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Research Article

Cancer Epidemiology, Biomarkers & Prevention

Interactions between Ultraviolet Light and *MC1R* and *OCA2* Variants Are Determinants of Childhood Nevus and Freckle Phenotypes

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Abstract

Background: Melanocytic nevi (moles) and freckles are well known biomarkers of melanoma risk, and they are influenced by similar UV light exposures and genetic susceptibilities to those that increase melanoma risk. Nevertheless, the selective interactions between UV exposures and nevus and freckling genes remain largely undescribed.

Methods: We conducted a longitudinal study from ages 6 through 10 years in 477 Colorado children who had annual information collected for sun exposure, sun protection behaviors, and full body skin exams. *MC1R* and *HERC2/OCA2* rs12913832 were genotyped and linear mixed models were used to identify main and interaction effects.

Results: All measures of sun exposure (chronic, sunburns, and waterside vacations) contributed to total nevus counts, and cumulative chronic exposure acted as the major driver of nevus development. Waterside vacations strongly increased total nevus counts in children with rs12913832 blue eye color alleles and facial freckling scores in those with *MC1R* red hair color variants. Sunburns increased the numbers of larger nevi ($\geq$2 mm) in subjects with certain *MC1R* and rs12913832 genotypes.

Conclusions: Complex interactions between different UV exposure profiles and genotype combinations determine nevus numbers and size, and the degree of facial freckling.

Impact: Our findings emphasize the importance of implementing sun-protective behavior in childhood regardless of genetic make-up, although children with particular genetic variants may benefit from specifically targeted preventive measures to counteract their inherent risk of melanoma. Moreover, we demonstrate, for the first time, that longitudinal studies are a highly powered tool to uncover new gene–environment interactions that increase cancer risk. *Cancer Epidemiol Biomarkers Prev; 23(12); 2829–39. ©2014 AACR.*

Introduction

As melanoma incidence rises, interest mounts about understanding the interactions between genetic and environmental factors that lead to its development (1). UV rays in sunlight are the principal environmental determinant of melanoma, whereas the number of melanocytic nevi, or moles, is the strongest host risk indicator (2–5). Patterns of UV exposure, such as waterside vacations, sunburns, and chronic exposure, during childhood and adolescence, correlate with melanoma incidence and nevi (2, 6). Moreover, most of the known genetic factors that predispose to nevi also confer melanoma risk (7), and UV-induced mutations in oncogenes such as *BRAF* are found at similar rates in nevi and melanoma (8). Therefore, the shared etiology of nevi and melanoma, and the shared role of UV in their genesis offer a unique opportunity where the study of nevus development in children may shed light on the gene–UV interactions that give rise to melanoma.

Pigmentation phenotypes, including fair or red hair, light skin and blue eye color, high nevus counts, and dense freckling, are known melanoma risk indicators (9–11), and many of the genetic loci responsible for these traits have now been identified and most are associated with melanoma (12). Freckles (or ephelides) are benign, usually small (1–2 mm) pigmented spots that appear on the

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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sun-exposed skin of young fair-skinned or red-haired children (13). Two major pigmentation genes, the *Melanocortin-1 receptor* (*MC1R*) and the *Oculocutaneous Albinism Type 2* (*OCA2*) gene, contribute to melanoma risk phenotypes (14–16). *MC1R*, encoding a G protein-coupled receptor, is the major red hair color (RHC) gene (17–19), whereas the *OCA2* locus encodes the P protein, an integral melanosomal protein of uncertain function (20). We and others showed that *MC1R* variants are associated with fair skin color, freckles, poor tanning, susceptibility to sunburn, and reduced nevus counts (16–18, 21). Much of the impact of *OCA2* on normal pigmentation may be attributable to a single SNP, rs12913832, which resides in a distal regulatory sequence within the neighboring *HERC2* gene (14, 20, 22, 23). rs12913832 modulates the level of OCA2 expression, accounting for >70% of blue versus brown eye color differences in Caucasians (22, 24). SNPs at this locus are associated with increased freckle and nevus counts, lighter hair and skin, reduced tanning capacity, and perhaps increased melanoma risk (16, 25, 26).

This study of a longitudinal cohort of children sought to evaluate the impact of different UV exposure patterns on both nevus and freckle formation in relation to genetic factors. Thus, we present data on differences in nevus counts and freckling scores in children with different sun exposures and different *MC1R* and *OCA2* genotypes.

Materials and Methods

Study population, pigmentation, and exposure variables

A cohort of 1,145 children born in 1998 participated in annual skin exams, and key phenotypic, environmental, and behavioral measures were recorded (27). Of these children, 509 provided DNA samples in 2007–2008 and were included in the analysis. Their parents completed surveys about the children's sun-behavioral patterns from mid-June to mid-October over the 2004–2008 time periods. The study population was restricted to include white (Hispanic and non-Hispanic) children ($n = 477$). This study protocol was approved by the Colorado Multiple Institutional Review Board and the Institutional Review Board for Kaiser Permanente of Colorado (Denver, CO). Both of these organizations adhere to the Declaration of Helsinki protocols for human subject research. Parents provided written informed consent and children provided written assent beginning at the age of 7 years.

