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PRKCZ methylation is associated with sunlight exposure in a North American but not a Mediterranean population

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Abstract

Sunlight exposure has been shown to alter DNA methylation patterns across several human cell-types, including T-lymphocytes. Since epigenetic changes establish gene expression profiles, changes in DNA methylation induced by sunlight exposure warrant investigation. The purpose of this study was to assess the effects of sunlight exposure on CD4+ T-cell methylation patterns on an epigenome-wide scale in a North American population of European origin ($n = 991$). In addition, we investigated the genetic contribution to epigenetic variation (methylQTL). We used linear regression to test the associations between methylation scores at 461 281 cytosine-phosphate-guanine (CpG) sites and sunlight exposure, followed by a genome-wide association analysis (methylQTL) to test for associations between methylation at the top CpG locus and common genetic variants, assuming an additive genetic model. We observed an epigenome-wide significant association between sunlight exposure and methylation status at cg26930596 ($p = 9.2 \times 10^{-8}$), a CpG site located in protein kinase C zeta (*PRKCZ*), a gene previously shown to be

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DECLARATION OF INTEREST

The authors have no conflicts of interest.

entrained by light. MethylQTL analysis resulted in significant associations between cg26930596 and two intergenic single nucleotide polymorphisms on chromosome 3, rs4574216 ($p = 1.5 \times 10^{-10}$) and rs4405858 ($p = 1.9 \times 10^{-9}$). These common genetic variants reside downstream of *WWTRI*, a transcriptional co-activator of *PRKCZ*. Associations observed in the North American population, however, did not replicate in an independent Mediterranean cohort. Our preliminary results support the role of sunlight exposure in epigenetic processes, and lay the groundwork for future studies of the molecular link between sunlight and physiologic processes such as tumorigenesis and metabolism.

Keywords

Epigenetics; methylation; protein kinase C; sunlight

INTRODUCTION

Epigenetics play an important role in establishing gene expression profiles in a tissue- and time-specific manner (Jaenisch & Bird, 2003). Epigenetic changes, such as DNA methylation, provide a molecular link between the genotype, environmental influences and physiologic processes including tumorigenesis and metabolism (Milagro et al., 2012). Among the environmental cues that have been shown to induce epigenetic changes is sunlight exposure. Specifically, evidence points to altered DNA methylation patterns in sun-exposed human skin (Gronniger et al., 2010) and the suprachiasmatic nucleus in murine hypothalamus (Azzi et al., 2014).

T lymphocytes are particularly relevant to studying the effects of sunlight exposure. Sunlight-induced vitamin D3 has been shown to signal T-cells to express CC chemokine receptor 10, thus programming their attraction to chemokine CCL27 (Sigmundsdottir et al., 2007). In addition, an *in vitro* study conducted in lupus patients showed that exposure to ultraviolet B (UVB) light triggered methylation changes in CD4+ T-cells by inhibiting DNA methyltransferase 1 activity (Wu et al., 2013). Therefore, assessing the effects of sunlight exposure on CD4+ T-cell methylation patterns on an epigenome-wide scale may offer unique mechanistic insights into the health effects of seasonal and geographic variation.

We hypothesized that sunlight exposure is correlated with DNA methylation patterns in CD4+ T-cells in 991 European-American participants of the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN). Our study was the first to interrogate associations between DNA methylation status of approximately 470000 cytosine-phosphate-guanine (CpG) sites across the genome and sunlight exposure in a large study. For CpG sites showing associations with sunlight exposure, we further investigated the potential genetic contributions to epigenetic variation *via* methylQTL analysis. To validate our top findings, we pursued replication analyses in an independent population of older Mediterranean participants of the Invecchiare in Chianti (InCHIANTI) study.

MATERIALS AND METHODS

Study populations

All participants provided written informed consent, and the experimental protocol conformed to international ethical standards (Portaluppi et al., 2010).

