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Associations between genetic variants in vitamin D metabolism and asthma characteristics in young African Americans: a pilot study

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Abstract

Introduction—Low vitamin D levels have been associated with asthma severity in children. Young, urban African Americans (AA) have high rates of hypovitaminosis D and asthma. Our objective was to determine associations between variants in vitamin D metabolism genes and asthma characteristics in a pilot study of young urban AAs.

Materials and Methods—Two urban AA cohorts of subjects aged 6 to 20 years (139 subjects with asthma and 74 subjects without asthma) were genotyped for 12 single nucleotide polymorphisms (SNPs) in 3 vitamin D metabolism genes: VDR (vitamin D receptor), CYP24A1 (cytochrome P450 vitamin D 24-hydroxylase), and CYP2R1 (cytochrome P450 vitamin D 25-hydroxylase). In a case-control analysis, SNPs were studied for associations with an asthma

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diagnosis. Within the asthmatic cohort, SNPs were analyzed for associations with quantitative asthma characteristics. All analyses were adjusted for age, gender, and BMI percentile.

Results—Only the CYP2R1 SNP rs10766197 homozygous minor genotype was associated with asthma ($P=0.044$). CYP24A1 SNP rs2248137 was associated with lower vitamin D levels ($P=0.006$). Within the asthma cohort, multiple significant associations between SNPs and asthma characteristics were identified; VDR SNP rs2228570 was associated with the higher nighttime asthma morbidity scores ($P=0.04$), lower baseline spirometric measures ($P<0.05$), ≥ 1 positive aeroallergen skin test ($P=0.003$), and increased IgE levels ($P<0.001$).

Discussion—This pilot study demonstrates that variants in vitamin D metabolism genes are associated with quantitative asthma characteristics in young, urban AAs. The collection of these associations provides evidence for the need for a large population-based study of vitamin D relevant SNPs in this cohort.

Keywords

asthma; vitamin D; African-American youth

Introduction

Asthma, a complex, multifactorial disease influenced by environmental and genetic factors¹, has become more prevalent over the past three decades for reasons that are unclear^{2, 3}. Its pathogenesis is characterized by an intricate immune response leading to inflammation, airway hyperresponsiveness, and mucus obstruction⁴⁻⁷.

Vitamin D has been long recognized as an important regulator of bone metabolism⁸, and recently, has also been linked to respiratory function, particularly in the setting of respiratory viral infections and asthma⁸⁻¹¹. The role of vitamin D in Th1/Th2 differentiation is of specific interest^{12, 13} as vitamin D deficiency has been linked to the development of other immune-mediated diseases such as Crohn's disease and type 1 diabetes^{14, 15}. In asthma, recent studies have shown that low vitamin D levels are linked to increased disease severity in older children¹⁶ and increased prenatal vitamin D intake may reduce childhood asthma incidence¹⁷.

The risk factors for both hypovitaminosis D and asthma are similar, and include urban living environments¹⁸, obesity¹⁹, and poor nutrition^{20, 21}. Additionally, both diseases occur at disproportionately high rates among young African Americans (AA), compared to other racial groups^{17, 22, 23}. Despite the growing link between these two highly prevalent conditions, there have been no studies investigating associations between variants within vitamin D metabolism genes and asthma in this population. Among the genes responsible for vitamin D metabolism, three are of particular interest. Variants within these three genes have been associated with asthma characteristics in Caucasians²⁴⁻²⁶: VDR (vitamin D [1,25-dihydroxyvitamin D₃; the most physiologically active form of vitamin D₃] receptor), CYP24A1 (cytochrome P450, family 24, subfamily A, polypeptide 1), and CYP2R1 (cytochrome P450, family 2, subfamily R, polypeptide 1). VDR, which has been shown to be expressed in respiratory epithelium²⁷, is the primary binding receptor for 1,25-dihydroxyvitamin D₃²⁸. It has also been mapped to chromosome 12q, an area of the genome with multiple loci previously associated with asthma²⁶. CYP24A1 encodes mitochondrial 1,25-dihydroxyvitamin D(3) 24-hydroxylase. Expressed in many tissues, including bronchial smooth muscle²⁹, it initiates the degradation of 1,25(OH)₂D₃ by hydroxylation³⁰. CYP2R1 encodes vitamin D 25-hydroxylase, a microsomal hydroxylase enzyme that converts vitamin D into the active ligand for the vitamin D receptor³¹. The localization and function of these gene products suggest an active role in airway function and disease (Table 1). Using the

twelve variants previously associated with asthma in Caucasians cohorts (Table 2), we hypothesize that these candidate SNPs are associated with asthma and quantitative asthma characteristics in young, urban African Americans.

Having already collected a cohort of 139 fully-phenotyped patients with asthma, we chose to conduct a pilot study to query the associations of known vitamin D-related SNPs with asthma characteristics. While this small sample size does not permit a full SNP association study, it does allow for initial inferences to be made that could potentially guide a larger population-based study.

