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Variation in cutaneous patterns of melanomagenesis according to germline *CDKN2A/CDK4* status in melanoma-prone families

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Abstract

CDKN2A and *CDK4* are well-established melanoma susceptibility genes, but their effect on tumor location/distribution is unknown. We used a case-case study design to assess for differences in tumor location between mutation carriers (*CDKN2A* =141 patients, 348 melanomas; *CDK4* =15 patients, 54 melanomas) and non-carriers (104 patients, 157 melanomas) in U.S. melanoma-prone families. Associations between groups were assessed with chi-square tests. Odds ratios (ORs) for tumor location were adjusted for diagnosis age, gender, and superficial spreading subtype. Models included random effects to account for within individual/family correlations. Compared to having a truncal melanoma, *CDK4* (vs. non-carriers: lower extremities OR=14.5, 95% CI, 5.02−42.0, $P<.001$; upper extremities OR=6.88, 95% CI, 2.37−19.9, $P<.001$; head/neck OR=18.6, 95% CI, 4.04−85.2, $P<.001$) and *CDKN2A* (vs. non-carriers: lower extremities OR=3.01, 95% CI, 1.56−5.82, $P<.05$; upper extremities OR=1.91, 95% CI, 1.03−3.52, $P<.05$; head/neck OR=5.40, 95% CI, 2.10−13.9, $P<.001$) carriers had higher odds of developing melanoma at all other sites.

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Conceptualization: MRS, RMP, XRY, MAT, AMG; Data Curation: MRS, MAT, AMG; Formal Analysis: MRS, RMP, XRY, MAT, AMG; Funding Acquisition: MAT, AMG; Investigation: MRS, RMP, XRY, MAT, AMG; Methodology: MRS, RMP, XRY, MAT, AMG; Project Administration: MRS, MAT, AMG; Resources: MRS, RMP, MAT, AMG; Software: MRS, RMP; Supervision: MRS, RMP, MAT, AMG; Validation: MRS, RMP, MAT, AMG; Visualization: MRS, RMP, AMG; Writing – original draft: MRS; Writing – review and editing: MRS, RMP, XRY, MAT, AMG

DATA AVAILABILITY

No datasets were generated or analyzed during the current study.

CONFLICTS OF INTEREST

The authors have no relevant conflicts of interest, financial or other, related to the contents of this article.

Similar findings were observed for analyses stratified by gender, age, and first vs. subsequent melanoma diagnoses. Further studies are needed to understand the biology underlying these genotype-associated patterns of tumor development, which could provide new insights into melanoma treatment and prevention.

Keywords

melanoma; tumor location; cyclin-dependent kinase inhibitor 2A (*CDKN2A*); cyclin-dependent kinase 4 (*CDK4*); cancer screening; family research

INTRODUCTION

Approximately 90,000 cases of melanoma are diagnosed annually in the United States. Between 5 and 10% of new diagnoses will occur in patients with a family history of melanoma. Further, among melanoma-prone families, germline mutations of *CDKN2A* will be identified in 20–40% of families, with the highest likelihood of a mutation occurring in families with 3 or more members with melanoma.(Florell et al., 2005, Goldstein et al., 2007, Hayward, 2003, SEER, 2018)

The *CDKN2A* locus encodes two proteins, p16 and p14(ARF), which function as tumor suppressors in the Rb/E2F and HDM2/p53 pathways respectively (OMIM: 600160). Mutations in *CDKN2A* are associated with a highly elevated risk for melanoma, and to a lesser degree, an increased susceptibility to pancreatic cancer. A subset of alterations including large deletions or mutations affecting both the pl6 and pl4 proteins may occasionally predispose patients to multiple cancer types in addition to melanoma and pancreatic cancer including malignant peripheral nerve sheath tumors, gliomas, breast cancer, and colon cancer.(Goldstein et al., 2006, Goldstein et al., 1995, Hussussian et al., 1994, Prowse et al., 2003, Randerson-Moor et al., 2001, Sargen et al., 2016, Vanneste et al., 2013)

Germline mutations of *CDK4* are also associated with an increased risk for melanoma, but are less common and have been reported in less than 20 melanoma-prone families. (Puntervoll et al., 2013, Soufir et al., 1998, Zuo et al., 1996) Pathogenic mutations in *CDK4* affect the p16 binding site of this protein, thereby allowing *CDK4* to remain in an activated state, which promotes cell division.(Zuo et al., 1996)

