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Published Version:

Moscarella E., Pellegrini C., Pampena R., Argenziano G., Manfredini M., Martorelli C., et al. (2019). Dermoscopic similarity is an independent predictor of BRAF mutational concordance in multiple melanomas. EXPERIMENTAL DERMATOLOGY, 28(7), 829-835 [10.1111/exd.13951].

Availability:

This version is available at: <https://hdl.handle.net/11585/801137> since: 2021-02-18

Published:

DOI: <http://doi.org/10.1111/exd.13951>

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Experimental dermatology 2019, 28 (7) 829-835

The final published version is available online at:

<https://doi.org/10.1111/exd.13951>

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DERMOSCOPIC SIMILARITY IS AN INDEPENDENT PREDICTOR OF *BRAF* MUTATIONAL CONCORDANCE IN MULTIPLE MELANOMAS

Running head: Dermoscopic similarity and BRAF concordance in multiple melanomas

Elvira Moscarella, MD;^{1*} Cristina Pellegrini, PhD;^{2*} Riccardo Pampena, MD;³ Giuseppe Argenziano, MD;¹ Marco Manfredini, MD;⁴ Claudia Martorelli, PhD;² Alessia Ciarrocchi, MD;⁵ Emi Dika, MD;⁶ Ketty Peris, MD;⁷ Ambra Antonini, MD;² Gianluca Cipolloni, MD;⁸ Roberto Alfano, MD;⁹ Caterina Longo, MD;^{3,4} Maria Concetta Fagnoli, MD²

*These authors equally contributed to this work and should be considered co-first authors

Dermatology Unit, University of Campania “Luigi Vanvitelli”, Naples, Italy; ²Department of Dermatology, University of L’Aquila, L’Aquila, Italy; ³Centro Oncologico ad Alta Tecnologia Diagnostica, Azienda Unità Sanitaria Locale – IRCCS di Reggio Emilia, Italy; ⁴Dermatology Unit, University of Modena and Reggio Emilia, Modena, Italy; ⁵Laboratory of Translational Research, Research and Statistic Infrastructure, Arcispedale Santa Maria Nuova-IRCCS, Reggio Emilia, Italy; ⁶ Dermatology, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Italy; ⁷ Institute of Dermatology, Catholic University, Rome, Italy;

⁸Department of Pathology, San Salvatore Hospital, L'Aquila, Italy; ⁹Department of Anesthesiology, Surgery and Emergency, Second University of Naples, Naples, Italy.

Corresponding author: Maria Concetta Fagnoli, MD, Department of Dermatology, University of L'Aquila, Via Vetoio, Coppito, 67100 L'Aquila, Italy. Tel: +39-0862-368519; Fax: +39-0862433433; email: mariaconcetta.fagnoli@univaq.it

ABSTRACT

Background: The association of clinical and dermoscopic features with *BRAF* mutational status has been poorly analysed in multiple primary melanomas (MPM).

Objective. To investigate if concordance of *BRAF* mutational status is associated with dermoscopic similarity in multiple melanomas of the same patient.

Methods. Dermoscopic images and corresponding tissue sections of 124 melanomas from 62 MPM patients were selected at 4 Italian Dermatology Departments. Similarity of dermoscopic appearance between multiple melanomas was evaluated according to the presence of the same prevalent dermoscopic feature. The *BRAF*^{V600} mutational status was analysed with allele-specific TaqManTM assays or pyrosequencing. Spearman's correlation, univariate and multivariate regression analysis were used for statistical analysis.

Results. A similar dermoscopic appearance was identified in 38.7% (24/62) of MPM patients and was correlated with older age at first diagnosis ($\rho: .26$; $p: .042$) and occurrence on sun-damaged skin ($\rho: .27$; $p: .037$). The *BRAF*^{V600} mutation was detected in 39.5% (49/124) of the tumours and a concordant *BRAF* mutational status between melanomas in 33/62 (53.2%) MPM patients. Dermoscopically similar melanomas showed 5.7-fold higher odds to be concordant for *BRAF* mutational status compared to dissimilar lesions (OR:5.7; 95%CI 1.7-19.5; $p: .005$).

