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Genome-wide association analysis of circulating vitamin D levels in children with asthma

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Abstract

Vitamin D deficiency is becoming more apparent in many populations. Genetic factors may play a role in the maintenance of vitamin D levels. The objective of this study was to perform a genome-wide analysis (GWAS) of vitamin D levels, including replication of prior GWAS results. We measured 25-hydroxyvitamin D (25(OH)D) levels in serum collected at the time of enrollment and at year 4 in 572 Caucasian children with asthma, who were part of a multi-center clinical trial, the Childhood Asthma Management Program. Replication was performed in a second cohort of 592 asthmatics from Costa Rica and a third cohort of 516 Puerto Rican asthmatics. In addition, we attempted replication of three SNPs that were previously identified in a large GWAS of Caucasian individuals. The setting included data from a clinical trial of childhood asthmatics and two cohorts of asthmatics recruited for genetic studies of asthma. The main outcome measure was circulating 25(OH)D levels. The 25(OH)D levels at the two time-points were only modestly correlated with each other (intraclass correlation coefficient = 0.33) in the CAMP population. We identified SNPs that were nominally associated with 25(OH)D levels at two time-points in CAMP, and replicated four SNPs in the Costa Rican cohort: rs11002969, rs163221, rs1678849, and rs4864976. However, these SNPs were not significantly associated with 25(OH)D levels in a third population of Puerto Rican asthmatics. We were able to replicate the SNP with the strongest effect, previously reported in a large GWAS: rs2282679 (*GC*), and we were able to replicate another SNP, rs10741657 (*CYP2R1*), to a lesser degree. We were able to replicate two of three prior significant findings in a GWAS of 25(OH)D levels. Other SNPs may be additionally associated with 25(OH)D levels in certain populations.

Introduction

Vitamin D is both a nutrient and a hormone. Environmental and behavioral determinants of vitamin D status vary widely, including exposure to the sun and time spent outdoors (Sahota et al. 2008; van der Mei et al. 2006), latitude, season, skin coverage (Webb 2006), skin color, age, diet, and supplement use (Sahota et al. 2008). As a nutrient, vitamin D is contained only in a few foods, including oily fish and fish liver oil, egg yolk, and offal (Lamberg-Allardt 2006). As such, excluding supplementation, food is not the primary way that we receive the necessary quantities of vitamin D. Sun exposure is one of the primary mechanisms by which we receive large amounts of vitamin D. Specifically, 7-dehydrocholesterol (7-DHC) is distributed in the skin and after exposure to sunlight, 7-DHC is converted to previtamin D₃, which is then transformed to vitamin D₃ by a thermally induced isomerization. Vitamin D₃ then undergoes hydroxylation in the liver to 25-hydroxyvitamin D₃ (henceforth, 25(OH)D) and then in the kidney to its biologically active form 1,25-dihydroxyvitamin D₃ (1,25[OH]₂D). Vitamin supplementation is another primary way that our bodies absorb vitamin D.

Serum 25(OH)D is the major circulating metabolite of vitamin D and is the standard measure of vitamin D status. Evaluations of most relations between vitamin D and health, and various disorders lead to the conclusion that a sufficient circulating vitamin D level for bone health is at least 20 ng/ml, but for other organ systems, is at least 30–40 ng/ml. Levels of 25(OH)D between 20 and 30 ng/ml are considered relatively insufficient and there are suggestions that levels even higher than 40 ng/ml may be necessary (Hollis et al. 2007; Taback and Simons 2007) for optimal immune functioning and overall health.

Vitamin D deficiency has been documented in many populations worldwide, and has been reported in individuals of all ages (Holick 2006; Nesby-O'Dell et al. 2002). Low vitamin D levels have been associated with a range of disorders. The association of low vitamin D and bone diseases such as rickets (Heaney 2003) and osteoporosis (Heaney 2004) is well known. While there are no controlled intervention studies, several lines of evidence have shown that vitamin D reduces the risk of colorectal cancer (Giovannucci 2005, 2006; Gorham et al. 2005). Other cancers that may be vitamin D-responsive include breast, lung, ovarian, and prostate cancer (Grant 2006). Other disorders in which the role of vitamin D is being actively investigated are the autoimmune disorders such as multiple sclerosis (MS), type 1 diabetes mellitus (1999b; Hypponen et al. 2001), and rheumatoid arthritis (Merlino et al. 2004). Our group is actively studying the effect of vitamin D on asthma pathogenesis and control (Brehm et al. 2009, 2010; Litonjua and Weiss 2007).