Germline mutations of *CDKN2A* and *CDK4* are associated with a significantly increased lifetime risk for melanoma.(Bishop et al., 2002, Puntervoll et al., 2013, Soufir et al., 1998, Zuo et al., 1996) In this study, we assessed differences in the anatomic distribution of melanomas between mutation carriers (*CDKN2A, CDK4*) and those without a mutation in either gene (non-carriers) from U.S. melanoma-prone families. If present, associations between genotype and tumor distribution could potentially further our understanding of melanoma tumor development among individuals with a familial predisposition to melanoma.

RESULTS

Clinical Characteristics of Carriers and Non-carriers in Melanoma-prone Families

We evaluated the anatomic location for 559 tumors (*CDKN2A* carriers having 348 tumors; *CDK4* carriers having 54 tumors; non-carriers having 157 tumors) obtained from 260 individuals with melanoma (141 *CDKN2A* carriers; 15 *CDK4* carriers; 104 non-carriers) (Figure 1) (Table 1). All three groups were similar with respect to the gender composition (Table 1). Compared to individuals without a known mutation (median age=45 years), mutation carriers were younger (median age: *CDKN2A* carrier, 31 years, P_{WMW} <.001; *CDK4* carrier, 35 years, P_{WMW} =0.003) and more likely to develop superficial spreading melanomas (Chi-square *P*<.001 for *CDKN2A* and *CDK4* carriers) than other histologic subtypes (Table 1).

Tumor Distribution in Melanoma-prone Families

Among melanoma-prone families, tumor occurrence at non-truncal sites was strongly associated with the presence of either a *CDKN2A* (*CDKN2A* carriers vs. non-carriers: 62% vs. 45%, Global *P*=.01) or *CDK4* (*CDK4* vs. non-carriers: 87% vs. 45%, Global *P*<.001) mutation after adjustment for age at diagnosis, gender, and superficial spreading melanoma subtype (Figure 1). Compared to having a melanoma of the trunk, mutation carriers had higher odds of developing melanoma at any non-truncal site (*CDKN2A* carriers vs. non-carriers, OR=2.77, 95% CI, 1.60 to 4.80, *P*<.001; *CDK4* carriers vs. non-carriers, OR=l1.6, 95% CI, 4.09 to 32.7, .*P*<.001) (Table 2). Statistically significant associations were also observed for each non-truncal anatomic region (lower extremities, upper extremities, head and neck) (Table 2). Similar findings were also observed for gender-specific (Table S1) and age-stratified (Table S2) comparisons.

First and Subsequent Tumor Diagnoses in Melanoma-prone Families

Among first melanoma diagnoses in melanoma-prone families, non-truncal melanomas were more common for *CDKN2A* (*CDKN2A* carriers vs. non-carriers: 57% vs. 44%, Global *P*=0.04) and *CDK4* (*CDK4* carriers vs. non-carriers: 79% vs. 44%, Global *P*=.03) carriers in adjusted models (Table S3). Compared to having a melanoma of the trunk, mutation carriers had higher odds of developing their first primary melanoma at any non-truncal site (*CDKN2A* carriers vs. non-carriers, OR=1.98, 95% CI, 1.07 to 3.67, *P*<.05; *CDK4* carriers vs. non-carriers, OR=6.17, 95% CI, 1.97 to 19.3, *P*<.05) (Table S3). Comparable associations and trends for anatomic distribution were observed for analyses of subsequent melanomas, that is, melanomas diagnosed after the patient's first primary melanoma (Table S3). Within each group (*CDKN2A* carriers, *CDK4* carriers, and non-carriers), anatomic distributions of tumors were similar (Chi-square *P>.05)* for the first and subsequent melanoma diagnoses (Table S3).

Body Surface Area and Tumor Distribution in Melanoma-prone Families

For each of the three groups (*CDKN2A* carriers, *CDK4* carriers, and non-carriers), there was a statistically significant difference (*P*<.05) in the observed and expected distribution of melanomas (Figure 2). Although based on smaller numbers of tumors, the overall O/E

(observed/expected) patterns for *CDK4* (truncal melanomas, O/E = 0.37) carriers differed more from the patterns seen for the two other groups (non-carriers, truncal melanomas, O/E = 1.52; *CDKN2A* carriers, truncal melanomas, O/E = 1.06).

