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Using genetic proxies for lifecourse sun exposure to assess the causal relationship of sun exposure with circulating vitamin D and prostate cancer risk

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Abstract

Background—Ecological and epidemiological studies have identified an inverse association of intensity and duration of sunlight exposure with prostate cancer, which may be explained by a reduction in vitamin D synthesis. Pigmentation traits influence sun exposure and therefore may affect prostate cancer risk. Because observational studies are vulnerable to confounding and measurement error, we used Mendelian randomization to examine the relationship of sun exposure with both prostate cancer risk and the intermediate phenotype, plasma levels of vitamin D.

Methods—We created a tanning, a skin color and a freckling score as combinations of SNPs that have been previously associated with these phenotypes. A higher score indicates propensity to burn, have a lighter skin color and freckles. The scores were tested for association with vitamin D levels (25-hydroxyvitamin-D and 1,25-dihydroxyvitamin-D) and PSA-detected prostate cancer in 3123 white British individuals enrolled in the Prostate Testing for cancer and Treatment (ProtecT) study.

Results—The freckling score was inversely associated with 25(OH)D levels (change in 25(OH)D per score unit −0.27; 95%CI: −0.52, −0.01), and the tanning score was positively associated with prostate cancer risk (OR 1.05; 95%CI: 1.02,1.09), after adjustment for population stratification and potential confounders.

Conclusions—Individuals who tend to burn are more likely to spend less time in the sun and consequently have lower plasma vitamin D levels and higher susceptibility to prostate cancer.

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Impact—The use of pigmentation related genetic scores is valuable for the assessment of the potential benefits of sun exposure with respect to prostate cancer risk.

Keywords

pigmentation; tanning; sun exposure; vitamin D; prostate cancer

Introduction

Prostate cancer is rapidly becoming the most common male cancer, with over 500,000 new cases each year worldwide(1). However, there is striking geographical variation, such that regional intensity of exposure to solar ultraviolet radiation (UVR) is inversely associated with prostate cancer incidence and mortality in fair-skinned populations(2). Furthermore, inverse associations of cumulative UVR exposure, adult sunbathing, childhood sunburn and regular holidays in sunny climates, with prostate cancer risk have been observed at the individual level(3,4).

The effects of UVR on prostate cancer may be mediated by circulating vitamin D levels, the main environmental source of which is sun exposure, which stimulates vitamin D synthesis in the deeper layers of the epidermis(5). Circulating 25-hydroxyvitamin-D (25(OH)D) results from the conversion of provitamin D (obtained from sun exposure or dietary intake) by the enzyme 25-hydroxylase (CYP2R1)(6). 25(OH)D is then converted into 1,25 dihydroxyvitamin-D (1,25$(OH)_2$D), the active form of the hormone, by the enzyme 1-α-hydroxylase (CYP27B1)(7). Vitamin D synthesis is influenced by skin pigmentation, with lighter skins producing more vitamin D than darker skins on exposure to sunlight(8). The ability to pigment in response to UVR, usually measured as skin type on the Fitzpatrick scale(9), has been both inversely and positively associated with prostate cancer(10–12). It has also been suggested that UVR may exert its beneficial effects through other vitamin D independent pathways(13,14).

Observational studies are prone to both confounding (by environmental and lifestyle factors associated with the exposures and outcomes of interest) and measurement error (e.g. due to the subjectivity and difficulty of measuring skin type and sun exposure over the lifecourse(15)), precluding causal inference. Mendelian randomization (MR) is a natural experiment which overcomes issues of confounding and measurement error by using genetic variants associated with a modifiable exposure (in this case skin type and sun exposure) as proxies for the latter in order to establish a causal relationship between exposure and outcome (in this case prostate cancer). Since alleles are randomly allocated from parents to offspring at gamete formation, associations between genotypes and outcome are not generally confounded by behavioural/environmental exposures. Thus, observational studies of genetic variants are similar (with some caveats) to intention-to-treat analyses in randomised controlled trials, in which people are randomly allocated different genotypes rather than therapeutic interventions(16). Similarly, those with genotypes related to skin type and sun exposure will have been, in effect, randomly allocated to their skin type or sun exposure levels across their lifecourse; hence genetic variation may be a better measure of exposure over a lifetime than a single measurement(16–18). Here we examine genetic factors strongly associated with pigmentation traits that affect response to sunlight, to evaluate the relationship of lifecourse UVR exposure with both screen-detected prostate cancer risk, and the potential intermediate phenotype, plasma levels of vitamin D.

