Vitamin D Receptor Polymorphisms and the Risk of Acute Lower Respiratory Tract Infection in Early Childhood

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To investigate associations of 2 vitamin D receptor (VDR) gene polymorphisms and acute lower respiratory tract infection (ALRI), we compared 56 young children hospitalized with ALRI and 64 children without a history of ALRI. The *Fok*I *ff* genotype was associated with an adjusted relative odds of ALRI that was ~7 times that of *Fok*I *FF*. A weaker association with the *Taq*I polymorphism was also found. These data provide preliminary evidence of associations of VDR polymorphisms with the risk of ALRI (predominantly viral bronchiolitis) in young children, consistent with a potential role of vitamin D in the immune response to respiratory tract infection.

The active metabolite of vitamin D, 1,25-dihydroxyvitamin D, has potent immunomodulatory actions via binding to the vitamin D receptor (VDR) and may influence the immune response to viral infections [1]. In North America, acute lower respiratory tract infection (ALRI) during early childhood usually has a viral etiology, most commonly respiratory syncytial virus (RSV). Clinical manifestations of RSV ALRI are mainly due to immunopathogenic mechanisms, suggesting that modulation of the host immune response is a potential approach to disease prevention or management [2].

To explore the hypothesis that vitamin D is implicated in the host response to childhood ALRI, we compared the distributions of 2 single-nucleotide polymorphisms (SNPs) in the gene encoding the VDR among young children admitted to the hospital for ALRI and among healthy control subjects. These SNPs, identified by the *Taq*I and *Fok*I restriction endonucleases, have been previously associated with the risk or severity of pulmonary tuberculosis in adults in some but not all populations studied [3, 4]. This study was a planned secondary analysis in a case-control study for which the primary association of interest was 25-hydroxyvitamin D concentration and ALRI risk.

Subjects, materials, and methods. Participants were recruited at the Stollery Children's Hospital in Edmonton, Canada (latitude 53°N), from 1 January through 31 March in 2005 and 2006. Cases were patients aged 1–24 months admitted with ALRI, clinically defined as bronchiolitis (rhinorrhea, coryza, cough, and/or fever of <2 weeks duration; wheezes and/or crackles on auscultation of the lung fields; and increased respiratory effort) or pneumonia (temperature of >38.0°C, respiratory distress, and consolidation or pleural effusion on a chest radiograph). Bronchiolitis and pneumonia cases were analyzed together because there is substantial clinical overlap between the 2 conditions and because both were likely to have a viral etiology in this setting. Cases were excluded if they had underlying conditions (e.g., pulmonary aspiration, oropharyngeal abnormality, or symptomatic congenital heart disease) that could complicate an ALRI. Controls were children aged 1 month to 2 years admitted for elective surgery who did not have any history of hospital admission for ALRI or any of the conditions used as exclusion criteria for the cases.

Analysis of VDR restriction fragment length polymorphisms (RFLPs) was performed in the Department of Pathology at the University of Alberta Hospital (Edmonton, Canada). Venous whole blood specimens were collected in EDTA tubes and stored at −20°C until processing. DNA was extracted from 200 μL of whole blood using the QIAamp DNA Blood Mini Kit (Qiagen). DNA sequences containing 2 previously described VDR SNPs identified by *Taq*I (rs731236) [5] and *Fok*I (rs10735810) [6] were amplified by PCR according to methods adapted from those described elsewhere [3]. The presence of a restriction site was designated by a lowercase letter and its absence by an uppercase letter: "*t*" and "*T*" were used for the *Taq*I site, and "*f*" and "*F*" were used for the *Fok*I site. Duplicate genotyping was performed on randomly selected samples to ensure reproducibility.

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The PCR-RFLP assays and assignments of genotypes were performed without knowledge of the participant identity or group.

The parent and/or caregiver of each participant provided informed consent and completed a written questionnaire concerning participant and family characteristics. The study was approved by the Human Research Ethics Board of the University of Alberta Health Sciences Faculties. Parents could refuse genetic testing but participate in other parts of the study.

