

Association of Vitamin D Receptor Gene Polymorphisms with Childhood and Adult Asthma

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Vitamin D receptor (VDR) polymorphisms have been associated with several immune-related diseases, and VDR and vitamin D itself modulate T cell differentiation. VDR maps to chromosome 12q, near a region commonly linked to asthma. We evaluated VDR as part of a 12q positional candidate survey, and in response to observations of VDR polymorphism associations with asthma and atopy in a founder population of Quebec. Twenty-eight loci in 7 positional candidates (7 in VDR) were genotyped in 582 families. Whereas other candidates demonstrated no association, the VDR *Apal* polymorphism demonstrated significant transmission distortion, with undertransmission of the C allele in a ratio of 4:5 ($p = 0.01$). This association was most prominent in girls, in whom distortion was more marked ($p = 0.009$). Sex-specific associations between multiple VDR polymorphisms and immunoglobulin E levels were also observed ($p = 0.006$ – 0.01). Asthma associations were replicated in a second cohort (517 females with asthma and 519 matched control subjects): 4 of 6 VDR variants demonstrated significant association ($p = 0.02$ – 0.04). The direction of association in this second cohort was opposite to the effects seen in the trios, but similar to findings in the Quebec study. These results suggest that VDR influences asthma and allergy susceptibility in a complex manner.

Keywords: association studies; asthma; genetics; replication; VDR

The vitamin D receptor (VDR; OMIM 601769) is the principal receptor that binds $1\alpha,25$ -dihydroxyvitamin D_3 [$1,25$ -(OH) $_2D_3$] and is a critical molecule in calcium metabolism and bone turnover. Rare nonsynonymous mutations in VDR cause vitamin D-resistant rickets (Type IIA), and more common variants contribute to variability in bone mineral density and susceptibility to osteoporosis. In addition to the central role of VDR in bone metabolism, it has long been recognized as an important contributor to a diverse array of cellular processes (1). VDR belongs to a family of nuclear proteins that act as transcriptional regulatory factors and is structurally similar to both steroid hormone and thyroid hormone receptors (2). Data suggest that vitamin D and

a normally functioning VDR are important for proper development of the immune system (3–5). Vitamin D supplementation in a murine model of pulmonary eosinophilic inflammation alters cytokine expression profiles, immunoglobulin E levels, and the pattern of airway eosinophilia during allergen sensitization, suggesting that the vitamin D pathway may influence the development of allergy and asthma (6). Importantly, VDR polymorphisms have been implicated in several immune and inflammatory disorders, including mycobacterial and human immunodeficiency virus susceptibility (7–9), diabetes (10, 11), psoriasis (12), and Crohn's disease (13), although the precise mechanisms of action of these diverse disease-related effects remain speculative.

VDR maps to the long arm of chromosome 12, a region that has been commonly linked to asthma and allergy-related phenotypes in genome-wide linkage analyses (reviewed in Raby and coworkers [14]). We and others have previously demonstrated linkage with asthma to a genomic region at the centromeric end of chromosome 12q that includes the VDR locus (14–16). In a companion article (17), Poon and colleagues report associations between VDR polymorphisms and haplotypes with asthma and atopy susceptibility in a founder population from northeastern Quebec. As part of our ongoing efforts to identify the genetic determinants of the asthma linkage on chromosome 12q, we screened several biologically relevant positional candidate genes, using a family-based association study design, including VDR (Table 1). Whereas variation in the other candidate genes evaluated did not demonstrate significant associations with asthma, VDR was significantly associated with asthma and related phenotypes. These effects were strongest in girls. Additional testing in a second cohort of women also revealed evidence of association between VDR and asthma. Together with the initial associations described in the Quebec cohort, these findings suggest that VDR polymorphisms contribute to asthma and atopic risk.

METHODS

Populations

The Childhood Asthma Management Program (CAMP) is a multicentered North American clinical trial designed to investigate the long-term effects of inhaled antiinflammatory medications in children with mild to moderate asthma (18, 19). Of the 1,041 children enrolled in the original clinical trial, 968 children and 1,518 of their parents contributed DNA samples. Five hundred and eighty-two complete nuclear families (including 55 families with additional affected offspring) are included in the analysis presented here: 471 are of non-Hispanic Caucasian descent, 64 are of African American descent, and 47 are of Hispanic descent. A diagnosis of asthma was based on methacholine hyperactivity (PC_{20} [provocative concentration causing a 20% fall in FEV_1] no greater than 12.5 mg/ml) and one or more of the following criteria for at least 6 months in the year before recruitment: (1) asthma symptoms at least two times per week, (2) at least two uses per week of an inhaled bronchodilator, and (3) daily asthma medication. Spirometry was performed according to American Thoracic Society recommendations, us-

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TABLE 1. ASTHMA POSITIONAL CANDIDATE GENES IN CENTROMERIC REGION OF CHROMOSOME 12q

Gene Name	HUGO Symbol	Position (Mb)*	SNPs Genotyped	Minimum FBAT p Value with Asthma	Evidence as Positional Candidate (Reference)
Vitamin D receptor	VDR	46.521–46.585	7	0.01	Implicated in T cell differentiation. Associated with asthma in French Canadian asthma cohort (17)
Signal transducer and activator of transcription-6	STAT6	55.775–55.791	2	0.11	Implicated in IL-4 and IgE production (63)
Interleukin-1 receptor-associated kinase-3	IRAK3	64.869–64.928	6	0.23	IL-1 and LPS receptor signal transduction and innate immunity gene (64)
Interferon- γ	IFNG	66.835–66.839	2	0.46	Critical modulator of T cell differentiation. Linkage and association of repeat marker and asthma previously demonstrated (60)
Interleukin-22	IL22	66.928–66.933	2	0.15	Inhibits Th2 lymphocyte production of IL-4 (65)
Carboxypeptidase M	CPM	67.534–67.613	6	0.91	Mediates bradykinin-induced airway reactivity (66, 67)
Stem cell factor/c-Kit ligand	KITLG	87.393–87.476	3	0.62	Induces mast cell degranulation and airway smooth muscle contraction (68)

Definition of abbreviations: FBAT = family-based association testing; HUGO = Human Genome Organisation; SNP = single-nucleotide polymorphism; Th2 = helper T cell Type 2.

* Position refers to the distance from the p-terminal end of chromosome 12q, based on the July 2003 freeze of the human genome (<http://genome.ucsc.edu>).

ing a volume displacement spirometer, and methacholine challenge was performed according to the Wright nebulizer tidal breathing technique (18). Total blood eosinophil counts were performed by center-specific methods, and total serum IgE was measured in radioimmunosorbent assays from blood samples collected during the screening sessions of the CAMP study.