Despite the relatively large amount that is known about the epidemiology of vitamin D, little is known about how genetics influence vitamin D levels. Family and twin studies suggest that a substantial portion of the variability in vitamin D can be attributed to genetics, with heritability estimates of 53 % (Hunter et al. 2001; Shea et al. 2009). Research identifying genetic variants that could influence vitamin D levels has been minimal until recently. Specific candidate genes have been studied in relation to vitamin D using relatively small and underpowered studies (Engelman et al. 2008; Ramos-Lopez et al. 2007). Recently, three genome-wide association studies (GWAS) of vitamin D were published (Ahn et al. 2010; Engelman et al. 2010; Wang et al. 2010), with the largest study using 15 cohorts of 33,996 individuals (Wang et al. 2010). That study identified several genetic variants for vitamin D including rs2282679 located in *GC* on chromosome 4p12 (overall $p = 1.9 \times 10^{-109}$); rs12785878 located near *DHCR7* on chromosome 11q12 ($p = 2.1 \times 10^{-27}$), and rs10741657 located near *CYP2R1* on 11p15 ($p = 3.3 \times 10^{-20}$). In this manuscript, we sought to further investigate genetic determinants of vitamin D levels, and to replicate the previous GWAS hits.

Methods

Initial study population

The Childhood Asthma Management Program (CAMP) was a multicenter clinical trial of the effects of anti-inflammatory medications in children with mild to moderate asthma. Detailed descriptions of subject recruitment and study protocol have been published elsewhere (1999a). All participants had asthma defined by symptoms greater than two times per week, the use of an inhaled bronchodilator at least twice weekly or the use of daily medication for asthma, and airway responsiveness to 12.5 mg/ml of methacholine. Children were 5–12 years of age at randomization, and were randomly assigned to one of three treatment arms (inhaled budesonide, nedocromil, or placebo). Children with severe asthma or other clinically significant conditions were excluded. In CAMP 1,041 asthmatic children were followed up for 4–6 years. Of these, 968 children and 1,518 of their parents contributed DNA samples. Our analysis was restricted to the non-Hispanic white children. Two final samples were generated: (1) A total of 422 of white (non-Hispanic) CAMP subjects and their parents were genotyped on Illumina's Human-Hap550 Genotyping

BeadChip. (2) An additional 150 CAMP probands who did not have parental DNA were genotyped with the Illumina Infinium HD Human610-Quad BeadChip. From these data, two separate datasets were generated: (1) 422 parent–offspring trios genotyped on Illumina’s Human-Hap550 Genotyping BeadChip, and (2) a merged HumanHap550/Human610-Quad data set composed of 572 CAMP probands. The CAMP was approved by the Institutional Review Boards of Brigham and Women’s Hospital and the other participating centers.

Phenotype

Serum 25-hydroxyvitamin D₃ (25(OH)D)—Serum levels of 25(OH)D are considered as the best circulating biomarker of vitamin D metabolic status and reflect contributions from all sources of vitamin D (i.e., diet and sun exposure) (Hollis 2005; Hollis and Wagner 2005). Two measures of vitamin D were available on 1,024 subjects (98 % of enrolled subjects) using a radioimmunoassay method in Dr. Bruce Hollis’ laboratory at the Medical University of South Carolina (Hollis et al. 1993; Hollis and Napoli 1985). This analysis was done on the 572 with both vitamin D values and GWAS data. Serum 25(OH)D was measured from stored samples drawn shortly prior to enrollment into the trial and at the end of the 4-year trial. Vitamin D levels have been shown to be relatively stable when specimens have been properly stored (Agborsangaya et al. 2010).

Genotyping and quality control

Genome-wide SNP genotyping was performed by Illumina, Inc. (San Diego, CA) on the HumanHap550v3 BeadChip for CAMP subjects and their parents. Genotype reproducibility was assessed by analyzing four subjects that were repeated once on each of the 14 genotyping plates; all replicates had at least 99.8 % concordance. The data were cleaned in several steps. 6,257 markers were removed due to low Illumina clustering scores (see supplementary file for additional information). An additional 1,329 markers were removed because their flanking sequences did not map to a unique position on the hg17 reference genome sequence. We used PLINK (Purcell et al. 2007) to further QC the remaining markers. All markers had greater than 90 % genotyping completion rate, while the average completion rate for each marker was over 99 %. 3,790 markers were removed because they were monomorphic in our sample. 2,445 markers were removed due to five or more parent–child genotype inconsistencies. No filtering was done based on Hardy–Weinberg equilibrium due to ascertainment of the cohort through affected probands. From 561,466 markers present on the BeadChip, 547,645 markers (97.54 %) passed quality control metrics. The 422 CAMP probands and their parents were successfully genotyped. The average genotyping completion rate for each subject was 99.75 %. SNPs with minor allele frequencies (MAF) <1 % were excluded. An additional 150 CAMP probands were available who had genotyping performed with the Illumina Infinium HD Human610-Quad BeadChip.