Maternal and paternal ethnicity were reported by caregivers as Aboriginal Canadian (i.e., First Nations, Inuit, or Métis), white (i.e., European or Caucasian), Chinese or East Asian, Indian (i.e., south Asian), Hispanic (i.e., Caribbean, Mexican, or Central or South American), black African, Middle Eastern, or other. For analysis, participants were divided into 1 of 4 categories, according to their parents' ethnicity: both Aboriginal Canadian, both white, 1 Aboriginal Canadian and 1 white, and other.

Differences in continuous variables were assessed by the Student *t* test for independent samples. Differences in proportions were assessed by means of the 2-tailed Fisher exact test. Hardy-Weinberg equilibrium was tested using the genhwcci command in Stata. Analyses were conducted using genotype as the independent variable, whereby the genotype homozygous for the 2 common alleles (*T* or *F*) served as the reference category. Logistic regression analysis was performed to obtain odds ratios and 95% confidence intervals for the association between VDR genotypes and ALRI, using unadjusted, ethnicity adjusted, and fully adjusted multivariate models. The latter model included the following covariates assumed to be confounders in the source population: age, sex, maternal and paternal education level (defined binarily as completion or noncompletion of high school), household crowding (defined as the number of people per bedroom), exclusive breastfeeding to 4 months of age, maternal smoking during pregnancy, and number of smokers in the home. To further minimize population stratification (confounding by ethnicity), the analysis was repeated in a subsample of participants with 2 white parents. A 2-sided *P* value of $<.05$ was considered to be statistically significant. Statistical analyses were conducted using Intercooled Stata, version 9.2 for Windows (Stata).

Results. Of 172 hospitalized children with ALRI, 116 were excluded from enrollment because a caregiver could not be contacted (22%), consent was refused (54%), exclusion criteria were present (9%), or a suitable blood specimen was unavailable (15%). The age and sex distributions of participating and nonparticipating cases were very similar (data not shown).

Of the 56 cases enrolled, 52 had bronchiolitis, and 4 had pneumonia. Routine viral analysis of nasopharyngeal aspirates revealed that 46 cases (82%) had RSV, 4 (7%) had parainfluenza or adenovirus, and 6 (11%) did not have a detectable virus. Compared with the 64 control participants enrolled, cases were younger, more likely to have an Aboriginal Canadian parent, and had a higher probability of reporting several conventional ALRI risk factors (table 1). The subsample of subjects with 2 white parents included 25 cases and 56 controls.

The *f* allele was overrepresented among children with 2 Aboriginal Canadian parents (*FF,* 15%; *Ff,* 60%; and *ff,* 25%), compared with its representation among children with 2 white parents (*FF,* 33%; *Ff,* 58%; and *ff,* 9% [$P = .062$]). However, the *Taq*I genotypic distributions in the group with 2 Aboriginal Canadian parents (*TT,* 65%; *Tt,* 30%; and *tt,* 5%) were to similar to those in the group with 2 white parents (*TT,* 44%; *Tt,* 48%; and *tt,* 7% [$P = .595$]).

The distribution of *Taq*I polymorphisms was similar among case and control groups in the complete sample but differed in the subsample (table 1). There was a statistically significant association between the *Taq*I *Tt* genotype and the odds of ALRI in the crude and adjusted analysis in the subsample but not in the complete sample (table 2).

The *Fok*I distributions were different between the case and control groups in both the complete sample and the subsample (table 1). In both the overall population and the subsample, the odds of ALRI for a child with the *ff* genotype were significantly increased relative to those for a child with the *FF* genotype in unadjusted and adjusted analyses (table 2). Effect sizes were similar and inferences unchanged upon exclusion of the subjects with pneumonia from the complete and subsample (data not shown).

Discussion. Among young Canadian children, a function-altering SNP in the gene that encodes the VDR (i.e., *Fok*I) was strongly associated with the risk of ALRI (predominantly RSV bronchiolitis). This association remained statistically significant after adjusting for ethnicity and other potential confounders. Within a subgroup with relative ethnic homogeneity (i.e., participants with 2 white parents), although the small sample size resulted in imprecise point estimates for the odds ratio, the confidence intervals excluded the null, suggesting that the *Fok*I-ALRI association was unlikely to have occurred by chance. There was a weaker and less consistent association of ALRI risk with an SNP (*Taq*I) that does not alter the VDR polypeptide.