The Nurses' Health Study (NHS) is a questionnaire-based longitudinal cohort study of 120,000 women, aged 30–55 years, initiated in 1976 (20–22). Between 1988 and 1996, 1,596 incident physician-diagnosed asthma cases were reported. An evaluation of the validity of a questionnaire-based asthma diagnosis revealed that only 5% of women who originally met diagnostic criteria raised issues suggesting misdiagnosis (23). DNA has been made available for 517 cases and 519 control subjects of European ancestry, matched for age. No lung function or allergy phenotypes are available for this population. To test for population stratification in this cohort, we tested 49 unlinked single-nucleotide polymorphisms (SNPs) for evidence of association, as suggested by Prichard and Rosenberg (24). The global χ^2 was 60.2, with a corresponding p value of 0.13, given 49 degrees of freedom (the number of loci tested). On the basis of this analysis, there is no evidence of significant population stratification in this data set.

Informed assent and consent were obtained from the study participants and their parents to collect DNA for genetic studies in CAMP. The Institutional Review Board of Brigham and Women's Hospital (Boston, MA), as well as those of the other CAMP study centers, approved the studies. Approval by the Institutional Review Board of Brigham and Women's Hospital was obtained for DNA collection in the NHS as well.

Polymorphism Genotyping

SNPs were selected from the SNP database (dbSNP; [see http://www.ncbi.nlm.nih.gov/SNP/](http://www.ncbi.nlm.nih.gov/SNP/)) that mapped to unique genomic positions, were double-hit SNPs (i.e., more than one submitter), and were not in repeat regions. Included were three VDR SNPs (*TaqI*, *ApaI*, and *FokI*) that have previously been implicated in other disorders (25, 26). SNP genotyping was performed on the basis of unlabeled minisequencing reactions and mass spectrometry analysis as implemented in the Sequenom MassARRAY platform (Sequenom, San Diego, CA), and using the 5' $\rightarrow$ 3' exonuclease assay as implemented in the TaqMan assay (Applied Biosystems, Foster City, CA) (27). Protocol details and primer data are available at <http://wchanning.bwh.harvard.edu/epigenetics/Projects>. Duplicate genotyping

was performed on about 10% of the sample to assess genotype reproducibility. Genotype data quality was assessed on the basis of several criteria including completeness of genotype data, degree of discordant duplicate genotyping, evidence of non-Mendelian marker inheritance, and Hardy–Weinberg disequilibrium among parental data.

Statistical Analysis

Parental (in CAMP) and control (in NHS) genotype data were used to assess Hardy–Weinberg equilibrium and pairwise linkage disequilibrium (LD). Hardy–Weinberg equilibrium was tested at each SNP locus, using an exact method (28). LD between each pair of SNP loci was evaluated by a maximum likelihood method (29) to infer phase for dual heterozygotes and was expressed as r^2 (30). Haplotypes were inferred by Bayesian methods (31) as implemented in the PHASE package (32). For the familial data, haplotypes were confirmed and determination of haplotype block structure was performed with Haploview (<http://www.broad.mit.edu/personal/jcbarret/haplo/>). Blocks were defined on the basis of the Gabriel definition ($D' > 0.9$; minimum allele frequency, 5%) (33).

In CAMP, ethnicity-specific evidence of association with asthma was evaluated by family-based association analysis with FBAT version 1.4 (34, 35), stratified by ethnic group. Quantitative trait analysis, stratified by ethnic group, was performed with PBAT (36, 37) for the following intermediate phenotypes: postbronchodilator FEV₁, bronchodilator (BD) response ([post-BD FEV₁ – pre-BD FEV₁]/pre-BD FEV₁), post-BD FEV₁/FVC ratio, airway hyperresponsiveness to methacholine (log-transformed PC₂₀), and log-transformed total serum IgE levels and total serum eosinophils. PBAT allows for variable transformation and covariate adjustment, stratified analysis, and tests for gene–covariate interaction. All variables were z-transformed, and the following covariate adjustments were included: (1) FEV₁, FEV₁/FVC, and BD response were adjusted for age, sex, and height to the second order; and (2) PC₂₀, IgE, and eosinophils were adjusted for age and sex. Gene–sex interactions also were evaluated. Evidence of haplotype-block association with asthma was assessed by the likelihood-ratio score test implemented in TRANSMIT (38). Tests for global significance of all haplotypes were performed. Haplotype analysis was restricted to the Caucasian cohort, given the limited sample size for the other ethnic groups in CAMP.

For the nested case–control association analysis among the NHS population, single-SNP associations were performed with SAS/Genetics

(version 8.2; SAS Institute, Cary, NC). SNPs were assessed for both genotypic and allelic associations, as well as the Armitage trend test. Significant associations ($p < 0.05$) were then explored further by specifying either dominant or recessive models by recoding genotypes 11 versus 12 or 22, and 11 and 12 versus 22, respectively. Haplotype analysis was performed with haplo.stat (39).

SAS/Genetics version 8.2 and Web-based bioinformatic tools (<http://innateimmunity.net>) were used to manage and analyze the data.

RESULTS

CAMP Genetics Cohort Characteristics

The baseline characteristics of the CAMP participants genotyped in this study are presented in Table 2. The cohort is primarily Caucasian and 62% male. Ninety percent of the cohort was atopic (defined as either having at least one positive skin-prick test [3 mm or more] or an elevated IgE [100 IU/ml or more]). Age of asthma symptom onset was significantly higher among girls, and girls had higher baseline lung function and tended to be less atopic than boys. There are no significant differences in asthma severity or lung function between those children included in this study compared with the original CAMP cohort (data not shown).

Positional Candidates for Asthma on Chromosome 12q

We genotyped 28 polymorphisms in 7 candidate genes that map to the centromeric region of chromosome 12q and tested for evidence of association with asthma (Table 1). Polymorphisms in IFNG, STAT6, CPM, KITLG, IL22, and IRAK3 did not show significant evidence of transmission distortion from parent to asthmatic offspring, suggesting a lack of association with disease status. Although we have tested only a limited number of SNPs at these loci, other groups have reported negative associations with several of these genes in other asthmatic cohorts, suggesting that variants in these genes are probably not important genetic determinants of asthma susceptibility, despite the documented functional importance of these genes in asthma-related biological pathways (40–42).

Association of VDR *Apal* SNP with Asthma in CAMP

Seven VDR polymorphisms were genotyped in 582 nuclear families. Genotype quality was high, with an average completion rate of 97.3%, no discordances on repeat genotyping of a random 10% of the cohort, and a low rate of Mendelian inconsistencies (less than 1/1,000 transmissions).