Melanocytic nevus counts, facial freckling density levels, hair, eye, and constitutive skin color were recorded during annual skin exams as previously described (27, 28). UV exposures and sun protection practices were assessed at the enrollment interview (2003/2004) and at each annual telephone interview from 2004 to 2007 (29) using a composite index that included shade seeking and sun-protective clothing, hat, and sunscreen use; the higher the composite score, the greater the protection behavior (29). Waterside vacations were considered cumulatively, with numbers taken from birth to the year before each skin exam. Cumulative levels of chronic exposure as of each interview year were computed by summing scores on this variable over time starting in 2004. Cumulative total number of sunburns as of each interview year was obtained by summing the number of prior year sunburns across years starting with 2004.

OCA2/HERC2 rs12913832 SNP genotyping and *MC1R* sequencing

DNA was collected and extracted from cheek cells using a commercial buccal swab kit (Epicentre Technologies). The *MC1R* coding region was sequenced as described (18). Alleles were classified as strong and weak red hair alleles, R and r, respectively, and were further grouped into three genotype groups as described (16): [R/R, R/r], [R/+, r/r], [r/+, +/+]. R alleles included D84E, R142H, R151C, R160W, and D294H, whereas r alleles were V60L, V92M, and R163Q. We detected additional rare variants, including T95M, A139V, I155T, A166V, and F196V, that we considered as r variants for this analysis. rs12913832 was genotyped using a custom-designed TaqMan endpoint genotyping assay shown in Supplementary Methods.

Data analysis

Preliminary analyses evaluating differences between subjects who did and did not provide DNA were performed using SPSS and SAS/STAT software (Version 9.3 for Windows, SAS Institute). Descriptive statistics (mean, SDs, proportions) were used to characterize participants in each year, and χ^2 and one-way ANOVA tests were used to assess differences in subjects with different MC1R and rs12913832 genotypes. Missing exposure data were imputed as described in Supplementary Methods. SAS PROC MIXED was used to analyze the impact of genotypes, exposures, and sun protection behaviors on total body nevus counts and nevi $\geq$2 mm. SAS PROC GLIMMIX was used to examine interactions among genotypes, exposures, and sun protection behaviors that impact facial freckling. Significant interaction effects for each model were plotted using SAS. A detailed analysis plan is included in Supplementary Methods.

Results

Characteristics and participation of study subjects

This study reports data collected annually in 2004–2008 for white children who provided DNA data in either 2007 or 2008 ($N = 477$). Study participants did not differ with regard to race/ethnicity, hair color, eye color, or gender from the other participants in the full study cohort who did not provide DNA (27). Participation was high with 68% of all 477 children completing a skin exam in all 5 years (age 6–10 years), 22% had four exams, 6% had three, and 4% had two or one exams (Supplementary Table S1). Ninety-four percent completed the survey in all 4 years, 4% completed three, and 2% completed two or fewer surveys.

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The number of nevi and freckle density grew in each successive year. The geometric mean total nevus counts increased almost 2.5-fold and the mean freckling score increased 1.75-fold from the age of 6 to 10 years (Supplementary Table S2A). Sun exposures also increased: the cumulative number of sunburns, waterside vacations, and chronic exposure grew 5-, 1.9-, and 3.7-fold, respectively (Supplementary Table S2B). Average sun protection frequency was approximately 12 on the 4–20 scale, and did not vary markedly each year.

Association of *OCA2*/*HERC2* rs12913832 SNP and *MC1R* with pigmentation phenotype

rs12913832 alleles (C/T) were found at frequencies of 0.69 and 0.31, respectively (Supplementary Table S3). As previously reported (16, 22, 30), the C allele was associated with lighter skin, hair, and eye color. The association with eye color was particularly striking where 62.5% of children homozygous for the C allele had blue eyes, and 89.8% of subjects homozygous for the T allele had brown eyes. Thus, the rs12913832 C allele is hereafter referred to as $OCA2^{blue}$ and the T allele as $OCA2^{Brown}$. $OCA2^{blue}$ was associated with increases in freckling, total body nevus count, and nevi $\geq$2 mm, similarly to previous reports. Sixty-two percent of children were in the *MC1R* grouped R/R, R/r category, 25% in R/+, r/r and 13% in r/+, +/+. As expected, *MC1R* R/R, R/r variants were positively associated with red hair, lighter skin, green eye color, and high facial freckle scores, but not total nevus counts or nevi $\geq$2 mm.

Because it is possible that sun exposure behaviors may be associated with pigmentation characteristics, the potential for significant associations between sun exposure and genotype was examined. Few associations were observed between genotype, sun exposure, and sun protection (Supplementary Table S4 and S5); although children in the $OCA2^{blue/blue}$ group experienced more sunburns in both 2004 and 2007, and those with *MC1R* R/R, R/r variants had more sunburns in 2006 and 2007.