Conclusion. Dermoscopic similarity of multiple melanomas represents an independent clinical predictor of a concordant *BRAF* mutational status in MPM patients.

Key words: multiple primary melanomas; dermoscopy; molecular analyses; *BRAF*.

INTRODUCTION

Multiple primary melanomas (MPM) are diagnosed in 1.2% to 8.2% of sporadic melanoma patients. [1,2] Patients with melanoma are at approximately 9-fold increased risk of developing an additional primary melanoma compared to the general population.[1,3-4] The risk is higher within the first year but remains elevated for more than 20 years after first diagnosis supporting a continued long-term surveillance in melanoma patients.[1,2,5]

Our group previously demonstrated that MPMs in a patient have almost the same chance of looking dermoscopically similar or different.[6] Synchronicity, older age, presence of sun damage, and comparable melanoma thickness were identified as clinical factors that may influence the occurrence of dermoscopically similar MPMs. [6]

Melanoma arises through the gradual accumulation of somatic abnormalities in specific driver genes, including *BRAF*, *NRAS*, *NF-1* and *c-KIT*. [7] The most common oncogenic alteration is a hot-spot mutation involving codon 600 of the *BRAF* gene, mainly the V600E change, occurring in 40%-50% of cutaneous melanomas. [8] A concordant *BRAF* mutational status (either V600-mutated or wild-type) between multiple melanomas within the same patient has been recently identified in 50% to 60% of patients, [9-12] independently of location and time of melanoma occurrence. This supports the hypothesis of molecular heterogeneity of multiple melanomas in MPM patients and has great impact in clinical practice when it is necessary to molecularly identify MPM subjects with discordant multiple melanomas for eligibility to target therapy with *BRAF* inhibitors.

BRAF mutational status has been associated with dermoscopic appearance of sporadic primary melanoma in a few studies. [13-15] Two observational retrospective studies showed a correlation between *BRAF* mutations and the presence of 'peppering', irregular streaks and ulceration. [13,14] In addition, Armengot-Carbò et al. [15] recently found an association of younger age at diagnosis and dermoscopic blue-white with *BRAF* mutation and developed a model based on these two features predicting with good accuracy the occurrence of *BRAF* mutational status. However, there is no knowledge about the association between dermoscopic aspects of multiple melanomas and *BRAF* mutational status. [16]

Herein, we investigated if dermoscopic similarity of multiple melanomas in the same patient is associated with concordance of *BRAF* mutational status in order to identify potential morphological predictors of *BRAF* status in subsequent melanomas, thus improving our understanding of the pathogenesis of multiple melanomas. We further analysed if specific clinico-pathological features of patients or melanomas were associated with dermoscopic similarity of multiple melanomas.

MATERIALS AND METHODS

Study population

Consecutive patients with MPM were retrospectively selected at 4 Dermatology Units in Italy (L'Aquila, Reggio Emilia, Bologna and Rome). Inclusion criteria were the availability of clinical and dermoscopic images and corresponding formalin-fixed paraffin-embedded (FFPE) tissue sections of the first and second melanoma of each MPM patient. In patients with more than 2 melanomas, only the first two were evaluated. Demographic and clinical data of patients (age at the first diagnosis and sex) as well as tumour information (anatomical site, Breslow thickness) and presence of sun damage at melanoma site (based on the presence of solar elastosis and thinning of the epidermis on histological slides) were retrieved.

Furthermore, data regarding synchronicity (defined as the occurrence of the subsequent primary lesion within 3 months after the first melanoma diagnosis) and site concordance (defined as the occurrence of multiple primaries on the same anatomical area) were tabulated for each patient. For concordance analysis, anatomical areas were grouped as follows: head/neck, thorax, back, abdomen, lower limbs and upper limbs. Melanomas located on acral sites and lentigo maligna melanomas were excluded.

