Published in final edited form as:
*J Am Acad Dermatol*. 2015 March ; 72(3): 496–507.e7. doi:10.1016/j.jaad.2014.11.014.

# Histological Features of Melanoma Associated with *CDKN2A* Genotype

**Michael R. Sargen, MD**[1], **Peter A. Kanetsky, PhD, MPH**[2], **Julia Newton-Bishop, MD, FMedSci**[3], **Nicholas K. Hayward, PhD**[4], **Graham J. Mann, MBBS, PhD**[5], **Nelleke A. Gruis, PhD**[6], **Margaret A. Tucker, MD**[7], **Alisa M. Goldstein, PhD**[7], **Giovanna Bianchi-Scarra, PhD**[8], **Susanna Puig, MD**[9,10], and **David E. Elder, MB, ChB, FRCPA**[11]

[1]Department of Dermatology, Emory University Hospital, Atlanta, Georgia, United States [2]Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, Florida, United States [3]Section of Epidemiology and Biostatistics, LICAP, University of Leeds, Leeds, United Kingdom [4]QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia [5]University of Sydney at Westmead Millennium Institute and Melanoma Institute Australia, Sydney, NSW, Australia [6]Department of Dermatology, Leiden University Medical Center, Leiden, Netherlands [7]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, United States [8]Department of Internal medicine and Medical Specialties (Di.M.I.) University of Genoa; Genetics of Rare Cancers. IRCCS AOU San Martino -IST l, Genoa, Italy [9]Hospital Clinic of Barcelona, University of Barcelona, Institut de Recerca Biomédica August Pi I Sunyer, Barcelona, Spain [10]Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBER ER), Instituto de Salud Carlos III, Barcelona, Spain [11]Department of Pathology and Laboratory Medicine at the Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, United States

## Abstract

**Background—**Inherited susceptibility genes have been associated with histopathologic characteristics of tumors.

**Objective—**To identify associations between histology of melanomas and *CDKN2A* genotype.

**Methods—**Case-control study design comparing 28 histopathologic tumor features among individuals with sporadic melanomas (N=81) and cases from melanoma families with (N=123) and without (N=120) *CDKN2A* germline mutations.

**Results—**Compared with *CDKN2A*-negative cases, mutation carriers tended to have histologic features of superficial spreading melanoma subtype including higher pigmentation ($p_{trend}$=0.02)



Corresponding Author/Address: 1525 Clifton Rd, Floor 1 Suite 100, Dermatology, Residency Training Program, Atlanta, GA 30322-4200, Phone: 610-324-4242, michael.sargen@emory.edu, Fax: 877-992-8778.

Conflicts of Interest: Graham J. Mann was a speaker for Roche and received honoraria. No relevant conflicts of interest to report.

and increased pagetoid scatter ($p_{trend}$=0.07) after adjusting for age at diagnosis, sex, and AJCC thickness category. Similar associations were observed when comparing mutation carriers to a combined group of *CDKN2A*-negative (wild type) and sporadic melanomas. The presence of spindle cell morphology in the vertical growth phase was also an important predictor of genotype. Of the fifteen cases with this phenotype, none were observed to harbor a *CDKN2A* mutation.

**Limitations—**Our study examined rare mutations and may have been underpowered to detect small, but biologically significant associations between histology and genotype.

**Conclusion—**Familial melanomas with *CDKN2A* mutations preferentially express a histologic phenotype of dense pigmentation, high pagetoid scatter, and a non-spindle cell morphology in the vertical growth phase.

### Keywords

Familial melanoma; sporadic melanoma; *CDKN2A*; histology; Classification and Regression Tree (CART) analysis; pigmentation; pagetoid scatter; genetic testing

## Introduction

Melanoma clusters within families in about 5-10% of cases, and *CDKN2A* germline mutations are found in 20-40% of familial melanoma kindreds.[1] In contrast, the prevalence of a *CDKN2A* germline mutation in sporadic melanomas is low ranging from 0.2-2.0%.[2-5] The *CDKN2A* locus codes for two proteins, p16INK4 and p14ARF, that function as tumor suppressors in the Rb/E2F and HDM2/p53 pathways respectively.[6,7] Previous research has shown that specific histopathologic features are associated with inherited genetics. Female *BRCA1* and *BRCA2* carriers are predisposed to medullary and lobular carcinomas of the breast, respectively; and the 6q22.2 and 6p21.32 genetic regions are associated with adenocarcinomas of the lung.[8-12]

To date, there has been limited information published as to the clinicopathologic subtypes of melanoma most likely to occur in familial melanoma kindreds, which are defined by the presence of 2 or more melanomas amongst first-degree relatives or 3 or more melanomas irrespective of degree of relationship.[5] Previous descriptive series have reported an overrepresentation of superficial spreading morphology among familial melanomas, but these studies were relatively small in size and did not report whether specific histologic features were associated with genotype.[13,14-16] Bastian and colleagues recently reported good correlation between melanoma histology and somatic mutation status of the oncogenes *BRAF* and *NRAS*, whose profiles broadly resembled those of superficial spreading and lentigo maligna type melanomas, respectively.[17] Amongst melanomas arising in individuals with *CDKN2A* germline mutations, the prevalence of *NRAS* and *BRAF* mutations is 16% and 37%, respectively.[16]

The purpose of this study was to determine if histologic features of melanoma are associated with inherited *CDKN2A* mutations, which are the most prevalent genetic alterations observed in melanoma families. We hypothesized that the majority of the melanomas diagnosed in *CDKN2A* mutation carriers would be melanomas of the superficial spreading subtype, and that histological markers of this tumor subtype would be observed at higher

proportions in this group. This hypothesis is based on our experience and that of others that suggest an increased prevalence of this subtype of melanoma in familial melanoma kindreds.[13,14-16,18]

## Methods

### Study Design

We performed a case-control study of the histopathologic features of familial melanomas from family members with (N=123) and without (N=120) *CDKN2A* germline mutations and sporadic melanomas (N=81). Hereinafter, melanomas from family members who carry a *CDKN2A* mutation are referred to as "*CDKN2A*-positive" and those from family members testing negative for a *CDKN2A* mutation are referred to as "*CDKN2A*-negative". Familial melanoma cases were obtained from individuals in families with 2 first-degree relatives diagnosed with melanoma or families with 3 or more cases of melanoma irrespective of degree of relationship.[5] Tumor samples were collected from Philadelphia, PA and Bethesda, MD (USA), Barcelona (Spain), Brisbane and Sydney (Australia), Genoa (Italy), Leeds (United Kingdom), and Leiden (Netherlands) for use in this Melanoma Genetics Consortium (GenoMEL, www.genomel.org) study. All melanoma specimens were fixed in formalin, stored in paraffin blocks, and slides were subsequently cut for pathologic review. For each melanoma family, only one case was selected for use in this study. All slides were stripped of patient identifiers to protect patient privacy. GenoMEL centers contributing tumor slides were asked to match sporadic and familial *CDKN2A*-negative melanomas to familial *CDKN2A*-positive melanomas on age at diagnosis, sex, and American Joint Committee on Cancer (AJCC) thickness categories to the best of their abilities. In practice however, matching was inconsistently applied across centers. This resulted in a collection of tumor slides that ranged from those selected without regard to any matching criteria to those matched to varying degrees dependent upon the number of familial melanoma specimens and/or availability of sporadic melanoma cases at a given center. The distribution of age at diagnosis, sex, and AJCC thickness category across the three comparison groups is presented in Supplementary Table 1.

### Pathology Review

All melanomas were independently reviewed by DEE and MRS, who were blinded to all patient and tumor characteristics including research group of origin and mutation status. Radial growth phase (RGP), vertical growth phase (VGP), and stromal histological features were recorded for each tumor. Grading of many of the features (listed and further discussed below) was based on the system developed and validated by Bastian's group.[17,19] Disagreements regarding histologic features were resolved by consensus after joint review of the case.