These findings are consistent with the hypothesis that vitamin D–related pathways are implicated in the host immune response to viral respiratory infection [1]. Furthermore, previous evidence suggests that an increased risk of ALRI associated with the *ff* genotype is biologically plausible. The *f* allele translates a VDR protein that is longer than that translated by the *F* allele and has decreased rates of transcription of VDR RNA [7]. In peripheral blood mononuclear cell (PBMC) cultures, the concentration of 1,25-dihydroxyvitamin D (1,25(OH)2D) required to cause 50% growth inhibition was directly related to the number of *f* alleles [8], and in vitro lymphoproliferation caused by exposure to mycobacterial antigen was more likely to be inhibited by 1,25(OH)2D if the cells expressed the *FF* genotype [9]. These observations suggest that, although the *f* allele encodes a less active VDR and may affect the host's ability to use vitamin D for antimicrobial activities or regulation of the inflammatory re-

Table 1. Characteristics and vitamin D receptor (VDR) polymorphisms in healthy controls and in cases with acute lower respiratory tract infection, overall and in a subsample with 2 white parents.

Characteristic	Overall			Subsample		
	Controls ($n = 64$)	Cases ($n = 56$)	P	Controls ($n = 56$)	Cases ($n = 25$)	P
Age, mean ± SD, months	13.1 ± 6.9	8.4 ± 5.8	<.001	13.2 ± 7.0	9.5 ± 6.8	.029
Male sex	48 (75)	33 (59)	.079	41 (73)	14 (56)	.197
Premature birth[a]	4 (6)	13 (23)	.009	3 (5)	6 (24)	.022
Exclusively breastfed to 4 months of age	21 (33)	6 (11)	.004	21 (38)	1 (4)	.001
Mother smoked during pregnancy	13 (20)	27 (50)	.001	7 (13)	11 (46)	.003
⩾1 smoker living in household	22 (34)	37 (69)	<.001	17 (30)	16 (67)	.003
Ethnicity of parents						
Both white	56 (88)	25 (45)		56 (100)	25 (100)	
Both Aboriginal Canadian	3 (5)	17 (30)	<.001	0	0	N/A
One Aboriginal Canadian, one white	3 (5)	8 (14)		0	0	
Other	2 (3)	6 (11)		0	0	
Completed high school						
Mother	53 (83)	35 (66)	.052	50 (89)	20 (83)	.477
Father	54 (84)	32 (60)	.006	50 (89)	19 (82)	.465
>1 person/bedroom in household	36 (56)	44 (81)	.005	29 (52)	19 (79)	.026
VDR locus, genotype						
*Taq*I[b]						
TT	32 (50)	24 (43)		30 (54)	6 (24)	
Tt	28 (44)	28 (50)	.742	22 (39)	17 (68)	.030
Tt	4 (6)	4 (7)		4 (7)	2 (8)	
*Fok*I[b]						
FF	24 (38)	14 (25)		22 (39)	5 (20)	
Ff	37 (58)	29 (52)	.010	33 (59)	14 (56)	.004
Ff	3 (5)	13 (23)		1 (2)	6 (24)	

NOTE. Data are no. (%) of subjects, unless otherwise indicated.

[a] Gestation period, <37 weeks.

[b] In the complete sample, the allelic distributions of *Taq*I and *Fok*I polymorphisms did not significantly deviate from the Hardy-Weinberg equilibrium among cases (*Taq*I: $\chi^2 = 1.197$ and $P = .274$; *Fok*I: $\chi^2 = 0.073$ and $P = .787$) or controls (*Taq*I: $\chi^2 = 0.432$ and $P = .511$; *Fok*I: $\chi^2 = 1.714$ and $P = .190$).

sponse, higher circulating 25(OH)D concentrations could theoretically overcome its hypofunctionality. Although some of the previous studies have suggested codominance (i.e., dose response with increasing copies of the *f* allele), the present study sample was too small to detect a significant effect of a single *f* allele.