North American population

We conducted our discovery analyses using data from the family-based GOLDN study, described in detail in previous publications (e.g. Aslibekyan et al., 2012; Corella et al., 2007; Hidalgo et al., 2014). Briefly, the study screened 1327 European American individuals from extended pedigrees from two sites of the National Heart, Lung and Blood Institute Family Heart Study in Minneapolis, MN, and Salt Lake City, UT. Of those, 1295 agreed to participate, and 1048 completed at least one study intervention. DNA samples used in this epigenome-wide methylation study were collected at the baseline visit, which took place between 26 August 2003 and 3 September 2005. Participants provided written informed consent, and the Institutional Review Boards at the following institutions approved the study protocol: University of Alabama at Birmingham, University of Minnesota, Tufts University, University of Utah, Washington University in St. Louis, University of Texas, University of Michigan and Fairview-University of Minnesota Medical Center.

Mediterranean population

The InCHIANTI study is a population-based epidemiological study aimed at evaluating the factors that influence mobility in the older population living in the Chianti region in Tuscany, Italy, described in previous publications (Ferrucci et al., 2000). Briefly, 1616 residents were selected from the population registries of Greve in Chianti (a rural area: 11 709 residents with 19.3% of the population older than 65 years), and Bagno a Ripoli (Antella village near Florence; 4704 inhabitants, with 20.3% older than 65 years). The epigenetic data collection involved participants with sufficient DNA at baseline (1998–2000) and at 9-year follow-up (2007–2009). The study population for this analysis included individuals who met the quality control criteria outlined below, as well as had complete data on baseline DNA methylation, sunlight exposure and covariates ($n = 476$). The Italian National Institute of Research and Care of Aging Institutional Review and Medstar Research Institute (Baltimore, MD) approved the study protocol.

Sunlight duration and vitamin D Intake measurements

In both discovery and replication cohorts, we used sunlight duration as a proxy for sunlight exposure, calculating the difference between the timing of sunrise and sunset on the day that each participant's blood sample was taken for the DNA methylation analysis. As such, our measure captured both seasonal and geographical variation. We expressed sunlight duration continuously.

Self-reported vitamin D intake in the North American population included both supplements and dietary calciferol and was estimated using the National Cancer Institute Diet History Questionnaire, which consists of 124 food items and includes both portion size and dietary supplement questions (Subar et al., 2001). In the Mediterranean population, 25-

hydroxyvitamin D (25-OH vitamin D) was measured by radioimmunoassay (DiaSorin Inc., Stillwater, MN), intra- and interassay coefficients of variation were 8.1% and 10.2%, respectively.

DNA isolation and epigenetic phenotyping

The procedures used to isolate CD4⁺ T-cells, extract DNA and prepare the North American samples for genome-wide methylation analyses are detailed in published manuscripts from our group (Absher et al., 2013; Irvin et al., 2014). Briefly, we harvested CD4⁺ T-cells from frozen buffy coat samples using antigen-specific magnetic beads (Invitrogen, Carlsbad, CA). We lysed the cells and extracted DNA using DNeasy kits (Qiagen, Venlo, the Netherlands). Prior to quantifying genome-wide methylation, we performed bisulfite conversion on 500 ng of genomic DNA (EZ DNA kit, Zymo Research, Irvine, CA).

Both cohorts used the Infinium Human Methylation450 array (Illumina, San Diego, CA) to measure DNA methylation at approximately 470000 CpG sites across the genome. After performing whole genome amplification, hybridization and imaging as prescribed by the manufacturer, we estimated β scores (proportion of total signal from the methylation-specific probe or color channel) and detection of p values (the probability that the total intensity for a given probe falls within the background signal intensity) using GenomeStudio software (Illumina).

Normalization and quality control

A description of quality control and normalization procedures used in the North American population is available in a prior manuscript from our group (Absher et al., 2013). Briefly, we first removed β scores with an associated detection p value greater than 0.01 and samples with more than 1.5% missing data points. We also excluded any CpG sites where probes either had annotation errors or failed to yield adequate intensity for more than 10% of samples. Our final analysis included 991 participants and 461281 autosomal CpGs sites.