Three major UV exposure variables interact to influence nevus counts in childhood

To evaluate the associations between exposure patterns such as waterside vacations, sunburns, and chronic exposure, and nevus counts among children with *MC1R* and rs12913832 variants, we analyzed the data using linear mixed modeling. A significant three-way interaction was detected between UV exposures that together were associated with log-transformed nevus counts (Table 1). This three-way interaction showed that the effects of waterside vacations and sunburns on nevus counts are different depending on whether children had low or high chronic exposures. In children with 24 or fewer hours of chronic exposure (mean = 18.25, 3rd quartile = 24), all UV exposures acted cumulatively to increase predicted nevus counts (Fig. 1A). Thus, among those with little chronic exposure, nevus count increased with each respective unit of exposure. As chronic exposure approached 28 hours, the combined effects of waterside vacations and sunburns started to diminish, and ultimately at higher chronic exposures, their impact appeared to reverse. Thus, the highest predicted rate of nevus accumulation was observed with increasing chronic exposure (>50 hours) in the presence of few sunburns and few waterside vacations (Fig. 1A, Group 1). In this context, the relationship between chronic exposure and nevus accumulation progressively diminished in individuals who had (i) few waterside vacations, many sunburns (Group 2), and (ii) many waterside vacations, few sunburns (Group 3), and (iii) many waterside vacations and many sunburns (Group 4).

Waterside vacations interact with $OCA2^{blue}$ to elevate childhood nevus counts

An interaction between rs12913832 genotype and cumulative number of waterside vacations was a significant predictor of log-transformed nevus counts (Table 1; Fig. 1B). At the average level of waterside vacations (2.21), geometric mean nevus counts were increased by 13% with each $OCA2^{blue}$ allele. Each additional waterside vacation increased that effect by another 3%. Thus, waterside vacations had the greatest effect in promoting nevus formation in children who carried $OCA2^{blue/blue}$ (Fig. 1B). In fact, $OCA2^{blue/blue}$ subjects increased their predicted geometric mean nevus counts around 2.5-fold when waterside vacations increased from the mean level (2.21) to 12. In contrast, predicted geometric mean nevus counts increased only 1.6-fold in $OCA2^{Brown/blue}$ children and 1.1-fold in $OCA2^{Brown/Brown}$ children who experienced the same increase in waterside vacations.

Genetic interaction of *MC1R* with *OCA2* in sunburned kids influences counts of nevi $\geq$2 mm

Because it is possible that nevi of different sizes develop via different pathways, the same potential interactions among *MC1R* and rs12913832 and the three different UV exposure variables were investigated using nevi $\geq$2 mm as the outcome (Table 2). In this model, a significant three-way interaction of *MC1R* and rs12913832 genotypes and number of sunburns was observed. Sunburns preferentially accelerated nevus counts $\geq$2 mm in children who were in the intermediate *MC1R* r/r, R/+ genotype group in combination with $OCA2^{Brown/Brown}$ (Fig. 2A). For this *MC1R*/rs12913832 combination, sunburns increased geometric mean nevus count 3.6-fold with 8 sunburns versus the overall average of 2 sunburns for the study period. The effect of sunburns on nevus counts among children with intermediate *MC1R* was progressively reduced as the number of $OCA2^{blue}$ alleles increased (Fig. 2B and C). Nevi also increased in response to sunburns in the *MC1R* R/R, R/r genotype group, and there was a tendency for this sunburn responsiveness to increase with each $OCA2^{blue}$ allele; however, this was not statistically significant. The *MC1R* +/+, r/+ genotype was minimally responsive to sunburns in any combination with rs12913832 genotype.

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Table 1. Linear mixed model analysis predicting log total nevus counts in children aged 6 to 10

	Estimate				
Predictor	**b**	**95% CI**	**Antilog (b)**	**R2**	***P***
Intercept	3.29		26.70		
rs12913832	0.12	0.04–0.20	1.13	0.02	0.01
MC1R					
R/R, R/r	−0.11	−0.27 to 0.05	0.90	0.006	0.19
r/r, R/+	0.05	−0.08 to 0.17	1.05	—	0.47
r/+, +/+	—	—	—	—	—
Gender					
Female	−0.10	−0.20 to 0.01	0.91	0.01	0.08
Male	—	—	—	—	—
Sun protection composite	0.004	−0.01 to 0.02	1.00	0.003	0.51
Water vacations (#, cum.)	0.01	−0.02 to 0.05	1.01	0.0004	0.46
Chronic exposure (cum.)	0.02	0.01–0.02	1.02	0.12	<0.001
Sunburns (#, cum.)	0.06	0.05–0.08	1.07	0.04	<0.001
Water vacations*rs12913832	0.03	0.01–0.05	1.03	0.004	0.01
Water vacations*chronic	−0.001	−0.002 to −0.0003	0.999	0.005	<0.01
Water vacations*sunburns	−0.005	−0.012 to 0.001	0.995	0.002	0.13
Chronic*sunburns	−0.002	−0.003 to −0.001	0.998	0.03	<0.001
Water vacations*chronic*sunburns	0.0002	0.000–0.0004	1.0002	0.004	0.03

Abbreviations: #, number; cum, cumulative.