### Description of Histopathologic Features

**Melanoma Subtype—**Melanoma cases were classified as superficial spreading melanoma (SSM), lentigo maligna melanoma (LMM), acral melanoma (AM), mucosal lentiginous melanoma (MLM), invasive melanoma with regressed RGP, or nodular melanoma (no RGP) subtype according to the World Health Organization (WHO) classification scheme and other

literature.[20-22] Definitions of radial and vertical growth phase were adopted from previously published works.[20,23-25]

**Pigment grade—**Pigment grade was based on a 0-3 scale.[17] In tumors with heterogeneous pigmentation, the pigment grade was based upon the area of most intense pigmentation. Grade 0 was assigned to amelanotic tumors. Grade 1 was assigned to tumors with faint pigmentation at low power (100X). Grade 2 was assigned to tumors with moderate pigmentation at low power but translucent cytoplasm. Grade 3 was assigned to tumors with high pigmentation at low power roughly equal to that of the nucleus.

**Epidermal (Pagetoid) Scatter—**Pagetoid scatter was graded on a 0-3 scale. Grade 0 was assigned to tumors with no pagetoid scatter. Grades 1 (“low”), 2 (“moderate”), and 3 (“high) were assigned to tumors in which 1-25%, 26-50%, and greater than 50% of the epidermal melanoma cells were above the basal layer of the epidermis, respectively.[17]

**Nesting Grade—**Nesting was defined as a clustering of five or more cells within the epidermis and was graded on a 0-3 scale. Grade 0 was assigned to tumors in which there was no nesting of melanoma cells within the epidermis. Grades 1 (“mild”), 2 (“moderate”), and 3 (“high”) were assigned to tumors in which 1-25%, 26-50%, and greater than 50% of the epidermal melanoma cells were located within nests, respectively.[17]

**RGP and VGP Cytologic Grade—**Low grade was used to describe tumor cells with nuclei similar in size to basal keratinocytes, regular shape, and no nucleoli. Intermediate grade was used to describe tumor cells with enlarged and slightly irregular nuclei, moderately clumped chromatin, hyperchromasia, and small or absent nucleoli. High grade was used to describe tumor cells with markedly pleomorphic and hyperchromatic irregular nuclei, and large eosinophilic or amphophilic nucleoli.

**VGP Cell Type—**Cells were classified as epithelioid, spindle, nevoid, or spitzoid. The ratio of the long to short axis for epithelioid and spindle classification was 1:1 and >2:1 respectively. Cells were classified as nevoid if they resembled those of a banal nevus. Spitzoid classification was assigned to tumors with very large plump spindle cells with abundant cytoplasm, large amphophilic nucleoli, and open chromatin.

**Tumor Infiltrating Lymphocytes—**Tumor infiltrating lymphocytes (TILs) were defined as lymphocytes in contact with melanoma cells in the dermis. TILs were graded on a 0-3 scale. Grade 0 was assigned to tumors with no TILs. Grades 1 (“low”), 2 (“medium”), and 3 (“high”) were assigned to tumors in which 1-25%, 26-50%, and greater than 50% of dermal melanoma cells were in contact with lymphocytes, respectively.

**Fibroplasia—**Concentric and diffuse fibroplasia, as defined by Clark et al, were graded using a 0-2 scale.[26] Grade 0 was assigned to tumors with no fibroplasia. Grade 1 (“slight”) was assigned to tumors with 1-25% of the epidermal tumor breadth demonstrating fibroplasia. Grade 2 (“definite”) was assigned to tumors with >25% of the epidermal tumor breadth demonstrating fibroplasia.[26]

**Perivascular and Diffuse Lymphocytes—**Perivascular and diffuse lymphocytes were each graded on a 0-3 scale. Grade 0 was assigned to tumors with no lymphocytic infiltrate. Grade 1 (“low”) was assigned to tumors with scattered lymphocytes in the tumor bed or around blood vessels. Grade 2 (“medium”) was assigned to tumors with a lymphocyte to melanoma cell ratio of 5:1 in the tumor bed or 1-2 concentric layers of lymphocytes around blood vessels. Grade 3 (“high”) was assigned to tumors with a lymphocyte to melanoma cell ratio of >10:1 in the tumor bed or >2 concentric layers of lymphocytes around blood vessels.

**Actinic (Solar) Elastosis—**Actinic Elastosis was graded on a 1-3 scale. Grade 1 (“mild”) was assigned to tumors with a background of scattered elastotic fibers lying as individual units between collagen bundles. Grade 2 (“moderate”) was assigned to tumors with a background of densely scattered elastotic fibers distributed predominantly as bushels. Grade 3 (“severe”) was assigned to tumors with a background of amorphous deposits of blue-gray material with loss of fiber texture.[17,19]

## Statistical Analysis

Using logistic regression, odds ratios (OR) and corresponding 95% confidence intervals (CI) were estimated for associations between groups defined by *CDKN2A* status (*CDKN2A*-positive versus *CDKN2A*-negative; *CDKN2A*-postive versus sporadic; *CDKN2A*-positive versus combined *CDKN2A*-negative and sporadic) and measured RGP, VGP, and stromal histologic features after adjusting for age at diagnosis, sex, and AJCC tumor thickness. Overall differences in the prevalence of histological variables were determined by chi-square analysis for nominal categorical variables or Mantel-Haenszel (M-H) test for trend for ordinal categorical variables. All histologic variables with a $p < 0.20$ were subsequently included in a forward selection multivariate logistic regression model to determine independent associations with mutation status, and these models were again adjusted for age at diagnosis, sex, and AJCC tumor thickness. We excluded melanoma subtype from the multivariate analysis because it is a morphologic classification scheme based upon the presence of specific histologic features and hence was highly collinear with the same features as variables incorporated into the multivariate models. Sporadic cases were combined with *CDKN2A*-negative familial cases for the third analysis because of their low prevalence for mutations at the *CDKN2A* locus.[3] All statistical analyses were performed using STATA v12.1 (StataCorp LP, College Station, TX) software.

## Classification and Regression Tree (CART) Analysis

Classification and regression tree (CART) analysis was performed to evaluate the ability of histopathologic features to predict *CDKN2A* genotype. CART analysis creates binary nodes with the objective of minimizing within-group heterogeneity at each branch point. [27,28] We used the computer software package, Salford Predictive Modeler (Salford Systems, San Diego, California), to perform each CART analysis. Each terminal node (branch point) has listed the estimated probability (%) with 95% confidence limits of a *CDKN2A* mutation.

## Results

Twenty-eight histopathologic features were recorded for 324 familial or sporadic melanoma cases, and comparisons among groups are reported in Table 1. Associations between histology and genotype were similar when analyzing invasive and melanoma *in situ* cases separately (Supplementary Tables 2 and 3).

### Familial Melanoma: *CDKN2A*-Positive vs. *CDKN2A*-Negative Cases

Compared with *CDKN2A*-negative cases, mutation carriers tended to have histologic features of superficial spreading melanoma subtype including higher pigmentation ($p_{trend}$=0.02) and increased pagetoid scatter ($p_{trend}$=0.07) after adjusting for age at diagnosis, sex, and AJCC thickness category. In multivariable models, higher pagetoid scatter ($p_{trend}$=0.01) and increased density of TILs ($p_{trend}$=0.02) were also associated with *CDKN2A* mutations after adjusting for age at diagnosis, sex, AJCC thickness category, pigmentation, associated junctional nevi, RGP cytologic grade, diffuse fibroplasia, VGP cell type, VGP cytologic grade, and VGP mitotic rate. Differences in VGP cell type (p=0.002) were also observed between the two groups.