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Materials and Methods

Subjects

This study is nested within a multicenter randomized controlled trial of treatments for localized disease: the Prostate Testing for cancer and Treatment (*ProtecT*) study (ISRCTN20141297). During recruitment to the ProtecT study (between 2001 and 2009) over 100,000 men aged 50–69 years at 337 general practices in nine UK centers (Birmingham, Bristol, Cambridge, Cardiff, Edinburgh, Leeds, Leicester, Newcastle, Sheffield) were offered a PSA test at a community-based "prostate check clinic", and those with raised levels (≥ 3 ng/ml) were offered a diagnostic biopsy. Detected tumors were all histologically confirmed and clinically staged using the TNM system(19). Cancer stages T1-T2 were categorized as "localized"; and T3-T4 as "locally advanced", because few tumours had metastasized. Histologic material obtained at biopsy was assigned a Gleason score by specialist uropathologists following a standard proforma and, for the purposes of this study, categorized as low- (score < 7) or high- (score ≥ 7) grade cancers. All men without evidence of prostate cancer were eligible for selection as controls; that is, men with a PSA test < 3 ng/mL or a raised PSA (≥ 3 ng/mL) combined with at least one negative biopsy and no subsequent prostate cancer diagnosis during the follow-up protocol for negative biopsies. We selected one stratum-matched control for each case from those men who had provided a nonfasting blood sample at the prostate check clinic. Controls were randomly selected from the same stratum—i.e., 5-year age-band (age at PSA test) and GP/family practice—as cases. All men provided written informed consent prior to inclusion in the study. Trent Multicenter Research Ethics Committee (MREC) approved the ProtecT study (MREC/01/4/025) and the associated ProMPT study which collected biological material (MREC/01/4/061).

Exposure data

Pigmentation and sun exposure variables—A self-administered diet, health and lifestyle questionnaire, completed at the prostate check clinic (i.e. typically prior to knowledge of the PSA level or diagnosis), included questions about pigmentation (skin reaction to sun, and skin and hair color) and behaviors affecting the level of sun exposure, such as total time spent outside in summer and use of sunscreen in the 2 years prior attendance to the prostate check clinic(12). Sunscreen use was categorized as: always, mostly, sometimes, seldom and never, in response to the question "When out in the sun for two hours or more, did you normally protect your skin from sun with clothes or sunscreen?"

'Time spent outside in summer' was derived from the questionnaire as the summation of the weighted number of hours spent outside in the summer across different ages (5-12, 13-19, 20-29, 30-39, 40-49 and 50-69 years) (weights: < 1hour/day = 0.5 hours; 1-3hours = 2 hours; >3 hours = 6 hours). Missing answers to any of these questions were assigned to zero. The distribution of this score was then split into thirds and labelled 'low', 'medium' and 'high', where 'high' indicates greater sun exposure(12).

