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Gain of *CCND1* May Occur Too Infrequently in Cutaneous Melanoma, and Too Late in Melanomagenesis, to Be Diagnostically Useful: Genomic Analysis of 88 Cases

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Abstract

Genomic analysis is an important tool in the diagnosis of histologically ambiguous melanocytic neoplasms. Melanomas, in contrast to nevi, are characterized by the presence of multiple copy number alterations. One such alteration is gain of the proto-oncogene *CCND1* at 11q13. In melanoma, gain of *CCND1* has been reported in approximately one-fifth of cases. Exact frequencies of *CCND1* gain vary by melanoma subtype, ranging from 15.8% for lentigo maligna to 25.1% for acral melanoma. We present a cohort of 72 cutaneous melanomas from 2017–2022 in which only 6 (8.3%) showed evidence of *CCND1* gain by chromosomal microarray. This *CCND1* upregulation frequency falls well below those previously published and is significantly lower than estimated in the literature ($P < 0.05$). In addition, all 6 melanomas with *CCND1* gain had copy number alterations at other loci (most commonly *CDKN2A* loss, followed by *RREB1* gain), and 5 were either thick or metastatic lesions. This suggests that *CCND1* gene amplification may be a later event in melanomagenesis, long after a lesion would be borderline or equivocal by histology. Data from fluorescence in situ hybridization, performed on 16 additional cutaneous melanomas, further corroborate our findings. *CCND1* gain may not be a common alteration in melanoma and likely occurs too late in melanomagenesis to be diagnostically useful. We present the largest chromosomal microarray analysis of *CCND1* upregulation frequencies in cutaneous melanoma, conjecture 3 hypotheses to explain our novel observation, and discuss implications for the inclusion or exclusion of *CCND1* probes in future melanoma gene panels.

Keywords

melanoma; *CCND1* ; histologically ambiguous melanocytic neoplasms; copy number variation

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INTRODUCTION

Melanomas, unlike nevi, often harbor genomic alterations at 8q24 (gain of *MYC*), 9q21 (loss of *CDKN2A*), 6q23 (loss of *MYB*), and 6p24 (gain of *RREB1*).[1,2] For histologically ambiguous melanocytic neoplasms, copy number variation (CNV) analysis at these loci can be decisive. Gain of the proto-oncogene *CCND1* at 11q13 has also been described as a recurrent alteration in melanoma, particularly of the acral lentiginous subtype.[3,4] *CCND1* is a 13,388 base pair proto-oncogene expressed ubiquitously in the skin and liver (National Library of Medicine, National Center for Biotechnology Information). Alternative splicing results in 2 major *CCND1* mRNA transcripts modulated by the A870G single nucleotide polymorphism[5] (Fig. 1). Both isoforms of cyclin D1, the protein encoded by *CCND1*, are involved in G1-S cell cycle progression by stimulating CDK4 and CDK6. Amplification of *CCND1* and overexpression of cyclin D1 can also promote melanomagenesis by inhibiting DNA repair, suppressing mitochondrial metabolism, promoting field cell proliferation, and activating the MAPK, PI3K, Akt, Wnt, and NF-kb oncogenic pathways.[6–10]

Previously Reported Frequencies of *CCND1* Gain in Cutaneous Melanoma

Previous literature estimates of *CCND1* gain in melanoma vary considerably (Fig. 2). To maximize generalizability and minimize bias, we analyzed data from the largest meta-analysis of *CCND1* amplification frequencies in melanoma, found in González-Ruiz et al. The authors analyzed 22 studies in 2096 melanomas (sample size range: 6–514 melanomas), with samples hailing from 5 continents and 11 countries.[11] Because we restricted our analysis to cutaneous melanomas, we excluded 5 of the 22 studies: 4 because mucosal melanomas were analyzed and 1 because both mucosal and uveal melanomas were analyzed. The 17 remaining studies comprised 1518 cutaneous melanomas (sample size range: 7–514 melanomas), with samples hailing from 5 continents and 9 countries (Table 1).

Among these 17 studies, estimates of *CCND1* gain in cutaneous melanoma varied considerably (mean 31.2%, SD 22.6%, range 5.7%–87.5%)[11] (Table 1). *CCND1* gain was most frequent in acral lentiginous melanomas (25.1%, 95% CI = 15.8–35.4%), followed by the nodular (22.7%, 95% CI = 3.7–48.3%), lentigo maligna (16.2%, 95% CI = 2.5–35.6%), and superficial spreading (15.8%, 95% CI = 3.4–32.9%) subtypes.[11]

MATERIALS AND METHODS

Cohort Selection

Institutional records were searched for all melanocytic neoplasms and metastatic melanomas diagnosed at our institution from 2017 to 2022. Inclusion criteria were as follows: (1) histologically confirmed diagnosis of cutaneous malignant melanoma; (2) known chromosomal microarray analysis (CMA)-determined copy number status for *RREB1*, *MYB*, *MYC*, *CDKN2A*, and *CCND1*; and (3) no evidence of mucosal or uveal melanoma. Our patient cohort hails largely from the Upper Valley of the Connecticut River (ie, MA, VT, and NH). Demographic and clinical details were gathered for each patient, including age, sex, primary versus metastatic status, Breslow depth, and histologic subtype (Table 2).

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All melanoma samples were reviewed by at least 1 board-certified dermatopathologist at an NCI-Designated Comprehensive Cancer Center.

Chromosomal Microarray (Array Comparative Genomic Hybridization)

DNA from the 72 melanoma samples was isolated using the QIAGEN QIAamp FFPE Tissue Kit (Qiagen, Valencia, CA). DNA quantity was measured through Qubit Fluorometer 3.0 and Qubit dsDNA High-Sensitivity assay kit (Thermo Fisher Scientific company, Waltham, MA). Samples were then subjected to CMA for quantitation of CNV in *CCND1*, *RREB1*, *MYC*, *CDKN2A*, and *MYB* following the protocol of the OncoScan FFPE Assay Kit (Affymetrix, a Thermo Fisher Scientific company, Santa Clara, CA). All CMA copy number gains and losses were confirmed by an expert in CMA analysis.

