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Title page

Factors related to nevus-associated cutaneous melanoma: a case-case study

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ABSTRACT

A proportion of cutaneous melanomas display neval remnants on histologic examination. Converging lines of epidemiologic and molecular evidence suggest that melanomas arising from nevus precursors differ from melanomas arising *de novo*. In a large, population-based study comprising 636 cutaneous melanomas subjected to dermatopathology review, we explored the molecular, host and environmental factors associated with the presence of neval remnants. We found nevus-associated melanomas were significantly associated with younger age at presentation, non-brown eye color, trunk site, thickness <0.5mm and $BRAF^{V600E}$ mutation. Compared with patients with *de novo* melanomas, those with nevus-associated tumors were more likely to self-report many moles on their skin as a teenager (OR 1.94, 95% CI 1.01-3.72) but less likely to report many facial freckles (OR 0.49, 95% CI 0.25-0.96). They also had high total nevus counts (OR 2.18, 95% CI 1.26-3.78). On histologic examination, nevus-associated melanomas exhibited less dermal elastosis in adjacent skin compared with *de novo* melanomas (OR 0.55, 95% CI 0.30-1.01). These epidemiologic data accord with the emerging molecular paradigm that nevus-associated melanomas arise through a distinct sequence of causal events which differ from those leading to other cutaneous melanomas.

ABBREVIATIONS

CI, Confidence Interval; LMM, Lentigo Maligna Melanoma; NM: Nodular Melanoma; OR, Odds-Ratio; SSM: Superficial Spreading Melanoma

KEYWORDS

cutaneous melanoma; epidemiology; melanocytic nevus; neval remnants; pigmentation; sun exposure

INTRODUCTION

The incidence of cutaneous melanoma, a potentially lethal cancer, has risen rapidly over recent decades in most countries with fair-skinned populations (Whiteman et al., 2016). Understanding the mechanisms through which these cancers arise is necessary for effective preventive strategies to be developed. Converging lines of evidence from epidemiologic and molecular studies suggest that cutaneous melanomas arise through at least two causal pathways: one of which is associated with chronic exposure to sunlight, and the other associated with host propensity to develop large numbers of melanocytic nevi (Shain and Bastian, 2016, Whiteman et al., 2011).

It has long been recognized that a proportion of cutaneous melanomas have evidence of contiguous neval remnants on histologic examination (hereafter “nevus-associated melanomas”), though the exact proportion is debated (Haenssle et al., 2016, Marks et al., 1990, Massi et al., 1999, Skender-Kalnenas et al., 1995). A recent meta-analysis of 38 observational studies reported a summary prevalence estimate of 29.1% of melanomas had adjacent neval remnants, although there was significant heterogeneity across studies (Pampena et al., 2017) . It was thus assumed that these nevus-associated melanomas arose by ‘carcinogenic evolution’ from the pre-existing benign lesion (Ackerman, 1980). Recent studies mapping the genomic profiles of melanocytic lesions displaying regions of benign, dysplastic and neoplastic architecture bear out this assumption (Shain and Bastian, 2016). A small number of earlier studies reported differences in phenotypic (Bevona et al., 2003, Carli et al., 1999, Chang et al., 2009, Haenssle et al., 2016, Kaddu et al., 2002) and environmental (Newton-Bishop et al., 2010, Purdue et al., 2005, Sergentanis et al., 2013) risk factors between nevus-associated melanomas and non-nevus melanoma, consistent with the two-pathway model. We sought to explore the molecular, host

and environmental factors correlated with nevus-associated melanomas in a large, population-based study, by comparing them with non-nevus melanomas.

RESULTS

Of 1456 melanoma patients invited, 808 gave consent to participate and pathology reports were retrieved for 807 patients. Figure 1 shows the details on the number of patients excluded upon pathology review and the subsequent study sample. After excluding all patients with lentigo maligna melanoma (n=99) from the primary analysis, as well as those with ineligible sites or types (n=42) and melanomas for which neval remnant status was unclassifiable due to insufficient adjacent tissue or artefact (n=30), our primary analysis study sample comprised 636 melanoma patients. *BRAF* mutation status was able to be determined in 393 tumors; the remainder either had insufficient material remaining in the blocks, or were not available for analysis.

There were 523 patients with trunk melanomas and 113 with head and neck melanomas. The average age of the patients was 56 years (SD 14.3) and two thirds (67%) were men. Most of the melanomas were of the superficial spreading (SSM) subtype (83%) and slightly over half (54%) had a Breslow thickness of 0.5mm or less. $BRAF^{V600E}$ mutations were detected in 102 of 393 melanomas (26%) for which sufficient material was available for somatic mutation analysis.

