Transethnic Evaluation Identifies Low-Frequency Loci Associated With 25-Hydroxyvitamin D Concentrations

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Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AADHS, African American Diabetes Heart Study; ARIC, Atherosclerosis Risk in Communities; ASW, Americans of African Ancestry in SW USA; BMDCS, Bone Mineral Density in Childhood Study; BMI, body mass index; BPRHS, Boston Puerto Rican Health Study; CEU, Northern Europeans From Utah; CHOP, Children's Hospital of Philadelphia; CHS, Cardiovascular Heart Study; GTEx, Genotype-Tissue Expression; GWAS, genomewide association study; Health ABC, Health, Aging and Body Composition; IRASFS, Insulin Resistance Atherosclerosis Study Family Study; JHS, Jackson Heart Study; LD, linkage disequilibrium; MESA, Multi-Ethnic Study of Atherosclerosis; METAL, Meta-Analysis Helper software; MXL, Mexican Ancestry From Los Angeles USA; NHANES, National Health and Nutrition Examination Survey; NHLBI National Heart, Lung, and Blood Institute; NIA, National Institute on Aging; NIH, National Institutes of Health; QC, quality control; SNP, single nucleotide polymorphism; SUNLIGHT, Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits; TRANSCEN-D, Trans-Ethnic Evaluation of Vitamin D; UV, ultraviolet.

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Context: Vitamin D inadequacy is common in the adult population of the United States. Although the genetic determinants underlying vitamin D inadequacy have been studied in people of European ancestry, less is known about populations with Hispanic or African ancestry.

Objective: The Trans-Ethnic Evaluation of Vitamin D (TRANSCEN-D) genomewide association study (GWAS) consortium was assembled to replicate genetic associations with 25-hydroxyvitamin D [25(OH)D] concentrations from the Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits (SUNLIGHT) meta-analyses of European ancestry and to identify genetic variants related to vitamin D concentrations in African and Hispanic ancestries.

Design: Ancestry-specific (Hispanic and African) and transethnic (Hispanic, African, and European) meta-analyses were performed with Meta-Analysis Helper software (METAL).

Patients or Other Participants: In total, 8541 African American and 3485 Hispanic American (from North America) participants from 12 cohorts and 16,124 European participants from SUNLIGHT were included in the study.

Main Outcome Measures: Blood concentrations of 25(OH)D were measured for all participants.

Results: Ancestry-specific analyses in African and Hispanic Americans replicated single nucleotide polymorphisms (SNPs) in *GC* (2 and 4 SNPs, respectively). An SNP (rs79666294) near the *KIF4B* gene was identified in the African American cohort. Transethnic evaluation replicated *GC* and *DHCR7* region SNPs. Additionally, the transethnic analyses revealed SNPs rs719700 and rs1410656 near the *ANO6*/*ARID2* and *HTR2A* genes, respectively.

Conclusions: Ancestry-specific and transethnic GWASs of 25(OH)D confirmed findings in *GC* and *DHCR7* for African and Hispanic American samples and revealed findings near *KIF4B, ANO6*/*ARID2,* and *HTR2A*. The biological mechanisms that link these regions with 25(OH)D metabolism warrant further investigation. **(*J Clin Endocrinol Metab* 103: 1380–1392, 2018)**

Vitamin D inadequacy, defined by 25-hydroxyvitamin D concentrations [25(OH)D] ≤20 ng/mL, affects approximately half of the adults in the United States, with an even higher prevalence in certain ethnic groups; >80% of African American adults and >60% of Hispanic adults have inadequate concentrations of 25(OH)D (1–3). Vitamin D inadequacy has been associated with elevated risk of autoimmune diseases, hypertension, dyslipidemia, cardiovascular events, and cardiovascular mortality (1, 4–6). Additionally, recent Mendelian randomization studies have suggested a causal relationship between vitamin D inadequacy and elevated risk of ovarian cancer, hypertension, lower cognitive function, multiple sclerosis, and all-cause and cancer mortality (7–12). Furthermore, some clinical trials have shown that vitamin D and calcium supplementation may reduce the risk of fracture in postmenopausal women (13–15). The high prevalence of vitamin D inadequacy in African and Hispanic Americans and the associated risk for adverse health outcomes could explain a portion of the health disparities between racial and ethnic groups (16). In fact, cross-sectional analyses from National Health and Nutrition Examination Survey (NHANES) data found that 25(OH)D concentrations explain one-quarter of the disparity in systolic blood pressure and more than one-third of the disparity in colorectal cancer mortality and peripheral artery disease between African Americans and people of European ancestry, even after adjustment for a wide range of factors typically related to health disparities (17–19).

Two genomewide association study (GWAS) meta-analyses of 25(OH)D concentrations in populations of European ancestry have been conducted identifying loci including group-specific component (vitamin D binding protein) gene (*GC*), nicotinamide adenine dinucleotide synthetase 1 gene (*NADSYN1*)/7-dehydrocholesterol reductase gene (*DHCR7*), vitamin D 25-hydroxylase gene (*CYP2R1*), and vitamin D 24-hydroxylase gene (*CYP24A1*). *GC* transports the vitamin D metabolites in the blood. *DHCR7* catalyzes the conversion of 7-dehydrocholesterol in the skin to previtamin D_3, a precursor to vitamin D_3. *CYP2R1* codes for a cytochrome P450 enzyme that hydroxylates vitamin D_2/D_3 to 25(OH)D. *CYP24A1* codes for another cytochrome P450 enzyme that degrades 25(OH)D to an inactive metabolite, 24,25-dihydroxyvitamin D. However, no large-scale GWAS has been performed in African or Hispanic populations (1, 20). One study reported that the heritability of 25(OH)D concentrations in African Americans was 28%, and in two Hispanic cohorts it ranged from 23% to 41% (21). A small GWAS in 229 Hispanic Americans found five independent single nucleotide polymorphisms (SNPs) (three from nongenic regions, one in *A2BP1,* and one in *GPR114*) that were associated with 25(OH)D concentrations, with replication in the full set of 1,190 Hispanic Americans (22). To provide additional evidence in African American and Hispanic populations, we assembled the Trans-Ethnic Evaluation of Vitamin D (TRANSCEN-D) GWAS consortium, including 12,026 subjects from 12 cohorts of African (n = 8541) and Hispanic (n = 3485) ancestry. Through a genomewide meta-analysis of multiethnic cohorts, we sought to confirm genetic associations found in the European ancestry population and identify genetic variants related to 25(OH)D concentrations in African and Hispanic populations.

