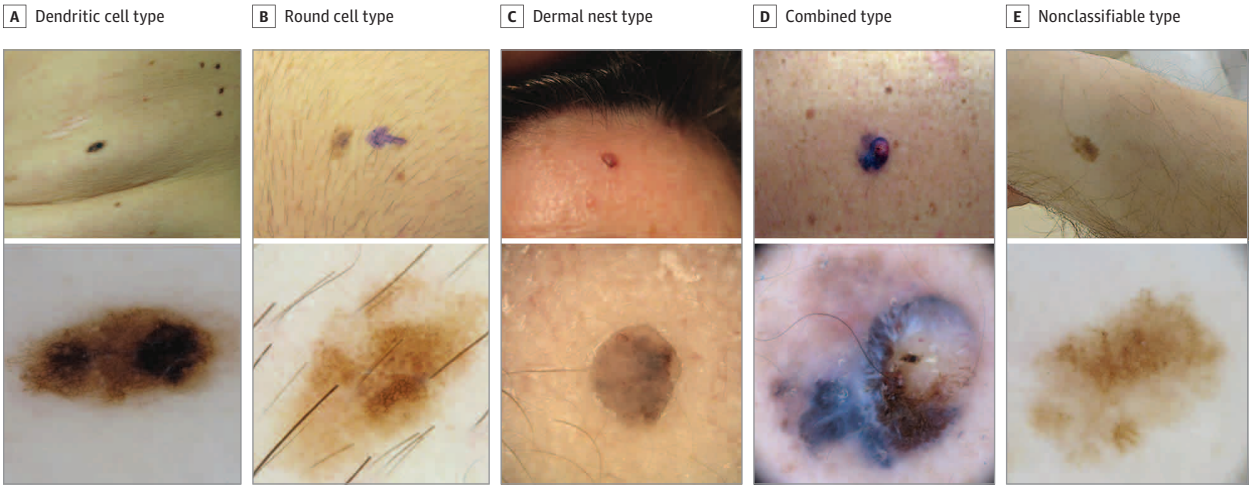
Description of Melanomas by RCM Pattern

Individuals with dendritic cell melanoma were a mean of 10.5 years older than patients with round cell melanoma (60.4 [17.5] years vs 49.9 [15.6] years, $P = .04$). Patients were older at first melanoma diagnosis in the dendritic cell group compared with the round cell group (53.6 [17.5] years vs 43.8 [14.8] years, $P = .053$). All patients with dendritic cell melanoma had phototype 2 or 3 disease. Compared with round cell type, patients had a more intense history of solar exposure after 18 years of age (10 of 23 [44%] vs 2 of 20 [10%], $P = .02$) and moderate to severe solar lentigines (7 of 18 [39%] vs 1 of 15 [7%], $P = .046$). Of the patients with dendritic cell melanoma, 14 of 22 (64%) had fewer than 100 nevi, and 15 of 23 (65%) had no family history of melanoma or pancreatic cancer. The frequency of *CDKN2A* mutations in this group was 5 of 23 (22%), and at least 1 *MC1R* variant allele was present in 16 of 23 patients (70%) (with or without the red hair phenotype). The comparative epidemiologic and clinical data between the 2 most prevalent types of melanoma on RCM, dendritic and round cell types, are given in **Table 2**.

Dendritic cell melanomas were located mainly on the trunk (10 of 23 [44%]) and extremities (7 of 23 [41%]). Although not the most frequent location, the dendritic cell type was the most prevalent confocal type in facial lesions (5 of 6 lesions [83%]). Most of the dendritic cell melanomas were between 5 and 10 mm. The most common histologic subtype found in dendritic cell tumors was superficial spreading melanoma (18 of 23 [78%]). However, the confocal pattern of dendritic cell melanoma was significantly associated with the lentigo maligna histologic subtype compared with other RCM types (5 of 6 [83]% vs 1 of 6[17%], $P = .03$). The mean (SD) tumor thickness in the dendritic cell type was 0.5 (0.1) mm. More than half (13 of 23 [57%]) of dendritic cell lesions were in situ melanomas. Regarding dermoscopic features, dendritic cell melanomas had an atypical pigmented network (18 of 23 [78%]) and atypical dots and globules (22 of 23 [96%]) (**Figure 2**). A multicomponent pattern was seen in 5 of 23 lesions (22%). The mean (SD) TDS of the dendritic cell type was 6.3 (0.8), which was not statistically different from the general score (mean [SD], 6.1 [0.6]; $P = .58$). The Barcelona confocal score achieved a mean (SD) value of 1.0 (1.0), and the mean (SD) Pellacani score was 4.9 (1.7).