Contiguous neval remnants were present in just over half of the melanomas (n=324, 51%). Patients with nevus-associated melanomas were younger on average (mean age: 53.7 years) than those with non-nevus melanomas (59.3 years). Nevus-associated melanomas were more common

on the trunk compared with the head and neck (54% vs 35% respectively within each site). Of the head and neck melanomas, 24 (21%) occurred on the scalp, and these melanomas had a lower prevalence of neval remnants (21%) than those arising on the face and neck (38%). Nevus-associated melanomas were also more common among SSM subtype than other subtypes, and were more likely to be thin (≤0.5mm) or to harbor $BRAF^{V600E}$ mutations than non-nevus melanomas (Table 1). All of these factors remained statistically significant after adjusting for age and sex.

With regard to phenotypic characteristics, when unadjusted, the prevalence of nevus-associated melanoma was slightly higher among patients with blond hair color and green/hazel eye color, but did not vary significantly with propensity to burning or tanning skin type (Table 2). The odds of nevus-associated melanoma increased significantly with numbers of moles on the skin as a teenager [self-reported] and with nevus counts [by skin examination], but decreased significantly with numbers of freckles on the face as a teenager [self-reported]. We found a lower prevalence of marked dermal elastosis in the skin adjacent to the nevus-associated melanomas compared with non-nevus melanomas (Table 2).

Of the various reported and derived measures of sun exposure, total hours of sun exposure and total hours of occupation sun exposure were inversely associated with nevus associated melanomas in unadjusted models, however the statistical significance was lost after adjustment for age and sex (Table 3). We observed no notable differences in the prevalence of nevus-associated melanomas across categories of recreational or childhood sun exposures, or numbers of sunburns in various periods of life.

To estimate the direct effects of the factors associated with nevus-associated melanoma, we developed multivariable models adjusting for the potential confounding effects of other factors. Our final model consisted of a reduced list of histological (anatomic site, tumor thickness and degree of dermal elastosis) and phenotypic factors (eye color, number of freckles and total body nevus counts), as well as *BRAF*^{V600E} mutation status. Not unexpectedly, several pairs of factors were highly correlated, requiring careful selection of terms to retain in the final model. For example, 'number of moles as a teenager' was dropped from the model when the term for 'total body nevus counts' was included. Given these two factors measure the same underlying trait (i.e. 'propensity to develop nevi'), we retained total nevus count in the model as the stronger term. Anatomical site and grade of dermal elastosis were also correlated (spearman rho 0.53, P<0.001), and both were statistically significant when included singly in the multivariable model, but each weakened the other when included together in the model. However, we retained both factors in the final model as they are measuring different characteristics despite their correlation, and each could strongly confound the other.

Thus, in the final model, factors significantly associated with nevus-associated melanoma were age, eye color, tumor thickness, freckles as a teenager and nevus count, while tumor site and dermal elastosis only marginally missed statistical significance at the 5% level (Table 4). The strongest predictor of nevus-associated melanoma was total nevus count, and the strongest predictors of non-neval melanomas were many facial freckles as a teenager, age ≥70 years, and marked dermal elastosis in the adjacent epidermis.

In analyses stratified by site of melanoma (trunk, head-neck), the distribution of factors according to nevus-association were similar for both sites (Table 5) except for self-reported number of nevi as a teenager, for which the odds ratios differed significantly across the sites. Although the observed association was stronger for nevus count and tumor thickness for trunk melanomas compared to head and neck, the limited number of nevus-associated melanomas on the head and neck meant that the analysis lacked statistical power for further assessment.

Supplementary analysis

We repeated our analyses including the 94 patients (5 LMMs were missing neval remnant classification, Figure 1) diagnosed with LMM who were excluded from the primary analysis (Supplementary Tables 1-5). Including the LMM cases made no difference to the list of factors associated with neval remnants when compared to the primary analysis, except for the inclusion of histological subtype (LMM subtype was significantly less likely to have contiguous neval remnants compared with SMM; Supplementary table 1). For all other factors, the effect estimates from the supplementary analysis were comparable to those from the primary analysis, except that the effects of age, anatomic site, dermal elastosis and nevus count were more pronounced.

