Vitamin D Receptor (VDR) Polymorphisms and Severe RSV Bronchiolitis: A Systematic Review and Meta-Analysis

J. Dayre McNally, MD, PhD,[1,2*] Margaret Sampson, PhD,[1] Loren A. Matheson, PhD,[2] Brian Hutton, PhD,[3] and Julian Little, PhD[4]

Abstract. Background: A number of small studies have suggested a relationship between vitamin D status and severe acute lower respiratory tract infection (ALRI), including RSV—bronchiolitis. The objective of this study was to evaluate the relationship between vitamin D receptor (*VDR*) polymorphism and severe RSV-bronchiolitis through a systemic literature review and meta-analysis. Methods: A comprehensive electronic literature search was conducted to identify all studies published before January 2013. Two reviewers independently screened all abstracts, followed by the full text of potential articles to evaluate eligibility. Study methodological quality was evaluated using the Newcastle Ottawa scale and individual component analysis. Meta-analysis evaluated associations at the allele and genotype levels. Results: Of 803 studies identified from our literature search, three met eligibility criteria. Two *VDR* polymorphisms were included in more than one study: *Taq*I (rs731236) and *Fok*I (rs2228570). All three reported a positive relationship between the *Fok*I minor allele and disease with random effects meta-analyses demonstrating a statistically significant relationship (OR 1.52, CI: 1.12, 2.05). Genotype analysis was highly suggestive of a dominant or incomplete dominance model with combined odds ratios for fF (OR 1.73, CI: 0.92–3.36) and ff (OR 2.24, CI: 0.98–5.14) compared to the FF genotype. No association between *Taq*I and severe RSV-bronchiolitis was evident at the allele or genotype level. Conclusions: Available literature supports an association between the *Fok*I polymorphism and severe RSV disease. Determination of *VDR* receptor polymorphism status could help predict high-risk infants who might benefit from preventive measures. **Pediatr Pulmonol.**

Key words: vitamin D; pediatrics; risk factors.

INTRODUCTION

Acute lower respiratory tract infections (ALRIs) are the leading cause of morbidity and mortality in children. It is well accepted that respiratory syncytial virus (RSV) is the most common pathogen in pediatric ALRI, defined here as pneumonia and brochiolitis.[1,2] RSV is a ubiquitous enveloped RNA paramyoxovirus that infects almost 100% of children within 2 years of birth.[3,4] Although most have subclinical infections, approximately 1 in 3 develop respiratory symptoms, 1 in 10 have disease severe enough to require acute medical attention, leading to between 5 and 10,000 hospital admissions per year in Canada alone.[5,6] The annual hospital costs in the United States have been estimated at approximately half a billion dollars.[7,8] In addition to acute morbidity and mortality, multiple studies have suggested long term consequences with higher rates of subsequent wheezing, asthma, and ALRI in children who had severe RSV infection in the first 2 years of life.[9–11]

Risk Factors, Prediction Tools, and Treatment Options

As an established public health problem, there has been significant interest in the behavioral, environmental, and

[1]Department of Pediatrics, Faculty of Medicine, University of Ottawa, Children's Hospital of Eastern Ontario, Ottawa, Canada.

[2]Research Institute, Children's Hospital of Eastern Ontario, Ottawa, Canada.

[3]Ottawa Hospital Research Institute Clinical Epidemiology Program, Ottawa, Canada.

[4]Department of Epidemiology and Community Medicine, University of Ottawa, Ottawa, Canada.

Human Genome Epidemiology (HuGE) Review.

*Correspondence to: James D. McNally, CHEO, 401 Smyth Road, Ottawa, ON, Canada K1S 3H2. E-mail: dmcnally@cheo.on.ca

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medical conditions that predispose children to severe RSV disease. Epidemiological studies on bronchiolitis have established multiple risk factors including age closer to birth, premature birth, congenital heart defects, parental smoking, ethnicity, older siblings, and day care attendance.[12,13] Knowledge of these predictors has proven valuable with the development of an intramuscularly administered RSV antibody. Monthly RSV antibody administration is well known to decrease hospital admission in extreme premature and congenital heart disease subpopulations,[14] and more recently to decrease wheezing in healthy infants born at 32–35 weeks gestation.[15] Due to drug and administration costs, current recommendations from the American Academy of Pediatrics and the Canadian Pediatric Society state that only very high risk populations (e.g., extreme prematurity, moderate prematurity with multiple additional risk factors, congenital heart disease requiring surgery) receive prophylaxis.[16] Presently, more than half, and up to 90% of children, who develop severe RSV and bronchiolitis do not fall into a very high risk group.[17] There remains considerable interest in the identification of RSV risk factors that are either modifiable, lead to novel interventions, or improve the accuracy of clinical prediction tools.