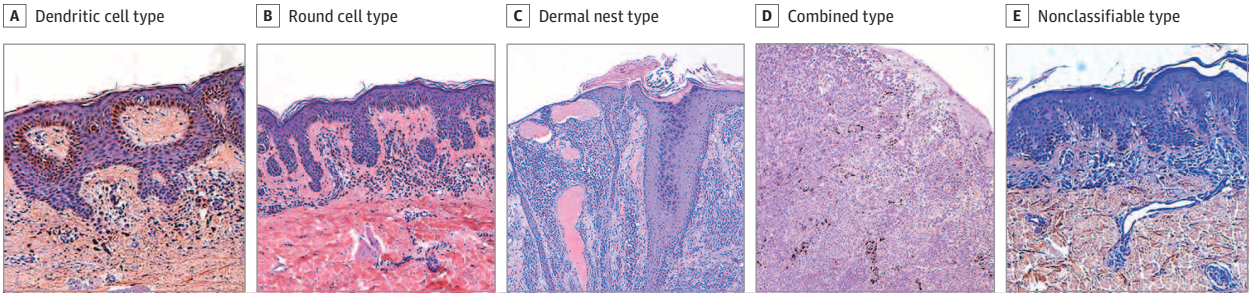
With confocal microscopy, dendritic cell melanomas frequently exhibited pagetoid cells larger than 20 µm (23 of 23 [100%] vs 21 of 34 [62%], $P = .001$) and pleomorphic cellular populations (20 of 23 [87%] vs 16 of 34 [47%], $P = .002$). Dendritic cell melanomas also presented a smaller nucleus of less than 10 µm (19 of 23 [83%] vs 11 of 34 [32%], $P < .001$) and a

Figure 2. Clinical and Dermoscopic Presentation According to Confocal Microscopy Classification



A (top), Dermoscopy reveals a melanocytic macular lesion on the left lateral trunk; A (bottom), reticular global pattern with atypical pigment network; B (top) melanoma on the right arm; B (bottom) globular pattern with irregular dots and globules and focal atypical network; C (top) slightly erythematous papular lesion on the frontal region; C (bottom) unspecific pattern with sparse atypical globules and vessels; D (top) palpable tumor on the infraescapular region; D (bottom) multicomponent pattern with atypical globules, blue whitish veil, blue regression, and short white streaks; E (top) flat melanocytic lesion on the forearm; and E (bottom) reticular pattern with atypical pigment network, structureless areas, and segmental streaks.

Figure 3. Histopathologic Presentation According to Confocal Microscopy Classification



A, Histopathologic presentation of an in situ superficial spreading melanoma; B, an in situ nevus-associated superficial spreading melanoma; C, a 1.1-mm-thick nevoid melanoma; D, an ulcerated 3.0-mm-thick superficial spreading melanoma; and E, a 0.2-mm-thick superficial spreading melanoma with more than 50% regression. Hematoxylin-eosin, original magnification ×100 (A), ×100 (B), ×40 (C), ×40 (D), and ×100 (E).

central ringed pattern more often than the other types (7 of 23 [30%] vs 3 of 34 [9%], $P = .04$) (eTable 2 in the Supplement).

Round cell melanomas were identified more often in a familial context (13 of 21 [61.9%]) than dendritic cell melanomas (8 of 23 [34.8%], $P = .07$). Phototype 1 was more prevalent than in the dendritic cell type (5 of 21 [24%] vs 0 of 23, $P = .02$), and 8 of 16 patients (50%) in this group had more than 100 nevi (Table 2). The most common location of round cell melanomas was on the trunk (13 of 21 [62%]). Most round cell melanomas (13 of 21 [62%]) were between 5 and 10 mm in diameter and were of the superficial spreading histologic subtype (15 of 17 [88%]) (Table 1). Most round cell melanomas (13 of 21 [62%]) were in situ, and the mean (SD) tumor thickness was 0.6 (0.5) mm (**Figure 3**). Only 3 round cell melanomas (14%) occurred in a *CDKN2A* mutation carrier. The frequency of any *MC1R* variant allele in this melanoma type was 80%. The most prevalent dermoscopic findings in round cell melanomas were atypical pigment network (17 of 21 [81%]) and atypical dots and globules (21 of 21 [100%]) (Figure 2). The global dermoscopic pattern was classified as multicomponent in 5 of 21 lesions (24%). The TDS presented a mean (SD) value of 6.4 (0.6). The mean (SD) RCM Barcelona score of 1.4 (0.7) was statistically higher than other groups (mean [SD], 0.7 [1.1]; $P = .006$). The mean (SD) round cell Pellacani score was 5.4 (1.1). Single confocal features, such as junctional thickening, dense dermal nests, and nucleated cells within papillary dermis, were more frequently found in round cell melanomas (17 of 21 [81%] vs 16 of 36 [44%], $P = .007$; 8 of 21 [38%] vs 4 of 36 [11%], $P = .02$; and 14 of 21 [67%] vs 13 of 36 [36%], $P = .03$, respectively) (eTable2 in the Supplement).