Waterside vacations enhance facial freckling in children with red hair color alleles

Despite the widespread belief that freckles appear with increasing sun exposure, and the recognition that both freckles and UV exposures are melanoma risk factors, the relationship between these two risk factors has not been previously described. Linear mixed model analysis was used to examine the impact of rs12913832, *MC1R* variants, and UV exposures on facial freckling (Table 3). No interaction was observed between rs12913832 and any of the UV exposure variables although there was a significant main effect of rs12913832, with each $OCA2^{blue}$ allele increasing the odds of being in both the high and medium freckle groups relative to the group with no freckles. Of the other UV exposure variables, chronic exposure and sunburns both elevated the odds of having higher freckling levels, and these effects were independent of *MC1R* and rs12913832 genotypes.

A significant interaction between waterside vacations and *MC1R* was detected. Children who had *MC1R* R/R, R/r variants were increasingly found in the highest freckle group as they experienced more waterside vacations (Table 3; Fig. 3). In fact, for *MC1R* R/R, R/r children, the predicted OR for being in the high versus no freckle group was negligible (0.1) in the absence of water vacations, and increased to over 7 when children experienced nine waterside vacations (Fig. 3). The odds of being classified in the high freckle group were unaffected by waterside vacations for youth in the other two *MC1R* groups.

Discussion

Much has been written about the power of longitudinal studies to uncover gene–environment interactions (31, 32); however, to our knowledge, our study is the first report of a longitudinal analysis where gene–environment interactions for cancer risk phenotypes are identified. With this in mind, our study uncovered a number of novel findings. We demonstrated that chronic exposure plays a central role in the formation of nevi: children with the highest chronic exposure had the highest nevus counts. Within the low range of chronic exposure, all UV exposures acted cumulatively to increase nevus number. However, at high levels of chronic exposure, the nevus promoting effect of waterside vacations and sunburns was reduced. Second, we identified interactions between waterside vacations, sunburns, and specific rs12913832 and *MC1R* genotypes that significantly enhanced total nevus counts, counts of nevi $\geq$2 mm, and the degree of facial freckling. Taken together, our data suggest that to the extent that nevi are a marker for melanoma risk, all children will benefit from sun protection. The results also indicate that a personalized sun protection strategy based on a child's genetic makeup may be of value in counteracting melanoma risk as a complement to current population-wide preventive efforts.

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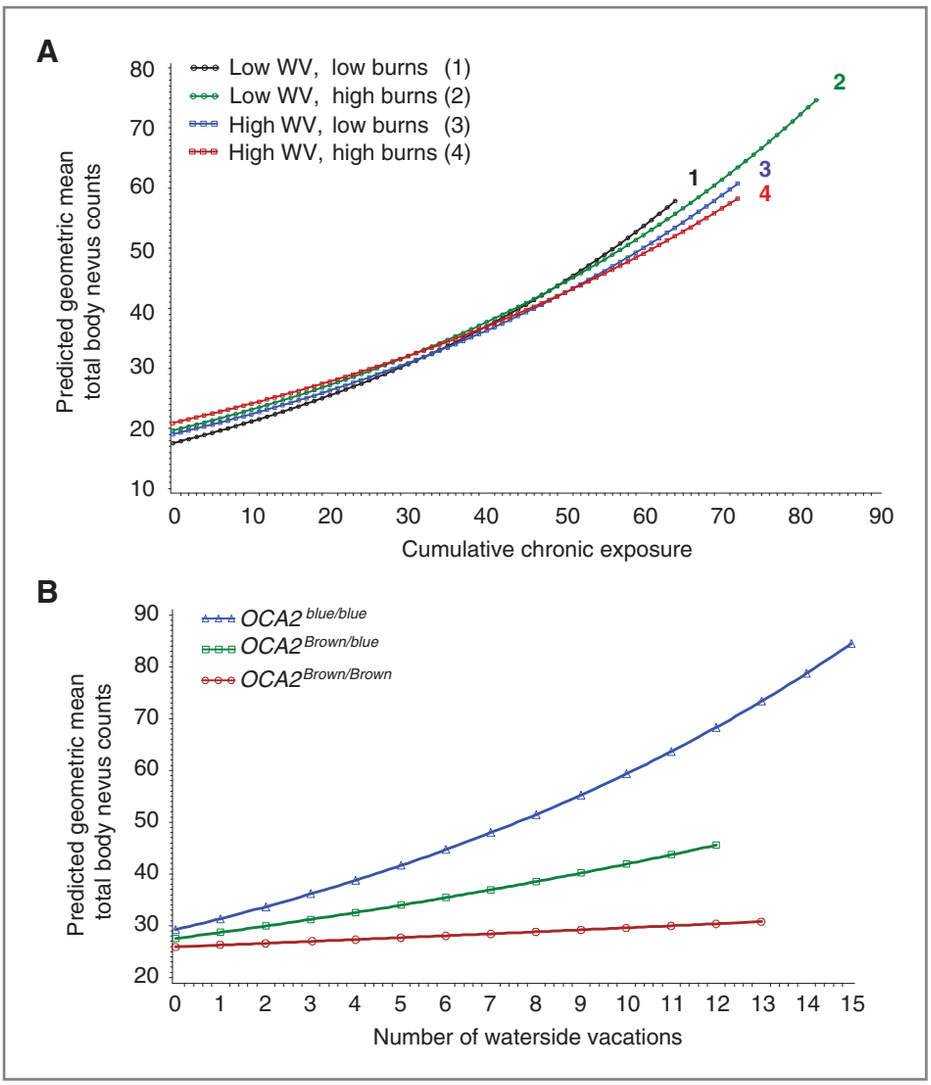