Table 3 shows the results of the family-based associations for the seven VDR SNPs in the Caucasian subgroup. In contrast to the lack of association with the other candidate genes tested, the intronic *Apal* polymorphism demonstrated evidence of associa-

tion with asthma: among informative matings, the C allele was undertransmitted to affected Caucasian offspring in a ratio approaching 4:5 (transmitted:untransmitted [T:U] ratio for the C allele, 199:251; $p_{\text{FBAT}} = 0.01$). Similar results were observed when the analysis was performed excluding the 38 Caucasian families with multiple affected offspring ($p_{\text{FBAT}} = 0.01$), confirming that the results were due to association, rather than to linkage at this region. Similar results were also observed when the analysis was performed with all ethnic groups together ($p_{\text{FBAT}} = 0.01$), but the subsets of other ethnicities were insufficiently powered to detect these effects when analyzed individually. Stratified analysis by sex revealed that the association was essentially restricted to girls: despite a marked reduction in sample size (with only 159 informative trios compared with 450 in the unstratified data), the *Apal* C allele demonstrated more striking transmission distortion, with a T:U ratio approaching 2:3 ($p_{\text{FBAT}} = 0.009$). In contrast, the degree of transmission distortion to the male offspring was minimal (T:U ratio, 136:155; $p_{\text{FBAT}} = 0.27$). No other VDR SNP demonstrated evidence of significant association with asthma (unstratified or sex stratified), although the rs2239185 C allele demonstrated trends of transmission distortion similar to those of the *Apal* C allele (T:U ratio of 4:4.8 in unstratified analysis, $p_{\text{FBAT}} = 0.09$; and T:U ratio of 2:2.6, $p_{\text{FBAT}} = 0.12$ among girls).

Family-based quantitative analyses for intermediate asthma-related phenotypes were performed with PBAT, which can accommodate adjustment for covariates and gene-by-environment interactions. Among the Caucasians, there were statistically significant (but weak) associations between VDR polymorphisms and asthma severity phenotypes. The *FokI* C allele was associated with greater baseline airways obstruction (i.e., lower pre-bronchodilator FEV₁/FVC ratio, $p = 0.009$), whereas G alleles in SNPs rs2239179 and rs1540339 were associated with lower bronchodilator responsiveness ($p = 0.009$ and $p = 0.02$, respectively). However, these effects were small, with locus-specific heritability estimates less than 1% each (data not shown). There were no significant associations detected in the other ethnic groups, but power was limited because of the small sample size. There were no significant associations between VDR polymorphisms and other tested phenotypes in unstratified analysis, including baseline FEV₁, methacholine responsiveness, total serum IgE levels, or total blood eosinophil counts.

Like the single-SNP associations with the binary asthma phenotype, stratification of the cohort by sex in the quantitative analysis revealed striking differences between boys and girls in the relationships between VDR SNPs and asthma-related intermediate phenotypes (Table 4). The most notable differences were seen in the impact of VDR polymorphisms on serum IgE

TABLE 2. BASELINE CHARACTERISTICS OF CHILDHOOD ASTHMA MANAGEMENT PROGRAM PROBANDS INCLUDED IN VITAMIN D RECEPTOR ANALYSIS

Variable	Girls (n = 238)	Boys (n = 399)	p Value
Race			0.36
Caucasian	197 (82.7%)	315 (79.9%)	
African American	25 (10.5%)	45 (11.3%)	
Hispanic	16 (6.7%)	39 (9.8%)	
Mean age, yr	8.2 (2.1)	8.1 (2.1)	0.41
Median age at onset of asthma symptoms, yr	4.00 (2.0–6.0)	3.00 (2.0–5.0)	0.007
Mean FEV ₁ , % predicted	104.9 (12.2)	101.9 (12.8)	0.003
Median PC ₂₀ , mg/ml	1.03 (0.72–1.49)	1.00 (0.73–1.49)	0.89
Median total serum eosinophils, cells/mm ³	436.5 (199.5–691.8)	467.7 (229.1–676.1)	0.94
Median total serum IgE, IU/ml	398.1 (128.8–1,258.9)	467.7 (204.2–1,122.0)	0.35
Total serum IgE level greater than 100 IU/ml, count	185 (78.1%)	336 (85.5%)	0.02
One or more positive skin prick tests, count	202 (84.87%)	358 (89.72%)	0.07

Definition of abbreviations: PC₂₀ = provocative concentration of methacholine causing a 20% fall in FEV₁.

TABLE 3. ASSOCIATION ANALYSIS OF VITAMIN D RECEPTOR WITHIN CHILDHOOD ASTHMA MANAGEMENT PROGRAM CAUCASIAN FAMILIES WITH ASTHMA

dbSNP rs#	Chromosome Position (Mb)*	Gene Location	Restriction Site	Alleles	Frequency [†]	Unstratified		Girls		Boys	
						T:U Ratio [‡]	p Value [§]	T:U Ratio [‡]	p Value [§]	T:U ratio [‡]	p Value [§]
rs2228570	46559162	Met1Thr	<i>FokI</i>	C > T	0.38	232:227	0.85	91:76	0.24	141:151	0.52
rs3782905	46552434	Intron 2	—	C > G	0.33	223:200	0.24	84:74	0.43	139:126	0.39
rs2239179	46544033	Intron 3	—	A > G	0.44	229:215	0.51	88:85	0.82	141:130	0.50
rs1540339	46543593	Intron 3	—	A > G	0.34	211:222	0.6	82:95	0.33	140:116	0.13
rs2239185	46530826	Intron 6	—	T > C	0.49	170:203	0.09	57:75	0.12	113:128	0.33
rs7975232	46525104	Intron 8	<i>Apal</i>	A > C	0.47	199:251	0.01	63:96	0.009	136:155	0.26
rs731236	46525024	Ile352Ile	<i>TaqI</i>	T > C	0.37	246:231	0.46	96:81	0.26	150:150	0.95

Definition of abbreviations: dbSNP = single-nucleotide polymorphism database; Ile = isoleucine; Met = methionine; rs# = dbSNP SNP reference number; T:U ratio = transmitted:untransmitted ratio; Thr = threonine.

* Chromosome 12 position (in megabases from the p-terminal), using Human Genome Browser Build 16 (July 2003).

[†] Frequency refers to that of the second allele listed.

[‡] T:U ratio refers to ratio of transmitted and untransmitted alleles of the second allele listed.

[§] p Values are those from the FBAT association test, assuming an additive genetic model.

levels. Whereas the unstratified analysis showed no significant relationship, four of seven VDR polymorphisms contribute to the variance in serum IgE levels in girls ($p = 0.006$ – 0.03), but not in boys ($p = 0.21$ – 0.81). To assess the stability of these results, we repeated the quantitative analysis, using IgE measurements from a follow-up visit during Year 4 of the clinical trial. Several SNPs (rs1540339 and *TaqI*) again demonstrated significant relationships to IgE levels in girls, but not in boys, suggesting that the relationship is consistent over time. Sex differences in the relationship between VDR and eosinophil level were also observed, but with fewer SNPs and without evidence of reproducibility at the Year 4 follow-up visit.

Haplotype Structure of VDR in CAMP

To better understand the relationship between the asthma-associated and IgE-associated SNPs, linkage disequilibrium analysis was assessed in the CAMP Caucasian parents. Strong evidence for LD was observed at the 3' end of the gene, with $r^2 = 0.97$ for SNPs rs2239185 and *Apal* (both with r^2 of 0.6 with *TaqI*). These three SNPs showed associations with asthma and IgE, and form a haplotype block associated with asthma (see below). SNPs rs3782905, rs2239179, and rs1540339 demonstrated some evidence of LD with each other and with three more 3' SNPs, but these relationships were weak ($r^2 = 0.14$ – 0.57). Two of these

three SNPs (rs1540339 and rs2239179) showed evidence of association with IgE. The *FokI* SNP was not linked to the other six in this cohort and showed no evidence of association with either asthma or IgE. This suggests an important haplotype block structure at the 3' end of VDR.

