

# Association of Vitamin D Receptor Genetic Variants with Susceptibility to Asthma and Atopy

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Genome scans for asthma have identified suggestive or significant linkages on 17 different chromosomes, including chromosome 12, region q13–23, housing the vitamin D receptor (VDR) gene. Through interaction with VDR, 1,25-dihydroxyvitamin D<sub>3</sub> mediates numerous biological activities, such as regulation of helper T-cell development and subsequent cytokine secretion profiles. Variants of the VDR have been found to be associated with immune-mediated diseases that are characterized by an imbalance in helper T-cell development, such as Crohn's disease and tuberculosis. The VDR, hence, is a good candidate to be investigated for association with asthma, which is characterized by enhanced helper T-cell type 2 activity. Here, we examined VDR genetic variants in an asthma family-based cohort from Quebec. We report six variants to be strongly associated with asthma and four with atopy ( $0.0005 \leq p \leq 0.05$ ). Analysis of the linkage disequilibrium pattern and haplotypes also revealed significant association with both phenotypes ( $0.0004 \leq p \leq 0.01$ ). The findings have been replicated by another research team in a second but not in a third cohort. These results identify VDR variants as genetic risk factors for asthma/atopy and implicate a non-human leukocyte antigen immunoregulatory molecule in the pathogenesis of asthma and atopy.

**Keywords:** genetic predisposition; polymorphism; vitamin D receptor

The interaction of 1,25-dihydroxyvitamin D<sub>3</sub> (1,25[OH]<sub>2</sub>D<sub>3</sub>) with the vitamin D receptor (VDR) modulates many biological activities of the neural, immune, and endocrine systems, including calcium and phosphorous homeostasis, apoptosis, and cell differentiation (reviewed in 1, 2). Once bound to 1,25(OH)<sub>2</sub>D<sub>3</sub>, VDR binds to specific DNA sequence elements in vitamin D-responsive genes, termed VDR response elements, to influence the rate of RNA polymerase II-mediated transcription (3–5). Vitamin D-dependent rickets and osteomalacia are classic manifestations of vitamin D deficiency (OMIM 601769).

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Abnormalities related to the pleiotropic functions of VDR underlie the pathogenesis of several diseases. Table E1 in the online supplement summarizes results of association studies of four VDR variants (*FokI*, *BsmI*, *Apal*, and *TaqI*) with serum osteocalcin levels, bone mineral density, prostate cancer, hyperparathyroidism, insulin-dependent diabetes mellitus, Crohn's disease, leprosy, tuberculosis, and acquired immunodeficiency syndrome (6–13). Among the biological effects of ligand-bound VDR, its influence on Th cell development (14) is of particular interest for diseases involving the immune system. In the mouse, 1,25(OH)<sub>2</sub>D<sub>3</sub> has been shown to inhibit Th1 development and interferon- $\gamma$  production and to stimulate Th2 cell development and the production of interleukin-4 and interleukin-10 (15–17). In humans, although vitamin D has been shown to inhibit Th1 responses, Th2 enhancement has not been demonstrated (18). However, several of the associated phenotypes in Table E1 are characterized by an imbalance in Th1/Th2 cell activity, for example, acquired immunodeficiency syndrome progression (19, 20), tuberculoid leprosy (12), lepromatous leprosy (12), Crohn's disease (11), and tuberculosis (13, 21–23). Because of the well-known immunoregulatory role of VDR and its known association with several immune-mediated diseases, VDR presents itself to be a candidate gene for asthma susceptibility. Because asthma is characterized by a shift of Th cell responses toward type 2, we hypothesized that VDR may function as a regulator of asthma and atopy susceptibility. We investigated this hypothesis through the characterization of genetic variants of VDR in an asthma family-based cohort from northeastern Quebec. Some of the results of this study have been previously reported in the form of an abstract (24).

## METHODS

### Cohort Description

Families are from the Saguenay-Lac-St-Jean region of northeastern Quebec, Canada. Probands were recruited if they fulfilled at least two of the following three criteria: (1) a minimum of three clinic visits for acute asthma within 1 year, (2) two or more asthma-related hospital admissions within 1 year, or (3) steroid dependency, as defined by either 6 months of oral or 1 year of inhaled corticosteroid use. Families were included for study if at least one parent was available for phenotypic assessment, at least one parent was unaffected, and all four grandparents were of French Canadian origin. When possible, grandparents and other relatives were also recruited to the study.