For replication studies of significant findings from prior GWAS studies, two additional SNPs were genotyped on the Sequenom platform, since they were not on the Illumina chip. SNPs rs10741657 (*CYP2R1*) and rs12785878 (*DHCR7*) were genotyped at the Children’s Hospital Boston Sequenom SNP Genotyping Facility. The genotyping completion rate for both these 2 SNPs was 99.2 % and there were no discordant genotypes.

Statistical methods

Since we had measured circulating vitamin D levels in the CAMP population at two time-points 4 years apart, we investigated the correlation between these two measures. We assessed the correlation between the two measures by calculating the Spearman correlation coefficient and by assessing the intra-class correlation (ICC) coefficient. We used mixed-effects regression models to estimate the ICC between the two values, adjusting for season of blood collection, using SAS version 9.1 (SAS Institute, Cary, NC).

We identified the most robust genetic associations for vitamin D by performing four genetic analyses: (1) a family-based association analysis of baseline vitamin D in 422 parent–offspring trios, (2) a family-based association analysis of vitamin D at year 4 in 422 parent–offspring trios, (3) a population-based analysis of baseline vitamin D in 572 probands, and (4) a population-based analysis of vitamin D at year 4 in 551 probands. Family-based association testing (FBAT) is a generalization of the transmission disequilibrium test (TDT), which allows for valid testing of association with any phenotype, sampling structure, and pattern of missing marker allele information. The FBATs have several attractive properties, including being inherently robust to population stratification. The genome-wide population-based analyses used linear regression analysis in PLINK (Purcell et al. 2007) to evaluate the association between SNPs and vitamin D. We used an additive model and adjusted for age, sex, season, and latitude. To correct for population stratification, we used Eigenstrat to generate the main eigenvectors that describe the underlying population substructure (Price et al. 2006), which were also included as covariates in the population-based analysis. The results from these analyses were then merged together and we identified SNPs with nominally significant *p* values and consistent direction of effect in all the four analyses. SNPs meeting these characteristics were then ranked by the average population-based *p* value and the top 50 SNPs were identified and selected to be replicated in the Costa Rican population. We focused on population-based analyses as these analyses contained additional probands, making these analyses substantially more powerful. SNPs that did not have a consistent direction of effect or nominally significant results in one of the analyses were excluded. HWE was checked for these 50 SNPs, and none of them were out of equilibrium.

Primary replication population

Replication genotyping was performed in 592 Hispanic asthmatic children participating in a study of the genetics of asthma in Costa Rica. Detailed information about this sample can be found elsewhere (Hunninghake et al. 2007). In brief, screening questionnaires were sent to the parents of 13,125 children aged 6–14 years enrolled in 113 schools in the Central Valley of Costa Rica from February 2001 to December 2006. Children were eligible for inclusion in the study of parent–child trios if they had physician-diagnosed asthma, at least two respiratory symptoms (wheezing, cough, or dyspnea) or a history of asthma attacks in the previous year, and high probability of having at least six great-grandparents born in the Central Valley of Costa Rica (as determined by the study genealogist on the basis of the paternal and maternal last names of each of the child’s parents) (Escamilla et al. 1996). Blood samples were obtained on all probands on entry into the study. Measurement of 25(OH)D levels was performed as in the CAMP study, and the residuals were calculated adjusting for age and gender. Replicate genotyping was performed using the Illumina BeadStation 500G system (Oliphant et al. 2002). The genotyping completion rate was >99.8 % with no discordance among replicate genotypes. Written parental consent was obtained for the participating children, for whom written assent was also obtained. The study was approved by the Institutional Review Boards of the Hospital Nacional de Niños (San José, Costa Rica), and Brigham and Women’s Hospital (Boston, MA).

For replication studies of significant findings from prior GWAS studies, three additional SNPs were genotyped. SNPs rs2282679 (*GC*), rs10741657 (*CYP2R1*), and rs12785878 (*DHCR7*) were genotyped using the Taqman assay in our laboratory. Genotyping completion rates for these SNPs were 99.4, 99.6, and 99.2 %, respectively. No discordant genotypes were noted in duplicate genotyping.

Second replication population

Replication studies were conducted in a study of asthma genetics and epidemiology in Puerto Rican children in Hartford (CT) and San Juan (Puerto Rico). At both the sites, the main recruitment tool was a screening questionnaire given to parents of children aged 6–14 years. All participants had to have four Puerto Rican grandparents. Children with asthma (cases) were selected on the basis of physician-diagnosed asthma and wheeze in the prior year. Controls had no physician-diagnosed asthma or wheeze in the prior year.