### *CDKN2A*-Positive vs. Sporadic Melanomas

Familial melanomas with *CDKN2A* mutations had a lower VGP cytologic grade ($p_{trend}$=0.04) and were more likely to have a non-mitogenic VGP (p<0.001) compared to sporadic cases after adjusting for age at diagnosis, sex, and AJCC thickness category. In multivariable models, these associations did not achieve statistical significance after additional adjustment for age at diagnosis, sex, AJCC thickness category, pigmentation, RGP mitotic rate, RGP breadth, associated junctional nevi, and VGP cell type. Differences in VGP cell type (p=0.01) were also observed between the two case groups.

### *CDKN2A*-Positive vs. Combined *CDKN2A*-negative and Sporadic Cases

Compared to a combined group of *CDKN2A*-negative familial and sporadic cases, *CDKN2A* mutation carriers were more likely to have higher pigmentation ($p_{trend}$=0.04), lower VGP cytologic grade ($p_{trend}$=0.03), and a non-mitogenic VGP (p<0.001) after adjusting for age at diagnosis, sex, and AJCC thickness category. These associations were not observed in the multivariable logistic regression model, which adjusted for age at diagnosis, sex, AJCC thickness category, pagetoid scatter, RGP breadth, RGP cytologic grade, RGP mitotic rate, associated junctional nevi, diffuse fibroplasia, and VGP cell type. Differences in VGP cell type (p=0.01) were also observed between the mutation carriers and the combined group of *CDKN2A*-negative and sporadic cases. VGP spindle cell morphology was exclusively seen within the *CDKN2A*-negative (N=9) and sporadic (N=6) groups.

### Classification and Regression Tree (CART) Analysis

CART analysis revealed that many of the histological variables that were associated with genotype in the logistic regression analysis were also predictive of mutation status. Amongst familial melanomas, pigmentation, VGP cell type, and TILs were important predictors of *CDKN2A* genotype (Figure 1). When comparing mutation carriers to sporadic cases, VGP mitoses was a strong predictor of mutation status (Figure 2). Similar findings from these two

analyses were observed when comparing the histologic features of mutation carriers to the combined control group of *CDKN2A*-negative familial and sporadic cases (Figure 3).

## Discussion

Consistent with our study hypothesis, we found that histologic features of SSM classification, including increased pigmentation and increased pagetoid scatter, were more common among familial melanoma cases with *CDKN2A* mutations compared to familial cases who were *CDKN2A*-negative.

Pigmentation in melanocytes is regulated by microphthalmia-associated transcription factor (MITF). MITF upregulates expression of tyrosinase in melanocytes resulting in increased synthesis of the pigment melanin.[29] MITF plays an important role in cell cycle regulation by binding to the *p16* (*CDKN2A* locus) promoter site where it induces gene transcription.[30] There is a negative feedback interaction between MITF and p16 whereby inactivation of the latter is associated with MITF amplification.[31] Therefore, the high pigmentation observed in *CDKN2A* mutation melanoma cases may reflect the loss of p16 negative feedback on the pigmentation regulator MITF.

Higher scatter grade was also associated with the presence of a *CDKN2A* mutation among familial melanomas after adjusting for potential confounders in our multivariate analysis, and this association trended towards statistical significance when mutation carriers were compared to the combined group of familial *CDKN2A*-negative and sporadic cases. Pagetoid scatter is a well-characterized feature of SSM, also referred to as pagetoid melanoma in the literature, and this association supports our original hypothesis.[17,19] VGP cell type was also an important predictor of mutation status. Of the fifteen cases with VGP spindle cell morphology, none were *CDKN2A* mutation carriers. Spindle cells are more characteristically seen in lentigo maligna type melanomas rather than superficial spreading melanomas. It was also observed amongst familial cases that melanomas harboring a *CDKN2A* mutation had an increased density of TILs after adjusting for potential confounders. This observation might suggest that the presence of a *CDKN2A* mutation confers a phenotype of increased immune surveillance accompanying the individual's predisposition for cancer, or perhaps that *CDKN2A*-mutated melanomas are more immunogenic.

Models comparing mutation positive to sporadic melanoma cases showed that melanomas harboring a *CDKN2A* mutation were more likely to have a lower VGP cytologic grade and to have a non-mitogenic VGP after adjusting for age at diagnosis, sex and AJCC thickness category. These associations are likely attributable to more frequent surveillance for melanoma within melanoma families.

To our knowledge, our study is the largest to date comparing the histologic features of sporadic melanomas and familial melanomas with and without *CDKN2A* germline mutations. A limitation of our study is that our analysis may have been underpowered to detect certain biologically important associations between histology and *CDKN2A* genotype.

In conclusion, our study demonstrates that histologic features of SSM are associated with the presence of a *CDKN2A* mutation. If these findings are validated, clinicians can use

histology in conjunction with clinical information to help determine which patients should be offered *CDKN2A* genetic testing.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

Jane Palmer (JP) assisted with the ascertainment of clinical information and tissue blocks at the QIMR Berghofer Medical Research Institute in Brisbane, Queensland, Australia. Patricia Van Belle, Althea Ruffin, and Jillian Knorr worked at the University of Pennsylvania in Philadelphia, Pennsylvania and helped to collect melanomas from individuals in melanoma families with *CDKN2A* germline mutations, and to coordinate the organization of the material received from all the sites, including blinded labeling of slides for pathology review. Wenting Zhou created and maintained a database containing all of the patient and pathology information for the study set and enabling real time data entry. Research nurse Linda Whitaker was responsible for collecting and organizing tissue blocks at the University of Leeds in Leeds, United Kingdom. Dr. Mark Harland performed mutational screening of individuals from melanoma families at the University of Leeds in Leeds, United Kingdom. Coby Out and Frans van Nieuwpoort were responsible for collecting and organizing tissue blocks at the Leiden University Medical Center in Leiden, Netherlands. Clinical and genetic information and tissue blocks were collected for this study in Sydney by Gayathri St. George and Elizabeth Holland and by Helen Schmid, who coordinated the GenoMEL project locally; they gratefully acknowledge the contribution of all research interviewers and lab researchers engaged in the study at Westmead. We are grateful to all families for their generous participation.

IRB Status: IRB approval was obtained at each participating institution.

Funding Sources: Research at each of the participating institutions is funded by the National Cancer Institute (NCI) of the U.S. National Institutes of Health (NIH) (R01 CA83115). The research at the Melanoma Unit in Barcelona is partially funded by Spanish Fondo de Investigaciones Sanitarias (grants 09/01393 and 12/00840); CIBER de Enfermedades Raras of the Instituto de Salud Carlos III, Spain; AGAUR 2009 SGR 1337 of the Catalan Government, Spain; European Commission under the 6th Framework Programme, Contract No. LSHC-CT-2006-018702 (GenoMEL). The Leeds research group is partially funded by Cancer Research UK (C588/A4994 and C588/A10589). Research at QIMR Berghofer Medical Research Institute is partially funded by the National Health and Medical Research Council of Australia. Work in Sydney was also funded by program grants of NHMRC (402761, 633004) and Cancer Institute NSW (05/TPG/1-01, 10/TPG/1-02).