Methods

Study Design

We conducted a case-control study paired with a cross-sectional study (within cases only) between April 2008 and June 2009 at Children's National Medical Center (CNMC), an urban pediatric medical center in Washington, DC. All cases and controls were studied in our Clinical Research Center. This study was approved by our Institutional Review Board. All participants and/or their guardians provided informed consent and all participants aged 6 to 17 years provided assent.

Study Cohorts

The Asthma Severity Modifying Polymorphisms (AsthMaP) Project based at CNMC provided the case data and samples for this study. AsthMaP is a single-center cross-sectional study designed to find associations among urban environments, genetics, and asthma. AsthMaP consists of a convenience sample of subjects aged 6 to 20 years, inclusive, with physician-diagnosed asthma for greater than one year. For this investigation, only self-identified AA subjects were included.

The control group consisted of a convenience sample of healthy urban AA children without asthma between the ages of 6 and 19 years, inclusive, enrolled in either a bone health study or a diabetes study at our medical center. None of the controls were receiving vitamin D supplementation at the time of inclusion in this study.

Phenotyping

Quantitative asthma characteristics were assessed within the asthma cohort. These included, but were not limited to, the following: 1) Pre- and post- short-acting beta-agonist (i.e. bronchodilator) spirometry measurements performed with a MedGraphics CPSP/D™ USB PC-based system (Medical Graphics Corporation, St. Paul, MN) using techniques validated in children³²; 2) Serum IgE and eosinophil measured using chemiluminescence with an Immulite 2000 system (Siemens Healthcare Diagnostics, Deerfield, IL); 3) standard aeroallergen skin prick/puncture testing using the MultiTest II device (Lincoln Diagnostics, Decatur, Illinois); 4) Parental interviews incorporating the Integrated Therapeutics Group's (ITG) Child Asthma Short Form^{33, 34} as a measure of asthma quality of life, National Institutes of Health, National Asthma Education and Prevention Program (NAEPP) 2007 criteria³⁵ to classify chronic asthma severity, Asthma Control Test™ (ACT) (QualityMetric Incorporated, Lincoln, RI) to assess asthma control, and additional asthma severity assessment questions.

Vitamin D Measurement

Circulating 25(OH)D is considered the best determinant of vitamin D sufficiency³⁶. The IDS 25-Hydroxy Vitamin D Direct EIA kit (Immunodiagnostic Systems Ltd., Fountain Hills, AZ) has been established as a reliable measure of both 25-hydroxyvitamin D isoforms

(i.e. D₂ and D₃)³⁷. Using this kit, direct enzyme immunoassay was performed on plasma from cases and controls to measure vitamin D levels. Simultaneous plasma and serum 25-hydroxyvitamin D levels measured by a similar chemiluminescent assay were reported to be equivalent³⁸.

Gene and Single Nucleotide Polymorphism Selection

As described above, candidate genes were chosen based on their role in vitamin D metabolism (Table 1)^{10, 29, 39, 40}. Twelve single nucleotide polymorphisms (SNPs) were selected from a SNP database (dbSNP; *see* <http://www.ncbi.nlm.nih.gov/SNP>) for analysis based on previously described asthma association studies in Caucasians cohorts (Table 2)^{24–26, 41}. These previous studies selected SNPs based on the following criteria: unique genomic position, more than one submitted validation, location not present in repeat regions, for tagging haplotypes and/or being functionally relevant.

Genotyping

Genomic DNA was isolated from blood using a Puregene® kit (Quigen, Valencia, CA). Genotyping was done using TaqMan allele discrimination assays (Applied Biosystems, Foster City, CA). Both alleles were detected simultaneously using allele-specific oligonucleotides labeled with different fluorophores, and genotypes automatically determined by the ratio of the two fluorophores used from the TaqMan Genotyping Universal PCR Master Mix (Applied Biosystems). PCR was performed on a 9700 Thermocycler (Applied Biosystems). Allele calling was performed on an ABI 7900HT (Applied Biosystems).

Population Stratification

A panel of 24 highly informative ancestry markers described by Kosoy et al. was genotyped in the case and control groups in order to determine whether population stratification exists within either group⁴². The existence of population clusters within the cases and controls was determined using STRUCTURE 2.3⁴³. The Bayesian Markov Chain Monte Carlo Method was run for different values of K (2 – 5) with a burn length of 50,000 and a run length of 50,000 replications after burn. The parameters of the runs included: 1) admixture model using prior population information; 2) independent allele frequencies; 3) INFERALPHA set to 1 and allowed to vary.

Asthma Characteristics Tested for SNP Associations

All twelve SNPs were tested for associations with the following selected characteristics: serum eosinophil level, serum IgE level, vitamin D level (log₁₀ transformed), ACT Score, ITG Composite Score, ITG Functional Limitations score, ITG Daytime Score, ITG Nighttime Score, ≥1 positive skin prick test. Additionally, the following spirometric studies were included: pre-bronchodilator testing for FEV₁ (% predicted forced expiratory volume in 1 second), FEV₁/FVC (% FEV₁ divided by forced vital capacity) and FEF_{25–75} (% predicted of forced expiratory flow [average] during expiration of 25–75% total forced expiratory volume) and change after bronchodilator testing for FEV₁ (% predicted), FEV₁/FVC (%) and FEF_{25–75} (% predicted).