We normalized the resulting β scores using the ComBat package (Boston, MA) (Johnson et al., 2007) in random subsets of 20 000 CpGs per run, with each array of 12 samples used as a “batch”. We normalized probes from the Infinium I and II chemistries separately, subsequently adjusting the β scores for Infinium II probes (Absher et al., 2013). Finally, we generated principal components (PCs) using β scores of all CpGs that passed quality control, aiming to adjust for CD4⁺ T-cell purity. The first four PCs explained most of the variation, with the first PC especially robustly associated ($r^2 = 0.85$) with predicted cell purity (Irvin et al., 2014).

In the Mediterranean cohort, we used the DASEN method in the wateRmelon package to perform quality control on the pooled 1022 samples from baseline and nine-year follow-up (Pidsley et al., 2013). We removed 251 CpG sites with the bead count <3 in at least 5% of the samples. In addition, we excluded three samples and 1893 CpG sites where at least 5% of detection p values were greater than 0.01. We applied background adjustment and quantile normalization to the filtered data set; the selected method normalized both methylated and unmethylated probes as well as Infinium I and II probes separately. After

filtering, 429 527 CpG sites in 1019 participants remained. Of those, 479 participants had complete baseline covariate and sunlight data and comprised the final analytic sample.

Genotyping

For the analyses of methylation quantitative trait loci (methylQTL) in the North American population, we used previously described data (Aslibekyan et al., 2012) on genome-wide sequence variation as ascertained by the Affymetrix Human Single Nucleotide Polymorphism (SNP) Array 6.0 and the Birdseed calling algorithm (Korn et al., 2008). We imputed untyped SNPs using MACH software, version 1.0.16 (Ann Arbor, MD with Human Genome Build 36 as a reference. After imputation, we created a hybrid data set of 2 543 887 SNPs, of which 584 029 were originally genotyped.

We genotyped the Mediterranean population using the Illumina 550K array as previously described (Melzer et al., 2008; Tanaka et al., 2009). Briefly, we genotyped 1210 subjects with a sample call rate 97%, heterozygosity rates 0.3 and correct sex specification. A total of 495 343 autosomal SNPs passed quality control (minor allele frequency (MAF) 1%, completeness 99%, Hardy–Weinberg equilibrium p value 10^{-4}). We used these SNPs and MACH software to impute additional variants (total ~2.5 million SNPs) with Human Genome Build 36 as a reference.

Statistical analysis

First, we fit linear mixed models to test for associations between methylation scores at each CpG site and sunlight exposure, adjusting for age, sex, study site, current smoking, the first four PCs capturing CD4+ T-cell purity as fixed effects and pedigree as a random effect. To evaluate putative mediation by vitamin D status, we further adjusted for self-reported vitamin D intake in another model. We set the genome-wide statistical significance level using a conservative Bonferroni correction of 1.1×10^{-7} ($\alpha=0.05/461\ 281$) and constructed a Manhattan plot to visualize the results.

We next conducted follow-up analyses for genome-wide significant CpG sites to evaluate contributions of common genetic variants to methylation at these loci. Using data from participants with both GWAS and Illumina 450K data ($n=715$), we tested for the association between methylation and common genetic variants by fitting linear mixed models assuming an additive genetic model. The models adjusted for age, sex, study site and the first four cell purity PCs. We did not adjust for ancestry because the North American participants were selected to be genetically homogeneous (Aslibekyan et al., 2012). We considered genome-wide significance at the Bonferroni-corrected threshold of 2.0×10^{-8} ($\alpha=0.05/2543887$). We used R statistical software, v. 3.1.0 (Vienna, Austria) for all analyses presented in this manuscript.