DISCUSSION

We assessed the prevalence of nevus-associated melanomas and explored the factors associated with their presence, on the assumption that these malignant melanocytic tumors evolve directly from pre-existing benign lesions. We found that approximately half (51%) of the invasive melanomas in this series from two anatomic sites had neval remnants. Overall, nevus-associated melanomas were more likely to occur in younger individuals, and those with green or blue eye

color, no or few freckles as a teenager, and high nevus counts. They were also significantly less likely than non-nevus melanomas to show signs of chronic sun damage. These associations were observed both for melanomas occurring on the trunk and on the head or neck, tending to confirm the associations reported previously (Bevona et al., 2003, Haenssle et al., 2016, Lee et al., 2006, Purdue et al., 2005, Shitara et al., 2014).

The prevalence of contiguous remnants in the present series was at the higher end of the range reported in the literature (9% to 58%) (Bevona et al., 2003, Carli et al., 1999, Haenssle et al., 2016, Lin et al., 2015), and was substantially higher than the summary prevalence estimate of 29.1% reported by Pampena et al (Pampena et al., 2017). It must be noted that our series was restricted to melanomas of the trunk and head and neck. Although Pampena et al (Pampena et al., 2017) did not report the pooled prevalence of continguous neval remnants according to the anatomic site of the melanoma, we reviewed the primary publications and derived summary site-specific prevalence estimates of 38% (range 23% to 64%) for melanomas of the trunk and 18% (range 0% to 30%) for melanomas of the head and neck. Of note, there was highly significant heterogeneity of nevus prevalence across studies, suggesting substantial variation in this feature. The site-specific prevalence of neval remnants observed in this population-based series of melanomas in Queensland broadly aligns with those observed elsewhere, being markedly higher on the trunk than the head and neck.

We intentionally restricted recruitment of patients to two anatomical sites, as *a priori* our objective was to quantify associations between melanomas grouped according to specific histologic characteristics. Assuming a fixed sample size, the greatest statistical power for

examining associations is gained by sampling participants from the extremes of a distribution. In this particular study of cutaneous melanoma, the extremes of exposure were 'habitually sun exposed sites' and 'habitually covered sites'. Typically, in Australian adults of both sexes, the limbs are uncovered by clothing and exposed to the sun to a greater extent than the trunk, but less than the face, ears, head and neck. Thus, for reasons of statistical efficiency, the sampling frame was restricted to patients with melanomas of the head or trunk only, and did not include patients with melanomas of the limbs, palms, soles or other sites. We also excluded melanomas of the lentigo maligna subtype from our primary analyses on the grounds that these were already known to be associated with chronic solar exposure and not with nevi. While our subsequent analysis including LMMs made no difference to our conclusions about the differences between nevus-associated and non-nevus melanomas, their inclusion naturally reduced the prevalence of contiguous neval remnants. For these reasons, the prevalence figures for nevus-associated melanoma reported here are not generalizable to all melanomas, and are only comparable to those case series which have reported site-specific prevalence of invasive, nevus-associated melanoma.

By restricting our sample only to patients with melanomas of the trunk or head and neck, we also may have limited the generalizability regarding factors correlated with nevus-associated melanoma. Specifically, it is conceivable that nevus-associated melanomas arising on the upper or lower limbs may differ from nevus-associated melanomas arising elsewhere, although there is no reason to assume so. Indeed, the effect sizes we observed for nevus-associated melanomas on the trunk were similar to those observed for melanomas on the head & neck, suggesting that the factors predicting nevus-associated melanomas (namely young age, thin lesions, nevus density

and *BRAF* mutations) operate independently of the anatomic site of the melanoma. Confirming these site-specific associations in statistically powered, purpose-designed studies would resolve any uncertainty in this regard.

Our understanding of the origins of cutaneous melanomas has progressed rapidly in recent years. Whereas in earlier generations of epidemiologic studies, patients with cutaneous melanomas were typically analyzed as a single homogenous group, it became apparent that not all cutaneous melanomas share the same causal origins. Descriptive (Elwood and Gallagher, 1998, Lachiewicz et al., 2008) and analytical (Whiteman et al., 2006, Whiteman et al., 2003) epidemiologic studies first suggested that melanomas may be grouped according to their age of onset, anatomical site and other host characteristics. More recently, molecular and genomic studies have mapped the pathways through which mutations in key driver genes drive neoplastic progression in melanocytes. There is now convincing evidence that a proportion of melanomas arise directly from benign precursors (nevi) that carry distinctive $BRAF^{V600E}$ mutations (Shain and Bastian, 2016, Shain et al., 2015). Most of these lesions remain in a state of induced senescence however a very small proportion of lesions acquire additional mutations in key driver genes (e.g. *CDKN2A*, *TERT*) which herald the onset of more several dysplastic properties. It appears that the steps to invasive and potentially metastatic melanoma requires only one or two additional mutations (e.g. *ARID*, *ATM*, *TP53*, *PTEN* etc) (Shain and Bastian, 2016). This sequential pathway of acquired somatic mutations appears to fit well with the model of progression from benign nevus to *in situ* melanoma to invasive melanoma. Our data, collated from a large population-based series of melanoma cases, demonstrate a significant association between

BRAF^{V600E} mutation and nevus-associated melanoma and thus accord with the molecular model described.