Statistical Analyses

Hardy-Weinberg equilibrium (HWE) was determined by χ^2 testing. Descriptive statistics and frequencies were tested by Student's T-tests and χ^2 testing. Two-tailed P Values ≤ 0.05 were considered significant. Population stratification was determined using an unpaired t-test. Linear regression analysis was performed to determine SNP associations for continuous phenotypic variables. Multinomial logistic regression analysis was performed to determine

case-control SNP associations. When appropriate, continuous variables were log₁₀ transformed to approximate a normal distribution. Odds ratios (OR), β coefficient (β) and P Values were corrected for age, gender, and body mass index (BMI) percentile in both genotypic and dominant model analyses. Additionally, vitamin D levels were adjusted for season of sampling. Only significant associations ($P < 0.05$) have been listed; any characteristic not listed was not significant. All statistical tests were performed with SPSS Statistics 17.0 (SPSS Inc., Chicago, IL). All associations were tested in the genotypic (i.e. analyze homozygous minor, homozygous major, and heterozygote genotypes individually) and dominant (i.e. combine heterozygote and homozygote minor and analyze separately from homozygous major genotypes) models.

Results

Descriptive Analyses

Of the AA children and adolescents in the AsthmaP cohort ($n=139$), 58% were male, the mean BMI percentile for age (SE) was 71.5% (2), and the mean age (SE) was 11.2 (0.3) years. Notable asthma-relevant characteristics included mean serum IgE = 583 (82) IU/mL, 56% prevalence of atopic dermatitis, 50% prevalence of parental history of asthma, and 40% prevalence of one or more positive allergen skin prick test. Based on National Institutes of Health, NAEPP criteria³⁵, 90% had persistent asthma. As previously reported, the median vitamin D level for a subset of our asthma cohort ($n=85$) was 18.5 ng/mL [interquartile range (IQR): 11.3, 25.1]⁴⁴. A detailed description of select measured asthma characteristics can be found in Table 3.

Controls

The control cohort ($n = 74$) had significantly fewer males (35%; $P < 0.001$) and a significantly higher mean BMI percentile of 86% (3) ($P < 0.001$) than the asthma cohort (Table 3). The mean age (SE) of 11.8 (0.5) years was not significantly different from the asthma cohort. As previously reported, the median vitamin D level for a subset of our control cohort ($n=21$) was 40.4 (IQR: 34.6, 49.5)⁴⁴.

Population Stratification

The smallest log probability estimated by STRUCTURE 2.3 was for $K=2$ for the control group, followed closely by $K=3$ and $K=4$ (both groups had similar log probabilities), and $K=2$ for the case group, followed by $K=3$. This suggests there is population structure beyond the self-reported African American ethnicity and that both cases and controls descended from at least two ancestral populations. The $K=2$ model appears to distinguish two major clusters, one with a combined African and European ancestry and another with combined Native American and East Asian ancestry. African/European ancestry represents 86.3% and 80.2% of ancestry in the control and case cohorts, respectively. Native American/East Asian represents 13.7% and 19.8% of ancestry in the control and case cohorts, respectively. There was no significant difference in European/African ancestry clusters ($P=0.1$) or Native American/Asian ancestry clusters ($P=0.1$) between cases and controls.

Vitamin D Metabolism Gene Variants Associated with Asthma Diagnosis

In the case-control study, all SNPs were evaluated for association with the diagnosis of asthma (Table 4). Only the CYP2R1 SNP rs10766197 homozygous minor genotype was significantly associated with asthma ($P=0.044$).

Vitamin D Metabolism Gene Variants Associated with Vitamin D Level

Within the asthma cohort, among subjects for which levels were measured, all SNPs were evaluated for associations with vitamin D level. Only CYP24A1 SNP rs2248137 was significantly associated with a decrease in vitamin D level. This was noted in both the genotypic [$B = -0.09$ (95% CI: -0.15 to -0.03); $P=0.006$] and dominant [$B = -0.06$ (-0.11 to -0.01); $P=0.012$] models.

Vitamin D Metabolism Gene Variants Associated with Quantitative Asthma Phenotype Traits

In the cross-sectional study (within the asthma cohort only) all SNPs were evaluated for associations with quantitative asthma characteristics.