We sought replication of the association between sunlight duration and the top CpG in an independent Mediterranean cohort of older adults using comparable statistical models. Namely, the replication analyses adjusted for age, sex, study site, white blood cell differential count and batch effects (total of 13 experimental batches). We assessed blood cell differential count on EDTA anti-coagulated whole blood using a Coulter Counter (LH

750 Hematology Autoanalyzer, Beckman Coulter Inc., Brea, CA) and expressed as percentages of neutrophils, lymphocytes, monocytes, eosinophils and basophils. We also pursued replication of the associations between common genetic variants and the methylation status of the top CpG finding using models that adjusted for age, sex and technical covariates as described above.

RESULTS

Table 1 summarizes the general characteristics of the discovery ($n = 991$) and replication ($n = 476$) populations. On an average, Mediterranean participants were older and more likely to report current smoking ($p < 0.0001$). Mean sunlight exposure in the North American population exceeded that of the Mediterranean population by two hours, with approximately equal variability in the distribution. It is important to note, however, that because of holidays, none of the methylation measurements in the Mediterranean cohort were conducted in August, a month characterized by relatively early sunrise and late sunsets. In contrast, the measurements in the North American population were evenly distributed throughout the year.

We observed an epigenome-wide significant ($p < 1.1 \times 10^{-7}$) association between the methylation status at cg26930596 and sunlight exposure ($\beta \pm \text{SE}$: 0.004 ± 0.0007 ; $p = 9.2 \times 10^{-8}$) in the discovery stage (Table 2 and Figure 1). This CpG site is located in the protein kinase C zeta (*PRKCZ*), the gene encoding an isoform of protein kinase C. We present the results from the methylQTL genome-wide association analysis for the cg26930596 site in Table 3. We observed genome-wide significant ($p < 2 \times 10^{-8}$) associations for rs4574216 ($\beta \pm \text{SE}$: -0.03 ± 0.005 ; $p = 1.5 \times 10^{-10}$) and rs4405858 ($\beta \pm \text{SE}$: -0.03 ± 0.005 ; $p = 1.9 \times 10^{-9}$), two intergenic SNPs on chromosome 3 in linkage disequilibrium (LD) ($r^2 > 0.80$). In addition, we report a nominally significant association between cg16922167 and sunlight exposure ($\beta \pm \text{SE}$: 0.003 ± 0.0006 ; $p = 2.3 \times 10^{-7}$). This CpG site is located in the *FGR* gene, a member of the Src family of protein tyrosine kinases. Further adjustment for vitamin D intake did not appreciably change the estimates of the top associations (Table 2).

Neither direction nor magnitude of the association between the *PRKCZ* locus and sunlight exposure replicated in the Mediterranean population ($\beta \pm \text{SE}$: -0.0002 ± 0.0008 ; $p = 0.85$). On an average, the cg26930596 CpG site was more methylated in the North American (methylation proportion: mean=0.69, median=0.75) than in the Mediterranean cohort (methylation proportion: mean=0.49, median=0.49). The variability of methylation at the locus of interest was also higher in the North American population (interquartile range: $0.83 - 0.56 = 0.27$ vs. $0.54 - 0.45 = 0.09$ in the Mediterranean population). In addition, we did not observe methylQTL associations for the cg26930596 site with rs4574216 ($\beta \pm \text{SE}$: -0.001 ± 0.005 ; $p = 0.84$) and rs4405858 ($\beta \pm \text{SE}$: -0.0007 ± 0.005 ; $p = 0.88$), despite similar minor allele frequencies (MAF ~6%) between the two populations. In both cohorts, adjustment for vitamin D had no substantial effect on the regression estimates.

DISCUSSION

We present the first epigenome-wide study of sunlight exposure and DNA methylation patterns in humans. We have identified a novel association between the methylation status at the cg26930596 site in the promoter region of *PRKCZ* and sunlight exposure. MethylQTL analysis further suggested that methylation at this CpG site is associated with two common genetic variants on chromosome 3. These findings made in a North American population, however, did not replicate in an independent Mediterranean population.