Dermoscopic evaluation

All images were evaluated in a random order by consensus of two expert dermoscopists (E.M., M.M.) for the presence of global aspects such as asymmetry, number of colours and dermoscopic structures. [17,18] The following eight dermoscopic criteria were evaluated: atypical network, inverse network, regression structures, irregular dots/globules, streaks, structureless pigmentation, irregular vessels and blue-white veil. [18]

Evaluators were asked to indicate if a dermoscopic feature was present and if it was the prevalent criterion in any given lesion. The prevalent criterion was considered as the most relevant dermoscopic feature for melanoma diagnosis and/or covering more than 40% of lesion surface. Two experienced observers (E.M., M.M.) analysed dermatoscopic features and a third investigator (C.L.) was involved in case of disagreement. Similarity of dermoscopic appearance between multiple melanomas was defined as the detection of the same prevalent dermoscopic feature in both melanomas.

Then, the association between dermoscopic similar appearance and *BRAF* mutational status (*BRAF* mutated concordance; *BRAF* wild type concordance; *BRAF* discordance) of the two melanomas was evaluated for each patient.

***BRAF* molecular analysis**

Genomic DNA was obtained by microdissection from 3 FFPE melanoma tissue sections (each of 10-micron in thickness) using a QIAmp Micro tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

The *BRAF*^{V600} mutational status was analysed by competitive allele-specific TaqManTM PCR for the detection of *BRAF*^{V600E} and *BRAF*^{V600K} mutations in multiple melanomas of 33 MPM patients (from L'Aquila and Rome) or by pyrosequencing in tissue samples of 29 MPM patients (Reggio Emilia and Bologna).

For competitive allele-specific TaqManTM PCR, the reaction containing 20 ng of DNA, 1X TaqManTM Mutation Detection Assays (assay Hs00000111_mu for *BRAF*^{V600E}; Hs000000002_rm for *BRAF*^{V600K}), 1X TaqManTM Genotyping Master Mix (Thermo-Fisher, Foster City, USA) and water to reach the final volume of 20 µl was amplified using the standard TaqMan protocol on 7500 Fast Real Time-PCR System (Thermo-Fisher).¹² The ΔC_t cut-off was calculated using 5 DNA samples extracted from FFPE sections of melanomas positive for *BRAF*^{V600E} or *BRAF*^{V600K} and 3 extracted from FFPE sections of normal skin of unaffected individuals.

For pyrosequencing, amplicons harbouring the position of *BRAF*^{V600} were obtained by PCR, using 50-100 ng genomic DNA, forward and reverse 5-biotinylated primers at the concentration of 0.5 µM, and 2U of Taq Gold Polymerase (Promega, Milan, Italy). [19] Preparation of the single-stranded DNA template was performed using the PSQ 96-sample preparation kit and the PSQ vacuum prep tool (Diatech, Ancona, Italy) according to the manufacturer's instructions. [20] The sequencing-by-synthesis reaction of the complementary strand was automatically carried out on a PyroMark Q96 ID instrument using PyroGold reagents (Diatech). Percentage of the mutated allele was then calculated by PyroMark Q96 ID software using the allele quantitation mode. Samples were considered *BRAF* mutated when the percentage of the mutated allele was above the threshold of 5%. Each run included no template control and positive and negative controls obtained by amplifying DNA extracted from BCPAP and TPC1 human cell lines, known to be homozygous and wild type for the *BRAF*^{V600E} mutation, respectively.

All experiments were performed in duplicate for both allele-specific PCR and pyrosequencing.

Statistical analysis

Scale variables were first checked for normality through Smirnov-Kolmogorov test and then compared through Student T test or Mann-Whitney U test. Pearson χ^2 test was used for qualitative variables. Absolute and relative frequencies were calculated for clinical and dermoscopic criteria.

To assess which factors, among demographic, clinical and tumour-related variables, were independently associated with the concordance of *BRAF* mutational status, Pearson χ^2 test and Spearman's rho correlation were first used to flag significant correlations, which were then quantified via univariate logistic regression analysis. A multivariate forward logistic regression model was finally constructed to assess independent predictors of *BRAF* mutational status concordance among the variables that showed a significant difference ($p < .10$) on univariate analysis, together with the notable intervariable interactions. Alpha level was set at 0.05.