After recruitment of probands and their family members, the affection status of all study participants was determined by clinical evaluation and the completion of a standard respiratory questionnaire that was modified to include questions about asthma and atopy severity, family history of asthma and/or atopy, age of onset, and the presence of other respiratory failure diagnoses (25). In 41 cases, the age of onset described by parents was below 2 years; because of the uncertainty of this information, we used a default class of 2 years. We defined participants as having asthma if (1) a reported history of asthma (questionnaire based) and a history of physician-diagnosed asthma (past/current) were available or (2) confirmation by a positive methacholine provocation

test was done only on subjects older than 12 years of age (*see* online supplement methods section for a description of the clinical tests performed). Subjects were deemed atopic if they had at least one positive response (wheal diameter  $\geq 3$  mm at 10 minutes) to skin-prick tests. The family participation rate was approximately 60%, and all subjects gave informed consent. A total of 223 independent families (1,139 individuals) with family size ranging from 3 to 17 and the number of affected family members (including probands) ranging from 1 to 10 were analyzed.

### Single Nucleotide Polymorphism Selection and Genotyping

We investigated 93 kb of genomic DNA harboring *VDR*, spanning from chromosome 12 position 46586093 to 46492363 on build 34 hg16 genome assembly released by the National Center for Biotechnology Information. An initial panel of 20 single nucleotide polymorphisms (SNPs) was selected from public databases (National Center for Biotechnology Information and the SNP Consortium) based on (1) the location in the gene, (2) relative distances to each other, (3) compatibility with the genotyping methods employed, which is dependent on types of base change and flanking sequences, and (4) the known associations with diseases. A final collection of 12 SNPs was tested for associations. The remaining eight SNPs were discarded because of low information content or unreliable genotyping data. SNPs described in this report are cited using their reference SNP identifier from the National Center for Biotechnology Information database, except for *VDR* SNPs having commonly used aliases (*ApaI*, *BsmI*, *FokI*, and *TaqI*).

SNP genotyping was performed using SNPstream UltraHigh Throughput Genotyping System (Orchid Biosciences, Princeton, NJ) (26). All protocols and reaction conditions are available in this journal's online supplement (*see* Table E3 in the online data supplement for oligonucleotides used in genotyping and Table E4 for those used in sequencing).

### Statistical Analysis

Hardy-Weinberg equilibrium was tested in a subset of DNA samples using MERLIN (27). These samples correspond to parents of probands whose DNA are available. These samples are independent so that unbiased estimates of Hardy-Weinberg equilibrium for the variants can be obtained.

Allele distribution patterns were assessed by the family-based association test (FBAT, version 1.4) (28, 29). This software uses an empirical variance-covariance estimator to account for the possibility of noninde-

pendent allelic transmission to affected sibs (30). Asthma and atopy phenotypes were tested separately under an additive genetic model.

Associations between *VDR* variants were assessed. Strength of linkage disequilibrium (LD) between pairs of SNPs was measured as  $D'$  (31), using Haploview (<http://www.broad.mit.edu/personal/jcbarret/haplo/documentation.php>). Regions of strongly associated markers (LD blocks) were inferred and modified from the definition proposed by Gabriel and colleagues (32) as implemented in Haploview. Specifically, the parameter confidence interval minima for strong LD was relaxed from the definition of Gabriel and colleagues, and the upper confidence level was set from 0.98 to 0.90.

Haplotype-specific associations were investigated using the "hbat" command implemented in FBAT (version 1.4) (28, 29). An empirical variance estimator was used (30). Asthma and atopy phenotypes were tested separately under an additive genetic model.

### VDR Resequencing

The promoter region and all exons were sequenced to detect novel coding SNPs using ABI PRISM BigDye Terminator (version 2) kit on an ABI 3,700 DNA sequencer (Applied Biosystems, Foster City, CA) as described elsewhere (33). The protocols, reaction conditions, and primers used are described in this article's online data supplement and at <http://www.genomequebec.mcgill.ca/VDR>.

## RESULTS

### Patient Characteristics

Clinical characteristics were obtained for 1,139 individuals (Table 1). At the time of recruitment, study participants were aged 3 to 88 years. There were 570 subjects with asthma, of which 223 are probands. The median age of onset for cases and their affected siblings is 5 years (2–46 years). We note that 419 of the 570 subjects (74%) with asthma and 218 of the 569 subjects (38%) without asthma were atopic. The sex ratio (male:female) for probands is 1:1.2, for affected family members is 1:1.4, and for unaffected family members is 1:1.2. Compared with their affected family members, the probands with asthma had higher immunoglobulin E levels and coexistence of atopy.