## References

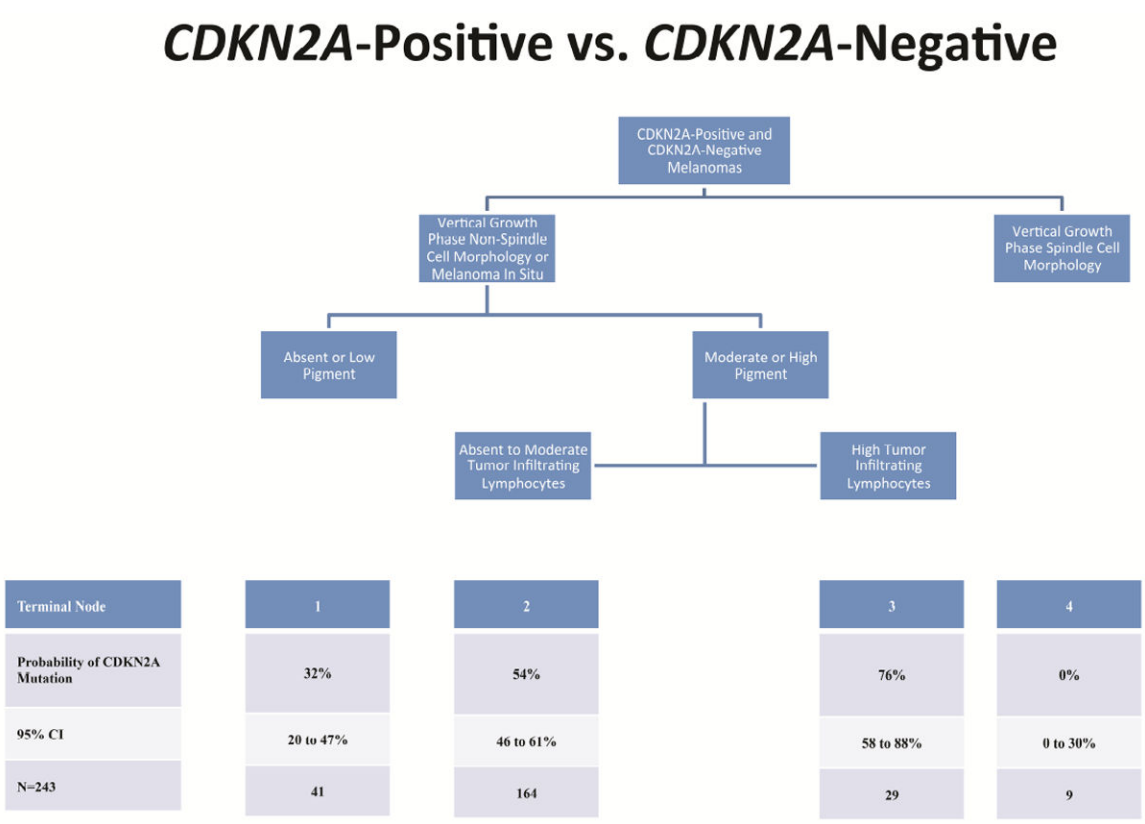
1. Kefford RF, Newton Bishop JA, Bergman W, Tucker MA. Counseling and DNA testing for individuals perceived to be genetically predisposed to melanoma: A consensus statement of the Melanoma Genetics Consortium. J Clin Oncol. 1999; 17(10):3245–3251. [PubMed: 10506626]
2. Kefford R, Bishop JN, Tucker M, et al. Genetic testing for melanoma. Lancet Oncol. 2002; 3(11): 653–654. [PubMed: 12424065]
3. Aitken J, Welch J, Duffy D, et al. CDKN2A variants in a population-based sample of Queensland families with melanoma. J Natl Cancer Inst. 1999; 91(5):446–452. [PubMed: 10070944]
4. Begg CB, Orlow I, Hummer AJ, et al. Lifetime risk of melanoma in CDKN2A mutation carriers in a population-based sample. J Natl Cancer Inst. 2005; 97(20):1507–1515. [PubMed: 16234564]
5. Leachman SA, Carucci J, Kohlmann W, et al. Selection criteria for genetic assessment of patients with familial melanoma. J Am Acad Dermatol. 2009; 61(4):677, e671–614. [PubMed: 19751883]
6. Tsao H, Chin L, Garraway LA, Fisher DE. Melanoma: from mutations to medicine. Genes Dev. 2012; 26(11):1131–1155. [PubMed: 22661227]
7. Christensen KD, Roberts JS, Shalowitz DI, et al. Disclosing individual CDKN2A research results to melanoma survivors: interest, impact, and demands on researchers. Cancer Epidemiol Biomarkers Prev. 2011; 20(3):522–529. [PubMed: 21307304]
8. Mavaddat N, Barrowdale D, Andrulis IL, et al. Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). Cancer Epidemiol Biomarkers Prev. 2012; 21(1):134–147. [PubMed: 22144499]

9. Armes JE, Venter DJ. The pathology of inherited breast cancer. Pathology. 2002; 34(4):309–314. [PubMed: 12190286]
10. Armes JE, Egan AJ, Southey MC, et al. The histologic phenotypes of breast carcinoma occurring before age 40 years in women with and without BRCA1 or BRCA2 germline mutations: a population-based study. Cancer. 1998; 83(11):2335–2345. [PubMed: 9840533]
11. Lan Q, Hsiung CA, Matsuo K, et al. Genome-wide association analysis identifies new lung cancer susceptibility loci in never-smoking women in Asia. Nat Genet. 2012; 44(12):1330–1335. [PubMed: 23143601]
12. Pathology of familial breast cancer: differences between breast cancers in carriers of BRCA1 or BRCA2 mutations and sporadic cases. Breast Cancer Linkage Consortium. Lancet. 1997; 349(9064):1505–1510. [PubMed: 9167459]
13. Carey WP Jr, Thompson CJ, Synnestvedt M, et al. Dysplastic nevi as a melanoma risk factor in patients with familial melanoma. Cancer. 1994; 74(12):3118–3125. [PubMed: 7982177]
14. Nagore E, Botella-Estrada R, Garcia-Casado Z, et al. Comparison between familial and sporadic cutaneous melanoma in Valencia, Spain. J Eur Acad Dermatol Venereol. 2008; 22(8):931–936. [PubMed: 18355200]
15. Chiarugi A, Nardini P, Crocetti E, et al. Familial and sporadic melanoma: different clinical and histopathological features in the Italian population - a multicentre epidemiological study - by GIPMe (Italian Multidisciplinary Group on Melanoma). J Eur Acad Dermatol Venereol. 2012; 26(2):194–199. [PubMed: 21429041]
16. Jovanovic B, Egyhazi S, Eskandarpour M, et al. Coexisting NRAS and BRAF mutations in primary familial melanomas with specific CDKN2A germline alterations. The Journal of investigative dermatology. 2010; 130(2):618–620. [PubMed: 19759551]
17. Viros A, Fridlyand J, Bauer J, et al. Improving melanoma classification by integrating genetic and morphologic features. PLoS Med. 2008; 5(6):e120. [PubMed: 18532874]
18. Hemminki K, Ji J, Brandt A, Mousavi SM, Sundquist J. The Swedish Family-Cancer Database 2009: prospects for histology-specific and immigrant studies. Int J Cancer. 2010; 126(10):2259–2267. [PubMed: 19642094]
19. Broekaert SM, Roy R, Okamoto I, et al. Genetic and morphologic features for melanoma classification. Pigment Cell Melanoma Res. 2010; 23(6):763–770. [PubMed: 20874733]
20. Clark WH Jr, From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer research. 1969; 29(3):705–727. [PubMed: 5773814]
21. Reed, RJ. New concepts in surgical pathology of the skin. New York: Wiley; 1976.
22. Elder, DE.; MG. Melanocytic Tumors of the Skin. Washington, DC: American Registry of Pathology; 2011. p. 209-276.
23. Guerry, Dt; Synnestvedt, M.; Elder, DE.; Schultz, D. Lessons from tumor progression: the invasive radial growth phase of melanoma is common, incapable of metastasis, and indolent. The Journal of investigative dermatology. 1993; 100(3):342S–345S. [PubMed: 8440920]
24. McGovern VJ, Mihm MC Jr, Bailly C, et al. The classification of malignant melanoma and its histologic reporting. Cancer. 1973; 32(6):1446–1457. [PubMed: 4757934]
25. Clark WH Jr, Mihm MC Jr. Lentigo maligna and lentigo-maligna melanoma. Am J Pathol. 1969; 55(1):39–67. [PubMed: 5776171]
26. Clark WH Jr, Hood AF, Tucker MA, Jampel RM. Atypical melanocytic nevi of the genital type with a discussion of reciprocal parenchymal-stromal interactions in the biology of neoplasia. Human pathology. 1998; 29(1 Suppl 1):S1–24. [PubMed: 9445124]
27. Breiman, L. Classification and regression trees. Belmont, Calif: Wadsworth International Group; 1984.
28. Breiman, L. Random Forests. 2001. http://oz.berkeley.edu/~breiman/randomforest2001.pdf
29. Shoag J, Haq R, Zhang M, et al. PGC-1 coactivators regulate MITF and the tanning response. Mol Cell. 2013; 49(1):145–157. [PubMed: 23201126]
30. Loercher AE, Tank EM, Delston RB, Harbour JW. MITF links differentiation with cell cycle arrest in melanocytes by transcriptional activation of INK4A. J Cell Biol. 2005; 168(1):35–40. [PubMed: 15623583]