Statistical Analysis

To assess CNV in *CCND1*, 13 of the 17 studies in the Gonzalez-Ruiz et al[11] meta-analysis used fluorescence in situ hybridization (FISH), 1 used qPCR, 1 used both FISH and qPCR, 1 used comparative genomic hybridization, and 1 used next-generation sequencing. *CCND1* gain was considered “positive” (evidence of *CCND1* gain) or “negative” (no evidence of *CCND1* gain) according to the methodology used in each study. Of all 1518 cutaneous melanomas in the meta-analysis, 329 had evidence for *CCND1* gain, yielding an overall literature estimate of 21.7% (Table 1).[11]

RESULTS

Patient Cohort

Seventy-two formalin-fixed, paraffin-embedded (FFPE) skin samples from 60 patients met inclusion criteria (Table 2). Seventeen patients (28.3%) were female. Mean age at the time of biopsy was 66.7 years (range 33–97, SD 12.2). Of the 72 cutaneous melanomas, 9 (12.5%) were superficial spreading, 8 (11.1%) nodular, 7 (9.7%) melanoma in situ, 6 (8.3%) nevoid, 3 (4.2%) acral lentiginous, 3 (4.2%) not otherwise specified, 2 (2.8%) lentigo maligna, 2 (2.8%) superficial spreading and spindle cell, 1 (1.4%) blue nevus–like, 1 (1.4%) spitzoid, 1 (1.4%) mixed desmoplastic and nodular, 1 (1.4%) mixed desmoplastic and spindled, 1 (1.4%) mixed desmoplastic, 1 (1.4%) subungual, 1 (1.4%) epithelioid, spindled, and clear cell, 1 (1.4%) dedifferentiated and spindled, and 1 (1.4%) metastatic versus amelanotic. The remaining 23 (31.9%) cases were metastatic lesions. Average Breslow depth for histologically confirmed primary cutaneous melanomas was 3.3 mm (range 0.2–16.0).

Comparison With Previous Literature Estimates

Of the 72 cutaneous melanomas in our cohort, 6 (8.3%) had evidence of *CCND1* gain by CMA. To compare our frequency of cutaneous melanomas with *CCND1* gain (8.3%) to the overall literature estimate of 21.7%, we used a 2-proportions *z*-test. We obtained a statistically significant difference between our frequency of *CCND1* gain in cutaneous melanoma (8.3%) and the overall literature estimate of 21.7% ($P < 0.05$) (Fig. 3). Because this 21.7% estimate combines melanomas from 17 individual studies, we calculated an additional literature estimate (from the same 17 studies in the meta-analysis) that accounts for combining data from heterogeneous sources. To do this, we fit a generalized mixed-

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effects model in which “individual study” was treated as a random effect and obtained a “corrected” overall literature estimate of 25.6%. We again saw a statistically significant difference between our frequency of *CCND1* gain in cutaneous melanoma (8.3%) and this “corrected” literature estimate of 25.6% ($P < 0.01$) (Fig. 3). All statistical tests were two-sided.

Prospective Versus Retrospective Analysis

For 67 of the 72 melanomas, CMA was performed prospectively (ie, for research, not clinical care). For these 67 cases, marked as “research” in Table 2, the melanoma diagnosis was secured by either hematoxylin and eosin staining alone (30 cases) or by hematoxylin and eosin staining and immunohistochemistry (37 cases). CMA was not involved in clinical care and was performed for research purposes after the melanoma diagnosis was established. All 6 *CCND1*-positive melanomas fall into this category, wherein the diagnosis was already clear.

For the 5 remaining melanomas, CMA data were collected retrospectively (ie, CMA had been performed for clinical care, not research) (Table 2). None of these 5 melanomas had evidence of *CCND1* gain by CMA.

Histopathology of Melanomas With *CCND1* Gain

All melanoma cases were assessed for dense (lymph node–like) inflammation, a prominent associated nevus, tumor necrosis, and hemorrhage. Of the 6 melanomas with *CCND1* gain in our cohort, 2 had both hemorrhage and necrosis, 2 had hemorrhage without necrosis, and 2 had neither hemorrhage nor necrosis. By histologic subtype, 3 of these 6 *CCND1*-positive melanomas were nodular (individual Breslow depths = 3.2 mm, 4.9 mm, and 9.8 mm), 1 was blue nevus–like (Breslow depth = 16 mm), 1 was superficial spreading and spindle cell (Breslow depth = 1.2 mm), and 1 was a metastatic lesion. Representative histologic images are shown in Figure 4.

Molecular Genetics of Melanomas With *CCND1* Gain

All 6 melanomas with *CCND1* gain also had copy number alterations in other chromosomal loci: 3 had *CDKN2A* loss, 1 had both *CDKN2A* loss and *RREB1* gain, 1 had both *RREB1* gain and *MYC* gain, and 1 had *CDKN2A* loss, *RREB1* gain, and *MYC* gain.

DISCUSSION

To the best of our knowledge, this study is the largest CMA analysis of *CCND1* amplification frequencies in cutaneous melanoma. *CCND1* gain is reported to be a common copy number alteration in cutaneous melanoma, with some studies[12] citing frequencies of up to 87.5%, but our results suggest otherwise. Notably, nearly all (67 of 72) melanomas had not required CMA data to establish a diagnosis. These 67 cases were not borderline melanocytic lesions. None of the 5 borderline lesions had evidence of *CCND1* gain. In our data set, that genomic feature only arose in melanomas wherein the disease was advanced and the diagnosis obvious. Our *CCND1* positivity rate is significantly lower than those of previous studies; we now turn to the question of why this has occurred in our cohort.