CDKN2A versus *CDK4* Carriers in Melanoma-prone Families

While mutation carriers (*CDKN2A* and *CDK4*) both demonstrated increased odds of developing non-truncal tumors compared to non-carriers in U.S. melanoma-prone families, *CDK4* carriers were also more likely to develop non-truncal tumors than *CDKN2A* carriers (*CDK4* carriers vs. *CDKN2A* carriers: 87% vs 62%, Global P<.001). Further, the likelihood of developing a non-truncal tumor (*CDK4* carriers vs. *CDKN2A* carriers: OR=4.18, 95% CI, 1.59 to 11.0, $P<.05$) was strongest for the lower extremities *(CDK4* carriers vs. *CDKN2A* carriers: OR=4.84, 95% CI, 1.89−12.4, $P<.05$) and upper extremities (*CDK4* carriers vs. *CDKN2A* carriers: OR=3.62, 95% CI, 1.36 to 9.65, $P<.05$) (Table 2). Similar associations and trends were observed for first and subsequent melanoma diagnoses (Table S3) as well as comparisons stratified by gender (Table S1) and age (Table S2).

Familial versus Non-Familial Cases from SEER

More than half of all melanomas diagnosed in non-carriers from melanoma-prone families were located on the trunk, which was significantly higher than the frequency of truncal tumors observed in predominantly non-familial SEER cases (non-carriers vs. SEER cases: 56% vs. 36%, Global $P=.04$) or in the familial mutation carrier groups (Table 3). *CDK4* carriers were less likely to develop truncal tumors than was observed in SEER, but these differences were not statistically significant (*CDK4* carriers vs. SEER cases: 21% vs. 36%, Global $P=0.10$). In contrast, the tumor distribution patterns for *CDKN2A* carriers and SEER cases were similar (global $P=0.24$).

DISCUSSION

In our analysis of U.S. melanoma-prone families, the anatomic distribution of melanomas differed substantially depending upon mutation status. *CDKN2A* and *CDK4* carriers were significantly more likely to have non-truncal tumors than individuals without a mutation.

Ascertainment for all three familial groups was similar. Mutation status was unknown at study entry for almost all families, except for a few recently ascertained kindreds (with known *CDKN2A* mutations). Tumor distributions according to genotype also showed consistent results even when analyses were stratified according to age at diagnosis, gender, and first versus subsequent melanoma diagnoses. Further, the study population was geographically diverse for all familial groups with individuals living across the United States (Northeast, Southeast, Midwest, Southwest, West Coast, Alaska, Hawaii).

Non-truncal sites are exposed to either chronic (head/neck) or intermittent (upper and lower extremities) ultraviolet (UV) radiation in contrast to the trunk, which is usually covered by clothing. Therefore, our results suggest that UV exposure is an important modifier of melanoma risk among individuals with germline *CDKN2A* and *CDK4* mutations, which is consistent with prior studies demonstrating increased melanoma penetrance among *CDKN2A* carriers living closer to the equator where the ultraviolet index is higher.(Bishop

et al., 2002) Consistent site-specific UV exposure data (UVA exposure, UVB exposure, number of blistering sunburns, tanning bed use, etc.) were not available for our analysis, and therefore, further investigation is needed to understand the potential modifying effect of UV radiation on melanoma tumor development among individuals with germline *CDKN2A* and *CDK4* mutations.

CDKN2A and *CDK4* carriers in melanoma-prone families also demonstrated a high-frequency of superficial spreading melanomas, which is consistent with prior studies.(Sargen et al., 2015, Taylor et al., 2016) This association suggests that alterations of the *CDKN2A(p16)/CDK4* pathway are important in the development of tumors with this histologic subtype. Among *CDKN2A* carriers, *BRAF* mutations occur at a lower frequency than observed for sporadic melanomas.(Jovanovic et al., 2010) Further, *BRAF*-wild type melanomas often occur on the extremities.(Maldonado et al., 2003) Therefore, *CDKN2A* carriers may be at risk for melanomagenesis at non-truncal sites given their increased susceptibility to *BRAF-wild* type tumors. To assess this hypothesis, tumor *BRAF* mutational status, which was not available for our analysis, should be assessed in follow-up studies evaluating tumor distribution patterns in melanoma-prone families.