VDR Gene Variants—In the VDR gene, six polymorphisms were significantly associated with quantitative asthma characteristics. SNP rs3782905 was significantly associated with three spirometric measures: a lower pre-bronchodilator FEV₁/FVC (% predicted), a greater % increase in FEV₁/FVC change with bronchodilator, and a lower pre-bronchodilator FEF_{25–75} (% predicted). SNP rs2228570 (also known as *FokI*) was significantly associated with three spirometric measures as well: a lower pre-bronchodilator FEV₁/FVC (% predicted), a greater increase in FEV₁/FVC % change with bronchodilator, and a lower pre-bronchodilator FEF_{25–75} (% predicted). Additionally, two VDR polymorphisms, rs2107301 and rs1540339, were associated with significantly higher serum eosinophil levels, and one SNP, rs2228570, was associated with significantly higher IgE levels (IU/mL). Associations for SNPs rs7975232 (known as *ApaI*) and rs22359185 are also listed in Table 5.

Cytochrome P450 Gene Variants—As previously stated, CYP24A1 SNP rs2248137 was the only variant of the 12 candidate SNPs to display a significant association with circulating levels of vitamin D. Additionally, CYP24A1 rs2248137 was also significantly associated with a higher pre-bronchodilator FEV₁/FVC (%), a higher pre-bronchodilator FEF_{25–75} (% predicted), and a decrease in FEV₁/FVC (%) change with bronchodilator. CYP2R1 SNP rs10766197 was significantly associated with lower pre-bronchodilator FEF_{25–75} (% predicted) (Table 6).

Discussion

This pilot study of variants in vitamin D metabolism genes among AA youth with asthma identified only a single homozygous minor genotype associated with the diagnosis of asthma, but found multiple other associations between variants in vitamin D metabolism genes and asthma characteristics. When examined individually, each significant association may be considered unremarkable, however as a group, the collection of SNP associations representing several aspects of the asthmatic phenotype is quite compelling. Together, this suggests that variants in vitamin D metabolism genes play a significant role in the variable phenotypic expression of asthma within AA youth.

With a 75% increased prevalence over the past thirty years, asthma currently affects more than 14 million people in the United States⁴⁵. This trend is disproportionately represented in specific racial groups, including AA children^{46, 47}. The prevalence of asthma among AA youth is 105 per 1,000 – significantly greater than the prevalence in all U.S. children (83 per 1,000)⁴⁸. Despite environmental, cultural and socioeconomic differences, the racial morbidity and mortality disparities that exist in asthma suggest a genetic influence. In particular, inner-city AA children have been shown to have more severe asthma (i.e. lower FEV₁ and increased bronchial hyperresponsiveness) than their inner-city Caucasian counterparts⁴⁹.

Additionally, asthma prevalence, hospitalization and emergency department visits decline with increasing income in non-AA children, but not in AA children, implying that lifetime income and socio-demographic characteristics do not explain the excess risks of asthma among AA children⁵⁰. Furthermore, children with severe asthma demonstrating a poor response to directly observed oral steroid therapy were more likely to be AA, suggesting that this racial group may be genetically predisposed to steroid resistant asthma⁵¹.

The epidemiologic patterns of vitamin D deficiency mirror many of those seen in asthma, including its link to obesity⁵², AA race²² and an inner-city environment¹⁸. Similar to the disparities seen in asthma, AA children are also disproportionately affected with hypovitaminosis D^{17, 22, 23, 38}. While vitamin D has been previously implicated in innate immunity through Th1-Th2 regulation^{12, 13}, recent studies have investigated the role of vitamin D in asthma. For example, dietary and serum vitamin D levels have been inversely associated with spirometric values, suggesting that current vitamin D status may have an effect on lung function⁵³. In two distinct birth cohorts, higher maternal vitamin D intakes during pregnancy were inversely associated with wheezing prevalence in children 3 and 5 year old, suggesting the timing (prenatal vs. post natal) of vitamin D administration may have an effect on respiratory disease^{9, 17}. In steroid resistant asthma, administration of vitamin D reversed resistance through induction of IL-10-secreting T-regulatory cells⁵⁴. Despite the similar risk factors and studies linking vitamin D and asthma within the AA population, no study has evaluated the contribution of vitamin D metabolism genes in asthma development and severity in a young AA population.

We performed a pilot study using a cohort comprised of young, urban African Americans – one with a high prevalence of both asthma and hypovitaminosis D – in whom we attempted to determine associations between variants in vitamin D metabolism genes and asthma characteristics⁴⁴. Several of the twelve candidate SNPs we studied have been previously associated with asthma characteristics in other racial populations (Table 1). A recent study of the VDR gene in Caucasian French Canadian families demonstrated associations between six alleles (rs3782905C, rs1540339A, rs2239185C, rs2239185G, *BsmIG*, and *TaqIT*) with asthma and four alleles (rs2239185C, *BsmIG*, *ApaIC*, and *TaqIT*) with atopy ($P < 0.05$), respectively^{25, 26}. In this same population, three alleles (rs2239185C, *ApaIC*, and *TaqIT*) were significantly associated with higher IgE levels. In the Childhood Asthma Management Program (CAMP; predominately Caucasian children) cohort, the *ApaI* SNP was significantly associated with asthma in the overall population ($P = 0.01$)²⁶. Additionally, in a German adult cohort, a 5 point frequent CYP24A1 haplotype (rs2296241; rs17219315; rs276942; rs2248137; rs2248359) was significantly associated with a diagnosis of asthma ($P = 0.001$) and total IgE ($P = 0.004$)²⁴. This study also reported a significant association between CYP2R1 SNP rs10766197 and IgE level ($P = 0.028$).