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Ethnic Composition of the Patient Cohort

Previous studies have shown that *CCND1* amplifications are less prevalent in white patients than in black patients for breast cancer (4.0% vs. 16.0%, $P < 0.05$), localized prostate cancer (1.9% vs. 5.2%, $P < 0.05$), and prostate cancer of all stages (3.2% vs. 6.0%, $P < 0.05$).[13,14] More than 90% of patients in our catchment area self-identify as white. It is possible that in cutaneous melanoma, as in breast and prostate cancers, *CCND1* gain is less common among white patients than among black patients. Notably, such a difference would be likely independent of processes related to sun exposure. If verified, this could explain the low frequency of *CCND1* gain in our cohort.

We also note that previous melanoma FISH studies were conducted in large, ethnically diverse cities, which may have contributed to the wide variation seen in previous estimates of *CCND1* gain in melanoma (mean 31.2%, SD 22.6%, range 5.7%–87.5%). Significantly, acral melanomas, in which *CCND1* gains are most prevalent, make up approximately 70% of melanomas among black patients but fewer than 5% of melanomas among white patients.[15] In our cohort of predominantly white patients, only 3 melanomas (4.2%) were of the acral subtype, none of which showed evidence of *CCND1* gain.

Type of Sunlight Exposure (Chronic vs. Intermittent)

In melanoma, *CCND1* gains are less common in patients exposed to intermittent UV light than in patients exposed to chronic UV light.[16–18] Our cohort lives predominantly in the Upper Valley region of the Connecticut River. This region receives very little direct sunlight and is covered by snow—which can backscatter as much as 80% of UV radiation—for many months of the year. Some patients, of course, do travel to other regions. But on a population scale, exposure to intermittent, as opposed to chronic, sunlight in our catchment area presents a possible explanation for our comparatively low melanoma *CCND1* amplification frequency (Table 3).

Is it a CMA Technical Error?

CMA is most effective when performed on euchromatic genes.[19] Because *CCND1* is pericentromeric (ie, located outside of 11q euchromatin), it is possible that user error may account for our anomalously low *CCND1* positivity rate (Fig. 5). This seems somewhat unlikely because all CMA runs were performed by multiple PhD scientists and technicians with years of experience running CMA. It seems similarly unlikely that user error would cause a systematic (ie, nonrandom) effect, only at the 11q pericentromeric locus, over a sustained period from 2017 to 2022. Furthermore, no other genes seem to be affected: Of the 72 melanomas for which CMA was performed, 39 (54.2%) had evidence of *RREB1* gain, 29 (40.3%) had evidence of *CDKN2A* loss, 23 (31.9%) had evidence of *MYC* gain, and 22 (30.6%) had evidence of *MYB* loss.

To further exclude the possibility of technical error, we reviewed FISH results for an additional 16 cutaneous melanomas, collected at our institution over the past 4 years (Table 4). None of these 16 cases were in the original cohort of 72 melanomas, as these 16 cases had FISH data (not CMA data) for CNV in *RREB1*, *MYC*, *MYB*, *CDKN2A*, and *CCND1*—i.e., they did not meet inclusion criterion (2). For these 16 melanomas, FISH had been

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performed at different laboratories across the United States, chiefly ProPath and the Mayo Clinic. Like the 5 melanomas in the retrospective CMA cohort, these 16 melanomas had CNV analysis performed as part of clinical care; the diagnosis was not obvious and required FISH studies.

If CMA technical error were occurring, we would expect a greater *CCND1* positivity rate by FISH than by CMA. However, only 1 (6.7%) of these 16 melanomas showed evidence of *CCND1* gain by FISH. This case was a spitzoid melanoma (Breslow depth = 0.3 mm) that was also FISH-positive for both *RREB1* gain and *MYC* gain. We found no statistically significant difference between our CMA-determined *CCND1* positivity rate of 8.3% and our FISH-determined *CCND1* positivity rate of 6.7%. These orthogonal data further reduce the likelihood that a CMA technical error occurred.

CCND1 Gain May Occur Too Late in Melanomagenesis to be Useful for Borderline Lesions.

In melanoma, the frequency of *CCND1* amplification is significantly greater in distant metastases than in primary tumors ($P < 0.05$).[11] *CCND1* gain may occur in the later stages of melanomagenesis, long after a lesion would be considered equivocal or borderline by histology. Our results are congruent with this idea. None of the 7 cutaneous melanomas with gain of *CCND1* (6 by CMA and 1 by FISH) were borderline lesions that required genomic analysis for clinical care; all were histologically unambiguous cases for which *CCND1* CNV analysis was performed prospectively. In addition, all 7 melanomas with *CCND1* gain also had alterations in other loci (most commonly *CDKN2A* loss, followed by *RREB1* gain), and 5 were either thick or metastatic lesions. Even if a melanoma gene panel without *CCND1* had been performed, it would have returned a positive result (ie, met the criteria for melanoma) for all 7 cases.

Collectively, our results suggest that gain of *CCND1* may occur too late in melanomagenesis to be diagnostically useful in borderline lesions, which are often atypical nevi or early stage melanomas. By the time *CCND1* amplification would occur in melanomagenesis, the tumor will have already progressed to a stage at which histopathology, or CNV in a different FISH locus, would suffice. At least in our cohort, it seems that *CCND1* amplification is present when least needed and absent when most needed.

Gain of *CCND1* in cutaneous melanoma, particularly in borderline lesions, may not be as common as previously reported. The cost of each additional melanoma FISH probe can be significant; thus, the resource should be used efficiently. In our cohort, removing the 11q13 (*CCND1*) probe would have had no effect on the sensitivity or specificity of CMA or FISH assays for melanoma. *CCND1* gene amplification may not be common enough, or occur early enough, in melanomagenesis to warrant routine inclusion of 11q13 probes in genomic analyses for all borderline melanocytic lesions.