Haplotype block analysis of the seven VDR SNPs was performed with Haploview and the block definition proposed by Gabriel and coworkers (33). Two major blocks were observed, each with three major haplotypes, accounting for more than 90% of all haplotypes detected (Figure 1). This structure is similar to that observed by Poon and coworkers in a French Canadian cohort of patients with asthma (17), suggesting a conserved haplotype structure of the VDR locus in Caucasians. Identical block structure was observed in the Hispanic group, although the frequency distributions of haplotypes within each block were different. Linkage disequilibrium in the African Americans differed slightly, with much weaker LD between rs2239185 and *Apal*. As a result, the block structure among the African Americans was different, with less block structure at the 3' end of the gene (Figure 1).

On the basis of this haplotype structure, we tested the VDR blocks for evidence of association with asthma, using TRANSMIT. Because of the limited number of non-Caucasian trios, haplotype analysis was limited to the Caucasian trios. The termi-

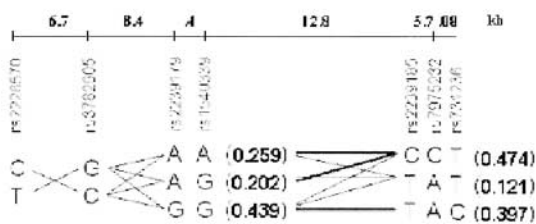
TABLE 4. QUANTITATIVE TRAIT ANALYSIS OF VITAMIN D RECEPTOR POLYMORPHISMS IN CHILDHOOD ASTHMA MANAGEMENT PROGRAM CAUCASIAN FAMILIES

Marker	Allele	Unstratified		Girls		Boys	
		No. Info. Fam.	p Value*	No. Info. Fam.	p Value*	No. Info. Fam.	p Value*
Log-transformed serum total IgE							
rs2239179	G	305	0.35	120	0.006	195	0.24
rs1540339	G	316	0.13	129	0.03	198	−0.81
rs2239185	C	268	−0.47	99	−0.03	172	0.37
TaqI	C	342	0.47	137	0.01	214	0.21
Log-transformed total blood eosinophils							
FokI	T	331	0.45	129	0.794	217	0.04
rs2239179	G	305	0.24	120	0.014	195	0.77
rs1540339	G	316	0.84	129	−0.046	198	0.65
FEV ₁ /FVC ratio							
FokI	T	331	0.009	128	0.38	217	−0.006
rs2239185	C	267	−0.81	98	0.68	172	−0.03

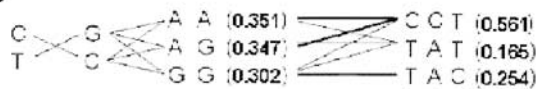
Definition of abbreviations: No. Info. Fam. = number of informative families.

* Negative sign indicates that the presence of the allele confers lower trait values; the absence of a negative sign indicates that the allele confers higher values. Analysis presented assumes additive genetic model.

Caucasian



Hispanic



African American

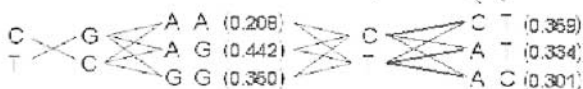


Figure 1. Haplotype block structure of vitamin D receptor in Childhood Asthma Management Program (CAMP). Inter-SNP (single-nucleotide polymorphism) distance is expressed as kilobases (kb). Haplotypes observed at greater than 1% are presented. Fractions in parentheses denote haplotype frequency observed in CAMP parents. Line thickness reflects frequency of adjacent block haplotype distribution (thick lines, > 10%; thin lines, > 1%).

nal three-SNP haplotype block (rs2239185–*ApaI*–*TaqI*) was associated with asthma in the Caucasian trios (global test of significance, $p = 0.02$), with overtransmission of haplotypes TAT and TAC, and with undertransmission of haplotype CCT. The consistency of these results with the single SNP analysis is not surprising, given that the *ApaI* C allele (shown to be significantly undertransmitted in the single SNP analysis) resides only on the CCT haplotype.

Linkage Disequilibrium, Haplotype Block Structure, and Association Testing in VDR Genomic Region

Within 200 kb of VDR reside other attractive biological candidates for asthma susceptibility genes (see Figure 2). Most notable is Type IIA collagen (COL2A1), located within 100 kb of VDR. Collagens (particularly Types I and III) are critical molecules in extracellular matrix repair and play important roles in airway remodeling—a process that contributes to asthma severity and remission (43). Although Type II collagen has not been implicated in asthma previously, the associations observed with the VDR SNPs here could be explained by linkage disequilibrium between these associated VDR SNPs and polymorphisms in adjacent asthma-related genes. To better understand the relationship between VDR and adjacent genes, and because the proximal portion of chromosome 12q has been linked to asthma in several genome-wide and regional linkage studies of asthma, we genotyped a set of polymorphisms across a 330-kb region centered around VDR to evaluate the extent of regional LD and the haplotype block structure and tested these polymorphisms for association with asthma in the CAMP cohort. Twenty-nine polymorphisms were selected from the dbSNP and genotyped in the nuclear families (a complete list of genotyped SNPs can be found with supplemental information at <http://wchanning.bwh.harvard.edu/epigenetics/Projects>). The median intermarker distance was 4.1 kb, with several large gaps flanking VDR (about 55 kb upstream and 89 kb downstream) because of reduced dbSNP availability of SNP data between genes in this region.

As can be seen in Figure 2, linkage disequilibrium across this 330-kb region was discrete, with clusters of LD observed within three gene clusters (P11–HDAC7A, VDR, and COL2A1), but not between them. Because of the lack of genotype information for the region immediately surrounding VDR, we were unable to characterize the precise extent of VDR blocks. However, there was no evidence of meaningful LD between VDR SNPs and those mapping near or within other candidate genes along the segment, indicating historical recombination around VDR, and suggesting that the VDR locus can be considered a distinct genetic unit in disease association studies. In keeping with this notion, testing of the surrounding non-VDR SNPs for association with asthma did not reveal any significant evidence of transmission distortion (data not shown), suggesting that the VDR single SNP and haplotype associations observed are unlikely to be due to effects of variation in neighboring genes.