TABLE 1. CLINICAL CHARACTERISTICS OF SUBJECTS

|                                               | Families Probands<br>(n = 223) | Members Affected<br>(n = 347) | Members Unaffected<br>(n = 569) |
|-----------------------------------------------|--------------------------------|-------------------------------|---------------------------------|
| Male:female ratio                             | 1:1.2                          | 1:1.4                         | 1:1.2                           |
| Mean age in years (range)                     | 18 (3–46)                      | 40 (2–83)                     | 48 (3–96)                       |
| Median age in years                           | 16                             | 41                            | 48                              |
| Mean age of onset in years (range)            | 7.8 (2–46)                     | 7.5 (2–75)                    | Not applicable                  |
| Median age of onset in years                  | 5                              | 5                             | Not applicable                  |
| Smoking status                                |                                |                               |                                 |
| Never                                         | 186 (83.4%)                    | 176 (50.7%)                   | 243 (42.7%)                     |
| Ex-smoker                                     | 12 (5.4%)                      | 105 (30.3%)                   | 199 (40%)                       |
| Smoker                                        | 25 (11.2%)                     | 66 (19%)                      | 127 (22.3%)                     |
| FEV <sub>1</sub> as percentage predicted (SD) | 92.2 (16.3)                    | 88.9 (22.7)                   | 98.9 (17.01)                    |
| PC <sub>20</sub> in mg/ml (SD)*               | 2.66 (3.33)                    | 3.36 (4.53)                   | 26.91 (3.04)                    |
| Serum IgE in mg/L (SD)*                       | 229.09 (4.61)                  | 157.4 (4.55)                  | 80.9 (3.72)                     |
| Number of persons with subphenotypes          |                                |                               |                                 |
| Asthma                                        | 223 (100%)                     | 347 (100%)                    | Not available                   |
| Atopy                                         | 182 (82%)                      | 237 (68.3%)                   | 218 (38.3%)                     |
| AHR†                                          | 169 (90%)                      | 217 (82.2%)                   | 64 (11.2%)                      |
| IgE > 100 mg/L                                | 141 (63%)                      | 185 (53.3%)                   | 155 (27.2%)                     |
| IgE > 280 mg/L                                | 95 (43%)                       | 106 (30.5%)                   | 70 (12.3%)                      |

Definition of abbreviations: AHR = airway hyperreactivity according to American Thoracic Society criteria; PC<sub>20</sub> = provocative concentration of methacholine inducing a 20% fall in FEV<sub>1</sub>.

Evaluated for 188 probands, 486 unaffected family members, and 264 affected family members.

\* Geometric mean

† Present asthma or past documented clinical history of asthma.

### Genetic Analysis of the VDR Locus

A final panel of 12 SNPs was used from an initial collection of 20 obtained from public databases for *VDR* characterization and association testing (see Table E2 in the online supplement for SNPs characteristics). Of the eight discarded SNPs, seven are uninformative, with minor allele frequencies of less than 0.025, and one SNP failed the genotyping assay. Among the 12-SNP panel are four widely studied variants: *FokI* C>T (rs2228570) (13, 22, 34), *Apal* A>C (rs7975232) (35, 36), *BsmI* G>A (rs1544410) (7, 37–40), and *TaqI* C>T (rs731236) (13, 21, 23). Of the 12 SNPs, *FokI* and *TaqI* reside in the coding region, whereas the remaining 10 SNPs are located in noncoding regions, between 4.8 kb upstream of the translation start site and 32 kb downstream of exon 9 (Figure 1A).

Of the 12 SNPs, only rs2239182 gave a significant deviation from Hardy-Weinberg equilibrium ( $p = 0.039$ ); given the number of SNPs tested, this deviation can be expected to occur by chance alone.

### Family-based Association Analysis of VDR with Asthma and Atopy

The 12 SNPs were tested individually for association with asthma and atopy (Figure 1B). In the absence of *a priori* evidence for transmission models at this locus, we tested allelic associations under an additive genetic model. Six alleles (rs3782905C, rs1540339A, rs2239185C, rs2239185G, *BsmI*G, and *TaqI*T) and

four alleles (rs2239185C, *BsmI*G, *Apal*C, and *TaqI*T) were significantly overtransmitted to offspring with asthma and offspring that were atopic ( $p < 0.05$ ), respectively.