Our findings contribute to an emerging body of evidence for the entrainment of *PRKCZ* by light. *PRKCZ*, protein kinase C (PKC) zeta, is a member of the PKC family of serine/threonine kinases, which are involved in cellular processes including proliferation, differentiation and secretion (Mellor & Parker, 1998). *Prkcz* mutant mice were found to have impaired light-induced gene expression in the suprachiasmatic nuclei, as well as attenuated circadian phase-shifting responses to light (Hankins et al., 2008). Other members of the PKC family participate in UVB-induced mitogen-activated protein kinase pathway signaling (Sodhi & Sethi, 2005). In addition, light was previously shown to entrain *PRKCA*, another member of the PKC family, and a gene homologous to the *PRKCZ* gene (Jakubcakova et al., 2007). In this study, we show that light entrainment for *PRKCZ* in humans could be mediated through epigenetic changes. In a recent candidate gene study, hypermethylation in seven sites in the *PRKCZ* promoter region in peripheral blood leukocytes was linked to a significantly reduced level of *PRKCZ* gene expression (Zou et al., 2013). Our data indicate that increased sunlight exposure is associated with increased methylation at this CpG site, and therefore a possible reduction in its expression. Future assessment of *PRKCZ* expression is warranted to verify the effect of methylation at this site.

MethylQTL analysis in the discovery cohort suggests that two common genetic variants influence methylation at the *PRKCZ* promoter CpG site, and reside in an intergenic locus on chromosome 3. These genetic variants in LD reside downstream of *WWTRI*, a transcriptional co-activator involved in muscle and fetal/neonatal cardiac development, and therefore could influence its functionality (Azzolin et al., 2012). Although it is not located proximally to the *PRCKZ* CpG site, *WWTRI* targets genes of the highly conserved hippo signaling pathway, which includes *PRKCZ*, and is involved in limiting organ size and also tumor suppression (Kanehisa & Goto, 2000). The observed associations between the common genetic variants and the methylation pattern indicate that the effect/risk alleles at these loci, or another causal variant in the LD block, are associated with decreased methylation at *PRKCZ* and possibly with increased gene expression.

A nominally significant association between sunlight exposure and methylation in the North American population was also determined in *FGR*, which encodes a non-receptor tyrosine-protein kinase. *FGR* is an oncogene, and its product is implicated in regulating immune responses in neutrophil, monocyte, macrophage and mast cells. *FGR* is further involved in cytoskeleton remodeling in response to extracellular stimuli. A mouse study indicates that ultraviolet light induces *fgr* following ultraviolet light treatment (Suzuki et al., 1992). Our data indicate that prolonged sunlight exposure is associated with increased methylation at this CpG site, and possibly gene attenuation. It is worth noting that methylation sites

identified in this analysis are located in the promoter regions of kinases, suggesting the role of sunlight exposure in the epigenetic programming of kinases involved in different signaling pathways.