From September 2003 to July 2008, the children were recruited from public schools enrolling Puerto Rican children in Hartford. Flyers with a study description were distributed to all parents of children in grades K to 8. Of the 640 children whose parents completed a screening questionnaire, 585 (91.4 %) were eligible; 449 (76.8 %) of these children agreed to participate. From March 2009 to June 2010, children were chosen from households selected by a multistage probability design in San Juan, using a scheme similar to that of a prior study (Bird et al. 2006). In an effort to reach a sample size of 700 children, a random sample of 783 of 1,111 eligible households was contacted; parents of 106 of these 783 households refused to participate or could not be reached, leaving 677 participants. The study was approved by the Institutional Review Boards of Connecticut Children's Medical Center (Hartford, CT), the University of Puerto Rico (San Juan, PR), Brigham and Women's Hospital (Boston, MA), and the University of Pittsburgh (Pittsburgh, PA).

Of the 1,126 participants at both study sites, 999 had blood samples and sufficient DNA for genotyping. Genome-wide genotyping was completed in these 999 children using the Illumina HumanOmni2.5-Quad v1.0 DNA Analysis BeadChip. After excluding subjects with >5 % missing genotypes, and SNPs with: missingness >2 %, extreme deviations from Hardy–Weinberg equilibrium, and minor allele frequency <1 %, there were 943 subjects and ~ 1.9 million SNPs available for analysis. Of these 943 children, 516 had asthma and were included in this analysis. Plasma 25(OH)D was measured with a liquid chromatography–mass spectrophotometry assay (Holick 2005). The association analysis of vitamin D was adjusted for study site (Hartford, CT vs. San Juan, PR), age, sex, season of blood draw, and GEM clusters (to adjust for population stratification) (Lee et al. 2010).

Results

The characteristics of children included in the three asthma cohorts are presented in Table 1. After data cleaning, there were 572 probands in CAMP and 592 parent–offspring trios in the Costa Rican population. Despite the disparity in climate and latitude, Table 1 illustrates the striking similarities between the CAMP and Costa Rican cohorts, particularly with regard to the vitamin D levels. The 25(OH)D levels were slightly lower in the Puerto Rico cohort compared with the other two cohorts. Other characteristics of the Puerto Rican children were similar to CAMP and Costa Rica. In the CAMP population, serum vitamin D levels were lower at the end of the 4-year trial than at the start. While the values were correlated, this was modest at best (Pearson correlation coefficient = 0.39, $p < 0.001$, Fig. 1), and the ICC coefficient adjusted for season of collection of the samples was only 0.33. The correlation did not change when the analysis was limited to the children who had their two measurements taken during the same season, 4 years apart (Pearson correlation coefficient = 0.39).

Supplementary Table S1 shows details of the genotyping process in CAMP, which indicates that there were 512,296 autosomal SNPs meeting our QC criteria for the family-based analyses. The “manhattan plots” and QQ plots at baseline and year 4 are shown in Figs. 2, 3, which indicate no substantial deviation from what is expected by chance (genetic inflation factor = 1.015 and 1.006 for baseline and year 4, respectively). None of the SNPs achieved

genome-wide significance after a Bonferroni correction for multiple comparisons; however, the QQ plots indicate that the top hits are greater than what is expected by chance. The average population-based p value was calculated and the top 50 SNPs with low p values and nominally significant family-based p values were identified and replicated in the Costa Rican cohort (Table 2). Four of these SNPs had nominally significant associations in the Costa Rican sample, as depicted in Table 3. Although none of these SNPs meet genome-wide significance, they have consistent nominal associations in both studies. However, none of these SNPs were associated with 25(OH)D levels in a Puerto Rican cohort, despite having the same direction of effect as in the other two cohorts (Table 3, all p values >0.1). Even when the evidences from the three cohorts were combined, Liptak p values did not reach genome-wide significance.

Three SNPs were previously reported to be associated with vitamin D levels in a recent GWAS study (Wang et al. 2010), published after we had completed our analyses. None of these SNPs were selected for replication in our analyses. However, after publication of that study, we evaluated the associations for these three SNPs using both the baseline and year 4 vitamin D measurements in the CAMP population. Significant associations were found with baseline and 4-year 25(OH)D levels in rs2282679 and rs10741657 (Table 4). Our population-based analyses found an extremely strong association with rs2282679 as was observed previously. In fact, rs2282679 was among the top 25 associated SNPs for all of the population-based analyses and was the 11th best p value at the baseline vitamin D measurement (see online supplement, Supplementary Table S2). In Costa Rica, all three SNPs were associated with 25(OH)D levels, with the same direction of effects as that in CAMP. Taken together, the results in our initial and primary replication cohorts are consistent with the findings that this SNP in *GC* had the strongest association with 25(OH)D levels. Finally, in the Puerto Rico cohort, only one of the three SNPs were genotyped, and rs2282679 was significantly associated with 25(OH) levels (p value = 6.3×10^{-09}), and the association was in the same direction as in the previous cohorts. The combined p value (Liptak) of the one-sided p values for this SNP from all three cohorts was $1.243\text{E}-14$.