No significant association was found between the presence of *CDKN2A* mutations and a specific confocal melanoma type being any RCM melanoma type present in *CDKN2A* carriers. The presence of at least 1 *MC1R* variant was associated with widespread pagetoid cells within the epidermis compared with wild-type status (12 of 42 [29%] vs 0 of 14, $P = .03$).

Discussion

In the present study performed on multiple primary and familial melanomas, we stratified melanomas into the 5 confocal types previously described; it was possible to associate specific phenotypic features with some types of melanomas. Dendritic cell melanomas appeared in older patients with a history of a more intense solar exposure and moderate to severe solar lentigines as previously described by Bassoli et al.[4] Round cell melanomas had a predisposition for fair-skinned individuals and tended to occur with a family history of melanoma. However, in this study, the association with a high nevus count or the presence of atypical nevi was not observed in the round cell melanoma type, as previously reported.[11]

Dendritic cell and round cell melanomas were not different regarding tumor thickness or tumor size, differing from that previously described.[11] These findings reinforce the hypothesis that at least 2 different routes of melanoma development exist. The first was associated with multiple nevi, predominance on the trunk, and younger age at onset, characterized via RCM by round cell pattern. The second was associated with long-term sun damage and older age at onset, characterized via RCM as a dendritic cell type. A third possible, less frequent route may exist, characterized by thick and fast-growing tumors with a nested pattern via confocal microscopy. More advanced lesions had a multicomponent pattern, probably reflecting lesion evolution with 1 of the 3 previous patterns. Finally, lesions with unspecific pattern are probably very early melanomas in which the pattern is as yet undefined.[21]

Mean Barcelona score was higher in round cell type, probably because of the higher frequency of atypical nucleated dermal cells. These findings support the behavior of round cell melanomas to aggregate into clusters at the dermal-epidermal junction and papillary dermis and also to spread as isolated cells into the dermis. The dendritic cell melanomas, adjusting for tumors of similar stage and thickness, may take a longer time for single cells to aggregate into nests and to disseminate into the dermis, possibly representing slow-growing melanomas as previously proposed.[11]

Considering TDS, RCM score, and tumor thickness, it is possible that the combined and nonclassifiable melanomas represent opposite ends of a morphologic spectrum. In fact, milder morphologic expression in dermoscopy and confocal examination of nonclassifiable type is characteristic of early-stage tumors, which are frequently difficult to diagnose by in vivo techniques.

CDKN2A mutation carriers may develop any RCM type of melanoma, even those less represented as dermal nests. It is possible that the low sample size was not able to demonstrate any differences in the distribution between different types, if they actually exist.

Conclusions

Specific phenotypic features of patients with multiple primary and familial melanomas were associated with some types of melanomas based on confocal microscopy classification. Morphologic expression of melanomas under dermoscopy and confocal examination may be associated with differences in tumor stage and biological behavior. Future studies are necessary to enhance our knowledge of the interaction of multiple coexisting causal factors that drive melanoma development.

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Author Affiliations: Hospital Clinic I Provincial de Barcelona, Universitat de Barcelona and Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain (Grazziotin, Alarcon, Carrera, Potrony, Aguilera, Puig-Butillé, Brito, Badenas, Alós, Malvehy, Puig); Department of Dermatology, Post-Graduation Program in Pathology, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil (Grazziotin, Bonamigo); Pontifícia Universidade Católica do Rio Grande do Sul, School of Medicine, Porto Alegre, Brazil (Grazziotin); Centro de Investigación Biomédica en Red en Enfermedades Raras, Barcelona, Spain (Carrera, Aguilera, Puig-Butillé, Badenas, Malvehy, Puig).