Figure 1. Interactions that predict geometric mean total body nevus counts in 6- to 10-year-old children as determined by the linear mixed model analysis presented in Table 1. A, cumulative hours of chronic exposure interact with cumulative numbers of water vacations and sunburns to predict geometric mean total body nevus counts in the study period. Low and high water vacations and sunburns are used to depict the relationships between these variables in predicting nevus counts. The terms Low and High refer to the 33rd and 66th percentile levels of both cumulative centered sunburns (−1.2 and 0.79) and cumulative centered waterside vacations (−0.88 and 0.12). Exponentiated simple slope estimates and 95% confidence intervals for the effects plotted for each represented exposure group are in Supplementary Table S6. B, the *OCA2*/*HERC2* rs12913832 SNP interacts with water vacations to predict geometric mean total body nevus counts. The two-way association between rs12913832 and water vacations is observed in subjects who experience an average level of sunburns (1.88) and chronic exposure (18.25) for the study period. Exponentiated simple slope estimates and 95% confidence intervals for the plotted *OCA2* genotypes are in Supplementary Table S7.

Although intense intermittent UV exposure is associated with increased nevus counts and melanoma (2, 33), the role of chronic exposure is less clear (2, 34). We present a new perspective for the role of chronic exposure in nevogenesis and melanoma risk. In children with low levels of chronic exposure, all types of UV exposure contribute to nevus counts in a cumulative manner. This relationship tended to reverse in children who experienced higher levels of chronic exposure, where the nevogenic effect of this exposure tended to be reduced along with that of waterside vacations and sunburns as their respective numbers increased. These observations suggest that high levels of chronic exposure may counteract the effects of intense intermittent UV exposure on melanoma risk (33–35), possibly by inducing an adaptive response of skin to UVR. Alternatively, there may be a threshold of UV that a child experiences, equivalent to around 30 to 40 hours of cumulative chronic exposure by the age of 10 years in our study, at which the effects of all types of exposures are dampened. Previous studies, including our own, have not reported such a strong effect of chronic exposure on nevus counts (29). This may be due to differences between cross-sectional versus longitudinal analytical approaches, failure to examine interactions between exposure types, and our multiyear prospective study design where yearly sun exposure information is collected annually, rather than more distant retrospective assessment of sun exposure behaviors based on locality of past residence or recall. Chronic exposure may be particularly hard to assess retrospectively, and analysis of a single year of chronic exposure data may underestimate the effects of this variable. That is, chronic exposure may act in a cumulative fashion over multiple years, and one year's exposure may not accurately represent this.

The total nevus count model showed that waterside vacations, rather than sunburns or chronic exposures,

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Table 2. Linear mixed model analysis predicting log total nevus counts ≥2 mm in children aged 6 to 10

	Estimate				
Predictor	**b**	**95% CI**	**Antilog (b)**	**R2**	***P***
Intercept	0.70	0.41–0.99	2.02		<0.001
rs12913832	0.19	0.07–0.30	1.21	0.01	0.001
MC1R					
R/R, R/r	0.11	−0.41 to 0.63	1.12	0.01	0.67
r/r, R/+	0.30	−0.01 to 0.62	1.35	—	0.06
r/+, +/+	—	—	—	—	—
Gender					
Female	−0.06	−0.18 to 0.06	0.94	0.002	0.35
Male	—	—	—	—	—
Sun protection composite	0.004	−0.014 to 0.02	1.00	0.0001	0.66
Water vacations (#, cum.)	0.03	0.01–0.05	1.03	0.01	0.01
Chronic exposure (cum.)	0.01	0.005–0.01	1.01	0.02	<0.001
Sunburns (#, cum.)	0.02	−0.05 to 0.09	1.02	0.005	0.54
rs12913832**MC1R*					
R/R, R/r	−0.11	−0.44 to 0.22	0.90	0.003	0.52
r/r, R/r	−0.12	−0.32 to 0.08	0.89	—	0.25
r/+, +/+	—	—	—	—	—
rs12913832**sunburns	0.01	−0.03 to 0.05	1.01	0.001	0.68
*MC1R**sunburns					
R/R, R/r	0.04	−0.11 to 0.20	1.05	0.004	0.57
r/r, R/+	0.14	0.03–0.24	1.15	—	0.01
r/+, +/+	—	—	—	—	—
rs12913832**MC1R**sunburns					
R/R, R/r	0.01	−0.08 to 0.10	1.01	0.005	0.81
r/r, R/+	−0.08	−0.15 to −0.02	0.92	—	0.01
r/+, +/+	—	—	—	—	—