Discussion

Vitamin D insufficiency has become a common problem for many individuals and is now linked to various diseases. This therefore has led to substantial interest in identifying determinants of vitamin D. As many of environmental determinants of vitamin D are known, identifying genetic determinants of vitamin D is likely to help in our overall understanding of the biologic processes which may increase or decrease vitamin D levels. In order to further elucidate the genetic determinants of vitamin D, we performed a genome-wide association study and replicated the top 50 findings in an independent population, then pursued replication of the top 4 SNPs in a third population.

We identified four SNPs associated with serum vitamin D in two independent cohorts of asthmatic children. These variants were in/near four genes that were identified in the CAMP population and had consistent nominal associations in the Costa Rican Cohort: (1) rs11002969 is located in an intronic region of *ZCCHC24* on chromosome 10q22.3 and has previously been associated with right ventricular cardiomyopathy; (2) rs163221 is located in an intronic region of *C18orf16/CHST9* (carbohydrate (*N*-acetylgalactosamine 4-0) sulfotransferase 9); (3) rs1678849 is located in an intronic region of *FUT6*; and (4) rs4864976 is located on chromosome 4q12 and is not proximal to any relevant candidate genes. None of these SNPs or the nearby genes has a known clear involvement in biologic pathways related to vitamin D. We then attempted replication of these four SNPs in an additional population. However, these SNPs were not associated in a third cohort of asthmatic Puerto Rican children. One likely explanation is that the findings in the first two

cohorts were due to chance alone. In addition, differences in genetic architecture between the Puerto Rico cohort and the previous cohorts is also possible, since Puerto Ricans have been reported to have a much higher proportion of African ancestry (18 %) (Tang et al. 2007) than Costa Ricans of Central Valley ancestry (3 %) (Celedon et al. 2002).

Three GWAS studies of vitamin D levels were recently published after we had completed our analyses. One study was conducted in Hispanic Americans and did not find any associations that were significant at a genome-wide level (Engelman et al. 2010). In the second study performed in five adult Caucasian cohorts, three SNPs from *GC*—rs2282679, rs7041, and rs1155563—were noted to be significantly associated with circulating vitamin D levels (Ahn et al. 2010). Additional analyses by the authors showed that the strongest signal came from rs2282679. Finally, a well-powered GWAS on insufficient vitamin D levels was published (Wang et al. 2010) with the strongest association being with rs2282679, similar to the previous study. We, therefore, examined the top SNPs from the report of Wang and colleagues, and examined these in our cohort. There was replication of the top findings from the recent vitamin D GWAS by Wang et al., with some notable discrepancies. SNP rs12785878 was not associated in CAMP, whereas it was associated in Costa Rica. The top association finding from the previous article, rs228269, had a very strong association in CAMP at baseline and year 4, and in Costa Rica in the correct direction. Finally, this SNP was also strongly associated with 25(OH)D levels in Puerto Rico. Therefore, our findings further substantiate all three of the initial associations to some degree, with the most consistent replication being for rs2282679, which was the strongest finding in the initial paper. We have confirmed this association, and the combined evidence from these three asthma cohorts was significant at a genome-wide level.

In the two recent studies of twins, vitamin D was noted to have high heritability (Karohl et al. 2010; Orton et al. 2008). However, we are not aware of any studies that have looked at the correlation between vitamin D levels over time in the same individuals. Since vitamin D levels are known to vary over time, a strength of our analysis is that we have measurements at two time-points in our initial population. As seen from the correlation analysis, the levels at the end of the 4-year time point were lower than the baseline, and the correlation between the two values were very modest, at best. The correlation coefficient value that we obtained, was similar to the correlation between dizygotic twins that have previously been reported (Karohl et al. 2010; Orton et al. 2008). Restricting the analysis to measurements obtained during the same season, 4 years apart, did not change the correlation. Thus, assessing the two measurements separately provides some robustness to the results obtained from this initial cohort, on which the choice of SNPs to be genotyped in the second population was based. In addition, since participants in these two populations arose from eight centers across North America and from Costa Rica, it provided for a reasonably wide range of latitudes. Despite this fact, vitamin D levels from CAMP participants were similar to levels from participants from Costa Rica, reinforcing the notion that behaviors are likely more important than simple latitude in determining the amount of sun exposure. With respect to the Puerto Rican cohort, an additional potential determinant of vitamin D levels is the higher African ancestry of the population.