A large proportion of melanomas, likely the majority, do not arise through the nevus pathway described above. Our data, and others, show that melanomas arising through the non-nevus pathway are associated with older age, nodular subtype, higher Breslow thickness, location on the head or neck, dermal elastosis and low nevus count (Pampena et al., 2017). The associations with age, habitually exposed body sites and dermal elastosis lead us to infer that high levels of cumulative sun exposure cause these non-nevus melanomas. Nodular melanomas have also been associated with high levels of sun exposure (Mar et al., 2013). Although the inverse association with nevus-prone phenotype could, in theory, be explained by age differences in the two groups of melanoma patients (given that nevus counts are highest in young adults and low in older people), we believe this explanation is unlikely as our analyses were adjusted for age.

Several explanations have been offered as to how non-nevus melanomas might arise. One explanation is that such melanomas do arise from a prior nevus, but then the benign lesion is overgrown by the invasive, malignant tumor, removing any trace of its prior existence. The association with higher Breslow thickness fits with this model. An alternative explanation, now supported by sequencing data, is that the non-nevus melanomas arise through different mutations in the *MAPK* pathway (Shain and Bastian, 2016, Shain et al., 2015). It seems that these melanomas can have a rapid clinical onset (as has long been clinically recognized for nodular melanomas).

What do these epidemiologic data offer by way of insights to the new molecular paradigm? First, they underscore the importance of host phenotype in governing susceptibility (or alternatively, resistance) to melanoma development. Of all the factors assessed here, it is the association with nevus number that was the strongest predictor of nevus-associated melanoma, and the most consistent with other studies. Understanding why some people are more prone to develop nevi than others has long been the focus of research. Despite the significant contributions of sun exposure, the heritability of nevus count has been estimated by twin studies to be in the range of 60-90% (Zhu et al., 1999). The latter high estimate is for adolescent twins all living in a high UV environment in Australia, where one presumes environmental differences in exposure are minimized. Within these twin collections, it has been recently shown that ~25% of the Australian and ~15% of British genetic variance for nevus count can be explained by a panel of 1,000 SNPs covering 32 genomic regions. Given that nevi are precursors to melanomas, at least in some people, unravelling the biology of these lesions remains an important research goal.

Second, these data remind us that a large proportion of melanomas are not associated with neval remnants, and arise on the skin of people who may not have the classical 'high-risk' phenotype for melanoma. Moreover, it appears that this subset of melanomas includes the majority of rapidly invasive, potentially lethal nodular melanomas. Together, these two points suggest that efforts to control melanoma mortality through early detection programs may have limited capacity to reduce mortality if risk stratification is based largely on phenotype.

The associations described in this report are strong, due in part to a large sample size compared with earlier studies. Care was taken to explore the correlations within the data, and we are

satisfied that the associations reported here are unlikely to reflect the confounding effects of other factors. Further strengths of this study include the population-based ascertainment of cases, and the systematic reporting of histologic criteria by expert dermato-pathologists who were unaware of patient phenotype or sun exposure histories. Although it can be argued that using self-reported phenotype data from patients is a limitation, we have shown that very similar data collection instruments that we have used in other studies have very high repeatability and validity for these items (Morze et al., 2012).

Understanding the origins of melanoma is essential for tailoring control programs to maximum effect. These observations align with recent developments in melanoma biology, and underscore the diversity of pathways through which malignancies can arise from melanocytes. Weaving such knowledge into melanoma prevention strategies will be essential to ensuring that such efforts will have the desired effects.

MATERIALS AND METHODS

The parent study was designed specifically to test for differences in causal factors between cutaneous melanomas arising on habitually sun-exposed body sites (*viz.* head, face and neck), and those melanomas arising on habitually covered body sites (viz. trunk). Thus, the study was a case-case design, and did not include population controls. This approach provides maximum statistical efficiency for addressing this research question when the sample size is fixed since it ensures that patients with melanoma are sampled from body sites at each end of the range of typical sun exposure. For the analyses in this report, patients with melanoma arising on either of these two anatomic sites were placed into two groups based on the contiguous neval remnant

status of their melanoma ('nevus-associated melanoma' and 'non-nevus melanoma') and then compared for risk factors. Patients with incident diagnoses of invasive primary cutaneous melanoma were prospectively ascertained from diagnoses made in the main pathology laboratories serving southern Queensland. The Human Research Ethics Committee of the QIMR Berghofer Medical Research Institute granted ethical approval for the study and all patients gave their written informed consent.