Interestingly, *CDK4* carriers were more likely to develop non-truncal tumors than *CDKN2A* carriers and non-carriers in melanoma-prone families. Overall, 87% (47 of 54 tumors) of all melanomas diagnosed among *CDK4* carriers were non-truncal. The highest number of tumors were observed on the lower extremities followed by the upper extremities. These patterns were consistent across all subset and sensitivity analyses. While these observations suggest that *CDK4* mutations are strongly associated with non-truncal tumor development, our sample size (54 tumors, 15 patients, 2 families) was relatively small, and therefore additional studies are necessary to confirm these findings.

Our results for U.S. melanoma-prone families are consistent with two prior studies that reported the phenotypic characteristics for *CDKN2A* (Taylor et al, N=670 individuals) (Taylor et al., 2016) and *CDK4* (Puntervoll et al, N=140)(Puntervoll et al., 2013) carriers from North America, Europe, and Australia. Both studies included less detailed patient/tumor data from a subset of U.S. melanoma-prone families that were analyzed in the current study. Interestingly, non-carriers (N=l, 258 individuals) reported by Taylor et al. were less likely to have melanoma occur on the trunk (38%) compared to our U.S. non-carriers (55%). However, country-specific data were not reported by Taylor et al. to assess whether non-carriers in specific countries/regions develop truncal melanomas at an increased frequency similar to what was observed for non-carriers in our U.S. melanoma-prone families. Melanoma occurrence on the trunk for U.S. non-carriers was also considerably higher than that observed for non-familial cases from SEER, 1973–2015 = 36%. Further studies are needed to examine whether a high occurrence of truncal melanomas is restricted to U.S. non-carrier melanoma-prone families.

Non-carrier patients/families, who are at increased risk for melanoma, may carry mutations in other melanoma susceptibility genes. In the vast majority of cases (n=96/104 patients), WES and WGS data were available, and single gene mutations in known high-penetrance melanoma susceptibility genes (e.g. *BAP1, POT1*) were not identified, suggesting that

melanoma in these families may be caused by mutations in novel high-penetrance genes or multiple genes with moderate/low penetrance and these unknown genes may interact with UV exposure in different ways compared with *CDKN2A/CDK4*.

Despite the tendency to develop non-truncal tumors, mutation carriers also developed a substantial number of melanomas on the trunk (*CDKN2A* carriers = 38%; *CDK4* carriers = 13%). The wide anatomic distribution of tumor sites among mutation carriers highlights the importance of performing full-body skin exams to screen for melanoma, which is the current standard of care for melanoma-prone families.(Johnson et al., 2017)

In conclusion, this study observed significant differences in the melanoma distribution patterns between mutation carriers (*CDKN2A, CDK4)* and individuals without a known mutation in U.S. melanoma-prone families with mutation carriers developing the majority of their tumors at non-truncal sites. Given the relatively small sample size of our study, it will be important for future studies to validate our findings before recommending any changes to melanoma screening for members of melanoma-prone families. Further investigation is also necessary to understand the biology underlying these genotype-associated patterns of tumor development, which could provide new insights into melanoma treatment and prevention.

MATERIALS AND METHODS

Study Population

Data for this study came from a non-population-based family study from the Division of Cancer Epidemiology and Genetics at the National Cancer Institute (NCI). Families with and without germline *CDKN2A* and *CDK4* mutations were ascertained through self or health professional referrals, and have been followed prospectively for up to 40+ years, starting in 1976. All patients self-identified as non-Hispanic white. Tumor diagnosis and location were ascertained from medical records (pathology reports, physician notes). Given the possibility of phenocopies (individuals who express a particular phenotype (eg. melanoma) without the associated genotype (eg. *CDKN2A* or *CDK4* mutation)) in melanoma-prone families, we included only individuals whose germline mutation status was known in our analyses.(Helgadottir et al., 2018) Phenocopies with known mutation status (i.e. *CDKN2A* or *CDK4* negative) were included in the non-carrier group. Additional details of the study population are available online and have been described in prior publications. (Goldstein et al., 2018, Goldstein et al., 2000, Goldstein et al., 2017, Tucker et al., 2018) Written informed consent was obtained from all participants or their legal guardians prior to participating in this NCI institutional review board-approved protocol (NCI 002-0211; ClinicalTrials.gov identifier).