IgE data were collected and analyzed both as quantitative and dichotomous traits, independent of atopy status. In a dichotomous trait model, subjects can be classified as either high or low responders according to their IgE levels. In this cohort, log (IgE) values were normally distributed, and a cut-off point of 100 mg/L divided the subjects into low (two-thirds of participants) and high (one-third) responders. Under an additive model, three alleles (rs2239185C, *Apal*C, and *TaqI*T) are associated with being a high responder at 0.006 less than  $p$  less than 0.02. The alleles associated with high IgE are the ones also seen associated with asthma and atopy. As a quantitative trait, the phenotype investigated is high IgE responder. When regressed on age and sex, two alleles (rs2239185C and *TaqI*T) are associated at  $p$  of less than 0.005.

### Intragenic LD Structure of VDR

Associations among the 12 SNPs were assessed by measuring pairwise LD using  $D'$  (Figure 2A). The two flanking upstream SNPs (rs2238136, *FokI*) and the most downstream SNP (rs757344) are not in significant LD ( $D' \leq 0.30$ ) with any other marker tested. Thus, the LD of the two central *VDR* blocks is unlikely to have extended to these SNPs, and they are likely to be outside the core *VDR* blocks. The remaining nine SNPs are distributed within a

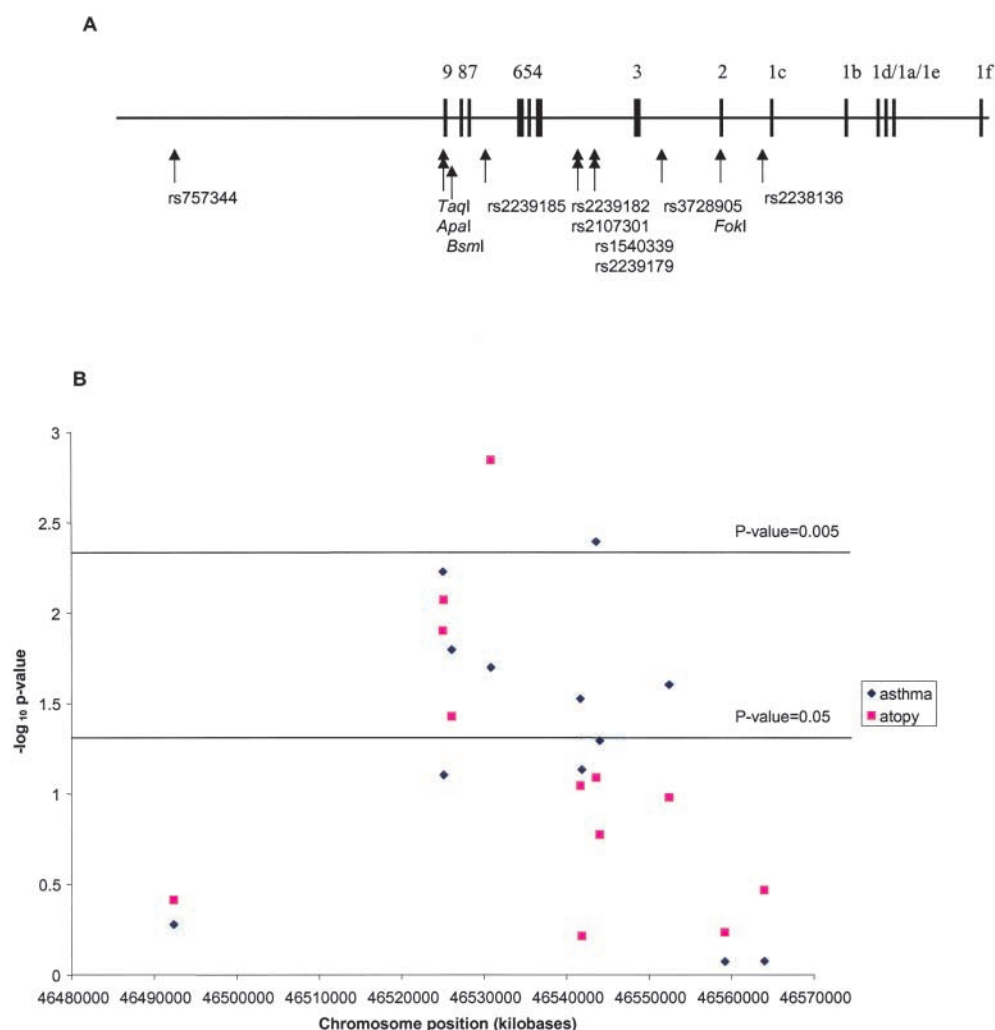


**Figure 1.** (A) Genomic organization of *VDR* and (B) association plot between *VDR* variants and the two phenotypes: asthma and atopy. Exons are represented by black boxes connected by a straight line representing introns and 3' and 5' noncoding regions. Locations of exons 1a–1f are described elsewhere (54). Positions and names of the 12 single nucleotide polymorphisms (SNPs) analyzed are represented by arrows below the gene structure. (B) Significance of association given as  $-\log_{10}$  ( $p$  value) is plotted against relative SNP position given in kilobases. Three levels of significance ( $p = 0.05$ ,  $p = 0.005$ , and  $p = 0.0005$ ) are indicated by straight lines drawn across the plot. Association with asthma is depicted by blue diamonds and atopy by pink squares. Chromosome positions are based on the July 2003 freeze of the University of California Santa Cruz genome browser (<http://genome.ucsc.edu/>).