Despite the given strengths of this study, there are certain limitations that must be discussed. First, the sample size of our initial and replication populations are underpowered given that we are testing over 500,000 SNPs. Secondly, in the analysis, we required that all genetic associations were nominally significant in the four analyses including both family-based and population-based analyses at baseline and year 4. Our intention of doing this was to identify genetic associations that are robust, and therefore limit the number of false positive findings. Although this approach is effective in limiting false positive associations, it is likely that we missed true genetic associations. This is likely what we observed with the SNP, rs2282679,

previously reported by Wang et al. In our analysis, this SNP had a very strong association in both the baseline and year 4 population-based analyses; however, it did not reach nominal significance in one of the family-based association analyses and therefore was not selected to be replicated in the top 50 SNPs. A better strategy would be to use a more liberal threshold for the family-based analysis, as these analyses are underpowered. Third, these findings are investigating genetic associations with vitamin D in general; however, the probands used in this analysis were ascertained for asthma. This can complicate the interpretation of some of the association, as there is evidence implicating vitamin D in the development of asthma. Significant associations between polymorphisms in the VDR gene with asthma have been reported in several genetic association studies (Poon et al. 2004; Raby et al. 2004), but has not been consistently replicated (Vollmert et al. 2004; Wjst 2005). Since research suggests that genetic variants for vitamin D may also influence asthma affection status, the association results that we have observed must be interpreted with caution, as these associations may be specific to asthmatic individuals. In particular, children with asthma may have different behaviors with regard to sun exposure than a sample from the general population.

In conclusion, we have performed a GWAS analysis on vitamin D in three independent samples of asthmatic individuals. We identified four SNPs that had consistent genetic associations in the first two cohorts, but not the third cohort. In addition, we performed analyses to verify the top three SNPs that were identified in the previously reported GWAS of vitamin D, with replication of the findings in the top SNP (rs2282679) from that analysis; there was weak evidence for replication of the second SNP (rs10741657). Therefore, we have confirmed the association of the GC SNP from prior GWAS studies. Other SNPs may be associated with 25(OH)D levels in certain populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Scatter plot of 25(OH)D values at baseline and at year 4 in children with asthma. *Trendline* corresponds to Pearson correlation coefficient = 0.39, $p < 0.001$

Fig. 2.

a, b Manhattan plot of the population based analyses at baseline and year 4. The y -axis has the $-\log(p \text{ value})$ while the x -axis has the 22 chromosomes. In this figure, while none of the findings reached genome-wide significance, there were several strong associations

Fig. 3.

a, b QQ plots for the population-based analyses at baseline and year 4. In both cases, this line does not differ significantly from what is expected by chance. The top p values do seem slightly higher than what is expected by chance, which would indicate that there are true positive findings among the SNPs more strongly associated with vitamin D

Table 1

Main characteristics of study participants

	CAMP	Costa Rica asthma cohort	Puerto Rico asthma cohort
<i>N</i>	572	592	516
Male gender	341 (59.6 %)	351 (59.3 %)	285 (55.2 %)
Age at enrollment, years [mean (SD)]	8.9 (2.1)	9.0 (1.8)	10.0 (2.7)
Circulating vitamin D at baseline, ng/ml [mean (SD)]	40.46 (15.75) range: 10.1–110.4	37.44 (11.79) range: 12.5–98.1	28.9 (8.7) range: 8–62
Circulating vitamin D at year 4 ^a , ng/ml [mean (SD)]	32.50 (13.50) range: 6.4–90.9		