Statistical analyses were performed using the IBM SPSS 22.0 package (Statistical Package for Social Sciences, IBM SPSS Inc., Chicago, Ill).

RESULTS

Study population

A total of 62 MPM patients (36 males and 26 females) were enrolled in this study (Supplementary Table 1). The majority of patients (48/62, 77.4%) were diagnosed with 2 melanomas, 11 with 3 melanomas (17.8%); 2 with 4 melanomas (3.2%) and 1 with 5 (1.6%). Mean age at the time of first melanoma diagnosis was 53 ± 18 years.

Overall, 124 melanomas with available dermoscopic images and FFPE tissue sections were evaluated (Supplementary Table 1). Seventy of 124 (56.4%) were invasive and the median Breslow thickness was 0.5 mm (range 0.3-0.8), with 0.6 mm (range 0.2-2.6) as the median thickness of the first melanomas and 0.48 mm (range 0.2-1.8) of the second

tumour. Regarding anatomical site, 50 melanomas (40.3%) were located on the back, 31 (25.0%) on the lower limbs, 17 on the upper limbs (13.7%), 12 on the abdomen (9.7%), 10 on the thorax (8.1%) and 4 (3.2%) on the head/neck region. The anatomical region of the first and second melanoma was the same in 21 patients (21/62, 33.9%).

Moreover, 18 patients (18/62, 29.0%) were diagnosed with synchronous melanomas and half of the patients (31/62, 50.0%) exhibited sun-damaged skin at melanoma site.

Dermoscopic evaluation

Dermoscopic examination of the 124 melanomas showed that the most frequently prevalent features were regression structures (46/124, 37.1%) and atypical network (40/124, 32.3%), followed by structureless pigmentation (12/124, 9.7%), irregular vessels (8/124, 6.5%), inverse network and blue-white veil (6/124, 4.8% each), irregular dots/globules (4/124, 3.2%) and streaks (2/124, 1.6%). Complete data on dermoscopic evaluation are reported in Supplementary Table 2. Asymmetry in two axes was observed in 82 melanomas (82/124, 66.1%); while 50 lesions (50/124, 40.3%) showed 3 colours, 42 (42/124, 33.9%) had 2, 28 (28/124, 22.6%) had 4 and 4 (4/124, 3.2%) only one colour.

Based on dermoscopic evaluation, we categorized each MPM patient as having multiple melanomas either similar or dissimilar, according to the prevalent criterion.

In detail, the same prevalent dermoscopic criterion between first and second melanoma was observed in 24 of 62 (38.7%) MPM patients. The prevalent dermoscopic criterion was represented by regression or atypical network in 11 of 24 cases each and by blue-white-veil and inverse network in 1 case each. Dermoscopic similarity between multiple melanomas in the same patient was correlated with an older age at first diagnosis [Spearman's coefficient (ρ):.26; p :.042] and with the occurrence of melanomas on sun-damaged skin (ρ):.27; p :.037). Conversely, sex, site concordance, Breslow thickness and synchronicity were not significantly correlated with the occurrence of dermoscopically similar or different melanomas (ρ):-.14, p :.283; ρ):-.08, p :.542; ρ):-.10, p :.445; ρ):.22, p :.084; respectively).

***BRAF* mutational status**

BRAF^{V600} mutations were detected in 39.5% (49/124) of melanomas with the following genotypes: V600E (48/49, 97.9%) and V600K (1/49, 2.1%) (Supplementary Table 1). No significant correlation was found between *BRAF* mutational status and Breslow thickness (ρ :-.16 p :.08). Concordance of *BRAF* mutational status between melanomas was observed in 33 of 62 (53.2%) MPM patients: both melanomas carried the *BRAF*^{V600E} mutation in 10 patients or were *BRAF* wild-type in 23 patients. In the remaining 29 (46.8%), there was no consistency between the mutation status of the first and subsequent melanoma. Illustrative cases of MPMs showing concordant or discordant *BRAF* mutational status are shown in Figs 1-3.

***BRAF* mutational status and clinico-dermoscopic features of MPM**

Results of the association analysis and of the Spearman's correlation between clinico-dermoscopic variables and *BRAF* mutational status concordance are detailed in Table 1.