Materials and Methods

TRANSCEN-D consisted of 12 cohorts: 9 African American (n = 8541) and 3 Hispanic from North America (n = 3485; Table 1). Briefly, the African American cohorts included the African American Diabetes Heart Study (AADHS; n = 531), the Atherosclerosis Risk in Communities (ARIC) study (n = 2658), the Bone Mineral Density in Childhood Study (BMDCS; n = 161), the Children's Hospital of Philadelphia (CHOP) cohort (n = 379), the Cardiovascular Heart Study (CHS; n = 303), the Health, Aging and Body Composition (Health ABC) study (n = 981), Mount Sinai BioMe BioBank (n = 361), the Jackson Heart Study (JHS; n = 2132), and the Multi-Ethnic Study of Atherosclerosis (MESA; n = 1035). The Hispanic American cohorts included the Boston Puerto Rican Health Study (BPRHS; n = 1360), Insulin Resistance Atherosclerosis Study Family Study (IRASFS; n = 738), and MESA (n = 1387). Additionally, as part of the transethnic meta-analysis, data from 16,124 participants in the Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits [SUNLIGHT, the largest European ancestry GWAS for 25(OH)D] were used. The data used in these analyses were collected under guidelines from the relevant institutional review boards, and all participants provided informed consent, including consent for use of genetic data. The TRANSCEN-D cohort characteristics are included in Table 1.

Each TRANSCEN-D cohort followed the sample and SNP quality control (QC) procedures specified in the TRANSCEN-D uniform analysis plan. Sample QC excluded samples with <95% call rates. Additional sample exclusion criteria applied by some cohorts are reported in Supplemental Table 1. SNP-level exclusion criteria (at the cohort level) included minor allele frequency (MAF) <0.01 (IRASFS cohort used MAF <0.05), call rate <95%, and Hardy-Weinberg equilibrium $P < 10^{-6}$. Imputation was done with IMPUTE or the Markov Chain–based haplotyper (MaCH). IMPUTE users excluded SNPs with a proper info <0.4, and MaCH users excluded SNPs with an r^2 <0.3. Each cohort performed imputation with the phase 1 reference panels of the 1000 Genomes Project (23). 25(OH)D values were natural log transformed because vitamin D values tend to be nonnormally distributed. SNPs that passed QC were tested for association with 25(OH)D concentrations via an additive genetic model adjusted for age,

Table 1. TRANSCEN-D Cohorts' Specific Characteristics

Ethnicity	Cohort	n	Female	Age, y [Mean (SD)]	BMI, kg/m^2 [Mean (SD)]	25(OH)D, ng/mL [Mean (SD)]
African American	AADHS[a,b]	531	56.8%	56.5 (9.6)	35.1 (8.5)	20.5 (11.8)
	ARIC[a]	2658	56.0%	56.3 (5.8)	30.0 (6.2)	19.0 (7.0)
	BMDCS[b]	161	53.4%	17.2 (4.2)	23.6 (5.1)	16.3 (7.8)
	CHOP[a]	379	55.1%	11.7 (4.7)	24.6 (9.1)	22.6 (13.1)
	CHS[a]	303	70.0%	72.7 (5.5)	28.4 (5.5)	18.1 (8.7)
	Health ABC[b]	981	57.2%	74.5 (2.9)	28.4 (5.5)	20.8 (9.0)
	Mount Sinai BioMe BioBank[b]	361	73.4%	55.6 (14.7)	30.4 (8.4)	22.9 (13.5)
	JHS[a]	2132	60.7%	49.9 (12.1)	32.3 (7.8)	14.9 (6.5)
	MESA[a]	1035	55.0%	62.3 (10.1)	30.1 (5.8)	19.1 (9.2)
Hispanic	BPRHS[b]	1360	70.1%	57.1 (7.5)	31.8 (6.6)	17.5 (6.8)
	IRASFS[b]	738	58.9%	40.6 (13.7)	28.3 (5.7)	16.5 (7.2)
	MESA[a]	1387	52.4%	61.4 (10.3)	29.5 (5.2)	24.7 (11.2)

Abbreviation: SD, standard deviation.

[a]Chromatographic 25(OH)D assay was used.

[b]Immune-based 25(OH)D assay was used.

sex, body mass index (BMI), ultraviolet (UV) index, and principal components 1 to 10, obtained from genotype data. Principle components were adjusted to account for population structure. Two cohorts, AADHS and IRASFS, were family studies and accounted for sample relatedness [AADHS: linear mixed model (Genome-wide Efficient Mixed Model Association, or GEMMA); IRASFS: variance component models (Sequential Oligogenic Linkage Analysis Routines, or SOLAR)] and admixture proportions instead of principal components. Site-specific UV index in the month of the blood draw was calculated for 58 US cities by averaging of the previous 3 months' UV indexes, based on data from the National Weather Service Climate Prediction Center (24). Cohorts used continuous UV index values for the city nearest the blood draw location.

The cohort-specific genomewide association results were meta-analyzed within each ancestral group with the sample size–weighted z score approach in Meta-Analysis Helper software (METAL) (25). The z score approach was used to adjust for the heterogeneity of assays used by the individual cohorts (1). The results from each cohort were corrected with the genomic control inflation factor, λ_{GC}, before meta-analysis. The ancestry-specific (African and Hispanic ancestry) meta-analysis results were then combined with previously published SUNLIGHT meta-analysis results via a sample size–weighted z score method of log-transformed 25(OH)D values, resulting in transethnic meta-analysis results (1). The sample size–weighted z score method was chosen over the inverse variance–weighted fixed-effects model because of the differences in linkage disequilibrium (LD) patterns between the common variants (MAF > 0.01) in the diverse ancestries and differences in 25(OH)D assays between the cohorts. The significance threshold was set to $P < 5 \times 10^{-8}$.