Similar to the aforementioned studies, the VDR gene analysis within our asthma cohort was suggestive of a more severe asthmatic phenotype, as evidenced by the six polymorphisms displaying associations with the worsening of at least one asthma characteristic. Within our cohort, rs7975232, rs2239185, rs2107301, rs1540339, rs3782905 and rs2228570 were significantly associated with lower pre-bronchodilator spirometric values as well as a significantly increased change in spirometric values after bronchodilator administration, both of which are attributes of increased asthma severity.

Two of these polymorphisms (rs7975232 and rs2228570) are known restriction sites, suggesting they confer their effect through gene regulation. Of particular interest in our study, rs2228570, an amino acid substitution (methionine>threonine) restriction site polymorphism, was significantly associated with asthma characteristics implicating several aspects of asthma heterogeneity including lung function (spirometry), immune response and

atopy (serum IgE and eosinophil levels, allergen skin testing), and asthma morbidity (ITG functional limitations score). Recent studies have implicated this variant in several diseases related to altered immune and hormone regulation such as ovarian⁵⁵ and thyroid⁵⁶ carcinoma, type 1 diabetes⁵⁷, and regulation of calcium homeostasis⁵⁸. To our knowledge, our study is the first to demonstrate significant associations between rs2228570 and asthma characteristics. The multitude of findings for rs2228570 in our cohort, coupled with the findings in other disease processes, strongly suggests that this mutation conveys an immunoregulatory effect through alterations in vitamin D metabolism.

With respect to the cytochrome P450 genes, CYP24A1 polymorphisms were significantly associated with decreased asthma control (rs2296241), higher baseline lung function (rs2248137), decreased response to bronchodilator (rs17219315, rs2248137, and rs2248359) and a decrease in 25(OH)D level (rs2248137). The one CYP2R1 polymorphism, rs10766197, located in the promoter region, was significantly associated with decreased baseline lung function (similar to the spirometric findings of the VDR polymorphisms) and implies lower baseline lung function.

The apparent contradictory findings noted for rs2248137 (decreased vitamin D levels and higher baseline lung function) appear to conflict with previous studies linking low vitamin D levels to increased asthma susceptibility^{16, 59}. Ultimately, our findings only highlight the fact that asthma is a complex disease influenced by many factors that play a role in disease severity and susceptibility.

We recognize that population stratification may be an issue in this study and our findings could be due to varying allele frequencies between our case and control cohorts. Our population stratification analysis demonstrated no statistically significant difference in genetic ancestry between cases and controls. Taking this population clustering a step further, future analysis replicated in a larger population may define unique risk groups based on genetic ancestry that are more likely to have SNPs related to vitamin D metabolism and the asthmatic phenotype.

One of the main limitations of this study is that several endpoints were tested for multiple SNPs. This could potentially weaken our study after correcting for multiple testing. For example, using the Bonferroni method to adjust for multiple testing, our p value for significance becomes 0.0003, suggesting that all the associations discussed above may not be significant. However, methods used to correct for multiple testing (i.e. Bonferroni method) assume independence among tests, which is not the case for the SNPs and asthma phenotype traits that we analyzed. Therefore, we have chosen to maintain a significance level at 0.05, and we have not adjusted for multiple testing, given the inherent interdependence of our variables.

Another concern was the use of healthy controls from other studies within our institution as a convenience sample for controls within our case-control study. Specifically, many of the control subjects were obese, and, as previously discussed, obesity is known to be associated with asthma and vitamin D deficiency. To address this issue, all analyses for this study were corrected for BMI percentile. No other co-morbid conditions were noted in our control cohort.

Another major limitation to our study is our sample size; we realize significantly larger cohorts are necessary for a case control SNP association study. However, our intentions were not to conduct a SNP association study, but rather to undertake a pilot study among fully phenotyped children in hopes of providing insight on this population for future genotypic analyses of large randomized control trials of vitamin D supplementation in

asthma. Our next step is to apply our results to a larger population-based study of AA youth with asthma and validate these findings in a replication population.

Conclusion

In summary, the presence of multiple associations between vitamin D metabolism gene polymorphisms and asthma characteristics supports the notion that vitamin D metabolism is important in asthma and may play a role in the phenotypic variability found in young AA with asthma. While we have validated previously identified associations between vitamin D metabolism genes and asthma characteristics found in other races, we have also identified several novel associations that suggest vitamin D metabolism contributes to alterations in lower airway obstruction, innate immunity, asthma morbidity, and asthma control. The fact that the genes selected for analysis comprise a small sample of those contributing to vitamin D metabolism suggests that we are observing only a fraction of the complex influence that vitamin D metabolism has on asthma. To fully appreciate the relationship between vitamin D and asthma, future research in this field should consider analyzing all vitamin D metabolism genes. Additionally, mechanistic studies exploring causality for specific polymorphisms, such as rs2228570, may implicate genetic alterations in vitamin D metabolism as an important medical issue for a specific subset of asthmatics. This type of discovery could potentially change clinical management for these patients.