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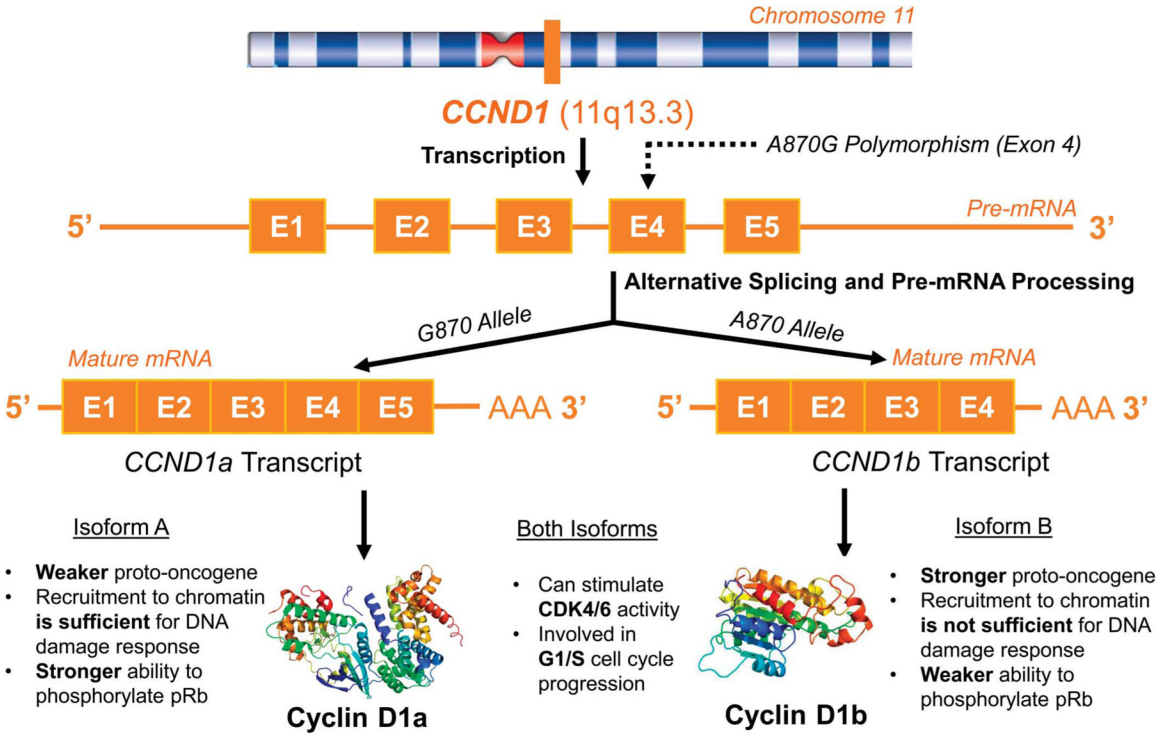

FIGURE 1.

The A870G single nucleotide polymorphism results in 2 major *CCND1* mRNA transcripts. Cyclin D1a has a stronger ability to phosphorylate pRb than cyclin D1b. In addition, recruitment of cyclin D1a, but not cyclin D1b, to chromatin is sufficient for DNA damage response.

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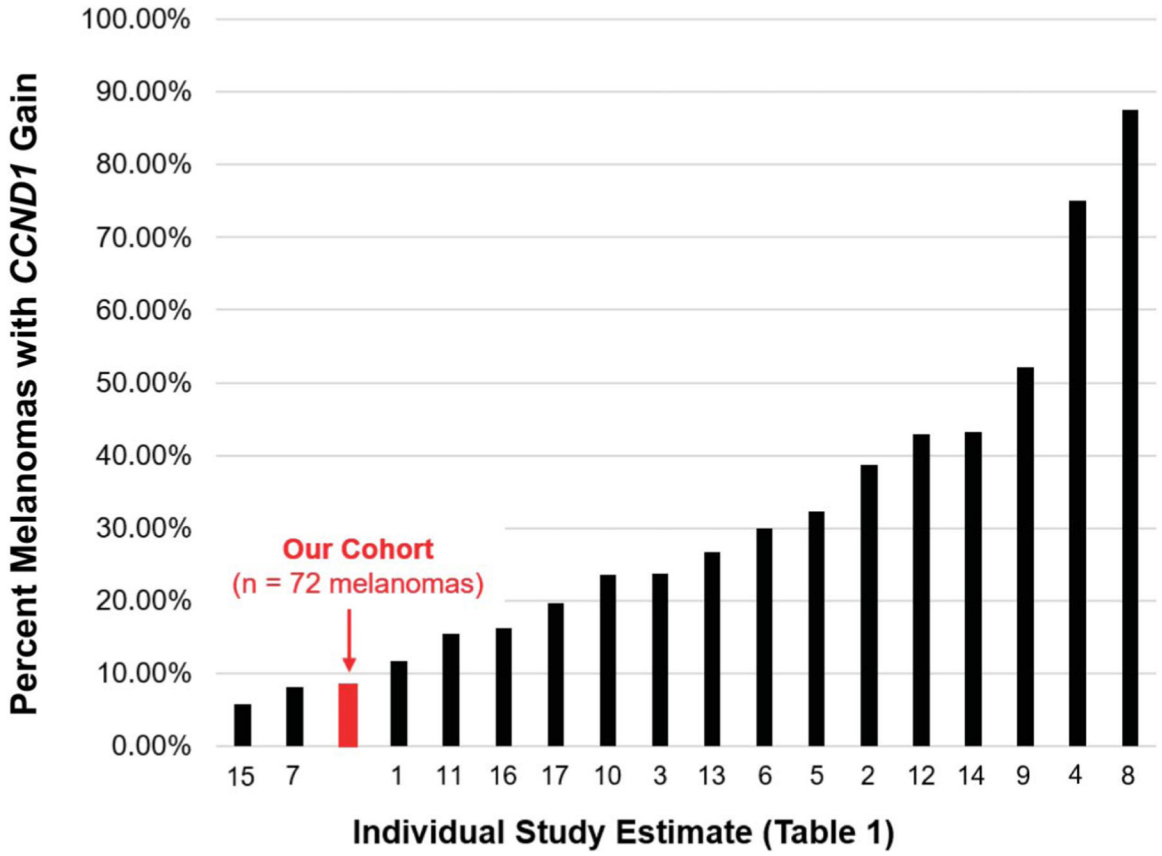

FIGURE 2.