We used R version 3.1.1 software to draw regional association plots showing $-\log_{10} P$ values in the y-axis for all SNPs in the region (within 250 kb of the index SNPs). The index SNP for the regional association plots was the SNP with the lowest P-value from the transethnic meta-analysis.

We calculated statistical power to identify SNPs at the significance threshold of 5×10^{-8} for a two-sided test, assuming an additive genetic model in Quanto version 1.2.4. With our transethnic sample size of 28,150, we have 80% power ($\alpha = 5 \times 10^{-8}$) to detect an effect size of 0.012 (units in log[25(OH)D]), assuming an average 25(OH)D level of 19 nmol/L (SE 0.136) and minor allele frequency of 0.1.

Results

Our transethnic and ancestry-specific meta-analyses replicated findings from previous vitamin GWAS studies, as summarized in Supplemental Table 3, and discovered potentially novel findings near three genes: *KIF4B, HTR2A,* and *ANO6/ARID2*.

Genetic variants in *GC* were associated with 25(OH)D concentrations in both the African and Hispanic ancestry analyses at levels of genomewide significance (Table 2). *GC* SNPs rs843005 ($P = 1.0 \times 10^{-12}$, n = 7255) and rs222040 ($P = 1.1 \times 10^{-12}$, n = 7258) were associated with 25(OH)D in the African ancestry cohort. These SNPs were in low LD with the top SUNLIGHT *GC* SNP, rs2282679 (LD as measured by coefficient of correlation $r^2 = 0.08$ for both rs843005 and rs222040, in 1000 Genomes Americans of African Ancestry in SW USA (ASW) (1). For the Hispanic ancestry cohort, four *GC* SNPs reached genomewide significance: rs1526692 ($P = 2.6 \times 10^{-10}$, n = 2095), rs377687 ($P = 6.2 \times 10^{-9}$, n = 2095), rs56003670 ($P = 6.7 \times 10^{-9}$, n = 2099), and rs3755967 ($P = 9.0 \times 10^{-9}$, n = 2725). These SNPs were not highly correlated with each other, and the LD between these four SNPs and the top SUNLIGHT *GC* SNP ranged from 0.11 to 0.93 in 1000 Genomes Mexican Ancestry From Los Angeles USA (MXL) and 0.097 to 1.0 in 1000 Genomes Puerto Ricans From Puerto Rico.

An SNP, rs79666294, near kinesin family member 4B gene (*KIF4B*) reached genomewide significance ($P = 2.7 \times 10^{-8}$, n = 3999) in the African ancestry cohort. This SNP was not genotyped, nor imputed, in

Table 2. Genomewide Significant Vitamin D SNPs in African and Hispanic Ancestry Populations

SNP	Chromosome	Position	Reference	Alternate	Reference Allele Frequency	*z*-Score	*P*	Sample Size	Nearest Locus
African ancestry GWAS meta-analysis									
rs843005	4	72616327	T	C	0.42	7.1	1.0×10^{-12}	7255	*GC*
rs222040	4	72616932	A	G	0.42	7.1	1.1×10^{-12}	7258	*GC*
rs79666294	5	154426706	C	T	0.99	5.6	2.7×10^{-8}	3999	*KIF4B*
Hispanic ancestry GWAS meta-analysis									
rs1526692	4	72578724	A	G	0.69	6.3	2.6×10^{-10}	2095	*GC*
rs377687	4	72569065	A	G	0.38	5.8	6.2×10^{-9}	2095	*GC*
rs56003670	4	72572154	A	C	0.75	5.8	6.7×10^{-9}	2099	*GC*
rs3755967	4	72609398	C	T	0.77	5.7	9.0×10^{-9}	2725	*GC*

Genomewide significance is defined as $P < 5 \times 10^{-8}$. The reference allele is the vitamin D raising allele. Position corresponds to build 37. The model was adjusted for age, sex, BMI, UV index, and principal components.

the SUNLIGHT consortium. The SNPs in this region were not associated with 25(OH)D concentrations in SUNLIGHT or the Hispanic ancestry meta-analysis (Supplemental Fig. 1).

The transethnic meta-analysis in METAL identified variants in or near *GC, DHCR7, HTR2A,* and *ANO6/ARID2* as significantly associated with 25(OH)D. Overall, 100 SNPs reached genomewide significance in the transethnic meta-analysis (Supplemental Table 2); however, many of these were correlated with each other. After we considered LD within each ancestry [ASW, MXL, and Northern Europeans From Utah (CEU)], using 1000 Genomes Project data to remove SNPs with ancestry-specific $r^2 > 0.05$ (26), 13 SNPs remained (Table 3). Nine of these SNPs were on chromosome 4 in or upstream of *GC;* two were upstream of *DHCR7,* one was located between *ANO6* and *ARID2,* and one was located near the *HTR2A* gene region.

Regional association plots highlight genomic intervals of interest near loci *GC, DHCR7, HTR2A,* and *ANO6/ARID2* (Figs. 1, 2, and 3 and Supplemental Fig. 2). The black diamond in each figure represents the most strongly associated SNP in that region from the transethnic meta-analysis results in Table 3. The regional association plot for the *GC* locus (Fig. 1) demonstrates strong associations but different top SNPs in this locus for each ancestry. For instance, the top SNP in this region in the European ancestry was rs2282679 ($P = 4.6 \times 10^{-63}$, MAF = 0.29), whereas the top SNPs in the African and Hispanic ancestries were rs843005 ($P = 1.0 \times 10^{-12}$, MAF = 0.42) and rs1526692 ($P = 2.6 \times 10^{-10}$, MAF = 0.31), respectively. The top African and Hispanic SNPs were in low LD with rs2282679 ($r^2 = 0.08$ and 0.17, respectively). The top SNP differs by ancestry, probably because of differences in the LD structure and variation in frequency of the underlying functional SNPs between ancestries.

At the *DHCR7* locus (Fig. 2), the top SNPs for the European (rs7944926; $P = 1.6 \times 10^{-13}$, MAF = 0.23) and African (rs12792306; $P = 4.8 \times 10^{-6}$, MAF = 0.41) ancestries were in strong LD ($r^2 > 0.8$) with the top SNP from the transethnic analysis (rs7938885; $P = 4.5 \times 10^{-16}$, MAF = 0.37). For the Hispanic ancestry, only one cohort (IRAFS) had this SNP, and the sample size dropped down to 738; therefore, power was probably insufficient to detect an association with this locus.