Synchronous melanomas were significantly more prevalent in patients with a *BRAF* concordant status than in those with a discordant status (14/33, 42.4% vs. 4/29, 13.8%; p :.028, respectively) with a significant correlation between these variables (ρ :.32; p :.013).

Concordance in the number of colours was more frequently observed among melanomas with a concordant *BRAF* mutational status as compared with those discordant (17/33, 51.5% vs. 7/29, 24.1%, respectively) with a borderline significance (χ^2 test p value: .052). However, since a significant correlation was observed between the variable "number of colours" and *BRAF* concordance status (ρ :.28; p :.027), we included this variable in the subsequent univariate logistic regression analysis.

No association was instead found for the other clinical variables, including age, sex, sun damage, anatomical site and asymmetry concordance.

Dermoscopically similar melanomas were significantly more frequent in the *BRAF* concordant group than in the discordant one (19/33 vs. 5/29, $p=.003$) and a significant correlation was found between the two variables.

Univariate logistic regression analysis quantified a significant association between *BRAF* concordance status and synchronicity (OR:4.6; 95%CI 1.3-16.3; $p=.018$), number of colours (OR:3.3; 95%CI 1.1-9.9; $p=.030$), and dermoscopic similarity (OR:6.5; 95%CI 2.0-21.3; $p=.002$) (Table 1).

The multivariate forward logistic regression model demonstrated that dermoscopic similarity was an independent predictor of *BRAF* mutational status concordance. Melanomas with the same prevalent criterion had a higher than 5-fold odd to be concordant for *BRAF* mutational status (OR:5.7; 95%CI 1.7-19.5; $p=.05$). In addition, in the multivariate model, the variable synchronicity only marginally reached statistical significance ($p=.052$), having synchronous melanomas a 3.8-fold odd (OR:3.8; 95%CI 1.0-14.6) of sharing the same *BRAF* mutational status compared to metachronous melanomas (Table 1).

The distribution of the prevalent criterion in dermoscopically similar melanomas showed that regression (present in 11 dermoscopically similar melanomas) was observed in 10 *BRAF* concordant (3 *BRAF*-mutated and 7 *BRAF* wild-type) and in 1 discordant case while atypical network (11 cases) in 8 *BRAF* concordant (2 *BRAF*-mutated and 6 *BRAF* wild-type) and in 3 discordant cases (Supplementary Table 3).

DISCUSSION

In our study, dermoscopic similarity was identified in 39% of MPM patients and was correlated with older age at diagnosis and occurrence on sun-damaged skin. Concordance of *BRAF* mutational status, either *BRAF* mutated or *BRAF* wild-type, between multiple melanomas was observed in 53% of MPM patients. Multiple melanomas appearing dermoscopically similar had a higher than 5-fold odd to be concordant for *BRAF* mutational status compared to dissimilar lesions.

Dermoscopically similar melanomas were detected in 38.7% of our MPM patients. In two different series of MPM patients, one from our group [6] (58 patients) and the other from Colombino et al. [10] (12 patients), a similar appearance of multiple melanomas was reported in 53% and in 66.7% of MPM patients, respectively, although differences in the criteria used to define dermoscopic similarity do not allow a direct comparison with our results.

Older age at diagnosis and occurrence of melanomas on photo-damaged skin were correlated to dermoscopic similar appearance. This is in line with our previous study reporting dermoscopic similarity in multiple melanomas of MPM patients aged >65 years, in lesions occurring synchronously or on photo-damaged skin. [6] Concordance of anatomical location did not significantly influence the occurrence of similar dermoscopic aspects in multiple melanomas in neither of the two studies.

Overall, we identified the *BRAF*^{V600} mutation in 39.5% of our samples, consistently with the frequency reported in previous studies in the Italian population. [9,12,21,22] Intra-patient concordance of the *BRAF* mutational status (either *BRAF* mutated or *BRAF* wild-type) between first and second melanoma was observed in 53.2% of our patients. A similar rate of *BRAF* mutational concordance ranging from 52.3% to 60% has been reported in previous studies. [9-12] These data support the intra-patient heterogeneity of *BRAF* mutational status in multiple melanomas of the same patient.