Patient eligibility and histologic criteria

Full details of patient recruitment and exposure measurement have been reported previously (Kvaskoff et al., 2015). Briefly, eligible patients were residents of greater Brisbane aged 18-79 years who were diagnosed with primary invasive cutaneous melanoma arising on the face, head, neck, or trunk between April 1, 2007 and September 30, 2010.

Patients with metastatic disease or a previous diagnosis of melanoma were ineligible. At the time of diagnosis, collaborating dermato-pathologists assessed tumor's anatomical site, histological type, tumor thickness, extent of dermal elastosis in the skin adjacent to the tumor and the presence of neval remnants in the skin surrounding each melanoma using a standard scoring sheet including definitions for scored items. Contiguous neval remnants were defined as the presence of nests, sheets, cords and/or single-file disposition of cytologically benign nevus cells in the dermis adjacent to or below (subjacent) the melanoma cells. Similarly, dermal elastosis was defined in four categories of none, mild (characterized by proliferation of elastic fibers in the papillary dermis), moderate or marked elastosis (papillary and upper reticular dermis is replaced by accumulations of thickened, curled and serpiginous fibers forming tangled masses which are

basophilic in HE-stained sections). Full details of the *BRAF* mutation analysis have been provided previously (Hacker et al., 2016), but briefly, tumor DNA was isolated from samples of melanoma material that had been dissected from formalin-fixed paraffin-embedded sections. Genotyping was performed on a mass spectrometric platform using an optimized multiplex assay (MelaCarta Panel, Agena Bioscience, San Diego, CA).

Phenotype and sun exposure

Participants completed a detailed self-administered questionnaire and then underwent a clinical examination by the study dermatologist. In addition to basic demographic details (including place of birth and age at migration to Australia, if applicable), participants self-reported their hair color as a teenager, burning tendency, tanning ability, facial freckling as a teenager and nevus burden as a teenager. To assess chronic sun damage, participants were asked to report the number of actinic keratoses that had been treated. We asked separately about treatments for "skin cancers", and recorded responses for the number of lesions that had been treated by freezing, creams, excision, and other means.

Participants were also asked to report patterns of sun exposure while attending elementary school and high school on week days and weekends, and to report the number of times they were sunburned to the point of blistering, soreness for 2 days or more, or peeling while attending elementary school, high school, and since leaving school. A comprehensive occupational sun exposure history was obtained (including periods of study and unemployment). Participants were asked to list the number of jobs, their start, end age and the number of days per week worked in each job including number of hours spent outdoor on work and non-workdays in three broad

categories Weights were assigned to each of the categories of outdoor sun exposure as follows: "<1 hour per day", 0.5 hours; "1 to 4 hours per day", 2 hours; ">4 hours per day", 6 hours. We calculated the hours of occupational and recreational sun exposure by multiplying the number of hours per day spent outdoors in the sun on workdays and non-work days respectively in each employment period. We summed hours of occupational and recreational sun exposures across all employment periods between 18 and 70 years of age to derive the total number of hours of sun exposure for each participant. These factors were categorized into quartiles for the analysis.

After completing the questionnaire, each participant was examined by a dermatologist (M.B.D.) who recorded hair and eye color and counted the number of melanocytic nevi and actinic keratoses according to standard protocols (English et al., 1990).

Statistical analysis

The primary aim of this analysis was to identify phenotypic and environmental risk factors for nevus-associated melanoma, and assess whether the factors differed by the anatomical site of the melanoma.

Missing dermal elastosis data

Information on dermal elastosis, a histological marker of chronic sun exposure, was missing for around 10% of our sample. Rather than exclude these participants from the analyses, we imputed data. For our primary analysis, we assumed the dermal elastosis data were missing at random and used multiple regression to impute the missing data on dermal elastosis. We used 25 imputations for stability. We also performed sensitivity analyses to assess the potential impact of missing

dermal elastosis data. We assigned all those with missing dermal elastosis data to the "none or mild" category, under the assumption that "not reported" equated to "no elastosis".