Individual study estimates of *CCND1* amplification in melanoma vary considerably. The frequencies of *CCND1* gain in melanoma for the 17 individual studies in Gonzalez-Ruiz et al, the largest meta-analysis of *CCND1* amplifications in melanoma, are shown in blue. Exact numerical values are found in Table 1. Our frequency of *CCND1* gain in melanoma (8.3%) is shown in red.

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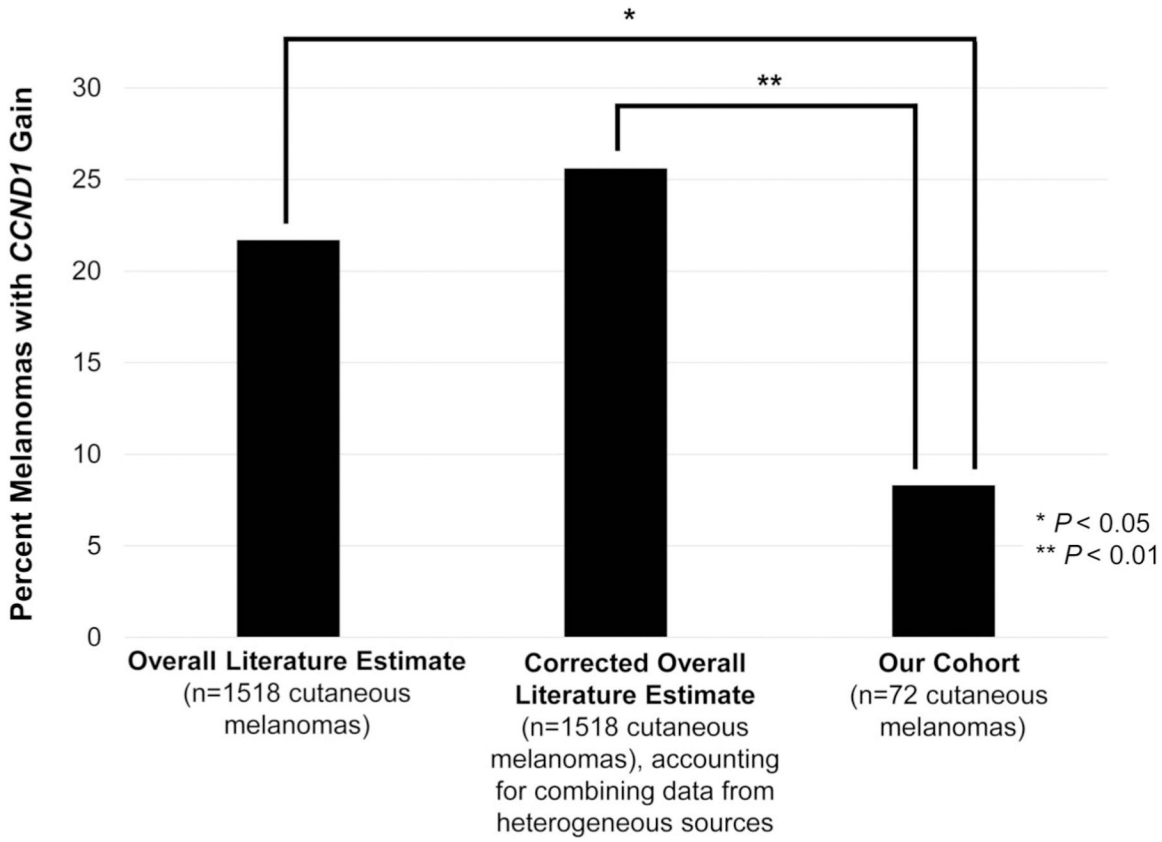

FIGURE 3.

Results of two-proportions *z*-test. We found a statistically significant difference between our *CCND1* positivity rate in melanoma (8.3%) and the overall literature estimate of 21.7% ($P < 0.05$). Statistical significance was maintained after accounting for combining data from heterogeneous sources ($P < 0.01$).