Replication of VDR–Locus Association with Asthma among Women

Given that we tested multiple candidate genes, multiple polymorphisms, and multiple phenotypes, our initial evidence of associations may be due to chance. To address this possibility and to evaluate whether VDR associations with asthma are more broadly generalizable, we genotyped six of the seven VDR SNPs in a second cohort of adult women with asthma and age-matched control subjects ascertained through the NHS. We were unable to reliably genotype the seventh, rs2239179. In contrast to the single SNP association noted in the CAMP cohort, four of the six SNPs tested demonstrated evidence of association with asthma in the NHS asthmatic cohort, particularly under an additive model of genetic risk, as assessed by the Armitage trend test (Table 5). The risk conferred by VDR polymorphisms was modest, and the greatest risk was noted among subjects carrying two copies of risk alleles. For example, homozygotes for a C allele at either *ApaI* or the tightly linked rs2239185 were at 1.4 times the risk of asthma compared with those with one or fewer copies (95% confidence interval, 1.03–1.91; $p = 0.02$). Interestingly, the minor allele of *TaqI* demonstrated protective effects in a recessive model (odds ratio, 0.68; 95% confidence interval, 0.47–0.98; $p = 0.04$), suggesting important haplotype effects. In fact, the rs2239185–*ApaI*–*TaqI* haplotype block associations seen in the CAMP cohort were also evident in this NHS cohort (Table 6), shedding some light on the single SNP associations: the CCT haplotype (which is the only one bearing the C alleles at rs2239185 or *ApaI*) was significantly overrepresented in the cases, whereas the TAC haplotype (the only one bearing the *TaqI* C allele) was significantly underrepresented.

Although significant associations are evident in both the CAMP and NHS cohorts, noteworthy differences in the nature of the associations were observed. Most importantly, compared with the associations in the CAMP cohort, the direction of the SNP and haplotype associations in the NHS appear to be reversed. For SNP *ApaI*, the C allele is overrepresented among NHS cases (49.9 versus 44.6%, $p = 0.03$), appearing to confer asthma risk, whereas this same allele is undertransmitted to affected asthmatic offspring in CAMP (thereby appearing protective). This difference is also observed at the haplotype level, with the CCT haplotype conferring risk in the NHS cohort, while undertransmitted in CAMP cohort. Of note, the direction of these SNP and haplotype associations in the NHS cohort is consistent with those observed by Poon and colleagues, who observe strong SNP and haplotype associations with asthma and atopy in asthma families from northeastern Quebec (17). Together these results suggest that although the VDR locus harbors variants that contribute to the development of asthma and allergy, the relationships are complex.

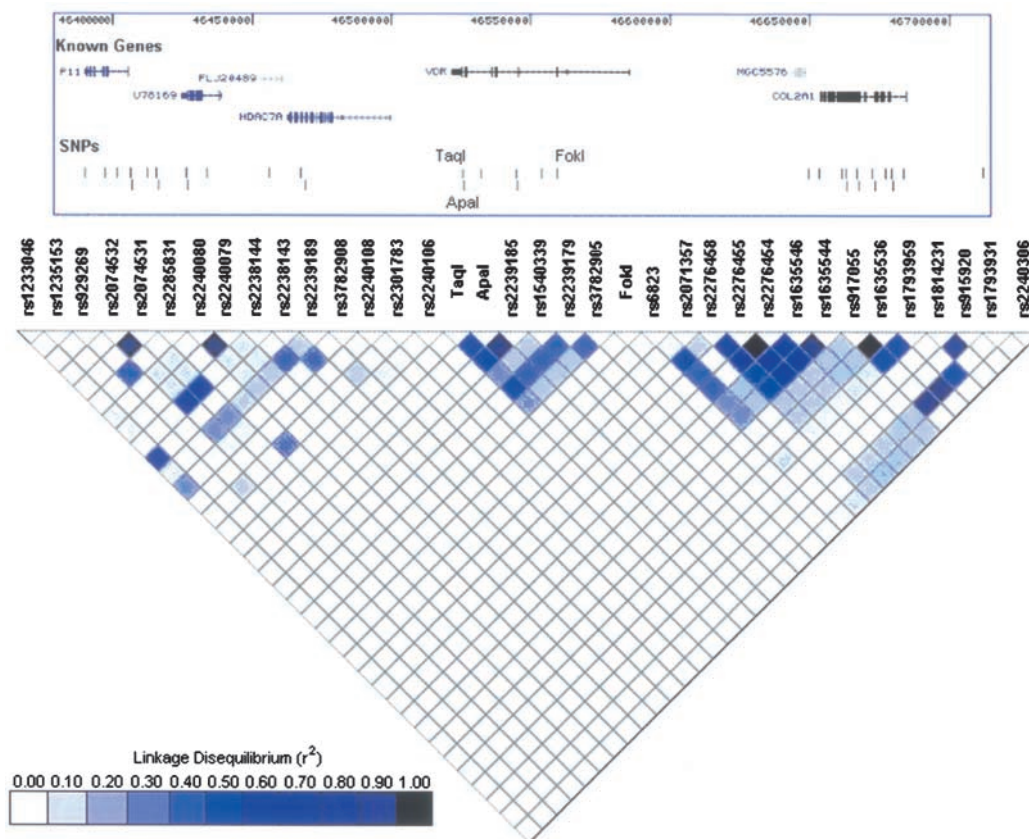


Figure 2. Linkage disequilibrium map across the genomic region surrounding VDR. Known genes mapped to chromosome 12q13.11 (bp46,389,340–46,722,774) in July 2003 freeze of the human genome. Gene symbols: P11 = 26 serine protease; EPAC = Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP; FLJ20489 = hypothetical protein FLJ20489; HDAC7A = histone deacetylase 7A; VDR = vitamin D (1,25-dihydroxyvitamin D₃) receptor; MGC5576 = hypothetical protein MGC5576; COL2A1 = collagen (Type II, α₁). Relative orientation of genes is denoted by intronic arrows. Strength of pairwise linkage disequilibrium (expressed as r^2) for parental data is plotted for the 36 SNPs typed in the CAMP cohort.

DISCUSSION

The results presented here provide independent replication of association of genetic variation at the VDR locus with asthma and related phenotypes in two cohorts. The results are similar to those observed in a family-based study in a French-Canadian founder population (17), and together suggest that VDR has a

role in the development of asthma and allergy. This is in keeping with the growing body of evidence that VDR and its ligand, 1α,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃], are important mediators of immunity and play a central role in the differentiation of naive T lymphocytes toward a helper T cell Type 2 (Th2; allergy-related) lineage. Experimental data suggest that 1,25-(OH)₂D₃ inhibits macrophage production of interleukin-12 (3),

TABLE 5. ASSOCIATION ANALYSIS OF VITAMIN D RECEPTOR SINGLE-NUCLEOTIDE POLYMORPHISMS IN NURSES' HEALTH STUDY ASTHMA COHORT