Vitamin D levels—A thousand three hundred and ninety-six individuals (699 cases and 697 controls) had their vitamin D levels measured in blood. Plasma samples drawn into heparinized tubes at the prostate check clinic were allowed to clot at room temperature, and then were centrifuged for 20 minutes within 2 hours of collection at 1,640 relative centrifugal force. Samples were stored at −80°C, until required for use. $25(OH)D_2$ and $25(OH)D_3$ were measured in plasma on a Tandem MS system as described previously in detail(20). The assay was standardized using NIST aligned standard material obtained from Chromsystems (UK). Between batch coefficients of variation for $25(OH)D_2$ were 4.2–5.5%, and for $25(OH)D_3$ were 4.5–5.7%, across the assay working range. Circulating concentrations of $25(OH)D_2$ and $25(OH)D_3$ were measured in nanograms per milliliter (ng/

ml) where 1 ng/ml = 2.5 nmol/l (nanomoles per liter). Total 25(OH)D (ng/ml) was calculated as the summation of $25(OH)D_2$ and $25(OH)D_3$. $1,25(OH)_2D$ samples were quantified by immunoassay(21) over a 2 month period using a single batch of reagents. $1,25(OH)_2D$ was measured in picomoles per litre (pmol/L) where 1pg/mL=2.6pmol/L.

Genotyping—Pigmentation-related single nucleotide polymorphisms (SNPs) were obtained from genomewide genotyping of ProtecT samples, carried out on 3,390 individuals at the Center National de Génotypage, Evry, France, using the Illumina Human660W-Quad_v1_A array (Illumina, Inc., San Diego, CA).

The quality control process performed before imputation excluded individuals on the basis of the following: sex mismatches, minimal (<0.325) or excessive heterozygosity (>0.345), disproportionate levels of individual missingness (>3%), cryptic relatedness measured as proportion of identity by descent (IBD > 0.1), and insufficient sample replication (IBD < 0.8). The remaining individuals were assessed for evidence of population stratification by multidimensional scaling analysis and compared with HapMap II (release 22) European descent (CEU), Han Chinese (CHB), Japanese (JPT) and Yoruba (YRI) reference populations; all individuals with non-European ancestry were removed. SNPs with a minor allele frequency below 1%, a call rate of < 95% or evidence for violations of Hardy-Weinberg equilibrium ($p < 5\times10^{-7}$) were discarded.

Autosomal genotypic data were subsequently imputed using Markov Chain Haplotyping software (MACH v.1.0.16(22)) and phased haplotype data from CEU individuals (HapMap release 22, Phase II NCBI B36, dbSNP 126) based on a cleaned dataset of 3,186 individuals and 514,432 autosomal SNPs. After imputation, all SNPs with indication of poor imputation quality (r^2 hat < 0.30) were removed. X chromosome imputation was performed on a cleaned dataset of 3,186 individuals and 10,092 X chromosome SNPs, using MACH v. 1.0.16 and MiniMac v 4.4.3, in conjunction with phased haplotype data from CEU individuals (HapMap 3 release 2, NCBI B36, dbSNP 126). The working dataset consisted of 3,123 individuals (1,136 cases and 1,791 controls, and 196 individuals with missing status). Genotypic dosages, which represent the expected number of one of the alleles and range from 0 to 2, were derived from genomewide data and used in the analysis. Dosages are continuous variables that incorporate the uncertainty of the imputation process.

Genetic scores—We computed 3 genetic scores from SNPs (selected *a priori*) that have previously been reported to be reliably associated with our pigmentation-related phenotypes of interest: skin color (skin color score = SCS), tanning ability (tanning score = TS) and freckling (freckling score = FS) ((23) and references therein). SNPs chosen had a minor allele frequency ≥ 0.05 in the CEU population, and the risk allele had been clearly established.

In the analysis scores are used instead of individual genetic variants because they are likely to explain a larger proportion of trait variability and thus represent strong, unconfounded proxies for the modifiable phenotype. Scores were calculated by summing up the dosages of all appropriate SNPs in each individual, after making sure that the ‘risk’ allele was the allele being counted in the dosage and there were no missing SNP data. Risk alleles were those associated with lighter skin color, burning rather than tanning, and having more freckles. Polymorphisms included in each score are shown in Table 1.