Abbreviations: #, number; cum, cumulative.

preferentially interacted with the $OCA2^{blue}$ allele to increase nevus counts. This finding builds upon the known association between $OCA2^{blue}$, blue eye color, and elevated nevus count (22), and is the first indication that these children are susceptible to nevi following UVR. Previously, the *methylthioadenosine phosphorylase* (*MTAP*) rs7023329 SNP was shown to interact with sunburns to increase nevus counts in adults (34). We also observed that sunburns acted on the intermediate *MC1R* genotype group, R/+, r/r, in combination with $OCA2^{Brown}$ alleles to increase counts of nevi ≥2 mm. These findings extend the previous report of interaction between *MC1R* and *OCA2* to include the effect of UV exposure on nevi (16). This interaction was not observed for total nevi, and altogether, these data may indicate that nevi of different sizes form through different mechanisms in response to different types of UV exposure.

Freckling is generally considered to be genetically determined (36), although a pre-established pattern of freckles may become visible following sun exposure (37, 38). We showed for the first time that the number of waterside vacations strongly interacted with *MC1R* RHC variants to enhance facial freckling in children. We note that *MC1R* is the major freckling gene, although variants in *ASIP*, *BNC2*, *IRF4*, *OCA2*, and *TYR* also contribute, albeit to a much lesser degree, to formation of this trait (13, 14). We did not identify any interaction between rs12913832 and any sun exposure variable, so freckling by this pathway may be entirely inherent. Further studies are needed to unravel the relative contributions of sun exposure and genes to formation and natural history of freckles.

Although our studies indicate that environment–environment and gene–environment interactions are associated with melanoma risk phenotypes in children, the extent to which these findings can be generalized to melanoma itself remains to be determined. The GEM study group previously reported an association between UV exposure and *MC1R* R variants (39), and we also observed a tendency toward increased nevi ≥2 mm in the $OCA2^{blue/blue}$ *MC1R* R/R, R/r group. Moreover, *MC1R* R/+ subjects are known to be at elevated risk for melanoma (40). *OCA2* variants, including rs12913832, are likely weakly penetrant for melanoma, as some studies show