Locus-based Analysis					Genotype-based Analysis				
Locus	Allele	Control Subjects (n = 519)	Cases (n = 517)	p Value	Genotype	Control Subjects (n = 519)	Cases (n = 517)	p Value	
rs2228570 (FokI)	C	627 (0.621)	598 (0.617)	0.84	CC	191 (0.378)	175 (0.361)	0.71	0.84
	T	383 (0.379)	372 (0.384)		CT	245 (0.485)	248 (0.511)		
					TT	69 (0.137)	62 (0.128)		
rs3782905	C	668 (0.669)	715 (0.718)	0.02	CC	228 (0.457)	254 (0.510)	0.04	0.02
	G	330 (0.331)	281 (0.282)		GC	212 (0.425)	207 (0.416)		
					GG	59 (0.118)	37 (0.074)		
rs1540339	G	634 (0.625)	599 (0.592)	0.12	GG	192 (0.379)	175 (0.346)	0.25	0.12
	A	380 (0.375)	413 (0.408)		GA	250 (0.493)	249 (0.492)		
					AA	65 (0.128)	82 (0.162)		
rs2239185	T	544 (0.554)	494 (0.501)	0.02	TT	157 (0.320)	132 (0.268)	0.07	0.02
	C	438 (0.446)	492 (0.499)		TC	230 (0.468)	230 (0.466)		
					CC	104 (0.212)	131 (0.266)		
rs7975232 (Apal)	A	551 (0.562)	495 (0.514)	0.03	AA	162 (0.331)	139 (0.288)	0.10	0.04
	C	429 (0.438)	469 (0.487)		AC	227 (0.463)	217 (0.450)		
					CC	101 (0.206)	126 (0.261)		
rs731236 (TaqI)	T	599 (0.587)	697 (0.633)	0.03	TT	181 (0.355)	230 (0.400)	0.09	0.04
	C	421 (0.413)	373 (0.367)		TC	237 (0.465)	237 (0.466)		
					CC	92 (0.180)	68 (0.134)		

TABLE 6. HAPLOTYPE ASSOCIATION ANALYSIS IN NURSES' HEALTH STUDY ASTHMA COHORT

Haplotype	Frequency		p Value
	Cases	Control Subjects	
CCT	0.495	0.442	0.02
TAT	0.137	0.137	0.9
TAC	0.358	0.414	0.01

Three-locus haplotype block characterized by rs2239185, *Apal*, and *TaqI*. Haplotype inference and association testing was performed with haplo.stat (39). p Values refer to haplotype-specific score tests. Global test for haplotype significance p value was 0.06.

inhibits peripheral blood lymphocyte production of interferon- γ (44), and suppresses helper T cell Type 1 (Th1) cytokine production while preserving Th2 cytokine expression, including interleukin-4 (4), thus favoring an allergic cytokine profile. VDR is expressed in activated T lymphocytes (45, 46), and splenocytes from mice deficient in VDR demonstrate a Th2 cytokine profile (reduced interferon- γ , increased interleukin-4), likely in part due to a shift in T cell lineage expansion from Th1 to Th2, supporting a critical role for VDR in immune regulation (5). Finally, circumstantial epidemiologic evidence suggests that the increase in allergic disease in developed nations may be due in part to vitamin D fortification of maternal and newborn diets (47), although more direct evaluation of this hypothesis is necessary.

The VDR associations observed in the CAMP cohort appear to be most pronounced in girls. Sex affects many important features of asthma and allergy, including lung growth and development (48, 49), age of symptom onset, and disease severity (50–52). There is evidence from twin studies that sex influences the heritability of asthma (53). Modification of genotype–phenotype correlation by sex is well documented, including the sex-specific effects of VDR polymorphisms on bone mineral density and skeletal growth (54, 55). However, sex also appears to influence the effects of VDR polymorphisms on other immune-mediated disease phenotypes, including Type 1 diabetes mellitus (10). These effects may be hormonally mediated, and the effects of VDR on the development of allergy and asthma also may be mediated in a similar fashion. In this study, the case–control population studied was composed of women only. Although positive associations were observed in this cohort as well, replicating the observations of VDR SNP association with asthma in women, we were not able to evaluate the role of VDR in asthma susceptibility in a second cohort of men, thereby precluding any further investigation for sex-specific effects in this study.

An important discrepancy in our findings is the direction of asthma risk conferred by given VDR alleles in the CAMP cohort compared with those observed in the NHS cohort (and the Quebec cohort). Whereas the *Apal* C allele seems to confer increased risk in both the Quebec and NHS cohorts (the C allele is overtransmitted to affected offspring in the Quebec cohort and is overrepresented in NHS asthma cases compared with control subjects), it is undertransmitted to affected offspring and thus appears protective in CAMP. Conflicting results such as these are unfortunately common in genetic association studies (56, 57). In one meta-analysis, reports of significant allelic effects in a direction opposite to the original report of association were observed in 11 of 25 gene associations examined (57). Moreover, similar contradictory results have been observed in studies of the vitamin D receptor and its role as a determinant of bone

mineral density (25, 54, 58, 59) and susceptibility to tuberculosis (reviewed by Poon and coworkers [17]). Although these outlier studies may be due to chance and publication bias (of positive results, regardless of direction of association), it is possible that both sets of associations are real and are dependent on other important (and unrecognized) covariates or variants.

There are differences between these cohorts that could explain these discrepant results. Although differences in the genetic composition of these cohorts are possible, this is an unlikely explanation for the observed results, for several reasons. Although the Quebec cohort represents a more homogeneous population (and may be genetically distinct), the associations observed in that cohort also were seen in the more heterogeneous NHS cohort. In addition, the allele frequencies, linkage disequilibrium patterns, and haplotype distributions across the three populations are similar, and the significant associations with common variants were seen in all three cohorts. Finally, although Poon and colleagues resequenced the coding regions of VDR, they were not able to identify additional variants that could explain their associations. However, we are not at this time able to conclude which (if any) of the genotyped variants are disease-causing, or whether these are in linkage disequilibrium with the true disease susceptibility variants.

Phenotypic heterogeneity and differing ascertainment schema are another explanation for the discrepancy. The most obvious difference between the CAMP and NHS (and Quebec) cohorts is the age of the populations: CAMP consisted only of children, NHS consisted of adults (with reported symptom onset in adulthood), and the Quebec cohort was mixed. It is difficult to speculate how age of cohort ascertainment might affect the results, although age-dependent effects may arise if there are important gene–hormone interactions, as proposed above to explain the observed sex-specific effects. It is also conceivable that VDR interacts with environmental exposures in a time-dependent manner to affect disease expression. Such factors may include determinants of circulating vitamin D levels (including sunlight exposure and milk intake) or exposure to atypical mycobacteria.

Although these results support a role for VDR in the development of asthma and allergy, it is evident that the precise relationship between VDR polymorphisms and asthma development remains unclear. Moreover, it is likely that variation at this locus is not the only asthma susceptibility gene on chromosome 12q. As described above, chromosome 12q is among the most frequently replicated regions of asthma linkage, with more than 10 groups reporting evidence of linkage with either asthma- or allergy-related phenotypes (reviewed in Reference 14). Two broad peaks of linkage have been identified, one centromeric (centered around interferon- γ), the other telomeric (surrounding nitric oxide synthase-1). VDR localizes to one end of the centromeric linkage, which spans more than 30 Mb. A gene with relatively weak effects is unlikely to have been observed in linkage studies so frequently, and would not generate such broad linkage signals. Moreover, the asthma linkage to centromeric 12q observed by most groups does not include VDR (42, 60–62). We have evaluated multiple polymorphisms in six other candidate genes across the broad linkage peak, but have not identified other associated variants. Although the positional candidate approach has helped in identifying VDR, we suspect that a more comprehensive screen of the region, using high-density linkage disequilibrium mapping, is required to localize the other asthma genes on chromosome 12q.

