**Supplemental Methods**

**Pigmentation Variables and Nevus Counts**

Melanocytic nevus counts, facial freckling density levels, hair, eye, and constitutive skin color were recorded during annual skin exams as previously described ([1](#_ENREF_1), [2](#_ENREF_2)). Trained physicians and nurses performed full body (with the exclusion of the genital area, buttocks and scalp) nevus counts that were coded by size (<2 mm and >2 mm) and documented on an anatomical map. They also assessed freckling density on the face, arms, and back with a 10-level chart ranging from 0-100, lower scores indicating lesser freckling ([3](#_ENREF_3)). Due to low levels of freckling on the total body, only facial freckling scores were used in the present analysis. Hair color was determined through the use of wigmaker samples, and coded as red, blonde, light brown, dark brown or black ([1](#_ENREF_1)). Eye color was classified as blue, green, hazel (combination of brown and another color), or brown ([1](#_ENREF_1)). Skin reflectance was measured on the unexposed, upper medial arm using a colorimeter (Minolta Chroma Meter CR-400; Konica Minolta Sensing, Inc., New Jersey)([1](#_ENREF_1), [2](#_ENREF_2)).

**Exposure Variables**

UV exposure and usual sun-protection practices were assessed at the enrollment interview (2003/2004) and at each annual telephone interview from 2004-2007 ([4](#_ENREF_4)). For waterside vacation exposure, parents were asked about the location and season of trips to sunny locations outside of the Denver-Boulder-Colorado Springs metropolitan areas. Locations were classified as “waterside” or “non-waterside” based on the presence of a large body of water (e.g., lake, ocean, or river) and whether the location presented opportunities for water-based recreational activities. Some locations (>27.5 °N) were only considered “waterside” in warmer seasons ([4](#_ENREF_4)). During enrollment, parents were asked about all trips taken since the child’s birth while in the annual interview, parents were asked about all trips taken since the previous interview. Waterside vacations were considered cumulatively, with numbers taken from birth to the year prior to the year each skin exam was conducted. Chronic, or routine daily UV exposure was assessed at each annual interview through parental report of the number of days per week the child spent more than 15 minutes outside between 11:00 a.m. and 3:00 p.m. and the usual number of hours spent outside during such occasions. Responses to these questions were multiplied to calculate the total number of hours per week in the summer that the child spends outside during midday (range = 0 to 24).

***OCA2/HERC2* rs12913832 SNP Genotyping and *MC1R* sequencing**

PCR amplification was performed directly from the DNA in the extraction solution.A Taqman endpoint genotyping assay was designed using the Biosearch Technologies genotyping design software (Novato, CA). A Forward 5’-CCTCGGCCCCTGATGATGATAG-3’ primer and a Reverse 5’-GGCTCTCTGTGTCTGATCCA-3’ primer were used to amplify a 90 bp fragment. A FAM-labeled oligo 5’-GTAATTCACGTTCAAGAC-3’ detecting the C allele and a CalFluor Orange-labeled oligo 5’-TTTACAGTTCAAGACGT-3’ detecting the T allele were employed. qPCR reactions were performed in duplicate on a Roche Lightcycler 480 Real Time PCR machine and analyzed using its endpoint genotyping software. Cases were coded as having 0, 1, or 2 C alleles and subsequently classified as TT, CT, or CC respectively. rs12913832 genotyping failed in 5 out of 509 children.

The *MC1R* coding region (951 bp) was amplified by PCR as previously described ([5](#_ENREF_5)) and the PCR products were purified with the GenElute PCR spinclean kit (Sigma) and sequenced with 5’-TCTGACGGGCTCTTCCTC-3’ and 5’-TCCAGCCTCTGCTTCCTG-3’ primers at Functional Biosciences (Madison, WI)([6](#_ENREF_6)). *MC1R* sequencing failed in 10 of 509 children.

**Data Analysis**

We used chi-square and t-tests in preliminary analyses to evaluate differences between participants who provided DNA data in 2007 or 2008 and those who did not and were therefore excluded. Descriptive statistics (means, standard deviations, proportions) were used to report demographic, pigmentation, and UV exposure characteristics of participants by year of data collection. Additional chi-square and one-way analysis of variance (ANOVA) tests were assessed for differences in these characteristics as a function of rs12913832 and *MC1R* genotypes. All analyses described above were carried out using SPSS Statistics program for Windows v. 19 (IBM company).

We evaluated changes over time in total body nevus counts, numbers of nevi >2 mm, and facial freckling density in relation to rs12913832 and *MC1R* genotypes, UV exposure (cumulative of waterside vacations, sunburns, and chronic exposure) and all two- and three-way interactions among genotypes and patterns of UV exposure. These studies were performed using linear and generalized linear mixed modeling ([7](#_ENREF_7)).