Model fitting

We analyzed presence or absence of contiguous neval remnants as a binary outcome. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression models to quantify the association between histologic, phenotypic factors, *BRAF* mutation status and presence of neval remnants. We first fitted simple models adjusting only for age and sex. Then we included in the model those factors significant at the 10% level of significance. (To rule out overfitting, we also constructed directed acyclic graphs to assess potential confounding. No changes were made to the model following this process, data not shown). Our final model consisted of age and variables that were statistically significant at the 10% level in the fully adjusted model. Where relevant, we performed tests for linear trend using an ordinal score for categories of ordinal factors. Each of the imputed datasets were analyzed separately and the estimates were pooled using Rubin's rules (Rubin, 2004). We report the pooled (imputed) estimates.

To test whether factors associated with contiguous neval remnants differed according to the tumor's anatomical site, we included interaction terms for each significant variable in the final model. We also performed site stratified analysis of variables in our final multivariable model and tested for homogeneity in the odds ratios across anatomical sites (Hosmer and Lemeshow, 2000).

Statistical analyses were performed using the SAS statistical package, version 9.3 (SAS Institute, Inc, Cary, NC).

Supplementary analysis

Our primary analysis excluded LMMs as they are known to be related to chronic sun exposure and rarely with contiguous neval remnants, and to occur predominantly on the head and neck. By including them, sun exposure related factors were more likely to take precedence over other factors on the outcome. However, most previous studies assessing factors associated with neval remnants included LMMs. Hence we performed supplementary analysis by repeating our analysis including 94 LMMs with complete data on neval remnant status so our results could be compared with the other published studies.

CONFLICT OF INTEREST

The authors state no conflict of interest

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Table 1. Distribution of age, sex and histological factors and association with nevus-associated melanoma (n=636)

	Presence of Nevus			Age and sex adjusted
	No (n=312)	Yes (n=324)		
Variables	**n (%)**	**n (%)**	**P value**	**OR (95% CI)**
Age at diagnosis				
<50	67 (21.5)	121 (37.3)		Reference
50-59	72 (23.1)	69 (21.3)		0.53 (0.34 – 0.84)
60-69	94 (30.1)	88 (27.2)		0.52 (0.34 – 0.80)
≥70	79 (25.3)	46 (14.2)	<0.001	0.32 (0.20 – 0.53)
Sex				
Female	93 (29.8)	114 (35.2)		Reference
Male	219 (70.2)	210 (64.8)	0.15	0.99 (0.70 -1.43)
Anatomical site				
Head and Neck	74 (23.7)	39 (12.0)		Reference
Trunk	238 (76.3)	285 (88.0)	<0.001	2.35 (1.52 – 3.64)
Histological type				
SSM	249 (79.8)	278 (85.8)		Reference
NM	25 (8.0)	11 (3.4)		0.42 (0.20 – 0.89)
Other	38 (12.2)	35 (10.8)	0.03	0.80 (0.49 – 1.32)
Tumor thickness				
≤0.05mm	147 (47.1)	195 (60.2)		Reference
>0.05mm	165 (52.9)	129 (39.8)	<0.001	0.58 (0.42 – 0.80)
BRAF^{V600E} mutation				
No	167 (41.9)	124 (37.5)	<0.001	Reference
Yes	34 (8.5)	68 (20.5)		1.98 (1.18 – 3.31)
Missing	198 (49.6)	139 (42.0)		1.13 (0.80 – 1.60)

Table 2. Distribution of phenotypic factors and association with the nevus-associated melanoma (n=636)