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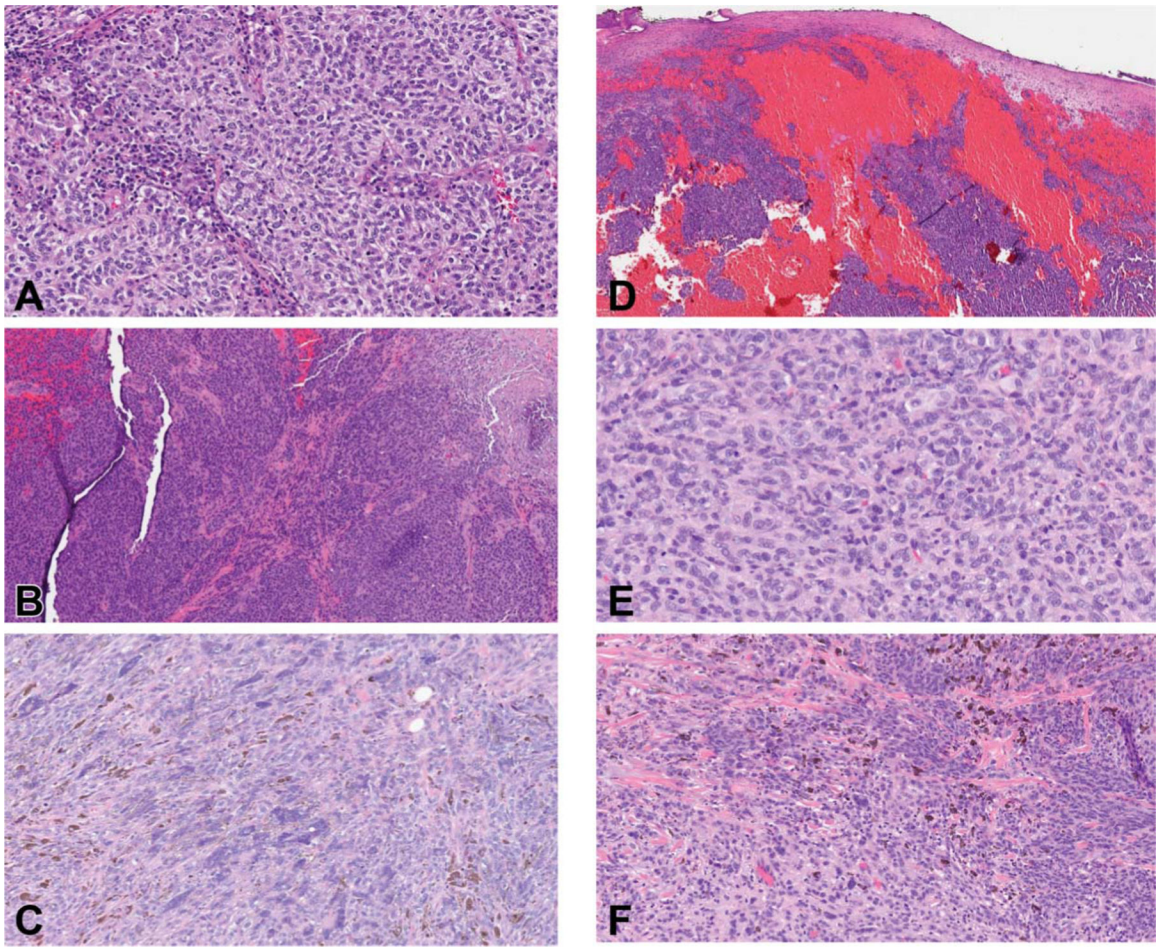

FIGURE 4.

Representative images of all cases with evidence of *CCND1* gain by CMA in our cohort. The melanomas are characterized by clearly malignant features, such as sheet-like growth (A–C), ulceration and hemorrhage (D), mitotic activity and a lack of maturation (E), or marked cytologic atypia (F). In each of these, CMA was performed for research purposes alone; the diagnosis of melanoma was already clear from morphology.

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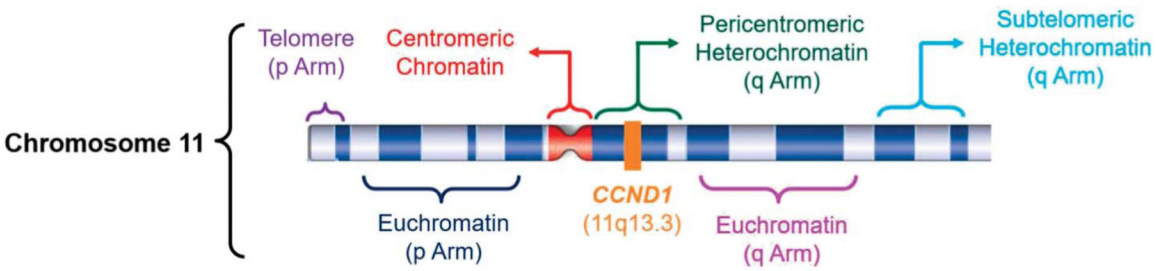

FIGURE 5.

The *CCND1* gene is located close to 11q pericentromeric heterochromatin. CMA is most effective when performed on euchromatin. We considered the possibility of whether this location, and/or technical error, could cause the results. Orthogonal FISH indicates otherwise.

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TABLE 1.

Individual Study Estimates of *CCND1* Gene Amplification Frequencies in Cutaneous Melanoma

No	Study	Country	Method for CNV Detection	No of Melanomas With *CCND1* Gain	Total No of Melanomas Analyzed	% Melanomas With *CCND1* Gain	Histologic Subtypes of Cutaneous Melanomas
1	Sauter et al, 2002	United States	FISH	12	102	11.76%	NM (17), SSM (57), LMM (18), and ALM (10)
2	Utikal et al, 2005	Norway	FISH	12	31	38.7%	NM (13), SSM (4), LMM (1), ALM (1), and metastatic (12)
3	Takata et al, 2005	Japan	FISH	5	21	23.8%	ALM (10) and metastatic (11)
4	Morey et al, 2009	Australia	FISH	15	20	75.0%	NM (3), SSM (6), metastatic (10), and unknown (1)
5	Lázár et al, 2009	Hungary	qPCR and FISH	24	74	32.35%	NM (26), SSM (42), and metastatic (6)
6	Gerami et al, 2009	United States	FISH	3	10	30.0%	NOC (10)
7	Nai et al, 2010	Brazil	FISH	5	62	8.06%	SSM (26), NM (13), LMM (12), ALM (10), and NOC (1)
8	Requena et al, 2012	Spain	FISH	7	8	87.50%	Spitzoid (8)
9	Nathanson et al, 2013	Australia and United States	CGH	11	21	52.17%	Metastatic (21)
10	Diaz et al, 2014	Spain	FISH	8	34	23.53%	ALM (34)
11	Young et al, 2014	Australia	FISH	22	143	15.38%	NOC (143)
12	Romano et al, 2016	United States	FISH	3	7	42.85%	ALM (3), NM (2), MIS (1), and unknown (1)
13	Kong et al, 2017	China	qPCR	137	514	26.65%	ALM (514)
14	Su et al, 2017	China	FISH	19	44	43.18%	ALM (44)
15	Sini et al, 2018	Italy	FISH	15	262	5.72%	NOC (118) and metastatic (144)
16	Haugh et al, 2018	United States	FISH	7	43	16.27%	ALM (19), SSM (19), LMM (1), and NM (4)
17	Yeh et al, 2019	United States	NGS	24	122	19.67%	ALM (122)
Total				329	1518	21.67%	

All studies were taken from Gonzalez-Ruiz et al, the largest meta-analysis of *CCND1* upregulation frequencies in cutaneous melanoma.