31. Garraway LA, Widlund HR, Rubin MA, et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. Nature. 2005; 436(7047):117–122. [PubMed: 16001072]

## Capsule Summary

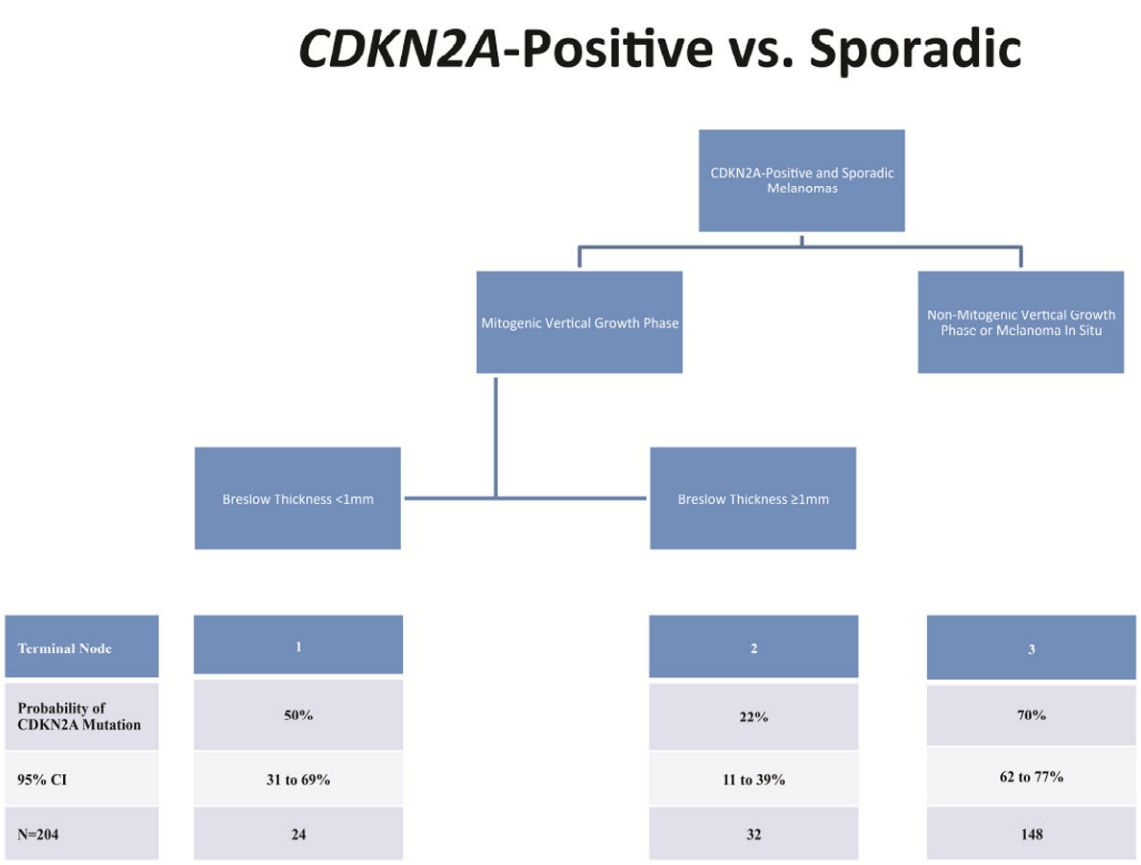
- It is unknown whether *CDKN2A* mutations are associated with specific histopathological features of melanomas arising within melanoma families.
- Familial melanomas with *CDKN2A* mutations preferentially express a non-spindle cell morphology, dense pigmentation, and high pagetoid scatter.
- If these findings are validated, clinicians can use histology to help determine which patients should be offered *CDKN2A* genetic testing.




| Terminal Node | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Probability of CDKN2A Mutation | 32% | 54% | 76% | 0% |
| 95% CI | 20 to 47% | 46 to 61% | 58 to 88% | 0 to 30% |
| N=243 | 41 | 164 | 29 | 9 |

**Figure 1.**

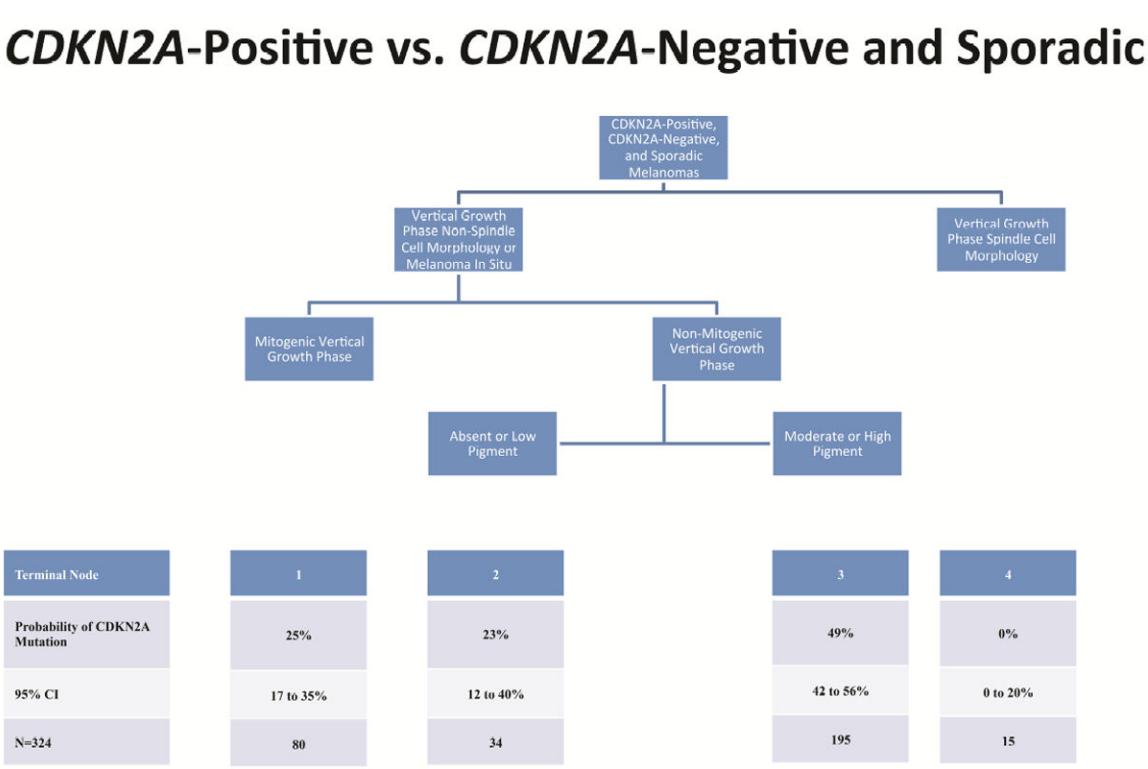
Classification and Regression Tree (CART) for familial melanomas with (N=123) and without (N=120) *CDKN2A* mutations. The probability (%) of a *CDKN2A* mutation, its 95% confidence limits, and the number of cases for each terminal node are reported in the table beneath the figure.