We assessed patterns of missing data on the outcome measures by examining the degree to which mean nevus counts and freckling scores systematically varied by the number of skin exams a child completed or by the child’s age at last exam. The extent of missing data on the yearly exposure variables was small, ranging from n=18 (3.8%) for 2005 reports of chronic UV exposure levels to n=13 (2.5%) for 2007 reports of waterside vacations. Furthermore, the impact of the imputations on the means and standard deviations of all annual and cumulative variables was minimal. This examination therefore suggested that data were missing at random. Linear and generalized mixed modeling procedures, which were used in the primary analyses of the nevus count and freckling outcomes, yield unbiased results when data are MAR ([7](#_ENREF_7)). Missing data on the yearly exposure variables were imputed using the methods recommended by Engels and Diehr ([8](#_ENREF_8)). Missing data in 2004 were imputed using the ‘next observation carried back’ (NOCB) method, while in 2005 and 2006 they were imputed through a combination of averaging the last known and next known values, and using the ‘last observation carried forward’ (LOCF) and NOCB methods. Missing data in 2007 were imputed based on the LOCF. SAS PROC MIXED was used for the analysis of total body nevus counts and for nevi >2 mm, both of which were subjected to a natural logarithmic transformation to correct for positive skew. We also examined bivariable associations between genotypes and nevus counts to assess the linearity of those relationships. rs12913832 genotype demonstrated a linear association with both nevus counts and was therefore treated as an ordinal variable in the analyses (i.e., 0=TT, 1=CT, 2=CC). *MC1R* was not linearly related to either nevus count measure so indicator variables were used in the nevus count analyses as follows: R/R and R/r (0/1) and r/r and R/+ (0/1) were compared to the +/+ and r/+ reference group. Inspection of residuals plots confirmed that all statistical assumptions (normality, linearity, homoscedasticity) were satisfactorily met and assessment of influence statistics revealed no outliers or other observations that were influential in determining the presence or magnitude of the higher order interaction effects that emerged.

Initial examination of the continuous facial freckling density measure revealed that the distributional assumptions of the linear mixed model were not satisfied even after applying natural logarithmic and logit transformations. We subsequently used quartile values to create a four-level facial freckling variable (representing no [0], low [10], moderate [20-30], and high [40-100] freckling groups) for analysis with SAS PROC GLIMMIX, assuming an underlying multinomial distribution. Results of likelihood ratio tests (LRTs) demonstrated that the model fit was superior when the four-level facial freckling variable was assumed to follow a nominal compared to ordinal distribution. Both the rs12913832 and *MC1R* genotypes were linearly related to the four-level facial freckling variable and therefore used as ordinal variables for this analysis (i.e., 0=TT, 1=CT, 2=CC; 0="+/+, r/+" 1="r/r, R/+" 2="R/R, R/r", respectively). Inspection of residuals plots confirmed that all statistical assumptions were satisfactorily met.

In the analysis of all outcomes, child gender and genotype variables were considered fixed effects whereas sun protection level and the three UV exposure variables were treated as time varying covariates. The latter were lagged such that the cumulative score through a given year (e.g., number of waterside vacations from birth through 2004) was used as a predictor of outcomes measured in the following year (e.g., total nevus count recorded in 2005). Study participation was a random effect. All participants in the analysis sample who provided data at one or more time points between 2004 and 2008 were included.

Model selection proceeded using a manual backward elimination approach whereby the initial model for each outcome included all main effects and all two- and three-way interaction terms involving rs12913832, *MC1R*, and the three cumulative UV exposure variables. To improve interpretability and reduce multicollinearity, the main effect and interaction variables involving the UV exposure variables were centered at the value of the mean of the variable over all subjects and times of observation ([7](#_ENREF_7)). Gender and annual sun protection level were included as covariates. Model testing proceeded iteratively by removing the least significant highest order interaction term on each step, except for those terms that were components of significant higher-order interaction terms. Reduced models were re-estimated until all eligible interaction terms had either been retained or removed due to lack of statistical significance. Main effects were retained in the model regardless of statistical significance.

The last stage of each analysis involved re-estimating the final, reduced model after adding a group of phenotypic covariates, first one at a time and then altogether as a set. Those variables included Hispanic ethnicity, constitutive skin color, hair color (with indicator variables for blonde, blondish brown to light brown, red, and black [reference = medium to dark brown]) and eye color (with indicator variables for blue and green or hazel [reference=brown]). In no case did the addition of these covariates appreciably change the estimates for the other predictor variables in the final models. Thus, we report the results of the final models without these covariates. All mixed model analyses were conducted using SAS/STAT software, Version 9.3 of the SAS System for Windows (SAS Institute Inc., NC, USA).

**Graphical Representation of Models and Model Interpretation**

Significant interaction effects were demonstrated by plotting predicted nevus counts in SAS after first calculating the simple slope of the x-axis variable (e.g., cumulative waterside vacations) at each level of the z-axis variable(s) (e.g., rs12913832 genotype). Predicted scores were calculated in a regression equation that included the overall model intercept, estimates for all main effects, lower order interactions, and the higher order interaction of interest. To enhance the clarity of the plots, scores on the outcome variables were back-transformed (exponentiated) to show predicted median counts of total nevi and counts of nevi >2 mm as well as predicted odds of freckling. For example, the regression equation used for the plot of predicted median total body nevus counts by waterside vacations and rs12913832 was based on the model intercept, the main effect of waterside vacations (regression coefficient \* centered vacation values), the main effect of rs12913832 (regression coefficient \* genotype group value), and the waterside vacations \* rs12913832 interaction (simple slope coefficient \* centered vacation values). Local maxima were identified so that scores on the exposure variables (e.g., waterside vacations) never exceeded their observed range within each level of the moderator variable (e.g., rs12913832). This ensured that predicted values were made only within the range of the observed data for the various subgroups of interest.

**References for Supplemental Methods**

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