ALM, acral lentiginous melanoma; CGH, comparative genomic hybridization; LMM, lentigo maligna melanoma; NGS, next-generation sequencing; NM, nodular melanoma; SSM, superficial spreading melanoma; NOC, cutaneous melanoma, not otherwise classified; MIS, melanoma in situ; qPCR, quantitative polymerase chain reaction.

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Table 2.

Clinicopathologic Features of Melanoma Cases in Our Cohort for Which CMA Was Performed

Case No	Sex	Age at Biopsy (yr)	*RREB1* Gain	*MYB* Loss	*MYC* Gain	*CDKN2A* Loss	*CCND1* Gain	Necrosis	Hemorrhage	Histologic Subtype	Breslow Depth (mm)	Research or Clinical?
1	M	87	+	—	—	+	—	Yes	No	Metastatic	N/A	Research
2	M	87	+	+	—	—	—	NR	NR	Metastatic	N/A	Research
3	M	65	+	—	+	—	—	Yes	No	Metastatic	N/A	Research
4	M	65	+	—	+	—	—	Yes	Yes	Metastatic	N/A	Research
5	M	52	+	+	—	+	—	NR	NR	Metastatic	N/A	Research
6	M	52	+	+	—	+	—	NR	NR	Metastatic	N/A	Research
7	F	53	+	—	—	—	—	Yes	Yes	Metastatic	N/A	Research
8	F	53	+	—	+	—	—	Yes	Yes	Metastatic	N/A	Research
9	F	53	+	—	+	—	—	NR	NR	Metastatic	N/A	Research
10	F	53	+	—	+	—	—	NR	NR	Metastatic	N/A	Research
11	M	33	—	+	+	—	—	No	No	Locoregional metastasis/ recurrence	N/A	Research
12	M	64	+	+	—	+	—	No	No	Nodular	1.6	Research
13	M	67	+	—	—	+	—	Yes	Yes	Not otherwise specified	N/A	Clinical
14	M	55	+	—	+	—	—	No	No	Nevoid	N/A	Clinical
15	M	64	—	—	—	+	—	No	No	Mixed (desmoplastic and nodular)	N/A	Clinical
16	F	51	—	—	—	—	—	No	No	Melanoma in situ and spitzoid	N/A	Clinical
17	M	48	—	+	+	—	—	No	No	Nevoid	2.3	Research
18	F	81	+	+	+	+	—	Yes	No	Metastatic	N/A	Research
19	F	68	—	—	+	—	—	No	No	Metastatic	N/A	Research
20	M	56	+	—	+	—	—	Yes	Yes	Metastatic	N/A	Research
21	M	56	+	—	+	—	—	Yes	Yes	Metastatic	N/A	Research
22	F	71	—	—	+	+	—	No	No	Metastatic	N/A	Research
23	F	71	—	—	+	+	—	No	No	Metastatic	N/A	Research
24	F	68	—	+	+	—	—	No	No	Metastatic	N/A	Research
25	M	67	—	+	—	+	—	No	Yes	Metastatic	N/A	Research
26	M	67	—	+	—	+	—	No	No	Metastatic	N/A	Research

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Case No	Sex	Age at Biopsy (yr)	*RREB1* Gain	*MYB* Loss	*MYC* Gain	*CDKN2A* Loss	*CCND1* Gain	Necrosis	Hemorrhage	Histologic Subtype	Breslow Depth (mm)	Research or Clinical?
27	M	67	—	+	—	+	—	No	Yes	Metastatic	N/A	Research
28	M	77	—	—	—	+	+	Yes	Yes	Metastatic	N/A	Research
29	M	73	+	—	—	+	—	No	No	Multiple (epithelioid, spindled, and clear cell)	4.1	Research
30	F	65	+	+	—	—	—	No	No	Acral	1.5	Research
31	F	65	+	+	—	—	—	No	No	Acral	0.9	Research
32	F	65	+	+	—	—	—	No	No	Acral	1.2	Research
33	M	97	+	+	—	+	—	No	No	Subungual	2.7	Research
34	F	83	+	+	—	+	—	Yes	No	Nevoid	4.8	Research
35	F	83	+	+	—	+	—	Yes	Yes	Nevoid	4.8	Research
36	M	71	+	+	—	—	—	No	No	Melanoma in situ	N/A	Research
37	F	58	+	+	—	—	—	No	No	Superficial spreading	0.4	Research
38	F	63	+	—	—	—	—	No	No	Melanoma in situ	N/A	Research
39	M	83	+	—	+	+	+	No	Yes	Nodular	4.9	Research
40	M	97	—	—	—	+	+	No	Yes	Nodular	9.8	Research
41	M	63	+	—	—	+	—	No	No	Superficial spreading	0.8	Research
42	M	59	+	—	+	—	—	Yes	No	Nodular	4.2	Research
43	M	66	+	—	—	—	—	No	No	Lentigo maligna	0.2	Research
44	F	74	+	+	—	—	—	NR	NR	Melanoma in situ	N/A	Research
45	M	79	+	—	—	—	—	No	No	Lentigo maligna	0.4	Research
46	M	72	—	—	—	+	—	Yes	Yes	Dedifferentiated and spindled	6.4	Research
47	M	84	+	—	—	+	+	No	No	Superficial spreading and spindle cell	1.2	Research
48	M	69	+	—	—	—	—	No	No	Nevoid	1.2	Research
49	M	76	+	+	+	—	—	NR	NR	Nodular	2.5	Research
50	M	60	+	—	+	+	—	NR	NR	Nodular	5.0	Research
51	M	66	+	—	—	—	—	NR	NR	Superficial spreading and spindle cell	0.9	Research
52	M	70	+	—	—	—	—	NR	NR	Mixed spindled and desmoplastic	15	Research
53	F	67	—	—	—	+	—	NR	NR	Not otherwise specified	1.3	Research
54	M	64	—	—	—	+	+	NR	NR	Nodular	3.2	Research