Population stratification—The top 10 principal components that reflect the population’s genetic structure were estimated according to Price et al.(24) from genomewide SNPs genotyped, imputed and cleaned as described above. All 10 PCs were included as covariates in the regression models to account for confounding by population stratification.

Statistical analysis

The association of genetic scores with pigmentation and sun exposure variables, and plasma vitamin D levels, was assessed using one way ANOVA and linear regression. The analyses that involved vitamin D were adjusted for age (continuous variable), study center location (binary variable - North: Sheffield, Newcastle, Edinburgh, Leeds; or South: Bristol, Cardiff, Birmingham, Leicester, Cambridge) and season of blood draw (4 categories - winter: January, February, March; spring: April, May, June; summer: July, August, September; or autumn: October, November, December). Logistic regression was used to investigate the association of genetic scores with prostate cancer status, stage and grade, with adjustment for age at recruitment and population structure. Scores were included in the regression models as continuous variables in the majority of analyses, but we also assessed them as categorical variables (i.e. tertiles) in relation to prostate cancer risk. Stratification by time spent outside in the summer was carried out for plasma vitamin D and prostate cancer outcomes, to determine if we could replicate earlier studies which have shown that in men with low sunbathing scores, skin type I (always burn/never tan) was inversely associated with prostate cancer(10,11). A Wald test of interaction between genetic scores and sun exposure was performed. Numbers of individuals included in each analysis correspond to those with complete data on outcome, exposure and confounder variables. All analyses were carried out in Stata 12 (StataCorp LP, 2012, College Station, TX).

Results

Genetic scores

All scores were normally distributed. The skin color score ranged from 1.0 to 10.1, the tanning score from 4.0 to 19.0, and the freckling score from 2.0 to 14.3, a higher score indicating lighter skin color, skin that burns instead of tanning, and a greater likelihood of having freckles. Scores were correlated with each other after adjustment for population stratification (TS vs SCS, coefficient of determination $R^2 = 0.18$; TS vs FS $R^2 = 0.25$; SCS vs FS $R^2 = 0.01$; $p<0.001$), and mainly with the first principal component (all $R^2 \sim 0.02$; $p<0.001$) (Supplementary Table S1).

All scores were associated with study centre location (Supplementary Table S2). None of the scores was associated with age at recruitment and season of blood draw (data not shown).

Genetic scores, pigmentation and sun exposure variables

Associations between genetic scores and self-reported skin color, skin reaction and sun exposure are shown in Table 2. The strongest association was observed between the tanning score and skin reaction, although the skin color score and the freckling score were also associated with skin reaction in the direction expected, i.e. the lighter the skin and the higher the probability of having freckles, the greater the likelihood of burning when exposed to the sun. The tanning score was the best predictor of skin color, followed by the skin color and freckling scores. Sunscreen use in the two years preceding the clinic visit was strongly associated with the tanning score and weakly with the skin color score. No association was found between time spent outside in summer and any of the genetic scores.

Genetic scores and vitamin D levels

Table 3 shows associations of genetic scores with plasma 25(OH)D, adjusted for age, center, season of blood draw and principal components, with stratification by time spent outside in the summer. There was some evidence that the freckling was associated with levels of vitamin D (albeit weakly). Individuals with a higher score (i.e. more likely to have freckles) showed lower concentrations of 25(OH)D (change in vitamin D levels per unit increase in

freckling score −0.27 ng/ml; 95% CI −0.52, −0.01; p = 0.04). Little evidence of association with the tanning and skin color scores was observed. There was evidence of an interaction (p = 0.02) between the tanning score and time spent outside in the summer with respect to 25(OH)D levels, that showed an effect for individuals in the 'medium' and 'high' strata of sun exposure consistent with that obtained for the whole population; however, for those who experienced little exposure to sunlight, the effect appeared reversed. The scores were not associated with plasma $1,25(OH)_2D$ (data provided on request).