CDKN2A and *CDK4* Mutation Status

During this longitudinal study multiple techniques, including single-strand conformation polymorphism (SSCP), Sanger sequencing, array comparative genomic hybridization (aCGH), whole exome sequencing (WES), and whole genome sequencing (WGS), have been used to screen for *CDKN2A* and *CDK4* mutations depending upon the technologies that were available at the time of patients' enrollment or mutation testing. Testing for

mutations in *CDKN2A/CDK4* was performed on stored blood specimens. For the current study, we grouped individuals into "carriers" (*CDKN2A* and *CDK4* carriers) and "non-carriers" (no mutation identified).

Statistical Analysis

The primary objective of this study was to determine if tumor distribution patterns are associated with genotype for individuals in U.S. melanoma-prone families. We used chi-square and Wilcoxon-Mann-Whitney (WMW) tests to assess differences in characteristics between groups. We modeled the anatomical location of each melanoma as a function of carrier status of the patients and other attributes (age at diagnosis, gender, and whether or not tumors were superficial spreading subtype) using multinomial models with random effects to account for correlations of multiple melanomas within individuals and correlations of family members (PROC GLIMMIX SAS 9.4). Tumor locations were grouped into 4 regional categories (lower extremities; upper extremities; trunk; head/neck). The probabilities of having a tumor at a specific location were modeled using polytomous logistic regression to obtain odds ratios (ORs) for association of tumor location with carrier status, adjusted for age, gender, and superficial spreading subtype. Adjusted ORs are presented unless specified otherwise. Models were also stratified by gender and overall median age of diagnosis for all three groups (≤39 or >39 years at diagnosis). Since patients developed different numbers of melanomas, we conducted sensitivity analyses in which we restricted models to the first diagnosed melanoma for each patient.

The effect of body surface area on melanoma distribution was evaluated by assessing differences in the observed and expected number of melanomas (see equation below) for each anatomic region using a chi-square test. The Wallace Rule of Nines was used to estimate body surface area for each anatomic region.(Orgill, 2009)

$$y = ab$$
$$y = \text{Expected number of melanomas}$$
$$a = \text{Total observed melanomas for specific genotype}(eg.\ CDKN2A\ carriera)$$
$$b = \text{Body surface area of particular anatomic region}(eg.\ trunk)$$

For this evaluation, our null hypothesis was that no difference would exist in the observed and expected distributions of melanoma if body surface area were an important determinant of melanoma distribution.

Two-sided p-value cut-offs of $P<.05$ defined statistical significance. Statistical analyses were performed using SAS 9.4 (Cary, NC) and STATA 15 (College Station, TX).

Familial vs. Non-Familial Cases

In a secondary analysis, we used chi-square and WMW tests to compare differences in attributes (gender, median age of diagnosis) and tumor location between our familial groups (*CDKN2A* carriers, *CDK4* carriers, non-carriers) and predominantly sporadic cases (Surveillance, Epidemiology, and End Results (SEER) database).(SEER*Stat) For the familial groups, our analysis of tumor location was restricted to an individual's first melanoma diagnosis in order to be consistent with SEER data, which is composed of first