An association of ALRI risk with the *Taq*I *Tt* genotype but not the *tt* genotype emerged in the adjusted analysis of the complete sample and in subsample. The *Taq*I polymorphism has previously been linked to susceptibility to infectious diseases, including tuberculosis [3]. Although the *TT* genotype has been associated with levels of VDR expression in PBMCs that are higher than those for the *tt* genotype [10], the implications of *Taq*I polymorphisms for immune function are not known. In the present study, carriers of a single *t* allele curiously appeared to be at greater risk of ALRI than those with 2 *t* alleles; however, the estimated odds ratio for the *tt* genotype may have been unstable because of a low number of participants in that genotype group.

There are several candidate mechanisms by which activated vitamin D binding to the VDR could modulate viral lower respiratory tract disease, including down-regulation of the toll-like receptor 4 [11] to which RSV binds [2], suppression of T cell proliferation [12] or tumor necrosis factor–α synthesis [11], or stimulation of the production of an antimicrobial host protein [13]. Subclinical vitamin D deficiency was a significant independent risk factor for severe ALRI in children in India aged <5 years [14]; however, among the relatively vitamin D–replete participants in the present study, we did not find that 25-hydroxyvitamin D concentrations were associated with the risk of ALRI [15].

There were several methodological limitations in this study. First, it is possible that the finding of an association between the *ff* genotype and ALRI risk resulted from a low prevalence of the *ff* genotype in the control group that occurred by chance or nonrandom control selection; however, compared with the *Fok*I genotype distribution previously reported among white northern

Table 2. Analysis of the association between vitamin D receptor (VDR) genotypes and the risk of acute lower respiratory tract infection, overall and in a subsample of children with 2 white parents.

VDR locus, model, genotype	Overall (n = 120)		Subsample (n = 81)	
	OR (95% CI)	*P*	OR (95% CI)	*P*
*Fok*I				
Crude				
FF	1		1	
Ff	1.34 (0.59–3.05)	.480	1.87 (0.59–5.92)	.289
ff	7.43 (1.80–30.67)	.006	26.40 (2.58–271.09)	.006
Ethnicity adjusted				
FF	1		N/A	
Ff	1.40 (0.55–3.58)	.481	. . .	
ff	6.16 (1.31–28.95)	.021	. . .	
Fully adjusted[a]				
FF	1		1	
Ff	1.04 (0.29–3.77)	.955	1.83 (0.32–10.39)	.493
ff	7.38 (1.17–46.55)	.033	29.90 (1.93–463.72)	.015
*Taq*I				
Crude				
TT	1		1	
Tt	1.33 (0.63–2.81)	.449	3.86 (1.31–11.39)	.014
tt	1.33 (0.30–5.88)	.704	2.50 (0.37–16.89)	.347
Ethnicity adjusted				
TT	1		N/A	
Tt	1.79 (0.75–4.26)	.189	. . .	
tt	1.99 (0.38–10.35)	.416	. . .	
Fully adjusted[a]				
TT	1		1	
Tt	2.83 (0.85–9.43)	.090	10.42 (1.55–70.25)	.016
tt	2.06 (0.25–16.97)	.503	1.77 (0.096–32.44)	.700

[a] Adjusted for age, sex, maternal and paternal education level (defined binarily as completion or noncompletion of high school), household crowding (defined as the number of people per bedroom), exclusive breastfeeding to 4 months of age, maternal smoking during pregnancy, and number of smokers in the home. CI, confidence interval; OR, odds ratio.