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TABLE 1
CANDIDATE VITAMIN D METABOLISM GENES ASSOCIATED WITH ASTHMA

Gene Name	HUGO Symbol	Chromosome	Position(Mb)*	SNPs Genotyped	Evidence as Candidate Gene
Vitamin D Receptor	VDR	12	46.521–46.585	7	Associated with asthma and allergies [10, 25, 26, 39]. Expressed on respiratory epithelium [40].
Cytochrome P450, family 24, subfamily A, polypeptide 1 (Vitamin D 24-hydroxylase)	CYP24A1	20	52.203–52.224	4	Associated with asthma and serum IgE levels [58]. Expressed in bronchial smooth muscle [29].
Cytochrome P450, family 2, subfamily R, polypeptide 1 (Vitamin D 25-hydroxylase)	CYP2R1	11	14.878–14.865	1	Associated with asthma and serum IgE levels [58].

* Position based on dbSNP build 129 (<http://genome.ucsc.edu>)

TABLE 2
SELECTED POLYMORPHISMS IN CANDIDATE VITAMIN D METABOLISM GENES

Gene	dbSNP rs#	Position [*]	Location	Restriction Site	Alleles
VDR	rs731236	46,525,024	Ile352Ile	<i>TaqI</i>	C>T
	rs7975232	46,525,104	Intron 8	<i>ApaI</i>	A>C
	rs2239185	46,530,826	Intron 6	-	C>T
	rs2107301	46,541,837	Intron	-	T>C
	rs1540339	46,543,593	Intron 3	-	A>G
CYP24A1	rs3782905	46,552,434	Intron 2	-	G>C
	rs2228570	46,559,162	Met1Thr	<i>FokI</i>	C>T
	rs2296241	52,219,626	Ala184Ala	-	A>G
	rs17219315	52,221,853	Intron	-	A>G
	rs2248137	52,223,150	Intron	-	G>C
CYP2R1	rs2248359	52,224,925	Locus	-	T>C
	rs10766197	14,878,456	Unknown	-	G>A

^{*} Position based on dbSNP build 129 (<http://genome.ucsc.edu>)

[†] Frequency refers to that of the second allele listed for all samples analyzed.

TABLE 3

CLINICAL CHARACTERISTICS OF SUBJECTS

Characteristic	Cases (n=139)	Controls (n=74)	P Value
Gender, % male	58	35	0.001
Age, yr (SE)	11.2 (0.3)	11.8 (0.5)	<0.001
Body mass index percentile (SE)	71.5 (2)	85.8 (3)	0.33
25-OH Vitamin D ^Δ , ng/mL (IQR)	18.5 (11.3, 25.1)	40.4 (36.6, 49.5)	0.002
Age of asthma onset, yr (SE)	2.7 (0.3)		
Premature birth, %	25		
Allergic rhinitis, %	76		
Atopic dermatitis, %	56		
Parent with asthma, %	50		
Sibling with asthma, %	67		
FEV ₁ (% predicted) change with bronchodilator, mean (SE)	8.6 (1.3)		
Post-bronchodilator FEV ₁ (% predicted), mean (SE)	90.7 (1.4)		
FEV ₁ /FVC (%) change with bronchodilator, mean (SE)	7.0 (7.4)		
Post-bronchodilator FEV ₁ /FVC(%), mean (SE)	82.0 (1.5)		
FEF ₂₅₋₇₅ (% predicted) change with bronchodilator, mean (SE)	32.2 (3.4)		
Post-bronchodilator FEF ₂₅₋₇₅ (% predicted), mean (SE)	93.0 (3.5)		
Nasal wash eosinophils, % (SE)	47.6 (3.5)		
Nasal wash neutrophils, % (SE)	35.7 (3.3)		
Serum IgE, IU/mL (SE)	583 (82)		
Blood eosinophils, %	4.8 (0.4)		
One or more positive allergen skin prick tests, %	40		
NAEPP* step level, %			
1 - Intermittent	10		
2 - Mild Persistent	23		
3 - Moderate Persistent	35		
4 - Severe Persistent	32		
ACT [†] Score, mean (SE)	20.1 (0.3)		

Characteristic	Cases (n=139)	Controls (n=74)	P Value
ITG [‡] Scores, mean (SE)			
<i>Composite</i>	75.8 (1.6)		
<i>Daytime Symptoms</i>	70.3 (2.0)		
<i>Nighttime Symptoms</i>	74.0 (2.1)		
<i>Functional Limitations</i>	83.0 (1.8)		

* National Asthma Education and Prevention Program [35]

[‡] Asthma Control Test[™]

[‡] Integrated Therapeutics Group's ITG Child Asthma Short Form

[^] Cases; n=85; Controls n=21; Adjusted for age, gender, body mass index percentile, and season of sampling

SE: Standard error of the mean

IQR: Interquartile Range

P Values < 0.05 are indicated in **bold**.