Association Between Vitamin D and Lower Respiratory Tract Illness

Lower vitamin D levels have been postulated as a risk factor for respiratory illness.[18] This hypothesis was originally based on the recognized role for vitamin D in immunity and the well-established seasonality of ALRI during winter when UV-B production of vitamin D is low. Support for this hypothesis was initially discovered in research from developing countries showing an association between ALRI and vitamin D deficiency rickets.[19–21] Subsequent ALRI and bronchiolitis research in developed countries investigating subclinical vitamin D deficiency using 25 hydroxyvitamin D (25OHD) measurements have supported this hypothesis.[22–27]

Adequate vitamin D axis functioning is a complex process influenced by more than just blood 25OHD concentrations.[28,29] The biological functions of activated vitamin D (or 1,25 dihydroxyvitamin D) are mediated by the vitamin D receptor (*VDR*), a ligand activated transcription factor. Genetic alterations of the *VDR* have the potential to affect vitamin D signaling through impaired gene transcription, mRNA stability and translation, protein activity, and protein stability. To date, hundreds of genetic variants of *VDR* have been identified with the vast majority being rare.[30] A possible role for *VDR* polymorphisms is supported by a recent Danish study suggesting that up to 22% of susceptibility to RSV lower respiratory tract infections can be attributed to host genetic factors.[31]

VDR Gene, Polymorphisms, and Functional Significance

The *VDR* gene (E-Figure A) is located on chromosome 12q12–q14.[30,32] The *VDR* gene product is a ligand activated transcription factor responsible for mediating the biological activities of activated vitamin D or $1,25OH_2D$. Many of the common *VDR* gene variants exhibit high levels of linkage disequilibrium (LD) within one of three blocks.[33] Of the common polymorphisms, there is a group (including *Taq*I and *Bsm*I) that is in high LD (block B) and occurs at the 3′ end of the *VDR*. These two polymorphisms have been suggested to impact mRNA stability and *VDR* gene transcription resulting in reduced cellular activity of the vitamin D system. Only one common polymorphism, *Fok*I, is a C/T polymorphism in the translation initiation codon of VDR. The variant T (or "f") results on the presence of *Fok*I restriction enzyme site and translation of a 3 amino acid longer VDR than the C (or "F") allele.[34] The functional significance of this change is unclear; however, in vitro studies have shown that the Fok1-f SNP has moderately lower VDR transcriptional activity than the Fok1-F SNP.[34–36] Further, it has been speculated that vitamin D target genes could have varying sensitivities and interactions with Fok1 VDR protein variant.

A growing body of literature, including a number of systematic reviews and meta-analysis, suggests that *VDR* polymorphism can predispose to various bone, autoimmune disease, and cancer related disease.[30,32,37–41] In the infectious disease literature, both isolated studies and meta-analysis have identified associations with *VDR* polymorphisms and both the progression and treatment response for HIV, TB, Hepatitis C, and recently, urinary tract infections in children.[42–44]

Similar attempts have been made to evaluate for a relationship between *VDR* polymorphism and RSV-related disease. Individually, the studies on *VDR* and RSV have small sample size and low statistical power resulting in odds ratio (OR) with low precision. In an attempt to overcome the limitations of the small sample size we performed a systematic review and meta-analysis. The objective of the present study was to determine, through a meta-analysis, whether polymorphism variants of the *VDR* gene compared to homozygous variants is associated with hospitalization for RSV infection or bronchiolitis in neonates, infants, and young children.

METHODS

The reporting within this systematic review and meta-analysis is in accordance with the PRISMA guideline,[45] including development of a protocol, search and selection of studies, data extraction from the studies, and meta-analysis.

Selection Criteria

Articles from peer reviewed medical journals were included in this systematic review if they fulfilled three criteria. First, the study was performed using either a case–control, cohort, nested case–control, or cross sectional design. Second, the study evaluated the association between at least one *VDR* polymorphism and hospitalization due to RSV infection or the ALRI subgroup bronchiolitis; studies reporting on ALRI/bronchiolitis were eligible if more than 75% of the cases were bronchiolitis and RSV related. Third, OR and 95% confidence intervals (CIs) were reported or sufficient genotype/allele data was available to permit estimation of these quantities.

Identification of Studies

We performed electronic searches on MEDLINE including in process and other non-indexed citations (1948 to January 15, 2013), Embase (1980- to January 15, 2013), and the Cochrane CENTRAL Register of Controlled Trials (December 2012) using the Ovid interface. No date, language, or study design restrictions were imposed on the search. The MEDLINE search was developed by one librarian (MS) experienced in systematic review searching, and then peer reviewed by another, using the PRESS standard.[46] The search strategy was then adapted for use with Embase and CENTRAL. The search was run in two sessions, the first on May 24, 2012, and the update on January 16, 2013. E-Table A shows the Medical Subject Headings (MeSH) and approach for the MEDLINE search; terms were adapted as required for the remaining databases by the librarian (MS).