| Terminal Node | 1 | 2 | 3 |
|---|---|---|---|
| Probability of CDKN2A Mutation | 50% | 22% | 70% |
| 95% CI | 31 to 69% | 11 to 39% | 62 to 77% |
| N=204 | 24 | 32 | 148 |

**Figure 2.**

Classification and Regression Tree (CART) comparing familial melanomas with *CDKN2A* mutations (N=123) to sporadic melanomas (N=81). The probability (%) of a *CDKN2A* mutation, its 95% confidence limits, and the number of cases for each terminal node are reported in the table beneath the figure.




| Terminal Node | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Probability of CDKN2A Mutation | 25% | 23% | 49% | 0% |
| 95% CI | 17 to 35% | 12 to 40% | 42 to 56% | 0 to 20% |
| N=324 | 80 | 34 | 195 | 15 |

**Figure 3.**

Classification and Regression Tree (CART) comparing familial melanomas with *CDKN2A* mutations (N=123) to a combined group of familial melanomas without *CDKN2A* mutations (N=120) and sporadic melanomas (N=81). The probability (%) of a *CDKN2A* mutation, its 95% confidence limits, and the number of cases for each terminal node are reported in the table beneath the figure.

**Table 1**

Comparison of Histological Features Amongst Sporadic Melanomas and Familial Melanomas with (*CDKN2A*-Positive) and without (*CDKN2A*-Negative) *CDKN2A* Mutations.

| | | **CDKN2A-Positive** | ***CDKN2A*-Negative** | **Sporadic** | ***CDKN2A*-Positive vs. *CDKN2A*-Negative** | ***CDKN2A*-Positive vs. Sporadic** | ***CDKN2A*-Positive vs. *CDKN2A*-Negative and Sporadic** | ***CDKN2A*-Positive vs. *CDKN2A*-Negative** | ***CDKN2A*-Positive vs. Sporadic** | ***CDKN2A*-Positive vs. *CDKN2A*-Negative and Sporadic** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Melanoma Subtype and Thickness** | **Category** | ***n* (%)** | ***n* (%)** | ***n* (%)** | **OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | | **Multivariate OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | |
| Melanoma Subtype[8] | LMM | 7 (5.7) | 16 (13) | 5 (6.2) | | | | | | |
| | NM | 2 (1.6) | 0 | 5 (6.2) | | | | | | |
| | SSM | 112 (91) | 102 (85) | 66 (81) | | | | | | |
| | ALM | 1 (0.81) | 1 (0.83) | 3 (3.7) | | | | | | |
| | MLM | 0 | 1 (0.83) | 0 | | | | | | |
| | Regressed RGP | 1 (0.81) | 0 | 2 (2.5) | | | | | | |
| | Total | 123 (100) | 120 (100) | 81 (100) | | | | | | |
| **Radial Growth Phase** | | ***n*=123** | ***n*=120** | ***n*=81** | | | | | | |
| Ulceration | Absent | 120 (98) | 118 (98) | 77 (95) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Present | 3 (2.4) | 2 (1.7) | 4 (4.9) | 1.51 (0.21-10.7) | 1.54 (0.23-10.3) | 1.74 (0.36-8.53) | | | |
| | **P-value (df=1)[4]** | | | | 0.67 | 0.34 | 0.77 | | | |
| Pigmentation | Amelanotic/Faint | 13 (11) | 32 (27) | 19 (23) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) |
| | Moderate | 96 (78) | 77 (64) | 48 (59) | **2.76 (1.27-5.97)** | 2.03 (0.84-4.93) | **2.42 (1.19-4.92)** | **3.06 (1.02-9.15)** | 1.12 (0.36-3.52) | 1.61 (0.64-4.04) |
| | High | 14 (11) | 11 (9.2) | 12 (15) | 3.13 (1.00-9.86) | 2.07 (0.62-6.95) | 2.52 (0.93-6.86) | 4.85 (0.99-23.8) | 1.03 (0.24-4.43) | 1.85 (0.52-6.55) |
| | Unable to Assess[5] | 0 | 0 | 2 (2.5) | | | | | | |
| | **P-value for trend[6]** | | | | **0.02** | 0.19 | **0.04** | 0.06 | 0.75 | 0.20 |
| Horizontal Breadth (mm) | Mean | 5.31 | 5.63 | 6.53 | | | | | | |
| | **P-value** | | | | 0.47 | **0.01** | 0.08 | | | |
| Pagetoid Scatter | Absent/Low | 22 (18) | 36 (30) | 19 (23) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | 1 (Reference) |
| | Moderate | 24 (20) | 25 (21) | 11 (14) | 1.35 (0.59-3.06) | 1.96 (0.70-5.50) | 1.54 (0.72-3.28) | 0.93 (0.27-3.22) | | 1.12 (0.38-3.29) |
| | High | 77 (63) | 59 (49) | 50 (62) | 1.84 (0.94-3.60) | 1.30 (0.60-2.80) | 1.57 (0.85-2.89) | **4.27 (1.45-12.5)** | | 2.23 (0.90-5.54) |
| | Unable to Assess[5] | 0 | 0 | 1 (1.2) | | | | | | |

| | | CDKN2 *A*-Positive | *CDKN2 A*-Negative | Sporad ic | *CDKN2A* -Positive vs. *CDKN2A*- Negative | *CDKN2A*- Positive vs. Sporadic | *CDKN2A*- Positive vs. *CDKN2A*- Negative and Sporadic | *CDKN2A*- Positive vs. *CDKN2A*- Negative | *CDKN2A*- Positive vs. Sporadic | *CDKN2A*- Positive vs. *CDKN2A*- Negative and Sporadic |
|---|---|---|---|---|---|---|---|---|---|---|
| **Melanoma Subtype and Thickness** | **Category** | ***n* (%)** | ***n* (%)** | ***n* (%)** | **OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | | **Multivariate OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | |
| | **P-value for trend[6]** | | | | 0.07 | 0.68 | 0.18 | **0.01** | | 0.09 |
| Epidermal Nesting | Absent/Mild | 26 (21) | 24 (20) | 13 (16) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Moderate | 16 (13) | 25 (21) | 10 (12) | 0.58 (0.24-1.42) | 0.76 (0.25-2.30) | 0.66 (0.29-1.49) | | | |
| | High | 79 (64) | 71 (59) | 57 (70) | 0.89 (0.44-1.80) | 0.72 (0.31-1.68) | 0.82 (0.44-1.55) | | | |
| | Unable to Assess[5] | 2 (1.6) | 0 | 1 (1.2) | | | | | | |
| | **P-value for trend[6]** | | | | 0.96 | 0.47 | 0.69 | | | |
| Radial Growth Phase Cell Shape | Round | 120 (98) | 118 (98) | 77 (95) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Ovoid | 1 (0.81) | 0 | 1 (1.2) | N.E | N.E. | N.E. | | | |
| | Elongated | 1 (0.81) | 0 | 0 | N.E | N.E. | N.E. | | | |
| | Spindled | 0 | 2 (1.7) | 2 (2.5) | N.E | N.E. | N.E. | | | |
| | Unable to Assess[5] | 1 (0.81) | 0 | 1 (1.2) | | | | | | |
| | **P-value (df=3)[4]** | | | | 0.26 | 0.28 | 0.24 | | | |
| Radial Growth Phase Cytologic Grade | Low | 6 (4.9) | 2 (1.7) | 2 (2.5) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | 1 (Reference) |
| | Intermediate | 112 (91) | 114 (95) | 69 (85) | 0.28 (0.05-1.57) | 0.51 (0.09-3.11) | 0.38 (0.10-1.47) | N.E. | | N.E. |
| | High | 4 (3.3) | 4 (3.3) | 8 (9.9) | 0.19 (0.02-1.87) | 0.25 (0.03-2.31) | 0.23 (0.04-1.44) | N.E. | | N.E. |
| | Unable to Assess[5] | 1 (0.81) | 0 | 2 (2.5) | | | | | | |
| | **P-value for trend[6]** | | | | 0.15 | 0.21 | 0.13 | | | |
| Radial Growth Phase Mitoses | None | 109 (89) | 102 (85) | 64 (79) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | 1 (Reference) | 1 (Reference) |
| | ≥1/mm2 | 13 (11) | 17 (14) | 15 (19) | 0.67 (0.30-1.53) | 0.63 (0.26-1.53) | 0.64 (0.31-1.34) | | 0.90 (0.31-2.59) | 0.71 (0.28-1.82) |
| | Unable to Assess[5] | 1 (0.81) | 1 (0.83) | 2 (2.5) | | | | | | |
| | **P-value (df=1)[4]** | | | | 0.39 | 0.10 | 0.17 | | 0.78 | 0.52 |
| Associated Dermal Nevus | Absent | 92 (75) | 90 (75) | 66 (81) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Present | 31 (25) | 30 (25) | 14 (17) | 0.91 (0.49-1.68) | 1.25 (0.58-2.68) | 1.01 (0.57-1.77) | | | |
| | Unable to Assess[5] | 0 | 0 | 1 (1.2) | | | | | | |