Of note, we first demonstrated that multiple melanomas of a given patient were more likely to be dermoscopically similar when sharing the same *BRAF* mutational status. In other words, a dermoscopic similar pattern of MPM might predict the same *BRAF* mutational status, further corroborating the close correlation between morphologic (dermoscopy) and genetic profile (*BRAF* mutational status) of a given tumour. Indeed, melanomas having the same “prevalent criterion” had more than 5-fold odds to have a concordant *BRAF* profile.

We found that the majority of dermoscopically similar multiple melanomas showed atypical network or regression structures as the prevalent criterion, in line with our previous findings. [6] Recently, retrospective and prospective studies demonstrated that specific dermoscopic features of melanomas are associated with somatic *BRAF* mutational pattern, [13-15,23] suggesting a potential influence of *BRAF* alterations on the dermoscopic appearance. However, we could not identify a specific dermoscopic prevalent criterion for concordant mutation-positive or mutation-negative similar melanomas, probably due to the low number of concordant cases.

Synchronicity is defined as the occurrence of MPM within three months from the first diagnosis and up to 40% of MPM are reported to be synchronous. [6,24,25] In our patients, synchronicity almost significantly correlated with a consistent *BRAF* mutational status, with a 4-fold probability of sharing the same *BRAF* mutational profile compared to metachronous lesions. Three studies previously investigated the intra-patient *BRAF* concordance in synchronous versus metachronous multiple melanomas, [9,11,12] with no significant differences in two of the studies [9,11] and a positive trend (p:.08) in the third one. [12]

BRAF mutations have been associated with melanomas at specific anatomical sites, such as the trunk and non-chronically sun exposed areas. [7] We did not find any significant association between the concordance of *BRAF* mutational status and occurrence of multiple melanomas at the same body site, in line with previous reports. [9,11,12]

Our study has few limitations. Firstly, the concept of similarity in dermoscopy is difficult to evaluate and no method is validated by a consensus. Few and different approaches have been previously reported. [6,10] Here, we used a semiquantitative approach that focuses on the prevalent criterion observed in a given lesion with the advantage to consider the overall main dermoscopic feature morphologically characterizing the tumour. Secondly, we mostly included in the study thin melanomas that might be associated with technical difficulties in the identification of *BRAF* mutations. To overcome this issue, we performed *BRAF* mutational analysis using Real Time PCR or

Pyrosequencing, both techniques have a very low detection limit for detection of somatic mutations. The frequency of *BRAF* mutated melanomas was indeed in line with previous studies. [1,22] In addition, due to the retrospective and multicentre nature of the study, we could not centralize the genetic analysis. We therefore performed a test in 10 random samples that showed reproducible results. Finally, further genetic alterations at germline[23] or somatic level, such as *NRAS*, [13] might be correlated to dermoscopic appearance of melanoma; however, we focused on the *BRAF* gene due to its higher mutation frequency and clinico-therapeutic impact.

In conclusion, we found that dermoscopic similarity is an independently clinical predictor of *BRAF* mutational concordance status in multiple melanomas of the same patient. Our findings might suggest a similar molecular pathogenesis in dermoscopically similar melanomas and could be of interest in MPM patients.

Conflicts of interest:

None to declare

Source of funding:

Dr Pampena is the recipient of a research contract funded by Research Project NET-2011-02347213, Italian Ministry of Health.

Authors' contribution:

EM, CP, RP designed and performed most of the experiments, collected and analyzed data, and drafted the manuscript; CP, CM, performed molecular analysis and drafted the manuscript; EM, RP, GA, MM, AC, ED, KP, AA, GC, RA, CL, MCF collected clinical data, assisted with image analysis and drafted the manuscript; CP, RP performed statistical analysis, interpreted data and assisted with manuscript draft; GA, CL, MCF supervised the experimental design and assisted with data interpretation. All authors reviewed and approved the manuscript
Jabbar-Lopez ZK, Yiu ZZN, Ward V

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Table 1. Distribution of clinical and dermoscopic features according to concordance of the *BRAF* mutational status. Univariate and multivariate logistic regression analysis.