TABLE 4
ALLELIC AND GENOTYPIC ASSOCIATION ANALYSIS OF VITAMIN D METABOLISM GENES WITH ASTHMA

Gene	dbSNP rs#	Asthmatics (n=139)	Controls (n=74)	OR (95%CI)	Adjusted* P Value	Genotype	Asthmatics (n=139)	Controls (n=74)	Adjusted* P Value
VDR	rs731236					AA	52 (0.44)	40 (0.54)	-
	A	0.67	0.75	1.00	0.31	AG	55 (0.47)	31 (0.42)	0.43
	G	0.33	0.25	1.38 (0.74–2.57)		GG	11 (0.09)	3 (0.04)	0.25
	rs7975232					AA	55 (0.44)	35 (0.48)	-
	A	0.68	0.72	1.00	0.55	AC	59 (0.47)	33 (0.46)	0.73
	C	0.32	0.28	1.21 (0.65–2.25)		CC	11 (0.09)	4 (0.06)	0.28
	rs2239185					AA	42(0.35)	23 (0.32)	-
	A	0.61	0.58	1.00	0.85	AG	65 (0.53)	34 (0.48)	0.87
	G	0.39	0.44	0.94 (0.4–1.81)		GG	15 (0.12)	14 (0.20)	0.32
	rs2107301					GG	82 (0.66)	46 (0.03)	-
	G	0.80	0.80	1.00	0.96	AG	35 (0.28)	26 (0.35)	0.60
	A	0.20	0.20	0.99 (0.52–1.88)		AA	7 (0.06)	2 (0.62)	0.17
	rs1540339					CC	77 (0.62)	38 (0.54)	-
	C	0.78	0.76	1.00	0.47	CT	40 (0.32)	30 (0.43)	0.28
	T	0.22	0.24	0.79 (0.42–1.49)		TT	7 (0.06)	2 (0.03)	0.33
	rs3782905					GG	63 (0.52)	45 (0.62)	-
	G	0.72	0.79	1.00	0.26	GC	48 (0.40)	26 (0.36)	0.37
	C	0.28	0.21	1.44 (0.77–2.70)		CC	10 (0.08)	2(0.03)	0.28
	rs2228570					CC	76 (0.62)	42 (0.57)	-
	C	0.79	0.76	1.00	0.72	CT	41 (0.34)	29 (0.39)	0.73
CYP24A1	T	0.21	0.24	0.89 (0.47–1.68)		TT	5 (0.04)	3 (0.04)	0.90
	rs2296241					GG	29 (0.23)	19 (0.27)	-
	G	0.51	0.52	1.00	0.87	AG	71 (0.56)	36 (0.51)	0.94
	A	0.49	0.48	0.94 (0.45–1.97)		AA	26 (0.21)	16 (0.22)	0.57
	rs17219315					AA	121 (0.97)	70(0.96)	-
	A	0.98	0.68	1.00	0.57	AG	3 (0.02)	1 (0.01)	0.71
	G	0.02	0.03	0.63 (0.12–3.18)		GG	1 (0.01)	2 (0.03)	0.23
	rs2248137					GG	57 (0.47)	37 (0.51)	-

Gene	dbSNP rs#	Asthmatics (n=139)	Controls (n=74)	OR (95%CI)	Adjusted* P Value	Genotype	Asthmatics (n=139)	Controls (n=74)	Adjusted* P Value
CYP2R1	G	0.67	0.71	1.00		GC	49 (0.40)	30 (0.41)	0.80
	C	0.33	0.29	1.06 (0.57–1.97)	0.84	CC	16 (0.13)	6 (0.08)	0.29
	rs2248359					TT	47 (0.39)	34 (0.49)	-
	T	0.66	0.55	1.00		TC	65 (0.54)	26 (0.37)	0.13
	C	0.34	0.45	1.39 (0.73–2.62)	0.30	CC	9 (0.07)	10 (0.14)	0.43
CYP2R1	rs10766197					GG	84 (0.70)	49 (0.46)	-
	G	0.79	0.84	1.00		GA	22 (0.18)	21 (0.30)	0.19
	A	0.21	0.16	0.98 (0.50–1.94)	0.96	AA	15 (0.12)	1 (0.24)	0.044

Allelic and genotypic frequencies are shown in parentheses.

Adjusted P Values < 0.05 are indicated in **bold**.