The locus *HTR2A* (rs1410656) was identified by the transethnic GWAS (Fig. 3). In the European ancestry–specific meta-analysis, SNP rs1410656 is a low-frequency variant (MAF = 0.01) associated with 25(OH)D concentrations ($P = 1.2 \times 10^{-7}$) and the only strongly associated SNP in this region; no other SNPs with $r^2 > 0.3$ with rs1410656 are in this region. The plot for African ancestry shows a weaker association with this SNP ($P = 1.2 \times 10^{-3}$, MAF = 0.10) but a stronger association with two other SNPs near the *HTR2A* gene that are not in LD ($r^2 < 0.3$) with the top transethnic SNP. The top transethnic SNP was not available in the Hispanic ancestry cohorts, nor were there highly significant SNPs in this region.

Another locus, *ANO6/ARID2* (rs719700), was identified by the transethnic GWAS (Supplemental Fig. 2). In the European ancestry–specific meta-analysis, SNP rs719700 is a low-frequency variant (MAF = 0.01) associated with 25(OH)D concentrations ($P = 1.7 \times 10^{-7}$) and the only strongly associated SNP in this region; no other SNPs with $r^2 > 0.3$ with rs719700 are in this region. The plot for African ancestry shows a weaker association with this SNP ($P = 5.0 \times 10^{-3}$, MAF = 0.06) but a stronger association with several other SNPs in or upstream of the *ARID2* gene that are not in LD ($r^2 < 0.3$) with the top transethnic SNP. The top transethnic SNP was not available in the Hispanic ancestry cohorts, nor were there highly significant SNPs in this region.

Regional association plots for *KIF4B, CYP2R1,* and *CYP24A1* are included in Supplemental Figs. 1, 3, and 4. Quantile-quantile and Manhattan plots for ancestry-specific

Table 3. Genomewide Significant Vitamin D SNPs in a Transethnic Evaluation and Stratified by Ethnicity

SNP INFO						Transethnic *Z*-Score METAL			
SNP	Chromosome	Position	Reference	Alternate	Nearest Locus	Reference Allele Frequency	*z*-Score	*P*	Sample Size
rs2282679	4	72608383	T	G	*GC*	0.77	16.6	4.4×10^{-63}	24,497
rs7041	4	72618334	C	A	*GC*	0.44	14.8	1.6×10^{-49}	24,729
rs377687	4	72569065	A	G	*GC*	0.35	8.4	4.9×10^{-17}	23,402
rs6837549	4	72596821	T	G	*GC*	0.46	7.4	1.7×10^{-13}	21,193
rs1402155	4	72705908	G	A	*GC*	0.63	7.6	2.1×10^{-14}	24,431
rs17767445	4	72745267	A	G	*GC*	0.15	7.2	6.1×10^{-13}	20,417
rs13107347	4	72974748	C	T	*GC*[a]	0.33	6.1	8.0×10^{-10}	26,517
rs1352844	4	72647749	T	C	*GC*	0.13	5.7	1.5×10^{-8}	24,119
rs6814839	4	72751722	A	G	*GC*	0.88	5.6	2.0×10^{-8}	26,505
rs7938885	11	71170043	C	T	*DHCR7*[b]	0.63	8.1	4.5×10^{-16}	22,039
rs10898223	11	71219264	G	A	*DHCR7*[c]	0.84	5.6	1.9×10^{-8}	22,020
rs719700	12	46029209	T	C	*ANO6/ARID2*	0.96	5.6	2.8×10^{-8}	12,087
rs1410656	13	47542521	C	T	*HTR2A*	0.94	5.9	3.7×10^{-9}	12,841

(*Continued*)

and transethnic analyses can be found in Supplemental Figs. 5–10.

Discussion

In this study, we have examined ancestry-specific GWAS meta-analyses and a transethnic meta-analysis to evaluate the genetic determinants of 25(OH)D concentrations in people of African and Hispanic ancestries. In addition to confirming findings from previous studies, we have identified variants rs719700, near *ANO6/ARID2,* and rs1410656, near *HTR2A,* by transethnic analysis and a variant, rs79666294, near *KIF4B* by ancestry-specific GWAS meta-analyses in the African American cohorts (1, 20). Thus far little has been published on the genetic architecture underlying 25(OH)D concentrations in people of non-European ancestry. Therefore, this study makes an important contribution to our knowledge.

Variants in *GC, DHCR7, CYP2R1,* and *CYP24A1,* in order of significance, were the loci with the strongest associations with 25(OH)D concentrations in SUNLIGHT, a GWAS of 33,996 individuals of European ancestry (1). Our transethnic meta-analysis replicated the *GC* and *DHCR7* findings: several SNPs in or upstream of *GC* reached genomewide significance, and two additional genomewide significant SNPs were located upstream of *DHCR7* (rs7938885 and rs10898223), in a similar location to the top *DHCR7* SNP from SUNLIGHT (3 kb and 60 kb away, respectively). Additionally, ancestry-specific meta-analyses in African and Hispanic Americans replicated the *GC* findings, underscoring that the *GC* locus is associated with vitamin D concentrations for all three ancestries. Of note, the top African and Hispanic ancestry SNPs are in low LD with the top European SNP (r^2 = 0.08 and r^2 = 0.17, respectively). Although the tagging (genotyped) SNP associated with vitamin D concentrations varies by ancestry [because of differing LD (correlation) structures in different ancestries], the consistency of results indicates that *GC* is biologically relevant to vitamin D concentrations. Previously reported SNPs, rs4588 and rs7041, that are nonsynonymous variants were significantly associated with vitamin D levels in the transethnic evaluation [rs4588 ($P = 2.3 \times 10^{-11}$) and rs7041 ($P = 1.6 \times 10^{-49}$)] but not in the ancestry-specific evaluations [Hispanic: rs4588 ($P = 1.2 \times 10^{-6}$), rs7041 ($P = 1.6 \times 10^{-5}$), African (rs4588 ($P = 1.7 \times 10^{-7}$), and rs7041 ($P = 1.1 \times 10^{-7}$)]. Ancestry-specific meta-analyses replicated the *DHCR7* locus in African Americans with only suggestive evidence for replication in Hispanic Americans, probably because of a reduced sample size in our Hispanic ancestry sample. The top African ancestry SNP is in high LD (>0.5) with the top European SNP. Additionally, our Hispanic ancestry meta-analysis showed a consistent direction of effect for the five genomewide significant SNPs (rs10141935, rs1507023, rs4778359, rs9937918, and rs2806508) in a previously conducted Hispanic ancestry GWAS, although our Hispanic ancestry sample included 192 of the Hispanic Americans from the previous GWAS (22). None of the previously reported genomewide significant SNPs were found in the MESA cohort, and only two (rs9937918 and rs2806508) were in the BPRHS cohort. However, the direction of effect in the BPRHS cohort alone was consistent with the previous GWAS, providing some evidence for replication of two of the previously discovered SNPs.