Author Contributions: Drs Grazziotin and Puig had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Grazziotin, Bonamigo, Badenas, Puig.
Acquisition, analysis, or interpretation of data: Grazziotin, Alarcon, Carrera, Potrony, Aguilera, Puig-Butillé, Brito, Badenas, Alós, Malvehy, Puig.
Drafting of the manuscript: Grazziotin, Puig, Bonamigo, Brito, Badenas.
Critical revision of the manuscript for important intellectual content: Grazziotin, Alarcon, Bonamigo, Carrera, Potrony, Aguilera, Puig-Butillé, Alós, Malvehy, Puig.
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REFERENCES

1. Viros A, Fridlyand J, Bauer J, et al. Improving melanoma classification by integrating genetic and morphologic features. *PLoS Med*. 2008;5(6):e120.

2. Carrera C, Palou J, Malvehy J, et al. Early stages of melanoma on the limbs of high-risk patients: clinical, dermoscopic, reflectance confocal microscopy and histopathological characterization for improved recognition. *Acta Derm Venereol*. 2011;91(2):137-146.

3. Alarcon I, Carrera C, Palou J, Alos L, Malvehy J, Puig S. Impact of in vivo reflectance confocal microscopy on the number needed to treat melanoma in doubtful lesions. *Br J Dermatol*. 2014;170(4):802-808.

4. Bassoli S, Maurichi A, Rodolfo M, et al. CDKN2A and MC1R variants influence dermoscopic and confocal features of benign melanocytic lesions in multiple melanoma patients. *Exp Dermatol*. 2013;22(6):411-416.

5. Lovatto L, Carrera C, Salerni G, Alós L, Malvehy J, Puig S. In vivo reflectance confocal microscopy of equivocal melanocytic lesions detected by digital dermoscopy follow-up. *J Eur Acad Dermatol Venereol*. 2015;29(10):1918-1925.

6. Goldstein AM, Chan M, Harland M, et al; Lund Melanoma Study Group; Melanoma Genetics Consortium (GenoMEL). Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. *J Med Genet*. 2007;44(2):99-106.

7. Badenas C, Aguilera P, Puig-Butillé JA, Carrera C, Malvehy J, Puig S. Genetic counseling in melanoma. *Dermatol Ther*. 2012;25(5):397-402.

8. Nelson AA, Tsao H. Melanoma and genetics. *Clin Dermatol*. 2009;27(1):46-52.

9. Potrony M, Badenas C, Aguilera P, et al. Update in genetic susceptibility in melanoma. *Ann Transl Med*. 2015;3(15):210.

10. Cuéllar F, Puig S, Kolm I, et al. Dermoscopic features of melanomas associated with *MC1R* variants in Spanish *CDKN2A* mutation carriers. *Br J Dermatol*. 2009;160(1):48-53.

11. Pellacani G, De Pace B, Reggiani C, et al. Distinct melanoma types based on reflectance confocal microscopy. *Exp Dermatol*. 2014;23(6):414-418.

12. Argenziano G, Soyer HP, Chimenti S, et al. Dermoscopy of pigmented skin lesions: results of a consensus meeting via the Internet. *J Am Acad Dermatol*. 2003;48(5):679-693.

13. Nachbar F, Stolz W, Merkle T, et al. The ABCD rule of dermatoscopy: high prospective value in the diagnosis of doubtful melanocytic skin lesions. *J Am Acad Dermatol*. 1994;30(4):551-559.

14. Pellacani G, Cesinaro AM, Seidenari S. Reflectance-mode confocal microscopy of pigmented skin lesions: improvement in melanoma diagnostic specificity. *J Am Acad Dermatol*. 2005;53(6):979-985.

15. Segura S, Puig S, Carrera C, Palou J, Malvehy J. Development of a two-step method for the diagnosis of melanoma by reflectance confocal microscopy. *J Am Acad Dermatol*. 2009;61(2):216-229.

16. Puig S, Ruiz A, Castel T, et al. Inherited susceptibility to several cancers but absence of linkage between dysplastic nevus syndrome and CDKN2A in a melanoma family with a mutation in the CDKN2A (P16INK4A) gene. *Hum Genet*. 1997;101(3):359-364.

17. Puig S, Malvehy J, Badenas C, et al. Role of the *CDKN2A* locus in patients with multiple primary melanomas. *J Clin Oncol*. 2005;23(13):3043-3051.