Conflict of Interest Statement: B.A.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; R.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; E.K.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.L.

does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; C.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.W. served as a speaker at scientific meetings of GlaxoSmithKline, Wyeth, Boehringer, and Roche and has been doing joint research projects with Sequenom and Illumina; S.T.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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References

- Holick MF. Vitamin D: a millennium perspective. *J Cell Biochem* 2003;88:296–307.
- Baker AR, McDonnell DP, Hughes M, Crisp TM, Mangelsdorf DJ, Haussler MR, Pike JW, Shine J, O'Malley BW. Cloning and expression of full-length cDNA encoding human vitamin D receptor. *Proc Natl Acad Sci USA* 1988;85:3294–3298.
- D'Ambrosio D, Cippitelli M, Cocciolo MG, Mazzeo D, Di Lucia P, Lang R, Sinigaglia F, Panina-Bordignon P. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D₃: involvement of NF- κ B downregulation in transcriptional repression of the p40 gene. *J Clin Invest* 1998;101:252–262.
- Lemire JM, Archer DC, Beck L, Spiegelberg HL. Immunosuppressive actions of 1,25-dihydroxyvitamin D₃: preferential inhibition of Th1 functions. *J Nutr* 1995;125:1704S–1708S.
- O'Kelly J, Hisatake J, Hisatake Y, Bishop J, Norman A, Koefler HP. Normal myelopoiesis but abnormal T lymphocyte responses in vitamin D receptor knockout mice. *J Clin Invest* 2002;109:1091–1099.
- Matheu V, Back O, Mondoc E, Issazadeh-Navikas S. Dual effects of vitamin D-induced alteration of T_H1/T_H2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. *J Allergy Clin Immunol* 2003;112:585–592.
- Barber Y, Rubio C, Fernandez E, Rubio M, Fibla J. Host genetic background at CCR5 chemokine receptor and vitamin D receptor loci and human immunodeficiency virus (HIV) Type 1 disease progression among HIV-seropositive injection drug users. *J Infect Dis* 2001;184:1279–1288.
- Bellamy R, Ruwende C, Corrah T, McAdam KP, Thursz M, Whittle HC, Hill AV. Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. *J Infect Dis* 1999;179:721–724.
- Roy S, Frodsham A, Saha B, Hazra SK, Mascie-Taylor CG, Hill AV. Association of vitamin D receptor genotype with leprosy type. *J Infect Dis* 1999;179:187–191.
- Györfy B, Vasarhelyi B, Krikovszky D, Madacsy L, Tordai A, Tulassay T, Szabo A. Gender-specific association of vitamin D receptor polymorphism combinations with Type 1 diabetes mellitus. *Eur J Endocrinol* 2002;147:803–808.
- Pani MA, Knapp M, Donner H, Braun J, Baur MP, Usadel KH, Badenhop K. Vitamin D receptor allele combinations influence genetic susceptibility to Type 1 diabetes in Germans. *Diabetes* 2000;49:504–507.
- Park BS, Park JS, Lee DY, Youn JI, Kim IG. Vitamin D receptor polymorphism is associated with psoriasis. *J Invest Dermatol* 1999;112:113–116.
- Simmons JD, Mullighan C, Welsh KI, Jewell DP. Vitamin D receptor gene polymorphism: association with Crohn's disease susceptibility. *Gut* 2000;47:211–214.
- Raby BA, Silverman EK, Lazarus R, Lange C, Kwiatkowski DJ, Weiss ST. Chromosome 12q harbors multiple genetic loci related to asthma and asthma-related phenotypes. *Hum Mol Genet* 2003;12:1973–1979.
- Wjst M, Fischer G, Immervoll T, Jung M, Saar K, Rueschendorf F, Reis A, Ulbrecht M, Gomolka M, Weiss EH, et al. German Asthma Genetics Group. A genome-wide search for linkage to asthma. *Genomics* 1999;58:1–8.
- Malerba G, Laiciello MC, Scherpbier T, Trabetti E, Galavotti R, Cusin V, Pescollerung L, Zanoni G, Martinati LC, Boner AL, et al. Linkage analysis of chromosome 12 markers in Italian families with atopic asthmatic children. *Am J Respir Crit Care Med* 2000;162:1587–1590.
- Poon A, Laprise C, Lemire M, Montpetit A, Sinnett D, Schurr E, Hudson TJ. Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy. *Am J Respir Crit Care Med* 2004;170:967–973.
- Childhood Asthma Management Program Research Group. The Childhood Asthma Management Program (CAMP): design, rationale, and methods. *Control Clin Trials* 1999;20:91–120.
- Childhood Asthma Management Program Research Group. Long-term effects of budesonide or nedocromil in children with asthma. *N Engl J Med* 2000;343:1054–1063.
- Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. *J Womens Health* 1997;6:49–62.
- Romieu I, Willett WC, Colditz GA, Stampfer MJ, Rosner B, Hennekens CH, Speizer FE. Prospective study of oral contraceptive use and risk of breast cancer in women. *J Natl Cancer Inst* 1989;81:1313–1321.
- Stampfer MJ, Willett WC, Colditz GA, Rosner B, Speizer FE, Hennekens CH. A prospective study of postmenopausal estrogen therapy and coronary heart disease. *N Engl J Med* 1985;313:1044–1049.
- Camargo CA Jr, Weiss ST, Zhang S, Willett WC, Speizer FE. Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. *Arch Intern Med* 1999;159:2582–2588.
- Pritchard JK, Rosenberg NA. Use of unlinked genetic markers to detect population stratification in association studies. *Am J Hum Genet* 1999;65:220–228.
- Sainz J, Van Tornout JM, Loro ML, Sayre J, Roe TF, Gilsanz V. Vitamin D-receptor gene polymorphisms and bone density in prepubertal American girls of Mexican descent. *N Engl J Med* 1997;337:77–82.
- Chiu KC, Chuang LM, Yoon C. The vitamin D receptor polymorphism in the translation initiation codon is a risk factor for insulin resistance in glucose tolerant Caucasians. *BMC Med Genet* 2001;2:2.
- Holland PM, Abramson RD, Watson R, Gelfand DH. Detection of specific polymerase chain reaction product by utilizing the 5'→3' exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proc Natl Acad Sci USA* 1991;88:7276–7280.
- Haldane JBS. An exact test for randomness of mating. *J Genet* 1954;52:631–635.
- Hill WG. Estimation of linkage disequilibrium in randomly mating populations. *Heredity* 1974;33:229–239.
- Hill WG, Robertson A. Linkage disequilibrium in finite populations. *Theor Appl Genet* 1968;38:226–231.
- Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001;68:978–989.
- Stephens M, Li M. PHASE. Seattle, WA: Department of Statistics, University of Washington; 2001.
- Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, et al. The structure of haplotype blocks in the human genome. *Science* 2002;296:2225–2229.
- Rabinowitz D, Laird N. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. *Hum Hered* 2000;50:211–223.
- Laird NM, Horvath S, Xu X. Implementing a unified approach to family-based tests of association. *Genet Epidemiol* 2000;19:S36–S42.
- Lange C, Laird NM. On a general class of conditional tests for family-based association studies in genetics: the asymptotic distribution, the conditional power, and optimality considerations. *Genet Epidemiol* 2002;23:165–180.
- Lange C, DeMeo D, Silverman EK, Weiss ST, Laird NM. Using the noninformative families in family-based association tests: a powerful new testing strategy. *Am J Hum Genet* 2003;73:801–811.
- Clayton D. A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. *Am J Hum Genet* 1999;65:1170–1177.
- Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 2002;70:425–434.
- Gao PS, Mao XQ, Roberts MH, Arinobu Y, Akaiwa M, Enomoto T, Dake Y, Kawai M, Sasaki S, Hamasaki N, et al. Variants of STAT6 (signal transducer and activator of transcription 6) in atopic asthma. *J Med Genet* 2000;37:380–382.
- Heinzmann A, Grotherr P, Jerkic SP, Lichtenberg A, Braun S, Kruse S, Forster J, Kuehr J, Deichmann KA. Studies on linkage and association of atopy with the chromosomal region 12q13–24. *Clin Exp Allergy* 2000;30:1555–1561.
- Ober C, Tsalenko A, Parry R, Cox NJ. A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. *Am J Hum Genet* 2000;67:1154–1162.
- Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, Boulet LP, Hamid Q. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF- β , IL-11, IL-17, and Type