Interestingly, neither ancestry-specific GWAS replicated previous associations with loci in *CYP2R1* and *CYP24A1* from the SUNLIGHT consortium. The top SUNLIGHT SNP in *CYP2R1* was rs10741657 ($P = 3.3 \times 10^{-20}$, discovery plus replication samples, MAF = 0.40).

Table 3. Continued

AFA *Z*-Score METAL				HIS *Z*-Score METAL				SUNLIGHT *Z*-Score METAL			
Reference Allele Frequency	*z*-Score	*P*	Sample Size	Reference Allele Frequency	*z*-Score	*P*	Sample Size	Reference Allele Frequency	*z*-Score	*P*	Sample Size
0.91	3.6	2.7×10^{-4}	7097	0.80	4.9	1.0×10^{-6}	1361	0.71	16.8	4.6×10^{-63}	16,039
0.17	5.3	1.1×10^{-7}	7258	0.44	4.2	2.6×10^{-5}	1361	0.56	13.6	3.7×10^{-42}	16,110
0.34	2.5	1.3×10^{-2}	7258	0.38	5.8	6.2×10^{-9}	2095	0.34	6.8	8.0×10^{-12}	14,049
0.38	0.2	8.6×10^{-1}	5126	NA	NA	NA	NA	0.49	8.4	5.1×10^{-17}	16067
0.46	1.5	1.3×10^{-1}	8290	0.67	4.7	3.1×10^{-6}	2100	0.72	7.2	8.6×10^{-13}	14,041
0.07	2.1	3.4×10^{-2}	7258	0.26	3.1	1.9×10^{-3}	2099	0.19	6.7	1.6×10^{-11}	11,060
0.23	2.7	6.4×10^{-3}	8293	0.35	1.4	1.8×10^{-1}	2102	0.37	5.5	4.6×10^{-8}	16,122
0.13	1.1	2.5×10^{-1}	7258	0.07	2.4	1.8×10^{-2}	738	0.13	5.7	1.4×10^{-8}	16,123
0.77	1.4	1.7×10^{-1}	8293	0.93	1.1	2.9×10^{-1}	2101	0.94	5.8	5.2×10^{-9}	16,111
0.39	4.2	2.7×10^{-5}	7258	0.51	0.8	4.4×10^{-1}	738	0.77	7.0	2.1×10^{-12}	14,043
0.80	1.7	8.4×10^{-2}	7251	0.83	1.6	1.2×10^{-1}	738	0.86	5.5	4.5×10^{-8}	14,031
0.94	2.8	5.0×10^{-3}	6727	NA	NA	NA	NA	0.99	5.2	1.7×10^{-7}	5360
0.90	3.2	1.2×10^{-3}	7251	NA	NA	NA	NA	0.99	5.3	1.2×10^{-7}	5590

Genomewide significance is defined as $P < 5 \times 10^{-8}$. Position corresponds to build 37. Shading indicates that for the given race or ethnicity the SNP is in LD ($r^2 > 0.05$) with another SNP for that race; LD between the 106 SNPs was calculated for each ancestry (1000 Genomes data for MXL, ASW, and CEU populations were used) with the LD Link Tool (SNP Clip), provided by the National Cancer Institute.

Abbreviations: AFA, African ancestry; HIS, Hispanic ancestry; NA, not available (not genotyped or imputed).

[a] 304 kb upstream of *GC* in *NPFFR2*.

[b] 11 kb upstream of *DHCR7* in *NADSYN1*.

[c] 60 kb upstream of *DHCR7*.

This SNP was not significantly associated with 25(OH)D in the Hispanic (P = 0.17, MAF = 0.34) or African (P = 0.89, MAF = 0.27) ancestry-specific analyses. *CYP2R1* is responsible for conversion of vitamin D_2/D_3 to 25(OH)D; this conversion happens in the liver and should not differ by degree of skin pigmentation that can differ by ancestry. Therefore, a lack of association in non-European ancestry is unexpected. Power analyses for our transethnic sample (n = 24,443 for *CYP2R1*) indicate that we have statistical power ranging from 6.8% to 86.0% to detect an effect size in *CYP2R1* that is roughly equivalent to 0.06% to 0.17% of the trait variation explained by an SNP (R^2) with MAF 0.3575. This finding could explain why we did not observe replication of the *CYP2R1* results reported in SUNLIGHT. It is also likely that the SNP found in SUNLIGHT is in strong LD with the underlying functional variant in European ancestry but not in Hispanic or African ancestries, in which case the underlying functional variant was not adequately tagged by any genotyped or imputed SNP. The top SUNLIGHT SNP in *CYP24A1*, rs6013897 ($P = 6.0 \times 10^{-10}$, discovery plus replication samples, MAF = 0.21), was not replicated in the Hispanic and African ancestry-specific analyses (P = 0.02, MAF = 0.35 and P = 0.30, MAF = 0.24, respectively). *CYP24A1* encodes an enzyme that degrades 25(OH)D to 24,25-dihydroxyvitamin D, an inactive metabolite. Lack of replication is not unexpected for *CYP24A1* because the variant in this gene was found only in the combined discovery and replication sample (n = 33,996) in SUNLIGHT but not in a smaller GWAS meta-analysis by Ahn *et al.* (20) (n = 4501) or in the SUNLIGHT discovery sample (n = 16,124) (1). Calculated statistical power for *CYP24A1* is 0.01% for those of Hispanic ancestry (n = 738) and 0.41% for those of African ancestry (n = 7022), whereas the power is 65.72% for the SUNLIGHT (discovery plus replication samples) and 4.55% for the SUNLIGHT discovery sample (n = 14,020).