The robust associations between sunlight exposure, *PRKCZ* methylation and chromosome 3 sequence variation observed in the North American population were not statistically significant in the Mediterranean cohort. Although there is always a possibility that the initial findings were due to chance, the differences in cg26930596 methylation profiles suggest epigenetic heterogeneity that could preclude comparisons between the discovery and replication cohorts. The observed discrepancies may be due to several important factors including baseline characteristics, exposure misclassification and differences in biological samples. First, age is a major contributor to epigenetic processes, including changes in DNA methylation induced by sunlight exposure (Gronniger et al., 2010). As Table 1 shows, Mediterranean participants were on an average much older than North American participants. Furthermore, Mediterranean participants were also more likely to smoke, which has also been shown to alter DNA methylation patterns (Zeilinger et al., 2013). Although our analyses adjusted for age and smoking, the relationship between these factors, sunlight exposure and DNA methylation may be non-linear, leaving room for residual confounding. Second, sunlight duration does not necessarily estimate sunlight exposure, resulting in potential misclassification. A more accurate proxy for sunlight exposure is warranted in future studies. However, indirect evidence from other biomarker studies suggests that differences in exposure are unlikely to explain our failure to replicate. Specifically, the average 25-OH vitamin D value in the Mediterranean cohort was 56 nmol/L (23 ng/ml), which is comparable to the 25 ng/ml and 26 ng/ml values reported by the National Health and Nutrition Examination Survey (2001–2004) for white non-Hispanic men and women, respectively, in the 40–59 age range (Ginde et al., 2009). Third, the difference in biological samples, CD4+ T-cells in the discovery stage and whole blood in the replication stage, could be another important factor resulting in the observed discrepancy. If the effect of sunlight on *PRKCZ* methylation is in fact cell type-specific, that could bias the findings from the Mediterranean population toward the null.

Our study is the first to evaluate the correlation between sunlight and DNA methylation in human blood on an epigenome-wide scale. In addition to novelty, its strengths include large population size, which bolsters statistical power, as well as the choice of CD4+ T-cells in the discovery stage. The latter is relevant for both practical (CD4+ T-cells are the most abundant lymphocyte in humans) and biological reasons. A large body of evidence implicates the interplay between sunlight exposure and CD4+ T-cells in the etiology of autoimmune diseases, e.g. systemic lupus erythematosus (SLE) and multiple sclerosis (Absher et al., 2013; Correale & Farez, 2013). Specifically, two *in vitro* studies conducted in cells from SLE patients demonstrated induction of hypomethylation in CD4+ lymphocytes by UVB, suggesting a possible epigenetic mechanism (Li et al., 2010; Wu et al., 2013). T-cell expression of the gene containing our top methylation finding, *PRKCZ*, has been prospectively linked to infant allergic disease, highlighting its relevance to immune phenotypes (D’Vaz et al., 2012). Taken together, these studies imbue our preliminary

findings in CD4+ lymphocytes with biological plausibility and stress the importance of future replication studies conducted in the same cell type.

Our findings must be considered in context of several limitations. First, because of the hypothesis-free nature of our approach and the non-replication, the results from the North American cohort are to be considered strictly preliminary yet etiologically promising. Second, previously published findings linking epigenetic changes in CD4+ T-cells to UVB radiation may not be easily extrapolated to population exposure, because the main component of sunlight is ultraviolet A (de Gruijl, 2000). Third, as discussed above, our daylight duration measure does not necessarily capture the amount of time exposed to sunlight, the difference in ultraviolet exposure between sunny and cloudy days, and other relevant environmental factors. Finally, our methylQTL hits are located on a different chromosome from the methylation locus (*trans*-methylQTL). Although most effects of SNPs on CpG methylation occur at short distances, robust distal effects have also been reported in the literature (Gibbs et al., 2010). However, until our *trans*-methylQTL finding on chromosome 3 is successfully replicated, its validity remains to be established.

On balance, we present preliminary evidence for an association between the methylation of a CpG site in the *PRKCZ* gene and sunlight exposure in CD4+ T-cells from a middle-aged North American population, but not in whole blood samples from an older Mediterranean population. Our findings are mechanistically relevant but require independent replication in other population-based studies measuring methylation in CD4+ T-cells specifically. Once validated, our results lay the groundwork for future studies of gene-sunlight exposure interactions, particularly in the setting of immune dysfunction.

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FIGURE 1.

Manhattan plot of epigenome-wide results of testing for association between epigenome-wide methylation and twilight exposure. The X-axis displays the chromosome on which the methylation locus is located, the Y-axis displays $-\log_{10}(p)$ value. The top horizontal line indicates the threshold for epigenome-wide statistical significance after a Bonferroni correction.

TABLE 1

General characteristics of the study populations.