18. Badenas C, Torra R, San Millán JL, et al. Mutational analysis within the 3′ region of the PKD1 gene. *Kidney Int*. 1999;55(4):1225-1233.

19. Puig-Butillé JA, Carrera C, Kumar R, et al. Distribution of *MC1R* variants among melanoma subtypes: p.R163Q is associated with lentigo maligna melanoma in a Mediterranean population. *Br J Dermatol*. 2013;169(4):804-811.

20. Potrony M, Puig-Butillé JA, Aguilera P, et al. Increased prevalence of lung, breast, and pancreatic cancers in addition to melanoma risk in families bearing the cyclin-dependent kinase inhibitor 2A mutation: implications for genetic counseling. *J Am Acad Dermatol*. 2014;71(5):888-895.

21. Zalaudek I, Marghoob AA, Scope A, et al. Three roots of melanoma. *Arch Dermatol*. 2008;144(10):1375-1379.

NOTABLE NOTES

History of Podophyllin

Ismaël Maatouk, MD

Podophyllotoxin is the principal active compound from the resin mixture known as podophyllin. It is obtained from 1 of the 3 species of *Podophyllum*: *P peltatum* (from North America), *P hexandrum* (from India; previously referred to as *P emodi*), or *P pleianthum* (from Taiwan).[1] *Podophyllum peltatum* is an indigenous, North American, herbaceous perennial flowering in May and bearing fruit in late summer or fall. Other common names include May apple, mandrake, Indian apple, wild lemon, and duck's foot.[1]

One of the first recorded medicinal uses of this agent, mentioned in the pre-Conquest English medical book the *Leech Book of Bald* (AD 900-950), was in a salve for cancer.[1-3]Catesby, in his *Natural History of the Carolinas* (1731), described the May apple and noted that the root is "said to be an excellent emetic and is used as such in the Carolinas." Jacques Cartier reported usage of this agent both as a mortal poison and as a topical antidote for snake venom.[1-3] The root was considered both a medicine and a poison by the North American Indians, and it was used as a suicide agent among the Iroquois.[1,3] John Uri Lloyd stated that *Podophyllum* was used by the Cherokees for deafness and as an anthelmintic, and by the Wyandottes and Southern Indians as a cathartic.[1]

The early colonists learned of the medical properties of the root from the Indians, and it was used as a cathartic in the first *United States Pharmacopoeia* (1820).[1-3] It was listed until the 12th revision (1942), from which it was dropped. Stories of the new American drug spread to England and the continent of Europe.[1-3]The resin, podophyllin, was first separated from *Podophyllum* by John King in 1835.[1-3] With the preparation of podophyllin on a commercial scale in 1850 by Merrell the use of the resin supplanted that of the crude *Podophyllum*.[1] Between 1863 (4th revision of the *United States Pharmacopoeia*) and 1942 (12th revision), podophyllin was reported to be a cathartic, purgative, deobstruent, vermifuge, hydragogue, cholagogue, choleretic, and expectorant. It was recommended, either alone or in combination with other herbs, for diseases of the liver and kidneys, for scrofula, syphilis, gonorrhea, obstructed menstruation, urinary obstruction, dropsy, and coughs.[1-3]

With the increased production of podophyllin, reports of its toxic properties appeared in the literature: pain in the eyes; hyperemia of the iris, cornea, mucous membranes, and eyelids; and erythematous eruptions of the scrotum. Moreover, oral and parenteral administration of podophyllin has been followed by serious results. Topical podophyllin was introduced in 1942 and is still accepted today as an effective treatment for condyloma acuminatum.[3]

Author Affiliation: Department of Dermatology, Keserwan Medical Center affiliated with AUBMC, Beirut, Lebanon.

Corresponding Author: Ismaël Maatouk, MD, Department of Dermatology, Keserwan Medical Center affiliated with AUBMC, Beirut, Lebanon (ismaelmaatouk@gmail.com).

1. Lloyd JU. *Origin and History of All the Pharmacopeial Vegetable Drugs*. Christchurch, New Zealand: Caxton Press; 1929.

2. Kelly M, Hartwell JL. The biological effects and the chemical composition of podophyllin: a review. *J Natl Cancer Inst*. 1954;14(4):967-1010.

3. Nelson LM. Use of podophyllin (podophyllum resin) in dermatology. *AMA Arch Derm Syphilol*. 1953;67(5):488-495.