| | | CDKN2 *A*-Positive | *CDKN2 A*-Negative | Sporadic | *CDKN2A* -Positive vs. *CDKN2A*-Negative | *CDKN2A*-Positive vs. Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative and Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative | *CDKN2A*-Positive vs. Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative and Sporadic |
|---|---|---|---|---|---|---|---|---|---|---|
| **Melanoma Subtype and Thickness** | **Category** | ***n* (%)** | ***n* (%)** | ***n* (%)** | **OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | | **Multivariate OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | |
| | **P-value (df=1)[4]** | | | | 0.97 | 0.20 | 0.51 | | | |
| Associated Junctional Nevus | Absent | 84 (68) | 91 (76) | 63 (78) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) |
| | Present | 38 (31) | 27 (23) | 17 (21) | 1.83 (0.97-3.44) | 1.28 (0.63-2.61) | 1.61 (0.93-2.78) | **3.21 (1.18-8.77)** | 1.26 (0.48-3.30) | 1.96 (0.89-4.32) |
| | Unable to Assess[5] | 1 (0.81) | 2 (1.7) | 1 (1.2) | | | | | | |
| | **P-value (df=1)[4]** | | | | 0.15 | 0.12 | 0.08 | **0.02** | 0.65 | 0.10 |
| Associated Lentigo | None | 119 (97) | 112 (93) | 78 (96) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Actinic Lentigo | 4 (3.3) | 8 (6.7) | 3 (3.7) | 0.59 (0.14-2.48) | 0.53 (0.10-2.81) | 0.56 (0.15-2.13) | | | |
| | Lentigo Simplex | 0 | 0 | 0 | N.E. | N.E. | N.E. | | | |
| | **P-value (df=1)[4]** | | | | 0.22 | 0.86 | 0.36 | | | |
| Concentric Fibroplasia | None | 107 (87) | 96 (80) | 66 (81) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Slight | 5 (4.1) | 13 (11) | 6 (7.4) | 0.45 (0.15-1.35) | 0.55 (0.15-2.04) | 0.51 (0.18-1.44) | | | |
| | Definite | 10 (8.1) | 11 (9.2) | 5 (6.2) | 0.88 (0.34-2.28) | 1.11 (0.35-3.56) | 1.07 (0.45-2.53) | | | |
| | Unable to Assess[5] | 1 (0.81) | 0 | 4 (4.9) | | | | | | |
| | **P-value for trend[6]** | | | | 0.45 | 0.88 | 0.75 | | | |
| Diffuse Fibroplasia | None | 19 (15) | 10 (8.3) | 11 (14) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | 1 (Reference) |
| | Slight | 20 (16) | 23 (19) | 7 (8.6) | 0.36 (0.13-1.02) | 1.79 (0.52-6.18) | 0.62 (0.25-1.51) | 0.15 (0.02-1.18) | | 0.36 (0.07-1.75) |
| | Definite | 83 (67) | 87 (73) | 59 (73) | **0.34 (0.14-0.84)** | 0.86 (0.34-2.13) | 0.51 (0.24-1.06) | **0.16 (0.03-0.94)** | | 0.28 (0.08-1.06) |
| | Unable to Assess[5] | 1 (0.81) | 0 | 4 (4.9) | | | | | | |
| | **P-value for trend[6]** | | | | **0.04** | 0.46 | 0.08 | 0.09 | | 0.07 |
| Actinic Elastosis | Mild | 24 (20) | 20 (17) | 21 (26) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Moderate | 87 (71) | 76 (63) | 50 (62) | 1.25 (0.61-2.55) | 1.33 (0.61-2.87) | 1.24 (0.67-2.31) | | | |
| | Severe | 11 (8.9) | 23 (19) | 8 (9.9) | 0.91 (0.31-2.62) | 1.04 (0.31-3.50) | 0.87 (0.34-2.23) | | | |
| | Unable to Assess[5] | 1 (0.81) | 1 (0.83) | 2 (2.5) | | | | | | |
| | **P-value for trend[6]** | | | | 0.99 | 0.76 | 0.96 | | | |

| | | CDKN2A-Positive | *CDKN2A*-Negative | Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative | *CDKN2A*-Positive vs. Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative and Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative | *CDKN2A*-Positive vs. Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative and Sporadic |
|---|---|---|---|---|---|---|---|---|---|---|
| **Melanoma Subtype and Thickness** | **Category** | ***n* (%)** | ***n* (%)** | ***n* (%)** | **OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | | **Multivariate OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | |
| Regression | None | 107 (87) | 104 (87) | 63 (78) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Focal | 3 (2.4) | 7 (5.8) | 9 (11) | 0.48 (0.11-2.02) | **0.20 (0.05-0.83)** | 0.29 (0.08-1.04) | | | |
| | Extensive | 13 (11) | 9 (7.5) | 9 (11) | 1.30 (0.51-3.34) | 1.19 (0.42-3.38) | 1.21 (0.54-2.73) | | | |
| | **P-value for trend[6]** | | | | 0.81 | 0.67 | 0.83 | | | |
| Perivascular Lymphocytes | Absent | 2 (1.6) | 2 (1.7) | 1 (1.2) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Low | 41 (33) | 43 (36) | 20 (25) | 0.74 (0.09-6.00) | 1.46 (0.11-18.7) | 0.96 (0.14-6.42) | | | |
| | Medium | 45 (37) | 51 (43) | 35 (43) | 0.67 (0.08-5.51) | 1.24 (0.09-16.8) | 0.79 (0.12-5.40) | | | |
| | High | 35 (28) | 24 (20) | 24 (30) | 0.85 (0.10-7.12) | 1.15 (0.09-15.4) | 0.93 (0.14-6.36) | | | |
| | Unable to Assess[5] | 0 | 0 | 1 (1.2) | | | | | | |
| | **P-value for trend[6]** | | | | 0.85 | 0.65 | 0.86 | | | |
| Diffuse Lymphocytes | Absent | 12 (9.8) | 8 (6.7) | 10 (12) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Low | 48 (39) | 57 (48) | 23 (28) | 0.43 (0.14-1.31) | 2.67 (0.83-8.59) | 0.81 (0.32-2.06) | | | |
| | Medium | 41 (33) | 38 (32) | 29 (36) | 0.55 (0.17-1.76) | 1.93 (0.56-6.62) | 0.85 (0.32-2.27) | | | |
| | High | 22 (18) | 17 (14) | 18 (22) | 0.53 (0.15-1.89) | 1.54 (0.42-5.65) | 0.77 (0.27-2.19) | | | |
| | Unable to Assess[5] | 0 | 0 | 1 (1.2) | | | | | | |
| | **P-value for trend[6]** | | | | 0.94 | 0.79 | 0.75 | | | |
| **Vertical Growth Phase[7]** | | ***n*=91** | ***n*=81** | ***n*=66** | | | | | | |
| Vertical Growth Phase Cell Type | Epithelioid | 86 (95) | 65 (80) | 57 (86) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) |
| | Spindle | 0 | 9 (11) | 6 (9.1) | N.E. | N.E. | N.E. | N.E. | N.E. | N.E. |
| | Nevoid | 2 (2.2) | 4 (4.9) | 1 (1.5) | 0.70 (0.13-3.86) | 2.52 (0.18-36.1) | 0.86 (0.18-4.06) | N.E. | N.E. | N.E. |
| | Spitzoid | 0 | 0 | 0 | N.E. | N.E. | N.E. | N.E. | N.E. | N.E. |
| | Unable to Assess[5] | 3 (3.3) | 3 (3.7) | 2 (3.0) | | | | | | |
| | **P-value (df=3)[4]** | | | | **0.002** | **0.01** | **0.01** | | | |
| Vertical Growth Phase Cytologic Grade | Low | 9 (9.9) | 3 (3.7) | 2 (3.0) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | 1 (Reference) |