SD standard deviation^aOnly 551 individuals at the second time point

Table 2

Results for the top 50 SNPs from the 4 statistical analyses

SNP	Chromosome	BP	Related gene(s)	p value population-based baseline	p value population-based year 4	Average population-based p value	p value family-based baseline	p value family-based year 4
rs26673	5	116,107,966		3.3×10^{-4}	3.6×10^{-4}	$3.4E-04$	$4.5E-03$	$4.5E-03$
rs26674	5	116,107,928		3.1×10^{-4}	5.6×10^{-4}	$4.4E-04$	$3.4E-03$	$3.4E-03$
rs5753362	22	29,594,281	OSBP2	4.2×10^{-4}	6.6×10^{-4}	$5.4E-04$	$1.6E-03$	$6.7E-03$
rs739894	22	29,588,108	OSBP2	6.5×10^{-4}	1.3×10^{-3}	$9.6E-04$	$9.4E-04$	$3.2E-03$
rs6837397	4	55,828,386		5.1×10^{-4}	1.4×10^{-3}	$9.8E-04$	$1.9E-02$	$6.4E-03$
rs7231689	18	42,532,983	ST8SIA5	1.9×10^{-4}	3.5×10^{-4}	$1.1E-03$	$8.6E-03$	$2.4E-06$
rs1119208	5	76,524,369		1.8×10^{-4}	4.3×10^{-4}	$1.1E-03$	$3.2E-02$	$4.4E-02$
rs4920935	5	116,134,125		2.6×10^{-4}	2.6×10^{-3}	$1.4E-03$	$6.1E-03$	$3.4E-02$
rs73737310	13	41,198,323	KIAA0564	1.8×10^{-3}	1.3×10^{-3}	$1.5E-03$	$5.3E-03$	$4.2E-02$
rs680617	5	116,007,082		3.2×10^{-3}	8.7×10^{-6}	$1.6E-03$	$1.1E-02$	$1.2E-03$
rs1458825	4	55,804,268		1.1×10^{-3}	2.3×10^{-3}	$1.7E-03$	$1.4E-02$	$1.8E-02$
rs6554253	4	55,812,933		1.1×10^{-3}	2.3×10^{-3}	$1.7E-03$	$1.3E-02$	$1.6E-02$
rs4301169	4	55,826,496		1.2×10^{-3}	2.5×10^{-3}	$1.8E-03$	$2.5E-02$	$7.6E-03$
rs4864972	4	55,837,040		3.6×10^{-3}	1.8×10^{-3}	$1.9E-03$	$6.8E-03$	$1.8E-03$
rs340286	3	160,065,698		4.5×10^{-3}	5.7×10^{-5}	$2.3E-03$	$1.7E-03$	$1.1E-03$
rs1948429	5	116,022,674		4.3×10^{-3}	5.0×10^{-4}	$2.4E-03$	$2.3E-02$	$1.1E-02$
rs1111721	16	67,255,962	CDH3	2.7×10^{-4}	$4.8E-03$	$2.5E-03$	$2.6E-02$	$1.3E-02$
rs12897195	14	61,029,474	PRKCH	1.8×10^{-4}	$5.0E-03$	$2.6E-03$	$3.4E-04$	$1.8E-04$
rs1888557	13	41,297,445	KIAA0564	2.7×10^{-3}	$2.5E-03$	$2.6E-03$	$2.1E-03$	$3.9E-02$
rs13026792	2	156,324,389		3.7×10^{-4}	$4.9E-03$	$2.7E-03$	$3.2E-02$	$1.5E-02$
rs12136973	1	173,406,942	KIAA0040	2.3×10^{-3}	$3.1E-03$	$2.7E-03$	$1.5E-03$	$1.4E-02$
rs1585289	4	55,797,233		5.5×10^{-3}	$9.3E-05$	$2.8E-03$	$7.8E-03$	$2.1E-02$
rs11002969	10	80,850,968	C10orf56	1.9×10^{-3}	$3.9E-03$	$2.9E-03$	$3.1E-02$	$3.9E-02$
rs1467118	4	55,865,036		5.9×10^{-3}	$4.8E-05$	$3.0E-03$	$1.2E-02$	$1.4E-03$
rs7843995	8	2,301,430		3.6×10^{-3}	$2.5E-03$	$3.1E-03$	$3.2E-02$	$4.0E-03$