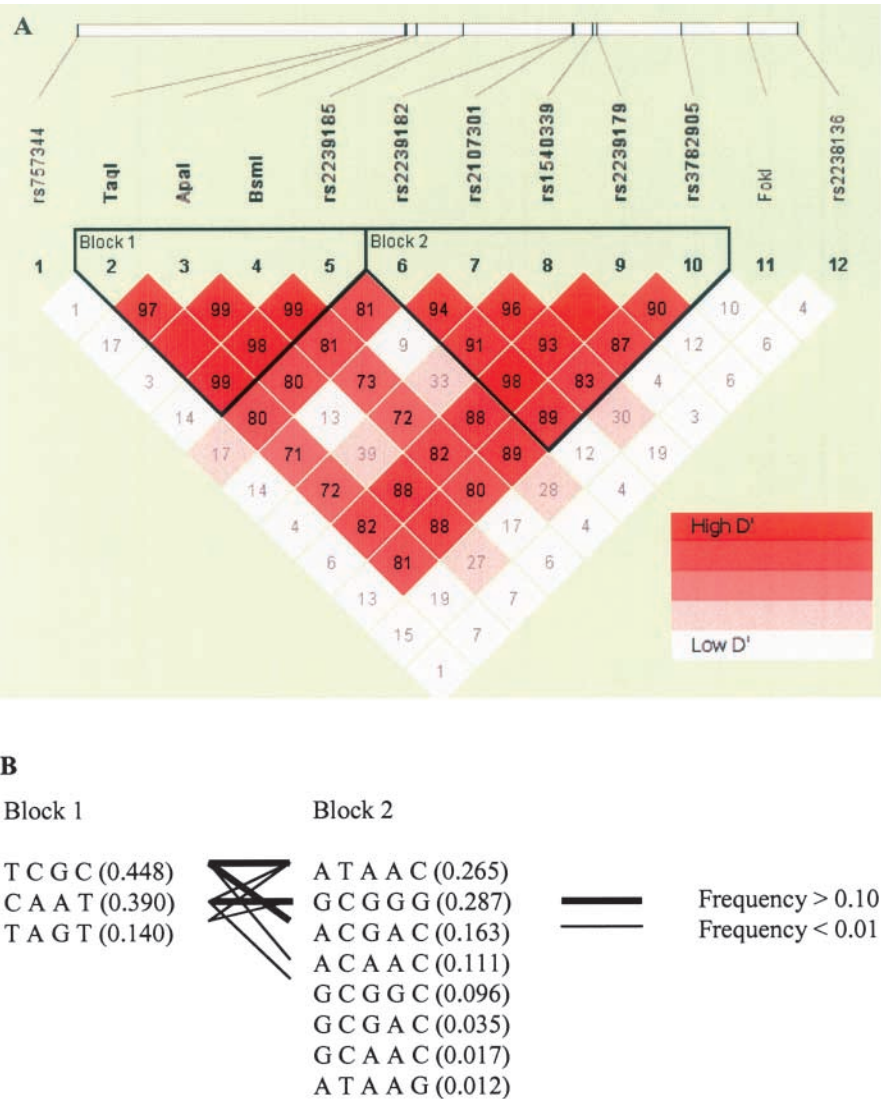


**Figure 2.** (A) Pairwise linkage disequilibrium (LD) pattern of *VDR* measured by  $D'$  and (B) the common haplotypes of the 28-kb LD region. The location of each tested SNP along the chromosome is indicated on top. The number in each diamond indicates the magnitude of LD ( $D' \times 10^{-2}$ ) between respective pairs of SNPs. For example, the pairwise  $D'$  for SNPs rs1540339 and rs2107301 is 0.96. Squares without  $D'$  written on them represent perfect LD ( $D' = 1.0$ ). The strength of LD is depicted by progression of color, for all  $D'$  with LOD of more than two, the color moves from red to light blue as  $D'$  runs from 1 to 0; for  $D'$  with LOD of less than two, it is represented by white. (B) Common haplotypes of the two blocks are listed with their frequencies within parentheses. Thick lines joining haplotypes from each block represent combined haplotypes with a frequency of more than 0.1 and thin lines for a frequency of less than 0.01.

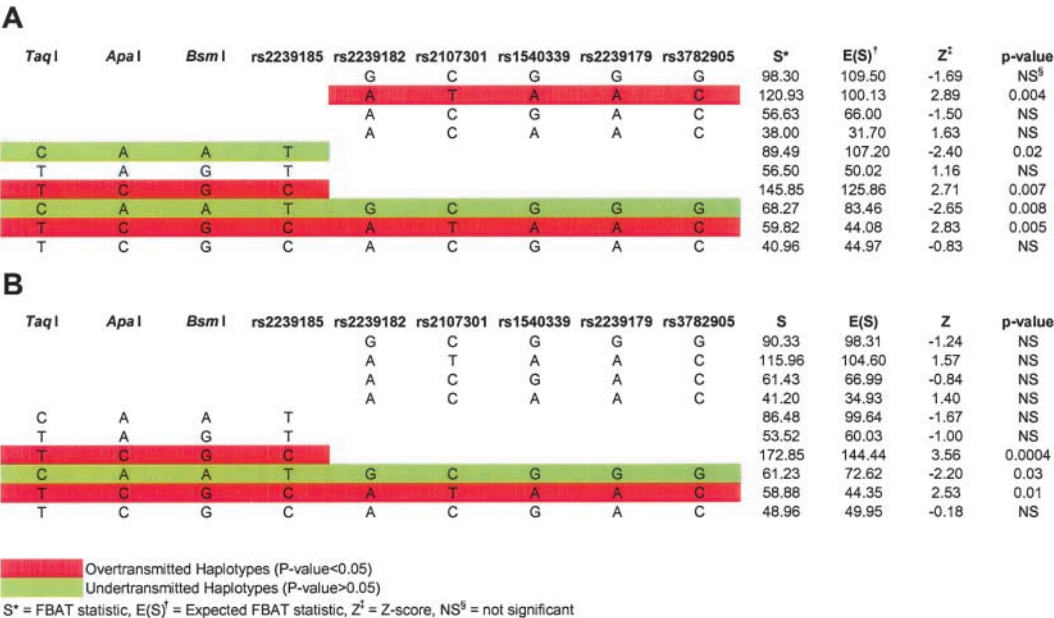


**Figure 3.** Haplotype transmission patterns for (A) asthma and (B) atopy. E(S) = expected FBAT statistic; S = FBAT statistic; Z = Z score.