Europeans (*FF*, 42%; *Ff*, 43%; and *ff*, 16%) [16], the prevalence of the *f* allele was relatively high (80%) among the cases in the subsample in this study. An important potential cause of confounding was that children with 2 Aboriginal Canadian parents were more likely to carry the *f* allele, were overrepresented among the cases, and had a higher probability of experiencing most of the traditional environmental risk factors for ALRI (data not shown). Regression analyses were adjusted to minimize population stratification and confounding by environmental risk factors; however, because there were no quantitative measures of population admixture, undetected heterogeneity between the groups may have caused confounding. An additional consideration is the possibility that the observed association was due to an association of ALRI with a nearby locus to which the VDR is closely linked. Finally, the sample size was small, leading to imprecise estimates (i.e., wide confidence intervals). The findings should thus be treated as preliminary, because false-positive associations are relatively common in case-control studies of genetic association.

In summary, the observed associations between VDR SNPs and the risk of ALRI in young Canadian children are consistent with the notion that vitamin D modulates the immune response to viral respiratory tract infections. Confirmation of these results in a larger sample, as well as further elaboration of the effect of interplay between circulating vitamin D metabolites and the VDR on the host response to ALRI, are required before firm conclusions can be drawn about the clinical or public health importance of these findings.

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References

1. Cannell JJ, Vieth R, Umhau JC, et al. Epidemic influenza and vitamin D. Epidemiol Infect **2006**; 134:1129–40.
2. Openshaw PJ, Tregoning JS. Immune responses and disease enhancement during respiratory syncytial virus infection. Clin Microbiol Rev **2005**; 18:541–55.
3. Wilkinson RJ, Llewelyn M, Toosi Z, et al. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis amongst Gujarati Asians in West London: a case-control study. Lancet **2000**; 355: 618–21.
4. Lewis SJ, Baker I, Davey Smith G. Meta-analysis of vitamin D receptor polymorphisms and pulmonary tuberculosis risk. Int J Tuberc Lung Dis **2005**; 9:1174–7.
5. Morrison A, Qi JC, Tokita A, et al. Prediction of bone density from vitamin D receptor alleles. Nature **1994**; 367:284–7.
6. Gross C, Eccleshall TR, Malloy PJ, Villa ML, Marcus R, Feldman D. The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. J Bone Miner Res **1996**; 11:1850–5.
7. Jurutka PW, Remus LS, Whitfield GK, et al. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Mol Endocrinol **2000**; 14:401–20.
8. Colin EM, Weel AE, Uitterlinden AG, et al. Consequences of vitamin D receptor gene polymorphisms for growth inhibition of cultured human peripheral blood mononuclear cells by 1, 25-dihydroxyvitamin D3. Clin Endocrinol (Oxf) **2000**; 52:211–6.
9. Selvaraj P, Chandra G, Jawahar MS, Rani MV, Rajeshwari DN, Narayanan PR. Regulatory role of vitamin D receptor gene variants of *Bsm*I, *Apa*I, *Taq*I, and *Fok*I polymorphisms on macrophage phagocytosis and lymphoproliferative response to mycobacterium tuberculosis antigen in pulmonary tuberculosis. J Clin Immunol **2004**; 24:523–32.
10. Ogunkolade BW, Boucher BJ, Prahl JM, et al. Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. Diabetes **2002**; 51:2294–300.
11. Sadeghi K, Wessner B, Laggner U, et al. Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. Eur J Immunol **2006**; 36:361–70.
12. Bhalla AK, Amento EP, Krane SM. Differential effects of 1,25-dihydroxyvitamin D3 on human lymphocytes and monocyte/macrophages: inhibition of interleukin-2 and augmentation of interleukin-1 production. Cell Immunol **1986**; 98:311–22.
13. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D–mediated human antimicrobial response. Science **2006**; 311:1770–3.
14. Wayse V, Yousafzai A, Mogale K, Filteau S. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 years. Eur J Clin Nutr **2004**; 58:563–7.
15. Roth DE, Jones AB, Prosser C, Robinson JL, Vohra S. Vitamin D status is not associated with the risk of hospitalization for acute bronchiolitis in early childhood. Eur J Clin Nutr **2007** [Epub ahead of print].
16. Langdahl BL, Gravholt CH, Brixen K, Eriksen EF. Polymorphisms in the vitamin D receptor gene and bone mass, bone turnover and osteoporotic fractures. Eur J Clin Invest **2000**; 30:608–17.