Genetic scores and prostate cancer

The tanning score exhibited a strong association with prostate cancer susceptibility. Individuals with higher scores had a 5% (95% CI: 2%, 9%) increased risk of disease per unit increase in tanning score (p = 0.004) (Table 4). A similar association was observed with the skin color score but the statistical evidence was weaker (p = 0.08). Additional adjustment for center location made no difference to the results (data not shown). Examination of tertiles of genetic scores showed considerably greater ORs for prostate cancer among individuals in the highest tertile compared to individuals in the lowest tertile for all scores although there was strong statistical evidence only for the tanning score (p-value for trend = 0.02) (Supplementary Table 3).

There was no association between any of the scores and disease stage or grade, except for a suggestive protective effect of the tanning score on Gleason grade. As with vitamin D levels, there was evidence of an interaction (p = 0.05) between the tanning score and time spent outside in the summer on prostate cancer risk, where being more prone to burning appeared to protect against prostate cancer among individuals with reduced sun exposure but increased disease risk if sun exposure was higher.

Discussion

Main findings

In this study we have used genetic polymorphisms associated with tanning ability, freckling and skin color to determine first, how well they correlate with the observational variables currently being used to assess skin color, skin reaction, and sun exposure; second, whether they are associated with 25(OH)D plasma levels; and third, whether they are associated with PSA-detected prostate cancer. We found strong associations between all three scores and skin reaction, and of the tanning and skin color scores with skin color, suggesting that they could potentially be used as proxies for self-assessed pigmentation variables. As expected, the use of sunscreen in the two years before recruitment was better explained by the tanning score than the other two scores. Individuals who reported always using sunscreen had higher propensity to burn. However, none of the scores was associated with total time spent in the sun. We found that having a higher freckling score was associated with lower 25(OH)D levels, but there was not enough statistical evidence to support a similar effect of the tanning and the skin color score. The effects uncovered were consistent, particularly those of the tanning and freckling scores. In accordance with our findings on vitamin D, participants with higher scores, exhibited higher rates of prostate cancer, with the tanning score showing the strongest association. When stratified by time spent outside in the summer, there was some indication that the relationship of tanning ability with vitamin D, and of tanning ability with prostate cancer risk, may be affected by how much an individual was exposed to sunlight during life.

Because prostate cancer rates vary by ethnicity and so do the allele frequencies of the SNPs that make up the scores there is the potential of obtaining spurious association results if the population under study is stratified(25). To overcome this problem we adjusted the

regression models for the first 10 principal components that summarize the population's variability. Effects were similar after adjustment suggesting that UVR exposure may play a causal role of on prostate cancer risk.

Comparison with previous literature

Prior assessment of the relationship of skin pigmentation with vitamin D has indicated that a lighter skin color is associated with higher levels of plasma vitamin D among individuals living in Australia, Canada and New Zealand(26–29). On the other hand, studies conducted in the UK and Denmark reported, in agreement with our findings, that fair skin types are more at risk of vitamin D deficiency or present with lower vitamin D levels than participants with darker skin(30,31). A possible explanation for these and our results is that fair-skinned sun-sensitive individuals tend to avoid the sun (and/or protect themselves with clothing and sunscreen) to prevent sunburn and skin cancer. Our findings also agree with earlier work that established that facultative (i.e. tanning ability) and not constitutive (i.e. skin color) pigmentation was an indicator of sunlight exposure and an important determinant of vitamin D(28).

Berry and colleagues(32) investigated the association of pigmentation SNPs and plasma vitamin D in the UK and found an effect of one *OCA2* polymorphism (rs7495174), which regressed towards the null after adjustment for a number of confounders, including time spent outside.