28-kb region, between intron 2 and exon 9 of *VDR*, and are in strong LD ( $D' \geq 0.8$ ) with at least one additional SNP (80% of the pairwise LDs are  $D' \geq 0.80$ ). Of the 36 pairwise LDs calculated between these nine SNPs, eight  $D'$ s are low ( $0.09 \leq D' \leq 0.73$ ), and they further separate the region into two blocks of tightly associated SNPs. We relaxed Gabriel's criteria for haplotype block definition: the outermost marker pair was required to be in LD with an upper confidence limit that exceeds 0.90 and a lower confidence limit that exceeds 0.7. Block 1 locates toward the 3' end of *VDR* and consists of SNPs *TaqI*, *Apal*, *BsmI*, and rs2239185, whereas block 2 locates toward the 5' end of *VDR* and comprises SNPs rs2239182, rs2107301, rs1540339, rs2239179, and rs3782905. Block 1 spans approximately 5.8 kb, and block 2 spans roughly 8.4 kb. The two blocks, which are separated by 10.8 kb, show moderate LD between blocks ( $D' = 0.77$ , data not shown). Three common haplotypes (frequency  $> 0.1$ ) are observed within block 1: haplotypes TCGC (frequency = 0.45), CAAT (0.39), and TAGT (0.15); within block 2, four common haplotypes are observed: haplotypes GCGGG (0.29), ATAAC (0.27), ACGAC (0.16), and ACAAC (0.11). Three common haplotypes of the nine SNPs that extended across the two blocks were observed: (3' to 5' SNPs) CAATGCGGG, TCGCATAAC, and TCGCACGAC (Figure 2B).