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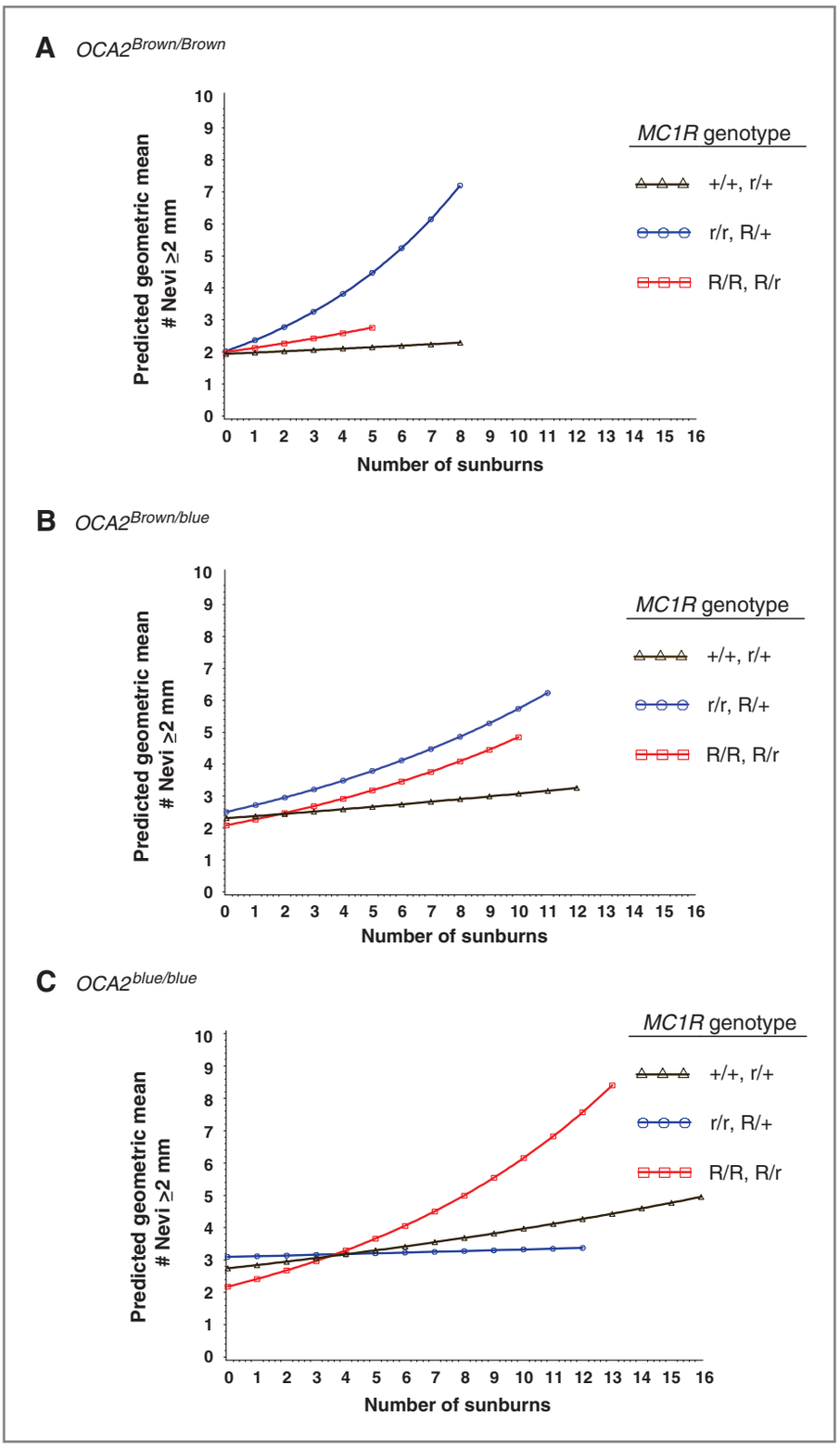

Figure 2. Three-way interaction that predicts geometric mean nevus counts ≥2 mm in 6- to 10-year-old children as determined by the linear mixed model analysis of longitudinal data presented in Table 2. A–C, a three-way interaction with cumulative number of sunburns (*x*-axis) predicts nevus counts in children from each combination of *OCA2*^{Brown} and *OCA2*^{blue} alleles together with each *MC1R* genotype grouping. Nevus counts were only plotted where actual sunburn data were available within each genotype group. Exponentiated simple slope estimates and 95% confidence intervals for each plotted *MC1R* and *OCA2* genotype group are shown in Supplementary Table S8.

an association (25, 26) but not others (12, 41). Our data suggest that associations between rs12913832 and melanoma may strengthen as future studies include UV exposure covariates. Our data also support the idea that GWA studies factoring in relevant exposure variables could uncover new cancer risk loci.

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Table 3. Generalized linear mixed model analysis predicting facial freckle level in children aged 6 to 10

	Parameter		
Predictor	**OR**	**95% CI**	***P***
Intercept			
High freckling	0.001	0.0001–0.01	<0.001
Medium freckling	0.07	0.01–0.41	0.003
Low freckling	0.43	0.13–1.39	0.16
rs12913832			
High freckling	3.40	1.50–7.75	0.004
Medium freckling	1.90	1.16–3.09	0.01
Low freckling	1.08	0.80–1.44	0.62
MC1R			
High freckling	17.65	8.07–38.60	<0.001
Medium freckling	4.51	2.77–7.34	<0.001
Low freckling	1.52	1.09–2.11	0.01
Female gender			
High freckling	1.10	0.39–3.06	0.86
Medium freckling	1.22	0.64–2.34	0.55
Low freckling	1.42	0.95–2.11	0.09
Sun protection composite			
High freckling	1.12	0.95–1.31	0.17
Medium freckling	1.00	0.64–2.34	0.97
Low freckling	1.00	0.95–2.11	1.00
Water vacations			
High freckling	0.73	0.55–0.97	0.03
Medium freckling	0.99	0.84–1.17	0.92
Low freckling	1.02	0.92–1.13	0.72
Chronic exposure			
High freckling	1.03	1.01–1.06	0.01
Medium freckling	1.02	1.00–1.04	0.05
Low freckling	1.01	1.00–1.03	0.04
Sunburns			
High freckling	1.38	1.17–1.63	0.000
Medium freckling	1.25	1.09–1.43	0.001
Low freckling	1.06	0.96–1.18	0.23
*MC1R**water vacations			
High freckling	1.45	1.11–1.88	0.01
Medium freckling	1.04	0.86–1.25	0.71
Low freckling	0.92	0.79–1.06	0.22

NOTE: Children with no freckles are used as the reference group.