	Presence of Nevus			Age and sex adjusted
	No (n=312)	Yes (n=324)		
Variables	**n (%)**	**n (%)**	**P value**	**OR (95%CI)**
Hair color as teenager				
Black/dark brown	107 (34.4)	93 (28.7)		Reference
Blond	60 (19.3)	84 (25.9)		1.47 (0.95 - 2.29)
Light brown	97 (31.2)	107 (33.0)		1.29 (0.87 – 1.92)
Red/auburn/strawberry	47 (15.1)	40 (12.3)	0.12	0.96 (0.57 - 1.61)
Eye color				
Brown	54 (17.3)	42 (13.0)		Reference
Blue/Grey	199 (63.8)	208 (64.2)		1.58 (0.99 – 2.51)
Green/Hazel	59 (18.9)	74 (22.8)	0.21	1.68 (0.97 – 2.89)
Burning reaction to skin				
Never/Rarely burns	28 (9.0)	30 (9.3)		Reference
Sometimes burns	92 (29.5)	98 (30.2)		0.81 (0.44 - 1.48)
Mostly burns	87 (27.9)	87 (26.9)		0.73 (0.39 - 1.35)
Always burns	105 (33.7)	109 (33.6)	0.99	0.71 (0.39 - 1.31)
Tanning reaction to skin				
No tan	32 (10.3)	34 (10.5)		Reference
Light tan	93 (29.8)	85 (26.2)		0.90 (0.50 - 1.60)
Moderate tan	141 (45.2)	147 (45.4)		1.05 (0.60 – 1.82)
Deep tan	46 (14.7)	58 (17.9)	0.64	1.28 (0.68 - 2.43)
Freckles on face as teenager				
None	104 (33.3)	115 (35.5)		Reference
Few	110 (35.3)	141 (43.5)		1.02 (0.70 - 1.48)
Some	57 (18.3)	49 (15.1)		0.66 (0.41 - 1.08)
Many	41 (13.1)	19 (5.9)	0.01	0.34 (0.18 - 0.64)
Moles on skin as a teenager				
None	71 (22.8)	43 (13.3)		Reference
Few	138 (44.2)	129 (39.8)		1.33 (0.84 – 2.11)
Some	75 (24.0)	99 (30.6)		1.71 (1.03 – 2.83)
Many	28 (9.0)	53 (16.4)	<0.001	2.32 (1.25 – 4.31)
Total nevus count by dermatologist				
<35	101 (32.4)	58 (17.9)		Reference
35-64	81 (26.0)	68 (21.0)		1.42 (0.89 - 2.28)
65-134	80 (25.6)	83 (25.6)		1.63 (1.01 - 2.63)
≥135	50 (16.0)	115 (35.5)	<.001	3.40 (2.03 – 5.72)

Variables	Presence of Nevus			Age and sex adjusted
	No (n=312)	Yes (n=324)		
	n (%)	n (%)	P value	OR (95%CI)
Degree of dermal elastosis				
Nil or Mild	137 (46.9)	188 (67.4)		Reference
Moderate	81 (27.7)	62 (22.2)		0.65 (0.42 – 1.01)
Marked	74 (25.3)	29 (10.4)	<0.001	0.34 (0.20 – 0.57)

Table 3. Distribution of sun exposure factors and association with the nevus-associated melanoma (n=636)

	Presence of Nevus			Age and sex adjusted
	No (n=312)	Yes (n=324)		
Variables	**n (%)**	**n (%)**	**P value**	**OR (95%CI)**
Total number of hours of sun exposure				
Quartile1	58 (18.6)	104 (32.1)		Reference
Quartile2	82 (26.3)	75 (23.1)		0.65 (0.40 - 1.06)
Quartile3	84 (26.9)	75 (23.1)		0.70 (0.42 - 1.19)
Quartile4	88 (28.2)	70 (21.6)	0.001	0.67 (0.38 - 1.17)
Number of hours of occupational sun exposure				
Quartile1	56 (17.9)	103 (31.8)		Reference
Quartile2	81 (26.0)	82 (25.3)		0.70 (0.43 - 1.14)
Quartile3	83 (26.6)	71 (21.9)		0.64 (0.38 - 1.06)
Quartile4	92 (29.5)	68 (21.0)	<0.001	0.60 (0.35 - 1.04)
Number of hours of recreational sun exposure				
Quartile1	80 (25.6)	79 (24.4)		Reference
Quartile2	84 (26.9)	69 (21.3)		0.85 (0.53 - 1.35)
Quartile3	70 (22.4)	89 (27.5)		1.14 (0.72 - 1.82)
Quartile4	78 (25.0)	87 (26.9)	0.26	1.12 (0.70 - 1.79)
Number of sunburns in primary school				
Never or 1-5 times	120 (38.6)	106 (32.8)		Reference
6-10 times	79 (25.4)	79 (24.5)		1.12 (0.74 - 1.69)
10+times	112 (36.0)	138 (42.7)	0.19	1.32 (0.91 - 1.91)
Number of sunburns in secondary school				
Never or 1-5 times	137 (48.2)	135 (43.7)		Reference
6-10 times	71 (25.0)	80 (25.9)		1.07 (0.72 - 1.61)
10+times	76 (26.8)	94 (30.4)	0.50	1.05 (0.70 - 1.57)
Number of sunburns since leaving school				
Never or 1-5 times	145 (46.6)	157 (48.8)		Reference
6-10 times	54 (17.4)	64 (19.9)		1.02 (0.66 - 1.58)
10+times	112 (36.0)	101 (31.4)	0.43	0.80 (0.56 - 1.15)

Table 4. Adjusted odds-ratios (ORs) and 95% confidence intervals (CIs) for factors associated with nevus-associated melanoma.