| | | CDKN2 *A*-Positive | *CDKN2 A*-Negative | Sporadic | *CDKN2A* -Positive vs. *CDKN2A*-Negative | *CDKN2A*-Positive vs. Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative and Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative | *CDKN2A*-Positive vs. Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative and Sporadic |
|---|---|---|---|---|---|---|---|---|---|---|
| **Melanoma Subtype and Thickness** | **Category** | ***n* (%)** | ***n* (%)** | ***n* (%)** | **OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | | **Multivariate OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | |
| | Medium | 70 (77) | 64 (79) | 42 (64) | 0.21 (0.04-1.03) | 0.27 (0.05-1.50) | **0.27 (0.08-0.93)** | N.E. | | N.E. |
| | High | 9 (9.9) | 10 (12) | 20 (30) | 0.16 (0.02-1.09) | **0.14 (0.02-0.94)** | **0.17 (0.04-0.75)** | N.E. | | N.E. |
| | Unable to Assess[5] | 3 (3.3) | 4 (4.9) | 2 (3.0) | | | | | | |
| | **P-value for trend[6]** | | | | 0.08 | **0.04** | **0.03** | | | |
| Vertical Growth Phase Mitotic Rate | 0/mm2 | 69 (76) | 51 (63) | 28 (42) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) |
| | ≥1/mm2 | 19 (21) | 27 (33) | 36 (55) | 0.48 (0.21-1.08) | **0.38 (0.16-0.91)** | **0.44 (0.21-0.92)** | 0.39 (0.13-1.20) | 0.46 (0.15-1.39) | 0.40 (0.15-1.01) |
| | Unable to Assess[5] | 3 (3.3) | 3 (3.7) | 2 (3.0) | | | | | | |
| | **P-value (df=1)[4]** | | | | 0.06 | **<0.001** | **<0.001** | 0.45 | 0.34 | 0.053 |
| Vertical Growth Phase Border | Partly Pushing (>50%) | 25 (27) | 26 (32) | 21 (32) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Entirely Pushing | 63 (69) | 52 (64) | 44 (67) | 1.35 (0.68-2.68) | 0.83 (0.38-1.84) | 1.12 (0.61-2.06) | | | |
| | Infiltrative | 0 | 0 | 0 | N.E. | N.E. | N.E. | | | |
| | Unable to Assess[5] | 3 (3.3) | 3 (3.7) | 1 (1.5) | | | | | | |
| | **P-value (df=1)[4]** | | | | 0.49 | 0.60 | 0.48 | | | |
| Neurotropism | Absent | 88 (97) | 78 (96) | 64 (97) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Present | 0 | 0 | 0 | N.E. | N.E. | N.E. | | | |
| | Unable to Assess[5] | 3 (3.3) | 3 (3.7) | 2 (3.0) | | | | | | |
| Vascular Invasion | Absent | 86 (95) | 77 (95) | 63 (95) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Present | 2 (2.2) | 0 | 1 (1.5) | N.E.[1] | 3.74 (0.25-55.0) | 6.87 (0.51-93.2) | | | |
| | Unable to Assess[5] | 3 (3.3) | 4 (4.9) | 2 (3.0) | | | | | | |
| | **P-value (df=1)[4]** | | | | N.E. | 0.76 | 0.31 | | | |
| Desmoplasia | Absent | 88 (97) | 78 (96) | 64 (97) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | | |
| | Present | 0 | 0 | 0 | N.E. | N.E. | N.E. | | | |
| | Unable to Assess[5] | 3 (3.3) | 3 (3.7) | 2 (3.0) | | | | | | |
| Tumor Infiltrating Lymphocytes | Absent | 23 (25) | 31 (38) | 10 (15) | 1 (Reference) | 1 (Reference) | 1 (Reference) | 1 (Reference) | | |

| | | CDKN2 *A*-Positive | *CDKN2 A*-Negative | Sporadic | *CDKN2A* -Positive vs. *CDKN2A*-Negative | *CDKN2A*-Positive vs. Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative and Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative | *CDKN2A*-Positive vs. Sporadic | *CDKN2A*-Positive vs. *CDKN2A*-Negative and Sporadic |
|---|---|---|---|---|---|---|---|---|---|---|
| **Melanoma Subtype and Thickness** | **Category** | ***n* (%)** | ***n* (%)** | ***n* (%)** | **OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | | **Multivariate OR (95% CI) for *CDKN2A* Mutation[1,2,3]** | | |
| | Low | 40 (44) | 33 (41) | 38 (58) | 1.44 (0.69-3.01) | 0.69 (0.27-1.77) | 1.07 (0.55-2.09) | **2.63 (1.01-6.88)** | | |
| | Medium | 15 (16) | 10 (12) | 9 (14) | 2.26 (0.82-6.25) | 1.29 (0.37-4.47) | 1.91 (0.77-4.70) | **4.62 (1.21-17.6)** | | |
| | High | 10 (11) | 4 (4.9) | 7 (11) | 2.43 (0.71-8.32) | 0.70 (0.20-2.48) | 1.44 (0.52-3.95) | **4.63 (1.02-21.0)** | | |
| | Unable to Assess[5] | 3 (3.3) | 3 (3.7) | 2 (3.0) | | | | | | |
| | **P-value for trend[6]** | | | | 0.06 | 0.93 | 0.22 | **0.02** | | |

[1] Includes histologic variables with p-values <0.20 in models adjusting for age at diagnosis, sex, and AJCC thickness category.

[2] All statistically significant values in Table 1 are bolded.

[3] The designation “N.E.” (not estimatable) was used for odds ratios that could not be estimated because of a zero count in either the numerator or denominator.

[4] Global p-values were determined by modeling variables as nominal categorical.

[5] Unable to evaluate because of poor slide quality.

[6] Mantel-Haenszel (M-H) p-values for trend were determined by modeling variables as ordinal.

[7] By definition cases of melanoma *in situ* lack a vertical growth phase (VGP) and were not included in the statistical analysis of VGP variables.

[8] Melanoma subtype was excluded from the multivariate analysis because it is a morphologic classification scheme based upon the presence of specific histologic features and hence was highly collinear with the same features as variables incorporated into the multivariate models.