primary tumors. SEER cases diagnosed in the time period of 1973 to 2015 were analyzed. (SEER*Stat)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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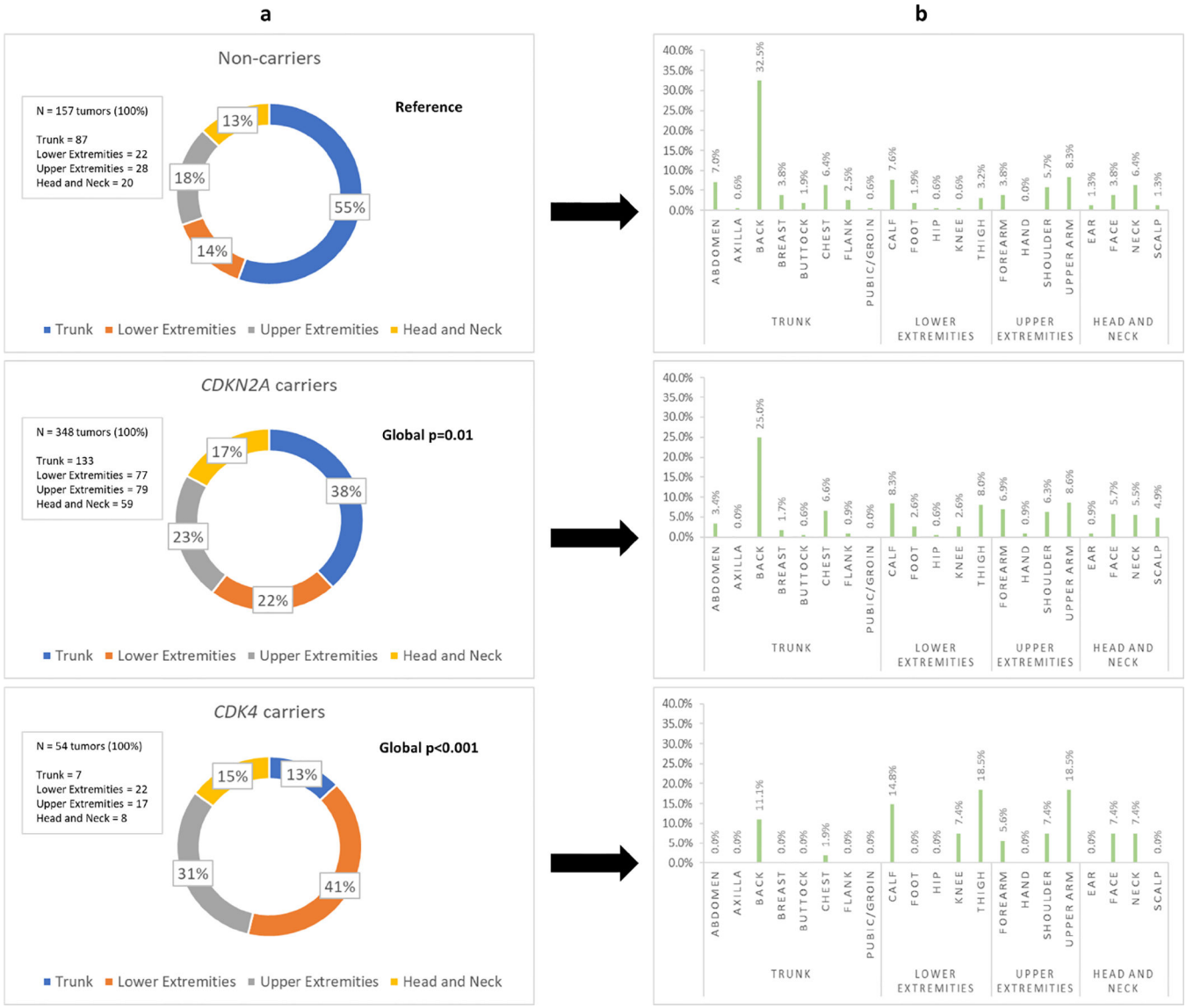

Figure 1: Cutaneous patterns of melanomagenesis in U.S. melanoma-prone families with and without germline *CDKN2A* and *CDK4* mutations.

The above figure illustrates the regional distribution (column “a”) and specific biopsy sites (column “b”) for melanomas diagnosed in individuals from U.S. melanoma-prone families according to genotype. Global p-values comparing differences in melanoma distribution between mutation carriers (*CDKN2A* or *CDK4*) and non-carriers (individuals without a *CDKN2A* or *CDK4* mutation) with adjustments for within individual and within family correlations, age at diagnosis, gender, and superficial spreading melanoma subtype are provided in column “a”.