In the models for nevi and freckles, no significant effect of sun protection was observed. The sun protection composite score incorporates information on hat and sunscreen use, sun-safe clothing use, and shade seeking behavior, and is likely to have an impact on our models by influencing the number of sunburns experienced by any individual (34). There is a moderate yet significant inverse correlation between sun protection composite score and number of sunburns experienced by subjects within the study period ($r = -0.07$–-0.13; $P = 0.038$–0.005; $n = 446$–457 from 2005 to 2007; 2004 was not significant). It is possible that new alternatives for intervention, perhaps including genetic information would show greater impact on these longitudinal models.

It will be useful to replicate the findings reported here using additional cases from the cohort being followed in this study. Such replication will not only help to validate the present findings, but may also reveal more robust associations, given a larger sample size and increased statistical power. Although the size of the current sample was sufficient for the analyses reported here, we did use imputation procedures to fill in the small amount of missing data that existed on our sunburn, waterside vacation, and chronic exposure variables. The imputation

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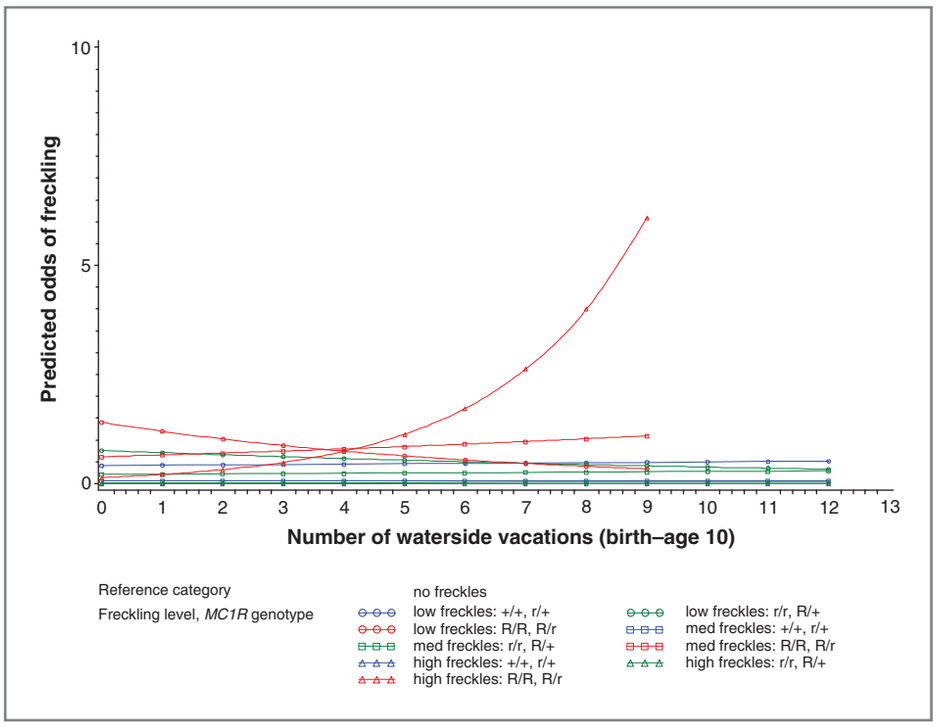

Figure 3. An interaction between waterside vacations and *MC1R* predicts facial freckle scores as determined by the model presented in Table 3. The ORs for being in each freckle group (low, moderate, high) versus the no freckles reference group for each *MC1R* genotype were plotted for each waterside vacation. Exponentiated simple slope estimates and 95% confidence intervals for each plotted freckle density level and MC1R genotype group are shown in Supplementary Table S9.

procedure clearly allowed us to maximize our analytic power with minimal negative impact on our findings.

It will also be important to confirm these findings in other locations where sun exposure patterns may vary from those experienced by children in Colorado. Colorado is characterized by a high altitude (>5,000 foot elevation) and a sunny climate (>300 days of sunshine per year), and a 30% higher ambient UV level compared with sea level. Repeat studies in other locations will determine whether differences exist in gene–environment interactions where ambient UV levels are substantially lower or higher, resulting in marked differences in the impact of chronic exposure.

Our understanding of the gene–environment interactions that influence melanoma risk phenotypes, and indeed melanoma itself, is in its infancy. Given that much of the sun exposure associated with later life melanoma risk may be accrued during childhood and adolescence, a clear understanding of the impact of different types of sun exposure is needed to engage the most effective prevention strategies. Although some studies suggest that sun protective behaviors may reduce nevus counts in children (42–45), a more efficient and consistent preventive strategy is needed. To this end, our unique longitudinal study of nevus development provides concrete evidence for what has been long suspected, that different patterns of sun exposure interact in different ways with each other and with different genetic factors to influence melanoma risk phenotypes. Our data suggest that all children may benefit from sun protection, and that a personalized sun protection strategy based on a child's genetic makeup may be of additional value in counteracting melanoma risk.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Interactions between Ultraviolet Light and *MC1R* and *OCA2* Variants Are Determinants of Childhood Nevus and Freckle Phenotypes

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