	All patients n= 62 (%)	BRAF mutational status concordance					Logistic regression analysis			
		No n= 29 (%)	Yes n= 33 (%)	#p value (x ² test)	Spearman's correlation		Univariate		Multivariate	
					rho	#p value	OR (95% CI)	#p value	OR (95% CI)	#p value
Age (years) (mean ± SD)	52.9 ± 18.4	48.1 ± 17.1	57.0 ± 18.8	.057	.24	.056	n.i.	n.i.	n.i.	n.i.
Sex (males)	36 (58.1%)	18 (62.1%)	18 (54.5%)	.733	.08	.557	n.i.	n.i.	n.i.	n.i.
Sun Damage	31 (50.0%)	14 (48.3%)	17 (51.5%)	>.99	.03	.803	n.i.	n.i.	n.i.	n.i.
Synchronous	18 (29.0%)	4 (13.8%)	14 (42.4%)	.028	.32	.013	4.6 (1.3-16.3)	.018	3.8 (1.0-14.6)	.052
Site concordance	21 (33.9%)	11 (37.9%)	10 (30.2%)	.716	-.08	.534	n.i.	n.i.	n.i.	n.i.
Asymmetry concordance	40 (64.5%)	17 (58.6%)	23 (69.7%)	.520	.12	.371	n.i.	n.i.	n.i.	n.i.
N. of colours concordance	24 (38.7%)	7 (24.1%)	17 (51.5%)	.052	.28	.027	3.3 (1.1-9.9)	.030	n.i.	n.i.
Dermoscopic similarity	24 (38.7%)	5 (17.2%)	19 (57.6%)	.003	.41	.001	6.5 (2.0-21.3)	.002	5.7 (1.7-19.5)	.005

#p<.05 in bold; SD: standard deviation; n.i.: not included, since a multi-step process was applied, variables which were not significantly associated with *BRAF* mutational status concordance at descriptive analysis or through Spearman's correlation were not included in the logistic regression analysis.

FIGURE LEGEND

Figure 1: Two synchronous melanomas in a 70-year old man. Both lesions harbored the *BRAF*^{V600E} mutation and shared the same prevalent criterion in dermoscopy. a. Clinical appearance of a heavily pigmented lesion located on the neck, a superficial spreading melanoma, 0.7 mm Breslow thickness. b. Dermoscopic appearance: blue-white veil was the prevalent dermoscopic criterion. c. Pyrogram with the *BRAF*^{V600E} mutation. d. Clinical appearance of a 1.9 mm Breslow thickness melanoma located on the back. e. Dermoscopic appearance: blue-white veil was the prevalent dermoscopic criterion. f. Pyrogram with the *BRAF*^{V600E} mutation.

Figure 2: Two metachronous melanomas, discordant for *BRAF* mutational status, in a young woman located on the thigh (at age 15 years) and on the back (at age 17 years), respectively. a. Clinical appearance of an in-situ melanoma located on the left thigh. b. Dermoscopic appearance: atypical network was the prevalent dermoscopic criterion. c. Allele specific Real-Time Assay with the *BRAF*^{V600E} mutation d. Clinical appearance of an in-situ melanoma located on the back. e. Dermoscopic appearance: irregular vessels was the prevalent dermoscopic criterion. f. Allele specific Real-Time Assay with the *BRAF* wild type.

Figure 3: Two synchronous melanomas located on the back and abdomen of a 74-year old man. Both lesions were *BRAF* wild type and shared the same prevalent criterion in dermoscopy. a. Clinical appearance of a 0.3 mm Breslow thickness melanoma located on the back. b. Dermoscopic appearance: regression was the prevalent dermoscopic criterion. c. Pyrogram with the *BRAF*^{V600E} wild type. d. Clinical appearance of the in-situ melanoma located on the thorax. e. Dermoscopic appearance: regression was the prevalent dermoscopic criterion. f. Pyrogram with the *BRAF*^{V600E} wild type.