A previous study on sun exposure and prostate cancer risk in ProtecT reported that men with olive/brown skin and those who burnt rarely/never had an increased risk of prostate cancer overall(12). Other studies that also looked at skin type, found an association between ability to tan and higher prostate cancer risk among those individuals with the lowest sun exposures(10,11). In contrast, when pigmentation was measured using a reflectometer, in the forehead (facultative pigmentation) and the upper underarm (constitutive pigmentation), it was found that darker facultative pigmentation and increasing darkness was associated with a reduced risk of prostate cancer(33). As far as we know, only two studies have considered pigmentation gene polymorphisms in relation to prostate cancer(34,35). The analysis of fair-skin associated SNPs *TYR* S192Y and *MC1R* R160W showed that while the *TYR* variant conferred risk, the *MC1R* variant was protective. The former was included in our skin color score, the latter has not been genotyped or imputed in the ProtecT samples, although we did have another *MC1R* red-hair and fair-skin associated variant: R151C. We did not uncover evidence of an effect of *TYR* S192Y or *MC1R* R151C (rs1805007) on vitamin D concentrations or prostate cancer status.

Studies on vitamin D levels and prostate cancer risk to date have been inconsistent, with several of them showing small or no effects(5), including one done on ProtecT samples that used plasma vitamin D-related genetic scores(36). Even though we detected an association of pigmentation scores with both vitamin D and prostate cancer risk, there is a possibility that these effects are unrelated and we are identifying independent influences of exposure to sunlight on vitamin D status and prostate cancer susceptibility. Because UVR may exert its effects on cancer development via routes other than vitamin D synthesis(13,14), this violates the exclusion restriction assumption in MR, of no link of the genotype with the outcome, other than via the exposure. Thus pigmentation scores cannot be considered suitable instruments for circulating vitamin D, only for skin color and response. Strong instruments for plasma vitamin D have already been identified(37,38), which were validated in ProtecT as well(36). The usefulness of pigmentation scores as instrumental variables for sun exposure will depend on the complex interrelation of cultural attitudes towards sun worship, skin cancer awareness, individual risk and behavior, and will have to be established for each population specifically.

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Limitations

The amount of variability in each pigmentation-related trait explained by the scores is less than 2%. Skin color associations with the scores were weaker than those of skin reaction, even for the skin color score. This does not necessarily imply that the scores are weak instruments but may indicate that self-reported skin complexion variables are not good proxies for actual skin pigmentation and tanning potential. In fact, the lack of correlation between recollection burning/tanning data and the minimal erythemal dose, which is the threshold UVR dose that produces sunburn, has been described by Rampen and colleagues(15) in the Netherlands.

Misclassification due to non-response to the questions related to time spent outside in the summer could have influenced the results obtained in the analysis of this variable but only if differential with respect to the genetic scores, which seems unlikely.

In addition, T3 and T4 tumours in ProtecT, while more locally advanced than the clinically localized T1 and T2 tumours are not “advanced” in the sense that clinically apparent tumours are. They were detected incidentally and were asymptomatic and it is difficult to predict when they might have become apparent. This could potentially have an important attenuating effect on whether an association between the genetic scores and cancer stage and grade was found.

Although we have carefully taken into account the confounding role of population stratification in a study such as this one, there is still a possibility of residual population stratification underlying the results.

Finally, because of the potential direct link between UVR exposure and prostate cancer we cannot determine unequivocally the role of vitamin D in this relationship.

Conclusions

Our results show that, among white British males, those who are more prone to burning than tanning, are more likely to have freckles and lighter skin color, use more sunscreen, have lower 25(OH)D levels and are at a higher risk of being diagnosed with PSA-detected prostate cancer. We also show that pigmentation genetic scores can be used as instrumental variables for skin reaction to overcome the problems generated by confounding and poor recall of self-reported sun exposure sensitivity. Nonetheless, replication of these findings is necessary before we can be certain of their importance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Skin color, tanning and freckling genetic scores.