The African American–specific GWAS meta-analysis uncovered a genomewide significant association with a low-frequency SNP, rs79666294, near *KIF4B* (Table 2), although its signal is slightly below the genomewide threshold in the transethnic meta-analysis ($P = 1.2 \times 10^{-6}$, n = 4737). This variant was not significantly associated with 25(OH)D concentrations in the Hispanic ancestry cohort (n = 738; P = 0.61; MAF = 0.03) and not genotyped or imputed in SUNLIGHT (MAF in the European 1000 Genomes population is 0.03), so the results appear to be driven by the African American cohorts (MAF = 0.01). Although the closest gene to SNP rs79666294 is *KIF4B*, this SNP is an expression quantitative trait locus for another nearby gene, *FAXDC2* [Genotype-Tissue Expression (GTEx) Portal accessed on 22 November 2017; GTEx Analysis Release V7, dbGaP Accession phs000424.v7.p2]. *FAXDC2* codes for the fatty acid hydroxylase domain–containing protein 2, which is involved in cholesterol and steroid biosynthesis. Given that cholesterol (7-dehydrocholesterol) is a precursor to vitamin D (previtamin D_3) produced in the skin, this finding could have meaningful biologic implications. According to the GTEx Project, the highest expression of

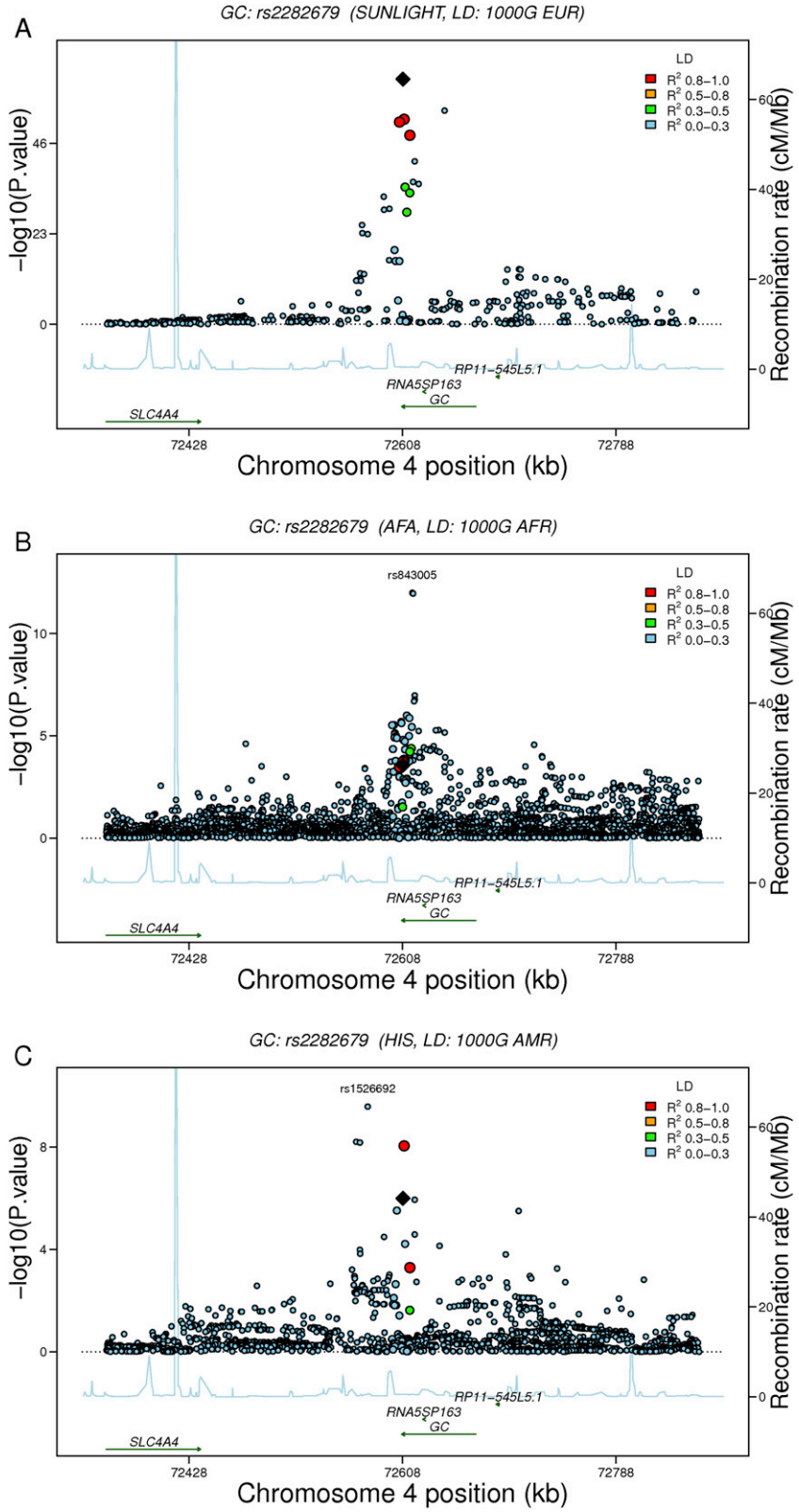

Figure 1. Regional association plots for *GC* SNPs on chromosome 4 for European (EUR), African (AFR), and Hispanic (AMR) ancestries. Log 10 *P* values of genotyped SNPs are plotted against their position in the genome (build 37). The top SNP from the transethnic analysis is shown with the black diamond, and shading of the circles corresponds to the LD with the top transethnic SNP (measured with r^2 and relevant populations from 1000G phase I data). Because of the highly significant *P* values, the SUNLIGHT regional association plot is on a different scale than the African and Hispanic plots are.

FAXDC2 is in pituitary tissue. This finding warrants replication in a larger African ancestry cohort, when available.