	North American (<i>n</i> =991)	Mediterranean (<i>n</i> =476)	<i>p</i> ^{<i>a</i>}
Age, years ^{<i>b</i>}	49 ± 16	63 ± 16	< 0.0001
Sex, % female	52	55	0.27
Current smokers, %	7	20	< 0.0001
Sunlight, hours/day	13 ± 2	11 ± 2	< 0.0001
Vitamin D			
Dietary, mcg/day	5 ± 4	Not available	–
Supplement, IU/day	156 ± 180	Not available	–
25-OH vitamin D, nmol/L	Not available	56 ± 34	–

^{*a*}Two-sided *p* values were calculated using a 2-sample *t*-test or the chi-squared distribution, for continuous and dichotomous variables, respectively.

^{*b*}Values are shown as mean ± SD or %.

TABLE 2

Top CpG methylation sites associated with sunlight exposure in the North American population ($n=991$).

CpG site	Chr	Position	Gene	β	SE	p^a	p^b
cg26930596	1	2 082 315	<i>PRKCZ</i>	0.004	0.0007	9.2×10^{-8}	9.2×10^{-8}
cgl6922167	1	27 961 746	<i>FGR</i>	0.003	0.0006	2.3×10^{-7}	2.4×10^{-7}
cg05095110	1	934 390	<i>HES4</i>	-0.0003	0.00007	2.7×10^{-6}	2.5×10^{-6}
cg21507487	14	71 941, 057	—	0.003	0.0007	3.6×10^{-6}	3.4×10^{-6}
cg03218988	11	62 341 521	<i>EEF1G</i>	-0.0004	0.00008	4.0×10^{-6}	3.9×10^{-6}
cgl6922167	16	88 522 439	<i>ZFPM1</i>	-0.0003	0.00009	5.1×10^{-6}	4.6×10^{-6}
cg05095110	8	123 793 873	<i>ZHX2</i>	-0.0003	0.00007	5.8×10^{-6}	5.8×10^{-6}
cg21507487	19	43 709 832	<i>PSG4</i>	-0.003	0.0006	8.2×10^{-6}	7.8×10^{-6}

^a Analysis adjusted for age, sex, study site, current smoking, the first four PCs capturing CD4+ T-cell purity as fixed effects and pedigree as a random effect.

^b Analysis further adjusted for vitamin D intake.

TABLE 3

Top methylation quantitative trait loci for cg26930596 in the North American population ($n=715$).

SNP	Chr	Position	Gene	Alleles	MAF	β	SE	p
rs4574216	3	150 642 446	–	A/C	0.06	–0.03	0.005	1.5×10^{-10}
rs4405858	3	150 661 351	–	G/T	0.06	–0.03	0.005	1.9×10^{-9}
rs2437807	15	84 945 996	<i>AGBL1</i>	G/A	0.28	0.01	0.003	1.5×10^{-6}
rs10221448	19	15 070 043	–	G/T	0.08	–0.02	0.005	2.6×10^{-6}
rs9505086	6	7 177 185	<i>RBE1</i>	C/T	0.34	0.01	0.003	3.3×10^{-6}
rs2714337	6	7 185 576	<i>RBE1</i>	T/A	0.32	–0.01	0.003	3.4×10^{-6}
rs2433639	12	22 902 750	–	A/G	0.05	–0.03	0.006	3.4×10^{-6}
rs339392	19	6 673 022	<i>C3</i>	G/T	0.23	–0.01	0.003	4.2×10^{-6}
rs163913	19	6 673 635	<i>C3</i>	C/T	0.23	–0.01	0.003	5.3×10^{-6}
rs13267369	8	26 869 077	–	C/T	0.45	–0.01	0.002	5.6×10^{-6}
rs4241719	4	181 510 121	–	T/C	0.46	–0.01	0.003	8.6×10^{-6}
rs2046187	8	26 868 351	–	A/G	0.41	–0.01	0.002	9.0×10^{-6}