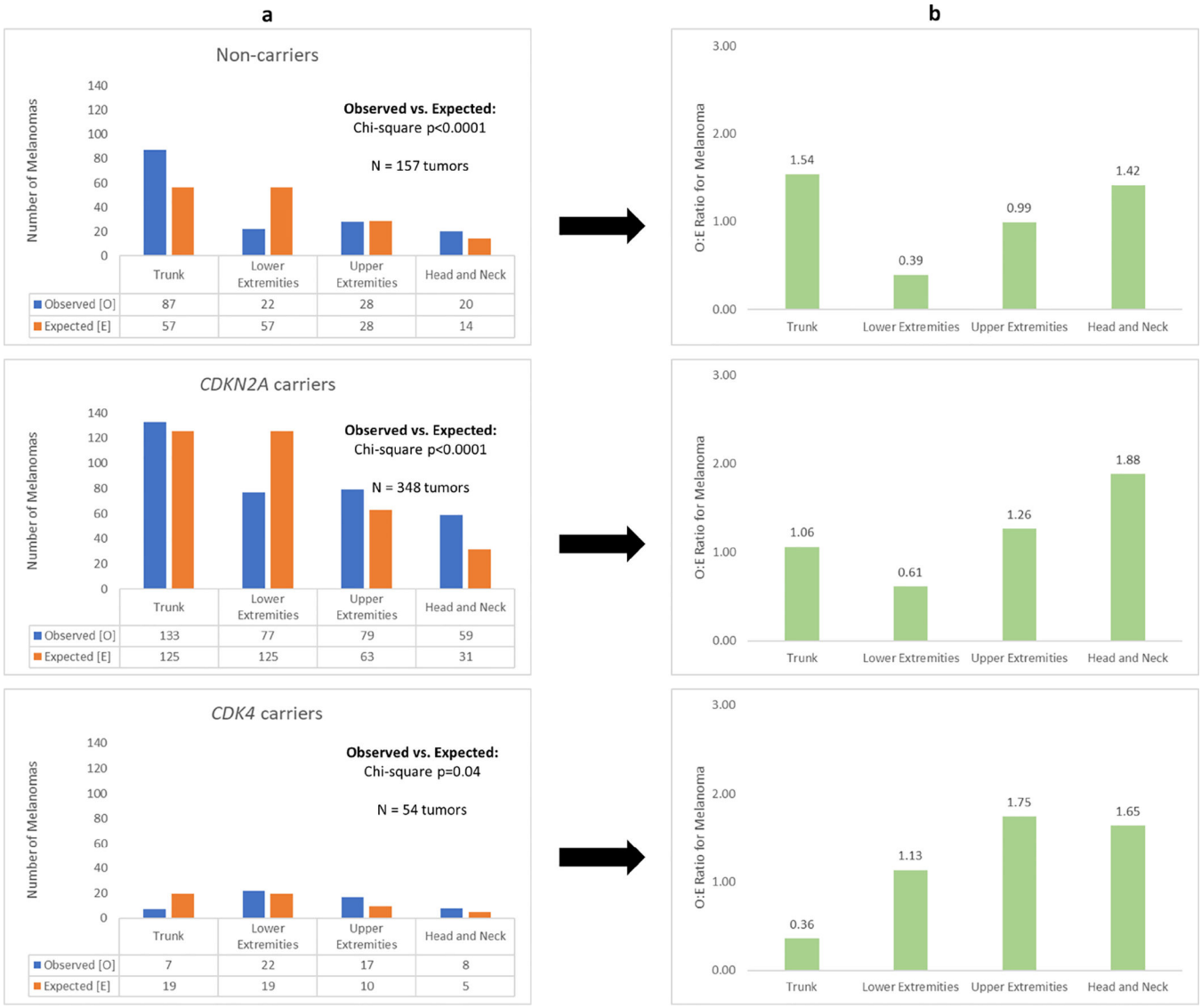

Figure 2: Body surface area and melanoma distribution.

The above figure illustrates the observed and expected number of melanomas for each anatomic region according to genotype. In column “a”, the total number of melanomas (N) for each group (non-carriers, *CDKN2A* carriers, *CDK4* carriers) was multiplied by the body surface area of each anatomic region to calculate the expected number of melanomas. Differences between the observed and expected melanoma distribution were assessed by chi-square test (column “a”). The ratio of observed to expected number of melanomas for each anatomic region is presented in column “b”.