Transethnic evaluation led to the identification of a low-frequency SNP, rs1410656, near the *HTR2A* gene. *HTR2A* encodes a serotonin receptor. Mutations in this gene affect serotonin levels, which are associated with mental health, such as schizophrenia, obsessive-compulsive disorder, and major depressive disorder (27). Additionally, vitamin D supplementation has been shown to reduce depressive symptoms and decrease the risk of schizophrenia (28, 29). However, the mechanism by which *HTR2A* functions warrants further inquiry. Additionally, the transethnic evaluation led to a second discovery, rs719700 (MAF = 0.04), on chromosome 12, which is downstream of *ANO6* and upstream of *ARID2* (Supplemental Fig. 2); this SNP was found to be associated with 25(OH)D in the African American and SUNLIGHT cohorts as well but was monomorphic in the Hispanic American cohort (Table 3). Although rs719700 is the top variant in this region in the transethnic analysis and in SUNLIGHT, the most statistically significant SNP in this region for African Americans is rs114330994. SNPs rs719700 and rs114330994 are not in LD, suggesting that the LD structure around a putative underlying functional variant is different between African and European ancestry. *ANO6* activates a multipass transmembrane protein that is involved in calcium transport primarily in the bones (30, 31). Scott syndrome is a rare congenital bleeding disorder caused by a mutation of this gene. There are no studies available examining 25(OH)D concentrations in this syndrome. However, *ANO6* has been shown to have differential expression in peripheral blood cells between the first and third trimester in pregnancy that is modified by underlying vitamin D concentrations (32). *ARID2* is a member of the adenine- and thymine-rich

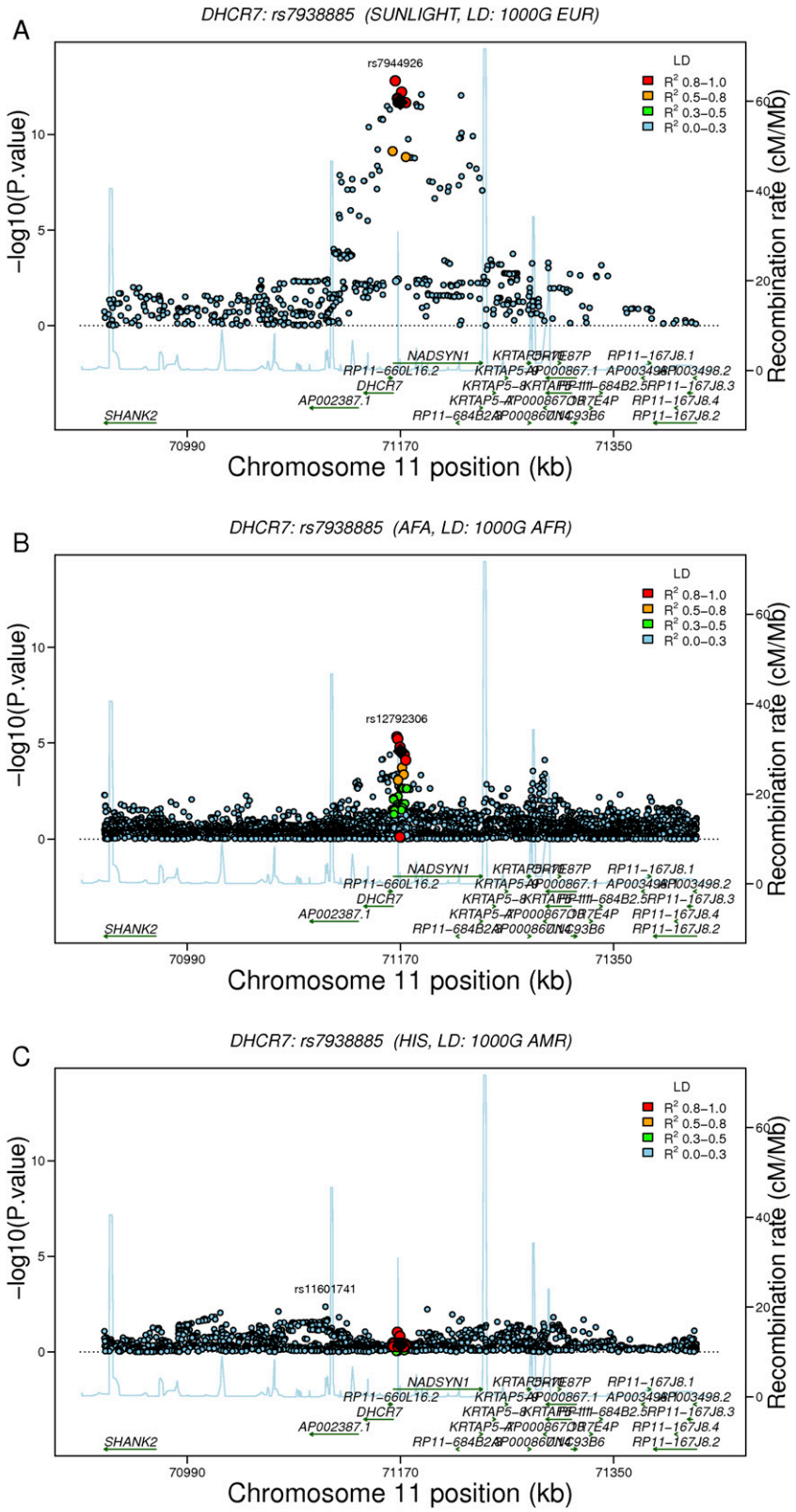

Figure 2. Regional association plots for *DHCR7* SNPs on chromosome 11 for European (EUR), African (AFR), and Hispanic (AMR) ancestries. Log 10 *P* values of genotyped SNPs are plotted against their position in the genome (build 37). The top SNP from the transethnic analysis is shown with the black diamond, and shading of the circles corresponds to the LD with the top transethnic SNP (measured with r^2 and relevant populations from 1000G phase I data).

interactive domain–containing family of DNA-binding proteins that is a subunit of a protein involved in ligand-dependent transcriptional activation by nuclear receptors. Mutations in this gene are associated with hepatocellular carcinomas (30, 31, 33). The relationship between this gene region and 25 (OH)D concentrations is unclear.