### Haplotype-specific Association Analysis

FBAT results and LD patterns indicate that associations between *VDR* variants and asthma/atopy occur across the two blocks within a 28-kb region. To characterize haplotype transmission in this region, 10 common haplotypes previously inferred using Haploview were assessed for nonrandom transmissions using FBAT, version 1.4 (28, 29) (Figure 3). For asthma, 5 of the 10 common haplotypes show nonrandom distribution of haplotypes. In block 1, the nonrandomly transmitted haplotypes were TCGC (overtransmitted,  $p = 0.007$ ) and CAAT ( $p = 0.02$ , undertransmitted). In block 2, haplotype ATAAC was overtransmitted ( $p = 0.004$ ). In the combined block, haplotype TCGCATAAC was overtransmitted ( $p = 0.005$ ), and haplotype CAATGCGGG was undertransmitted ( $p = 0.008$ ).

For atopy, 3 of the 10 common haplotypes show nonrandom distribution of haplotypes. In block 1, haplotype TCGC was overtransmitted ( $p = 0.0004$ ). In the combined block, haplotype TCGCATAAC was overtransmitted ( $p = 0.01$ ), and haplotype CAATGCGGG was undertransmitted ( $p = 0.03$ ).

### VDR Resequencing

To exclude the presence of a coding *VDR* polymorphism that was not seen in previous analyses of *VDR*, we sequenced the proximal promoter region housing the six alternatively spliced exons 1 (1a–1f), exons 2–9 and intron/exon boundaries of *VDR* locus from genomic DNA obtained from 24 cases; the selection was based on their haplotype diversity. A total of 15 SNPs were identified, including *FokI*, *Apal*, and *TaqI*. The remaining 12 SNPs were only seen in noncoding regions; these SNPs are listed in Table E5 in the online supplement.

### DISCUSSION

Using a family-based cohort, we observed association between common allelic variants of *VDR* and phenotypes of asthma and atopy in a French-Canadian founder population. Six SNPs, in introns 2, 3, 6, and 8 and exon 9 of *VDR* spanning approximately 28 kb of genomic sequence, were associated with asthma. These markers fall into two haplotype blocks, with very substantial LD among markers of both blocks. With our present LD pattern obtained from 12 SNPs, the precise boundaries of the two blocks are unknown, and additional SNPs might be needed to represent

better the LD pattern at this locus. Nevertheless, significant non-random segregation of marker haplotypes spanning this 28-kb region of *VDR* further confirmed association of this region with asthma and atopy.

Interestingly, the same 28-kb haplotypes spanning both core *VDR* LD blocks are associated with asthma and atopy. If considering the blocks individually, the same haplotypes show the same direction of over or undertransmission, but some of these haplotypes do not reach statistical significance for association with one of the phenotypes. At this point, the current level of information is not sufficient for us to assign a greater likelihood of association for any of the two haplotype blocks to either of the phenotypes. Given the high clinical correlation between both phenotypes and the similarity of their haplotype associations, we believe it likely that there is one or more functional variants at the 3' end of the *VDR* locus responsible for susceptibility to both asthma and atopy.

In a separate association study of *VDR* variants described in a companion article (Raby and colleagues) (41), association with asthma was evaluated in two study populations: a family-based cohort and a case control cohort. The family cohort is part of the Childhood Asthma Management Program (CAMP) study (42, 43), with families being recruited from eight centers across North America ([www.jhucc.com/camp/open/sites.htm](http://www.jhucc.com/camp/open/sites.htm)). The case control cohort is part of the Nurses' Health Study (44) and included only women.

In the Nurses' Health Study cohort, four of the five SNPs tested are associated with asthma (Table 2). Of these four SNPs, three are with the same alleles found to be associated in the Quebec cohort. For the remaining associated SNP *Apal*, the C allele was overtransmitted in both the Quebec and the Nurses' Health Study cohorts but only reaches the 95% significance level in the Nurses' Health Study cohort. In the Quebec cohort, SNP *Apal* showed significant association with the atopy phenotype. SNP *Apal* is the only tested SNP being associated with asthma in the CAMP cohort, but the distribution pattern of its alleles was different from both the Quebec and the Nurses' Health Study cohorts: allele A was overtransmitted in the CAMP cohort and was undertransmitted in the other two cohorts. Hence, the same *VDR* alleles were observed to be associated with asthma in two of three study populations.