skin color score					
gene	**SNP**	**chromosome**	**chromosomal location**	**major/minor alleles**	**risk allele**[a]
IRF4	rs12203592	6p25.3	341321	C/T	T
TYR	rs1042602	11q14.3	88551344	C/A	A
HERC2	rs12913832	15q13.1	26039213	G/A	G
MC1R	rs1805007	16q24.3	88513618	C/T	T
ASIP	rs4911414	20q11.22	32193105	G/T	T
ASIP	rs1015362	20q11.22	32202273	G/A	G
tanning score					
gene	**SNP**	**chromosome**	**chromosomal location**	**major/minor alleles**	**risk allele** [b]
PPARGC1B	rs32579	5q32	149191041	G/A	G
IRF4	rs12203592	6p25.3	341321	C/T	T
IRF4/EXOC2	rs12210050	6p25.3	420489	C/T	T
TYR	rs1393350	11q14.3	88650694	G/A	A
CALCOCO1/HOXC13	rs7969151	12q13.13	52445544	G/A	A
PAPOLA/VRK1	rs17094273	14q32.2	96173560	G/A	A
HERC2	rs12913832	15q13.1	26039213	G/A	G
CPNE7/DPEP1	rs154659	16q24.3	88194838	T/C	C
MC1R	rs1805007	16q24.3	88513618	C/T	T
DBNDD1	rs11648785	16q24.3	88612062	C/T	C
ASIP	rs4911414	20q11.22	32193105	G/T	T
ASIP	rs1015362	20q11.22	32202273	G/A	G
PRDM15	rs7279297	21q22.3	42100984	A/G	A
freckling score					
gene	**SNP**	**chromosome**	**chromosomal location**	**major/minor alleles**	**risk allele** [c]
IRF4	rs12203592	6p25.3	341321	C/T	T
BNC2	rs2153271	9p22.2	16854521	A/G	A
TYR	rs1042602	11q14.3	88551344	C/A	C
TYR	rs1393350	11q14.3	88650694	G/A	A
MC1R	rs1805007	16q24.3	88513618	C/T	T
ASIP	rs4911414	20q11.22	32193105	G/T	T
ASIP	rs1015362	20q11.22	32202273	G/A	G
EIF6	rs619865	20q11.22	33331111	G/A	A

[a]The risk allele is the allele associated with lighter skin pigmentation.

[b]The risk allele is the allele associated with higher propensity to burn.

[c]The risk allele is the allele associated with a greater likelihood of having freckles.

Table 2

Association between pigmentation genetic scores and self-reported skin color, skin reaction, and sun exposure.

N=2913[a]	skin color score	tanning score	freckling score
	mean ± SD	mean ± SD	mean ± SD
skin color			
fair/pale	5.20 ± 1.23	10.98 ± 2.12	6.20 ± 1.80
medium	5.06 ± 1.19	10.60 ± 2.04	6.09 ± 1.68
olive/brown	4.96 ± 1.03	10.68 ± 2.21	5.93 ± 1.88
p-value	0.004	1.34×10-5	0.11
F	5.61	11.16	2.18
R^2 (%)	0.38	0.76	0.15
skin reaction			
burns always/easily	5.31 ± 1.24	11.19 ± 2.10	6.33 ± 1.78
burns rarely/never	5.05 ± 1.19	10.63 ± 2.07	6.06 ± 1.75
p-value	4.13×10^{-8}	7.26×10^{-12}	7.28×10^{-5}
F	30.25	47.34	15.78
R^2 (%)	1.03	1.60	0.54
sunscreen use 2 years prior			
always	5.20 ± 1.21	11.01 ± 2.12	6.19 ± 1.78
mostly	5.11 ± 1.19	10.76 ± 2.11	6.19 ± 1.76
sometimes	4.99 ± 1.24	10.60 ± 2.11	6.02 ± 1.68
seldom	5.24 ± 1.18	10.71 ± 1.96	6.08 ± 1.86
never	5.13 ± 1.23	10.72 ± 2.07	6.16 ± 1.73
p-value	0.02	0.004	0.45
F	2.83	3.92	0.86
R^2 (%)	0.39	0.54	0.12
time spent outside in summer			
low	5.14 ± 1.19	10.89 ± 2.00	6.18 ± 1.78
medium	5.16 ± 1.28	10.78 ± 2.21	6.15 ± 1.75
high	5.13 ± 1.19	10.81 ± 2.10	6.14 ± 1.76
p-value	0.91	0.53	0.90
F	0.09	0.64	0.10
R^2 (%)	0.01	0.04	0.01