Table 1:

Clinical and histologic features of U.S. melanoma-prone families

	Non-carriers		*CDKN2A* carriers		*CDK4* carriers		*CDKN2A* carriers vs. Non-carriers	*CDK4* carriers vs. Non-carriers	*CDK4* carriers vs. *CDKN2A* carriers
	N	**%**	**N**	**%**	**N**	**%**	**P-value**	**P-value**	**P-value**
Total patients	104	100%	141	100%	15	100%			
Female patients	49	47%	70	50%	8	53%	0.70[1]	0.65[1]	0.79[1]
Median age of first melanoma diagnosis (range)	45 (18–95)		31 (10–69)		35 (24–64)		<0.001[2]	0.003[2]	0.21[2]
Average number of melanomas per patient (range)	1.5 (1–6)		2.5 (1–30)		3.6 (1–13)				
Total melanomas	157	100%	348	100%	54	100%			
Histologic Subtype							<0.001[3]	<0.001[3]	0.11[3]
Acral Lentiginous Melanoma	1	0.6%	1	0.3%	0	0%			
Lentigo Maligna Melanoma	17	11%	9	3%	0	0%			
Nodular Melanoma	12	8%	14	4%	0	0%			
Superficial Spreading Melanoma	94	60%	275	79%	51	94%			
Not Otherwise Specified	33	21%	49	14%	3	6%			

[1] Chi-square test comparing differences in gender (female vs. male) between groups

[2] Wilcoxon-Mann-Whitney test comparing differences in median age of first melanoma diagnosis between groups

[3] Fisher exact test comparing overall differences in histologic subtype between groups

Table 2:

Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for tumor development at specific anatomic site compared to trunk

Anatomic Site	*CDKN2A* carriers vs. Non-carriers		*CDK4* carriers vs. Noncarriers		*CDK4* carriers vs. *CDKN2A* carriers	
	OR[1]	95% CI	OR[1]	95% CI	OR[1]	95% CI
Trunk	Reference	Reference	Reference	Reference	Reference	Reference
Non-T runcal	2.77**	1.60–4.80	11.6**	4.09–32.7	4.18*	1.59–11.0
Lower extremities	3.01*	1.56–5.82	14.5**	5.02–42.0	4.84*	1.89–12.4
Upper extremities	1.91*	1.03–3.52	6.88**	2.37–19.9	3.62*	1.36–9.65
Head and neck	5.40**	2.10–13.9	18.6**	4.04–85.2	3.45	0.93–12.8

*$P<.05$

**$P<.001$

[1]Multinomial random effects model adjusting for within individual and within family correlations, age at diagnosis, gender, and superficial spreading melanoma subtype

Table 3:

Location of first melanoma diagnosis: Comparison of U.S. melanoma-prone families to Surveillance, Epidemiology, and End Results (SEER) data

	Non-carriers		*CDKN2A* carriers		*CDK4* carriers		SEER cases (1973–2015)		SEER cases vs. Non–Carriers	SEER cases vs. *CDKN2A* carriers	SEER cases vs. *CDK4* Carriers
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**P-value**	**P-value**	**P-value**
Total patients	104	100%	141	100%	15	100%	313,237	100%			
Female patients	49	47%	70	50%	8	53%	137,931	44%	0.52[1]	0.33[1]	0.56[1]
Median age of diagnosis (range)	45 (18–95)		31 (10–69)		35 (24–64)		62 (1–87)		<0.0001[2]	<0.0001[2]	<0.0001[2]
Total melanomas[4]	117	100%	161	100%	24	100%	313,237	100%			
Anatomic Site									0.04[3]	0.24[3]	0.10[3]
Trunk	65	56%	70	44%	5	21%	111,660	36%			
Lower extremities	16	14%	31	19%	6	25%	61,229	20%			
Upper extremities	22	19%	33	21%	9	38%	83,336	27%			
Head and neck	14	12%	27	17%	4	17%	57,012	18%			

[1] Chi-square test comparing differences in gender (female vs. male) between groups

[2] Wilcoxon-Mann-Whitney test comparing differences in median age of first melanoma diagnosis between groups

[3] Global p-value adjusting for age at diagnosis, gender, and superficial spreading melanoma subtype

[4] Analysis restricted to first primary melanomas for non-carriers, *CDKN2A* carriers, and *CDK4* carriers. Some noncarriers (N=8), *CDKN2A* carriers (N=15), and *CDK4* carriers (N=4) were diagnosed with multiple melanomas at the time of their first melanoma diagnosis.