- I and Type III collagen expression. *J Allergy Clin Immunol* 2003;111:1293–1298.
44. Reichel H, Koeffler HP, Tobler A, Norman AW. $1\alpha,25$ -Dihydroxyvitamin D₃ inhibits γ -interferon synthesis by normal human peripheral blood lymphocytes. *Proc Natl Acad Sci USA* 1987;84:3385–3389.
 45. Kizaki M, Norman AW, Bishop JE, Lin CW, Karmakar A, Koeffler HP. $1,25$ -Dihydroxyvitamin D₃ receptor RNA: expression in hematopoietic cells. *Blood* 1991;77:1238–1247.
 46. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. $1,25$ -Dihydroxyvitamin D₃ receptors in human leukocytes. *Science* 1983;221:1181–1183.
 47. Wjst M, Dold S. Genes, factor X, and allergens: what causes allergic diseases? *Allergy* 1999;54:757–759.
 48. Taussig LM, Cota K, Kaltenborn W. Different mechanical properties of the lung in boys and girls. *Am Rev Respir Dis* 1981;123:640–643.
 49. Hibbert M, Lannigan A, Raven J, Landau L, Phelan P. Gender differences in lung growth. *Pediatr Pulmonol* 1995;19:129–134.
 50. Burrows B, Bloom JW, Traver GA, Cline MG. The course and prognosis of different forms of chronic airways obstruction in a sample from the general population. *N Engl J Med* 1987;317:1309–1314.
 51. Skobeloff EM, Spivey WH, St Clair SS, Schoffstall JM. The influence of age and sex on asthma admissions. *JAMA* 1992;268:3437–3440.
 52. Magnusson CG. Cord serum IgE in relation to family history and as predictor of atopic disease in early infancy. *Allergy* 1988;43:241–251.
 53. Nieminen MM, Kaprio J, Koskenvuo M. A population-based study of bronchial asthma in adult twin pairs. *Chest* 1991;100:70–75.
 54. Suarez F, Zeghoud F, Rossignol C, Walrant O, Garabedian M. Association between vitamin D receptor gene polymorphism and sex-dependent growth during the first two years of life. *J Clin Endocrinol Metab* 1997;82:2966–2970.
 55. Tao C, Yu T, Garnett S, Briody J, Knight J, Woodhead H, Cowell CT. Vitamin D receptor alleles predict growth and bone density in girls. *Arch Dis Child* 1998;79:488–493; discussion 493–494.
 56. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet* 2001;29:306–309.
 57. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003;33:177–182.
 58. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone density from vitamin D receptor alleles. *Nature* 1994;367:284–287.
 59. Houston LA, Grant SF, Reid DM, Ralston SH. Vitamin D receptor polymorphism, bone mineral density, and osteoporotic vertebral fracture: studies in a UK population. *Bone* 1996;18:249–252.
 60. Barnes K, Freidhoff L, Nickel R, Chiu Y, Juo SH, Hizawa N, Naidu R, Ehrlich E, Duffy D, Schou C, et al. Dense mapping of chromosome 12q13.12-q23.3 and linkage to asthma and atopy. *J Allergy Clin Immunol* 1999;104:485–491.
 61. Dizier M, Besse-Schmittler C, Guilloud-Bataille M, Annesi-Maesano I, Boussaha M, Bousquet J, Charpin D, Degioanni A, Gormand F, Grimsfeld A, et al. Genome screen for asthma and related phenotypes in the French EGEA study. *Am J Respir Crit Care Med* 2000;162:1812–1818.
 62. Nickel R, Wahn U, Hizawa N, Maestri N, Duffy DL, Barnes KC, Beyer K, Forster J, Bergmann R, Zepp F, et al. Evidence for linkage of chromosome 12q15-q24.1 markers to high total serum IgE concentrations in children of the German Multicenter Allergy Study. *Genomics* 1997;46:159–162.
 63. Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, Nakanishi K, Yoshida N, Kishimoto T, Akira S. Essential role of Stat6 in IL-4 signalling. *Nature* 1996;380:627–630.
 64. Croston GE, Cao Z, Goeddel DV. NF- κ B activation by interleukin-1 (IL-1) requires an IL-1 receptor-associated protein kinase activity. *J Biol Chem* 1995;270:16514–16517.
 65. Xie MH, Aggarwal S, Ho WH, Foster J, Zhang Z, Stinson J, Wood WI, Goddard AD, Gurney AL. Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. *J Biol Chem* 2000;275:31335–31339.
 66. Chodimella V, Skidgel R, Krowiak E, Murlas C. Lung peptidases, including carboxypeptidase, modulate airway reactivity to intravenous bradykinin. *Am Rev Respir Dis* 1991;144:869–874.
 67. Dragovic T, Igic R, Erdos E, Rabito S. Metabolism of bradykinin by peptidases in the lung. *Am Rev Respir Dis* 1993;147:1491–1496.
 68. Undem BJ, Lichtenstein LM, Hubbard WC, Meeker S, Ellis JL. Recombinant stem cell factor-induced mast cell activation and smooth muscle contraction in human bronchi. *Am J Respir Cell Mol Biol* 1994;11:646–650.