* Adjusted for age, gender, and BMI percentile

TABLE 5
SIGNIFICANT VDR SNP ASSOCIATIONS WITH ASTHMA CHARACTERISTICS

dbSNP rs#	Characteristic	Genotypic Model		Dominant Model [‡]	
		Adjusted Parameter Estimate* (95%CI)	Adjusted* P Value	Adjusted Parameter Estimate* (95%CI)	Adjusted* P Value
rs7975232	FEF ₂₅₋₇₅ (% predicted) change with bronchodilator	11.2 (−0.4 to 22.7)	0.06	7.95 (0.63 to 15.27)	0.034
rs2239185	FEV ₁ /FVC (%) pre-bronchodilator	−3.0 (−7.3 to 1.3)	0.16	−3.3 (−6.2 to −0.4)	0.025
rs2107301	[†] Serum Eosinophil Level	0.20 (0.06 to 0.33)	0.004	0.09 (0.01 to 0.18)	0.028
rs1540339	[†] Serum Eosinophil Level	0.18 (0.05 to 0.32)	0.008	0.09 (0.01 to 0.18)	0.029
rs3782905	FEV ₁ /FVC (%) pre-bronchodilator	−3.9 (−8.3 to 0.5)	0.08	−3.2 (−6.0 to −0.4)	0.025
rs3782905	FEV ₁ /FVC (%) change with bronchodilator	4.7 (0.9 to 8.4)	0.016	2.7 (0.4 to 5.2)	0.022
rs3782905	FEF ₂₅₋₇₅ (% predicted) pre-bronchodilator	−8.8 (−16.6 to 1.0)	0.08	−7.8 (−14.1 to −1.6)	0.014
rs2228570	≥1 Positive Skin Prick Test		N/A [^]	3.8 (1.57 to 9.18)	0.003
rs2228570	[†] Serum IgE Level (IU/mL)	0.34 (0.12 to 0.56)	0.002	0.24 (0.11 to 0.37)	<0.001
rs2228570	[†] ITG Functional Limitations Score	−0.04 (−0.1 to −0.002)	0.043	−0.03 (−0.52 to 0.0)	0.052
rs2228570	FEV ₁ /FVC (%) pre-bronchodilator	−5.9 (−10.9 to −0.9)	0.022	−3.7 (−6.8 to −0.7)	0.017
rs2228570	FEV ₁ /FVC (%) change with bronchodilator	5.1 (0.8 to 9.5)	0.022	2.4 (−0.3 to 5.1)	0.08
rs2228570	FEF ₂₅₋₇₅ (% predicted) pre-bronchodilator	−11.5 (−23.0 to −0.1)	0.049	−7.0 (−13.9 to 0.0)	0.050

[‡] Dominant model: Presence of at least 1 minor allele for the given SNP

* Adjusted for age, gender, and BMI percentile

Parameter estimate for linear regression analyses expressed as B

Parameter estimate for logistic regression analyses expressed as Exp(B)

[^] Only dominant model analysis was performed for outcomes with nominal values

[†] Outcome was transformed to a log10 scale

Adjusted P Values ≤ 0.05 are indicated in **bold**.

TABLE 6
SIGNIFICANT CYTOCHROME P450 SNP ASSOCIATIONS WITH ASTHMA CHARACTERISTICS

Gene	dbSNP rs#	Characteristic	Genotypic Model		Dominant Model [‡]	
			Adjusted Parameter Estimate* (95%CI)	Adjusted* P Value	Adjusted Parameter Estimate* (95%CI)	Adjusted* P Value
CYP24A1	rs2296241	Asthma Control Test™	-0.60 (-1.60 to 0.40)	0.24	-0.80 (-1.58 to -0.02)	0.044
	rs17219315	FEV ₁ /FVC (%) change with bronchodilator	-15.5 (-25.6 to -5.5)	0.003	-12.5 (-19.0 to -6.0)	<0.001
	rs2248137	FEV ₁ /FVC (%) pre-bronchodilator	4.2 (0.2 to 8.3)	0.041	2.8 (-0.1 to 5.7)	0.055
	rs2248137	FEV ₁ /FVC change with bronchodilator	-4.8 (-8.3 to -1.4)	0.007	-2.6 (-5.1 to -0.06)	0.045
	rs2248137	FEF ₂₅₋₇₅ (%) predicted/pre-bronchodilator	9.7 (0.5 to 18.9)	0.039	6.2 (-0.3 to 12.7)	0.63
	rs2248359	FEV ₁ /FVC (%) change with bronchodilator	-5.1 (-9.2 to -1.1)	0.014	-3.7 (-6.2 to -1.2)	0.005
CYP2R1	rs10766197	FEF ₂₅₋₇₅ (%) predicted/pre-bronchodilator	-10.4 (-19.2 to -1.7)	0.020	-6.8 (-13.5 to -0.4)	0.049

[‡] Dominant model: Presence of at least 1 minor allele for the given SNP

* Adjusted for age, gender, and BMI percentile

Parameter estimate for linear regression analyses expressed as B

[†] Outcome was transformed to a log10 scale

Adjusted P Values < 0.05 are indicated in **bold**.