SNP	Chromosome	BP	Related gene(s)	<i>p</i> value population-based baseline	<i>p</i> value population-based year 4	Average population-based <i>p</i> value	<i>p</i> value family-based baseline	<i>p</i> value family-based year 4
rs6847603	4	55,812,539		6.2×10^{-3}	1.0E-04	3.2E-03	6.6E-03	1.1E-02
rs163221	18	22,763,991	C18orf16	4.9×10^{-4}	6.0E-03	3.2E-03	1.4E-02	6.0E-03
rs1398765	3	132,964,104	CPNE4	6.7×10^{-4}	2.8E-05	3.4E-03	1.2E-02	4.5E-02
rs1319922	2	126,291,398		6.8×10^{-3}	3.0E-05	3.4E-03	6.1E-03	1.5E-02
rs4077725	4	55,851,899		2.3×10^{-3}	5.8E-03	4.0E-03	9.8E-03	1.5E-02
rs1389164	5	116,027,419		8.0×10^{-3}	3.3E-04	4.2E-03	-3.1E-02	-1.6E-02
rs3793825	10	71,357,498	COL13A1	1.6×10^{-3}	7.0E-03	4.3E-03	1.7E-03	3.0E-02
rs1739644	20	36,460,639		1.4×10^{-4}	7.6E-03	4.5E-03	2.2E-04	3.1E-03
rs4237510	10	120,983,651	GRK5	6.4×10^{-3}	2.5E-03	4.5E-03	-1.4E-02	-7.7E-05
rs4864977	4	55,855,356		1.7×10^{-3}	7.7E-03	4.7E-03	8.9E-03	1.8E-02
rs1678849	19	5,787,964	FUT6	8.2×10^{-3}	1.3E-03	4.7E-03	3.9E-02	8.6E-03
rs205651	2	74,709,631	LOC130951	9.5×10^{-3}	2.3E-05	4.7E-03	-1.6E-02	-2.4E-04
rs4864976	4	55,848,489		3.6×10^{-3}	6.3E-03	5.0E-03	1.7E-02	1.9E-02
rs17831158	8	57,630,069		6.8×10^{-3}	3.6E-03	5.2E-03	1.8E-04	1.8E-03
rs1406381	11	61,825,261	SCGB1D4	2.7×10^{-3}	7.6E-03	5.2E-03	-3.1E-03	-2.2E-02
rs2025323	9	110,162,093		7.9×10^{-3}	2.6E-03	5.2E-03	1.7E-02	3.4E-02
rs12785918	11	100,218,437		8.6×10^{-3}	2.2E-03	5.4E-03	-3.3E-02	-3.3E-02
rs1780634	20	36,463,239		1.7×10^{-3}	9.3E-03	5.5E-03	1.2E-04	8.9E-04
rs388383	5	111,731,534	EPB41L4A	4.9×10^{-4}	1.1E-02	5.5E-03	1.2E-02	4.6E-02
rs11013514	10	23,799,607		1.2×10^{-4}	1.1E-02	5.6E-03	-7.4E-04	-9.1E-03
rs13103626	4	99,536,274	RAP1GDS1	9.5×10^{-3}	2.2E-03	5.8E-03	1.5E-02	6.6E-03
rs232319	18	22,754,149	C18orf16	1.4×10^{-3}	1.2E-02	5.9E-03	3.3E-02	6.3E-03
rs2470911	15	42,834,426	RNF36	1.1×10^{-2}	6.6E-04	5.9E-03	-1.1E-02	-8.6E-04
rs137906	22	48,827,920		8.2×10^{-3}	3.9E-03	6.1E-03	3.9E-03	1.9E-03
rs26670	5	116,116,302		1.2×10^{-3}	1.1×10^{-2}	6.2E-03	-1.6E-02	-4.3E-03

SNP single nucleotide polymorphism, *BP* base pair distance

Table 3

SNPs that replicate in the Costa Rican cohort, with attempted replication in the Puerto Rico cohort

SNP	Chr	BP	Gene(s)	CAMP		Costa Rica asthma cohort			Puerto Rico asthma cohort			Combined (Liptak) p value
				β for pop-based	Average pop-based p value	MAF	β estimate	p value	β estimate	p value	MAF	
rs11002969	10	80,850,968	C10orf56/ ZCCHC24	-2.137	2.9E-03	0.46	-0.013	4.4E-02	0.44	0.8428	0.34	1.6E-02
rs163221	18	22,763,991	C18orf16, CHST9	-2.011	3.2E-03	0.46	-0.015	3.8E-02	0.43	0.1031	0.43	3.8E-04
rs1678849	19	5,787,964	FUT6	2.459	4.7E-03	0.29	0.019	3.6E-02	0.15	0.0649	0.26	2.7E-02
rs4864976	4	55,848,489	-	2.864	5.0E-03	0.15	0.019	4.6E-02	0.13	0.8248	0.24	7.4E-04

β estimates are obtained from linear regression models in PLINK

SNP: single nucleotide polymorphism, CHR: chromosome, BP: base pair distance, MAF: minor allele frequency

Table 4

Replication of the genome-wide significant SNPs found in Wang et al

SNP	CHR	Gene	Wang et al. <i>p</i> value	Direction of effect in Wang et al.	CAMP		Costa Rica asthma cohort		Puerto Rico asthma cohort		Combined (Liptak) <i>p</i> values	Direction of effect in CAMP, Costa Rica, and Puerto Rican cohorts		
					S3 pop- based <i>p</i> value*	E48 pop- based <i>p</i> value*	<i>p</i> value*	MAF	<i>p</i> value ^a	MAF				
rs2282679	4p12	<i>GC</i>	1.9E-109	Minor allele	undertransmitted	6.0E-06	3.8E-03	0.29	9.3E-04	0.22	3.2E-09	0.21	2.06E-14	Minor allele is undertransmitted
rs10741657 ^a	11q12	<i>CYP2R1</i>	2.1E-27	Minor allele	overtransmitted	3.7E-03	1.2E-04	0.43	0.0119	0.32	–	–	2.47E-4	Minor allele is undertransmitted
rs12785878 ^a	11p15	<i>DHCR7</i>	3.3E-20	Minor allele	undertransmitted	9.5E-01	9.7E-01	0.26	2.4E-05	0.47	–	–	0.037	Minor allele is overtransmitted

SNP single nucleotide polymorphism, *CHR* chromosome, *BP* base pair distance, *MAF* minor allele frequency

* One-sided *p* values

^a rs10741657 and rs12785878 were not genotyped in the Puerto Rico asthma cohort