We note that the CAMP study recruited patients with median age of onset of three (boys) and four (girls) (ranging from 2 to 6 years), comparable to that of the Quebec probands (5 years, with a wide range of 2 to 46 years). However, the median age of recruitment of the Quebec probands was older (16 years),

**TABLE 2. COMPARISON OF ASSOCIATIONS OBSERVED IN THE QUEBEC, CHILDHOOD ASTHMA MANAGEMENT PROGRAM, AND NURSES' HEALTH STUDY COHORTS**

| Variant     | Quebec           | Childhood Asthma Management Program | Nurses' Health Study |
|-------------|------------------|-------------------------------------|----------------------|
| Rs3782905   | S                | NS                                  | S*                   |
| Rs1540339   | S                | NS                                  | NS                   |
| Rs2239182   | S                | NT                                  | NT                   |
| Rs2239185   | S                | NS                                  | S*                   |
| <i>BsmI</i> | S                | NS                                  | NT                   |
| <i>Apal</i> | NS (S for atopy) | S†                                  | S                    |
| <i>TaqI</i> | S                | NS                                  | S*                   |

Definition of abbreviations: NS = not significant; NT = not typed; S = statistically significant association ( $p < 0.05$ ).

\* Same allele of association as the Quebec cohort.

† Different allele of association from both the Quebec and the Nurses' Health Study cohorts.

which correlates with a longer duration of disease and/or recurrence in adulthood. The Nurses' Health Study cohort includes female nurses with asthma present in adulthood (44). The lack of *VDR* associations in the CAMP study may be explained by differences in phenotypes related to age or duration of disease and/or sample size issues (45, 46).

By sequencing the promoter, exons, and their surrounding regions, we excluded novel missense polymorphisms that could have been responsible for the observed associations. Of the SNPs associated with asthma/atopy, only *TaqI* resides in the coding region (exon 9), and the polymorphism does not result in an amino acid change. *TaqI* has been shown to be associated with many metabolic and immune-mediated diseases. The T allele, which is associated with asthma and atopy in our cohort, is also associated with tuberculosis (13, 21–23), chronic hepatitis B infection (23), lepromatous leprosy (12) and type 1 diabetes in Eastern Europeans (47) and South Indians (39). The C allele, on the other hand, is associated with Crohn's disease (11), tuberculoïd leprosy (12), and type 1 diabetes in Germans (48). The functional consequence of this polymorphism is not fully understood. In lymphocytes, the *TaqIC* allele is 30% less abundant (49), whereas in pituitary adenomas and transfected green monkey kidney (COS-7) cells, the same allele is associated with higher mRNA levels (7, 37).

Similar to variant *TaqI*, SNPs *ApaI* and *BsmI* have been widely studied and have been shown to be associated with many diseases. Alleles *BsmIG* and *ApaIC* are associated with asthma/atopy in this cohort; the same alleles are also associated with high bone mass density (7) and sporadic hyperparathyroidism in females (37), whereas allele *BsmIA* is associated with fast acquired immunodeficiency syndrome progression (6) and type 1 diabetes risk per se and acute-onset type 1 diabetes (40).

Given that association of asthma with *VDR* involves variants from intron 2 to exon 9, spanning approximately 28 kb and that no SNPs giving rise to an amino acid change were found, it is possible that the functional variant that confers susceptibility to asthma is a regulatory SNP (50), located in a *VDR* intron. Because *VDR* is a known immunoregulatory switch molecule and many of the associated phenotypes have the characteristics of Th1/Th2 imbalance, the mechanism of *VDR* in immune-mediated diseases may involve varying levels of *VDR* in immune cells on stimuli.

In summary, we identified a strong association between genetic variants at the *VDR* locus and asthma/atopy in a Quebec cohort. Along with other known asthma risk genes identified such as *ADAM33* (51), *TNFA* (52), *RANTES* (53), and *GPRA* (54), the addition of *VDR* involvement in the understanding of asthma/atopy pathogenesis will shed light for better control and treatment.

**Conflict of Interest Statement:** A.H.P. 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.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; A.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; D.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; E.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; T.J.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. A report of invention is being reviewed by McGill University Office of Technology Transfer.

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