Loci *HTR2A, ANO6/ARID2,* and *KIF4B* were not associated with 25(OH)D concentrations in the SUNLIGHT European ancestry analysis. SNP rs79666294 (*KIF4B*) was not genotyped, and no other SNPs in the region were significant in SUNLIGHT. SNPs rs1410656 (*HTR2A*) and rs719700 (*ANO6/ARID2*) were not identified in the European ancestry analysis because it was not selected for replication in SUNLIGHT because it did not meet the study's criteria for replication (1).

There are limitations to our study. Although the sample sizes are the largest to date, they are small, especially for the Hispanic American cohort (N = 3485). Of note, our Hispanic cohorts come from Mexican and Puerto Rican backgrounds, and this heterogeneity in the Hispanic samples could have limited our ability to detect associations. Additionally, not all cohorts included in the analyses used the same assay for 25(OH)D measurement. We accounted for this limitation by combining test statistics in the form of *z* scores rather than performing a fixed-effect meta-analysis of the effect estimates. Finally, the imputation used may not have been dense enough to capture the functional SNP or in some cases (*i.e.*, CYP2R1 and CPY24A1) the SNPs in LD with the functional SNP.

Conclusion

Investigators from the TRANSCEN-D consortium performed the largest multiethnic GWAS for genetic determinants of 25(OH)D concentrations to date. TRANSCEN-D consists of data from 12 cohorts of African and

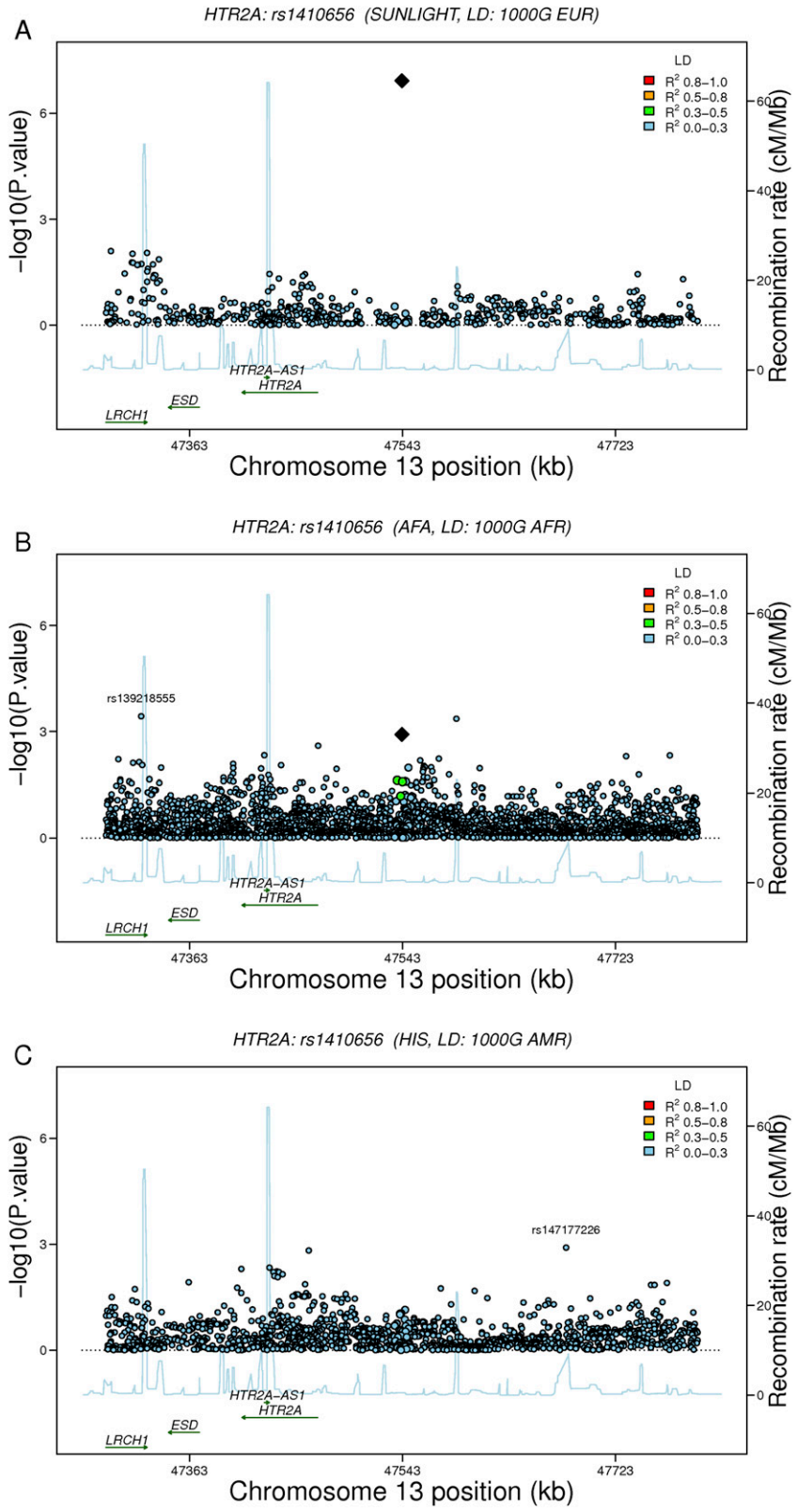

Figure 3. Regional association plots for *HTR2A* SNPs on chromosome 13 for European (EUR), African (AFR), and Hispanic (AMR) ancestries. Log 10 *P* values of genotyped SNPs are plotted against their position in the genome (build 37). The top SNP from the transethnic analysis is shown with the black diamond (this SNP was not genotyped for the Hispanic ancestry, so no black diamond is displayed), and shading of the circles corresponds to the LD with the top transethnic SNP (measured with r^2 and relevant populations from 1000G phase I data). The LD information is sparse in this regional association plot because of the low minor allele frequency of *HTR2A*.

Hispanic ancestry. By using the *z* score approach, the transethnic evaluation replicated previous associations between both *GC* and *DHCR7* and 25(OH)D. The evaluation of individual non-European cohorts and the transethnic meta-analysis identified SNPs near *ANO6/ARID2* and *HTR2A* and an SNP near *KIF4B* for African ancestry. Additional inquiry into the biological relationship between 25(OH)D and these regions is warranted.

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Health ABC

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Mt. Sinai SM BioMe Biobank

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