Variables[1]	OR (95%CI)
Age at diagnosis	
<50	Reference
50-59	0.62 (0.38 – 1.02)
60-69	0.77 (0.46 - 1.28)
≥70	0.58 (0.32 – 1.06)
Anatomical site[3]	
Head and Neck	Reference
Trunk	1.61 (0.95 - 2.76)
Tumor thickness	
≤0.5mm	Reference
>0.5mm	0.61 (0.43 - 0.85)
Eye color	
Brown	Reference
Blue/Grey	1.80 (1.11 - 2.94)
Green/Hazel	1.84 (1.04 - 3.28)
Freckles on face as teenager	
None	Reference
Few	1.08 (0.73 - 1.61)
Some	0.84 (0.50 - 1.41)
Many	0.49 (0.25 - 0.96)
Moles on skin as a teenager[2]	
None	Reference
Few	1.19 (0.73 - 1.94)
Some	1.36 (0.79 - 2.33)
Many	1.94 (1.01 – 3.72)
Total nevus count by dermatologist[2]	
<35	Reference
35-64	1.27 (0.78 - 2.09)
65-134	1.23 (0.74 - 2.04)
≥135	2.18 (1.26 - 3.78)
Degree of dermal elastosis[3]	
Nil or Mild	Reference
Moderate	0.69 (0.44 - 1.09)
Marked	0.55 (0.30 – 1.01)
BRAF^{V600E} mutation[4]	
No	Reference

Variables[1]	OR (95%CI)
Yes	1. 87 (1.08 – 3.23)

[1]ORs are adjusted for all variables in the table except for [2]moles and total nevus count not adjusted for each other.
[3]The OR (95%CI) for anatomical site in absence of degree of dermal elastosis in the model was 2.07 (1.31 -3.28). Similarly, the effect of dermal elastosis in absence of anatomical site in the model was 0.66 (0.42 – 1.03) for moderate and 0.42 (0.25 – 0.71) for marked elastosis. [4]BRAF missing category was included in the model as a separate category.

Table 5. Adjusted odds-ratios (ORs) and 95% confidence intervals (CIs) for factors associated with nevus-associated melanoma, stratified by body site.

Variables	Trunk OR (95%CI)	Head and Neck OR (95%CI)
Age at diagnosis		
<50	Reference	Reference
50-59	0.54 (0.32 - 0.93)	1.60 (0.39 – 6.64)
60-69	0.79 (0.45 - 1.38)	0.65 (0.17 - 2.57)
≥70	0.60 (0.31 - 1.18)	0.45 (0.10 - 2.10)
Tumor thickness		
≤0.5mm	Reference	Reference
>0.5mm	0.55 (0.38 - 0.80)	0.88 (0.387 - 2.11)
Eye color		
Brown	Reference	Reference
Blue/Grey	1.87 (1.10 - 3.16)	1.60 (0.36 – 7.17)
Green/Hazel	1.72 (0.92 - 3.21)	2.47 (0.49 - 12.41)
Freckles on face as teenager		
None	Reference	Reference
Few	1.13 (0.73 - 1.74)	0.88 (0.31 - 2.51)
Some	0.85 (0.49 - 1.49)	0.59 (0.14 - 2.52)
Many	0.49 (0.23 - 1.05)	0.48 (0.11 – 2.16)
Moles on skin as a teenager[1]		
None	Reference	Reference
Few	1.26 (0.73 - 2.17)	1.02 (0.33 - 3.12)
Some	1.69 (0.94 - 3.07)	0.21 (0.04 - 1.14)
Many	2.14 (1.05 - 4.36)	1.19 (0.18 - 7.78)
Total nevus count by dermatologist		
<35	Reference	Reference
35-64	1.29 (0.74 - 2.25)	1.16 (0.38 - 3.59)
65-134	1.38 (0.79 - 2.42)	0.80 (0.22 - 2.95)
≥135	2.40 (1.32 - 4.36)	1.51 (0.29 - 7.80)
Degree of dermal elastosis		
Nil or Mild	Reference	Reference
Moderate	0.69 (0.42 - 1.12)	0.44 (0.11 - 1.80)
Marked	0.51 (0.24 - 1.09)	0.47 (0.13 - 1.70)
BRAF^{V600E} mutation		
No	Reference	Reference
Yes	1.73 (0.95 - 3.17)	2.45 (0.58 - 10.26)

[1]ORs are adjusted for all variables in the table except for [2]moles and total nevus count were not adjusted for each other.

Figure legend

Figure 1. Flow chart of study participation and resulting sample for analysis