[a]Includes 1040 cases, 1717 controls and 156 individuals with unknown disease status.

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Table 3

Association between pigmentation genetic scores and plasma vitamin D, adjusted for age, centre, season of blood draw and population stratification; stratified by time spent outside in the summer.

vitamin D (ng/ml)	skin color score	tanning score	freckling score
change in plasma 25(OH)D per unit increase in genetic score	−0.06	−0.16	−0.27
95% CI	−0.42, 0.29	−0.37, 0.06	−0.52, -0.01
p-value	0.73	0.15	0.04
N=1396 (699 cases/697 controls)			
low sun exposure			
change in plasma 25(OH)D per unit increase in genetic score	0.48	0.26	−0.31
95% CI	−0.25, 1.21	−0.19, 0.72	−0.82, 0.19
p-value	0.20	0.26	0.22
N=362 (179 cases/183 controls)			
medium sun exposure			
change in plasma 25(OH)D per unit increase in genetic score	−0.51	−0.56	−0.20
95% CI	−1.11, 0.09	−0.92, −0.21	−0.65, 0.26
p-value	0.10	0.002	0.40
N=401 (202 cases/199 controls)			
high sun exposure			
change in plasma 25(OH)D per unit increase in genetic score	−0.17	−0.15	−0.33
95% CI	−0.71, 0.37	−0.47, 0.16	−0.70, 0.05
p-value	0.54	0.34	0.09
N=633 (318 cases/315 controls)			
p-value for interaction	0.12	0.02	0.94

Table 4

Association between genetic scores and prostate cancer status (stratified by time spent outside in the summer), stage and grade, adjusted for age at recruitment and population stratification.

prostate cancer	skin color score	tanning score	freckling score
status (cases/controls)			
OR[a]	1.06	1.05	1.03
95% CI	0.99, 1.12	1.02, 1.09	0.99, 1.08
p-value	0.08	0.004	0.17
N=2927 (1136/1791)[b]			
low sun exposure			
OR[a]	0.97	0.98	1.01
95% CI	0.85, 1.10	0.91, 1.06	0.93, 1.10
p-value	0.61	0.67	0.74
N=790 (276/514)			
medium sun exposure			
OR[a]	1.06	1.11	1.03
95% CI	0.94, 1.19	1.03, 1.19	0.95, 1.13
p-value	0.37	0.004	0.47
N=734 (268/466)			
high sun exposure			
OR[a]	1.14	1.07	1.05
95% CI	1.03, 1.25	1.01, 1.13	0.98, 1.12
p-value	0.01	0.02	0.15
N=1233 (496/737)			
p-value for interaction	0.11	0.05	0.74
stage (0=localised/1=locally advanced)			
OR[a]	0.99	0.99	1.02
95% CI	0.85, 1.15	0.90, 1.08	0.92, 1.13
p-value	0.91	0.77	0.77
N=1136 (1004/132)			
Gleason grade (0:<7/1:≥7)			
OR[a]	0.98	0.95	0.99
95% CI	0.88, 1.08	0.89, 1.01	0.92, 1.06
p-value	0.65	0.09	0.71
N=1135 (794/341)			

[a] Odds ratio = change in odds per unit increase in genetic score.

[b] The difference in sample size with the full dataset (N = 3123) is due to the exclusion of 196 participants who lacked information on disease status.