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Case No	Sex	Age at Biopsy (yr)	*RREB1* Gain	*MYB* Loss	*MYC* Gain	*CDKN2A* Loss	*CCND1* Gain	Necrosis	Hemorrhage	Histologic Subtype	Breslow Depth (mm)	Research or Clinical?
55	M	62	—	—	—	+	—	NR	NR	Nodular	3.3	Research
56	F	63	—	—	+	—	—	NR	NR	Not otherwise specified	1.9	Clinical
57	M	69	—	—	+	+	—	NR	NR	Metastatic	3.8	Research
58	M	45	+	—	+	—	+	NR	NR	Blue nevus–like	16	Research
59	M	64	—	—	—	+	—	No	No	Mixed desmoplastic	15	Research
60	M	75	—	+	+	+	—	NR	NR	Superficial spreading	2.9	Research
61	M	70	—	—	—	—	—	NR	NR	Superficial spreading	2.2	Research
62	M	74	—	—	—	—	—	Yes	Yes	Superficial spreading	3.0	Research
63	M	74	—	—	—	—	—	No	No	Melanoma in situ	N/A	Research
64	F	83	—	—	—	—	—	No	No	Melanoma in situ	N/A	Research
65	F	47	—	—	—	—	—	No	No	Melanoma in situ	N/A	Research
66	F	68	—	—	—	—	—	No	No	Melanoma in situ	0.7	Research
67	M	64	—	—	—	—	—	No	No	Superficial spreading	0.3	Research
68	M	57	—	—	—	—	—	No	No	Superficial spreading	0.3	Research
69	M	80	—	—	—	—	—	NR	NR	Nevoid	0.7	Research
70	M	71	—	—	—	—	—	NR	NR	Superficial spreading	0.7	Research
71	M	59	—	—	—	—	—	NR	NR	Spitzoid	0.6	Research
72	M	60	—	—	—	—	—	NR	NR	Superficial spreading	0.4	Research

“Clinical,” CMA was performed for clinical care, not research (retrospectively), and was used to establish the melanoma diagnosis; “Research,” CMA was performed for research, not clinical care (prospectively). N/A, not applicable; all copy number alterations were assessed through CMA; NR, not recorded; “+,” copy number alteration was detected by CMA; “−,” copy number alteration was not detected by CMA.

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TABLE 3.

Genomic Alterations in Melanoma Because of Varying Levels of Sun Exposure

	Chronic	Intermittent Sun Exposure	Minimal Sun Exposure	No Sun Exposure
Affected body parts	Face and hands	Trunk, arms, and legs	Soles and palms	Mucosal membranes
Chromosomal gains	6p, 11q13, 17q, and 20q	6p, 7, 8q, 17q, and 20q	5p13, 5p15, 6p, 7, 8q, 11q13, 12q14, 17q, and 20q	1q31, 4q12, 6p, 7, 8q, 11q13, 12q14, 17q, and 20q
Chromosomal losses	6q, 8p, 9p, 13, and 21q	9p, 10, and 21q	6q, 9p, 10, 11q, and 21q	3q, 4q, 6q, 8p, 9p, and 10
Relative level of sun exposure	High	Moderate	Low	None

11q13 harbors the *CCND1* proto-oncogene.

Table adapted from Nguyen and Watson.[20]

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Table 4.

Clinicopathologic Features of Melanoma Cases for Which FISH Was Performed

Case No	Sex	Age at Biopsy (yr)	*RREB1* Gain	*MYB* Loss	*MYC* Gain	*CDKN2A* Loss	*CCND1* Gain	Histologic Subtype	Breslow Depth (mm)	Research or Clinical?
1	F	41	+	—	+	—	+	Spitzoid	0.3	Clinical
2	M	86	+	—	+	—	—	Not otherwise specified	0.4	Clinical
3	M	75	—	—	+	—	—	Not otherwise specified	0.5	Clinical
4	F	57	+	—	—	—	—	Superficial spreading	0.6	Clinical
5	F	41	+	—	+	—	—	Spitzoid	0.4	Clinical
6	M	49	+	—	—	—	—	Superficial spreading	0.3	Clinical
7	F	67	—	—	—	—	—	Melanoma in situ	N/A	Clinical
8	M	41	—	—	—	—	—	Not otherwise specified	0.3	Clinical
9	F	70	—	—	—	—	—	Not otherwise specified	NR	Clinical
10	F	55	+	—	—	—	—	Not otherwise specified	0.6	Clinical
11	M	37	+	+	+	—	—	Melanoma in situ	N/A	Clinical
12	M	80	—	—	—	—	—	Not otherwise specified	2.4	Clinical
13	F	45	—	—	—	—	—	Not otherwise specified	0.3	Clinical
14	F	55	—	—	—	—	—	Melanoma in situ	N/A	Clinical
15	F	67	—	—	—	—	—	Melanoma in situ	N/A	Clinical
16	M	80	—	—	—	—	—	Not otherwise specified	0.3	Clinical

No cases in this cohort were included in the original CMA cohort of 72 melanomas. All copy number alterations were assessed through fluorescence in situ hybridization (FISH).

“+,” copy number alteration was detected by FISH; “−,” copy number alteration was not detected by FISH; “Research,” CMA was performed for research, not clinical care (prospectively); “Clinical,” CMA was performed for clinical care, not research (retrospectively), and was used to establish the melanoma diagnosis.

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