For the level one screening of abstracts, all relevant citations identified through the electronic search were independently reviewed on the basis of the title, keywords and abstract by two of the authors (J.D.M. and L.A.M.). Those citations not meeting all level one criteria were rejected (E-Table B). Full text of the identified articles was assessed using level two criteria (E-Table C) to determine eligibility for confirmation of inclusion in the systematic review. A focused gray literature search was performed including the related article function in PubMed, review of references from studies meeting level one screening, and contacting the corresponding authors of included articles. Furthermore, we appraised the full text and references of all review articles meeting level one screening criteria 2 through 4. Finally, we searched specifically for genome wide association studies of RSV or bronchiolitis using the catalog of published genome wide association studies.[47]

Data Collection

Data from eligible studies were independently extracted on a structured data collection form by two reviewers (J.D.M. and L.A.M) using HuGE review and STREGA guidelines for reporting on genetic association.[48] Discrepancies were resolved by discussion and consensus. Information collected included: study type, case and control definition and eligibility criteria, recruitment and loss to follow-up, *VDR* polymorphisms, genotyping method, validation of genotyping method, genotype and allele frequencies, ORs with CIs, group age and gender proportions, geography, blinding of research staff, adjustment for relevant RSV risk factors.

Study Quality Assessment

Methodological quality of the included studies was evaluated using the Newcastle Ottawa scale as it has been used in genetic association studies and is one of the few scales previously recommended by a Cochrane working group.[49–52] In addition, because the Newcastle Ottawa scale was not specifically designed for genetic association studies, we used an individual component approach and data were extracted from the articles on items commonly recommended for genetic association studies.[48,53–55] To encourage consistency, we included the same components evaluated by Flores et al.[56] in their quality assessment of genetic association studies on acute lung injury. Features specific to genetic association studies were: Hardy Weinberg equilibrium (HWE), population stratification and quality control procedures for genotyping. As it is often performed incorrectly, genotype data from controls were reanalyzed for departure from HWE using the Chi-square goodness of fit.[57,58] Given that this analysis was performed on a small number of small studies, an assessment of bias via funnel plots and Egger's tests were not undertaken.

Data Presentation and Analysis

Since there was no strong biological evidence to indicate the most appropriate genetic model, the primary analysis considered the per allele model for each polymorphism. In an exploratory analysis we evaluated genotype data for recessive, incomplete and dominance models. Because all of *VDR* polymorphisms are diallelic SNPs, ORs and corresponding 95% CIs were used to assess the strength of association between each *VDR* variant and RSV bronchiolitis. Due to inherent heterogeneity of observational studies, the meta-analyses calculated ORs and corresponding 95% CIs were calculated through random effects models.[59] Consistency of the gene effect size across studies was assessed using a test of heterogeneity (Q) and the I^2 statistic. Meta-analysis was performed using Comprehensive Meta-analysis (Version 2, Biostat, Englewood, NJ).

RESULTS

Extent of Available Evidence

The results of the literature search and both level one and level two screening are shown in Figure 1. The electronic literature search yielded 803 citations, of which 550 were determined to be unique after Reference Manager duplicate removal. Through screening of titles and abstracts, 17 citations passed level one screening, with 3 meeting full criteria for inclusion in the systematic review. Only one of three corresponding authors responded to an inquiry requesting knowledge on related or additional reports; they were unaware of any other published or unpublished studies on this subject.

Characteristics and Quality of Included Studies

The main characteristics of the three studies are shown in Table 1. The identified publications were case–control studies, published between 2007 and 2011, and they were undertaken in three different geographical and ethnic settings. In total, genotype or allele information was available for 822 cases and 1,185 controls. For the two largest studies, cases represented RSV positive bronchiolitis. For the third study, 52 of the 56 cases (93%) had bronchiolitis, and 82% of all cases were RSV positive. The following *VDR* polymorphisms were investigated: *Fok*I (three studies, F/f), *Taq*I (two studies, T/t), and *Bsm*I (one study, B/b). A different method for genotype determination was used in each study and all three reported the SNPs to be in HWE. Re-calculation using available genotype data failed to confirm HWE ($P = 0.02$) for the *Fok*I polymorphism in the study by Roth et al.[60]

Methodological quality was assessed using the Newcastle-Ottawa scale, with the scores ranging from 4 to 7 out of a maximum nine points (E-Table D). The component evaluation also suggested moderate methodological quality for all studies (E-Table E). More specifically, all three studies were determined to be at risk for selection bias, as no information was available on recruitment rate and biological sample non-availability for cases or controls. The study determined to be at most risk for bias was a large-scale genotyping study designed to evaluate 384 SNPs from 220 candidate genes, including

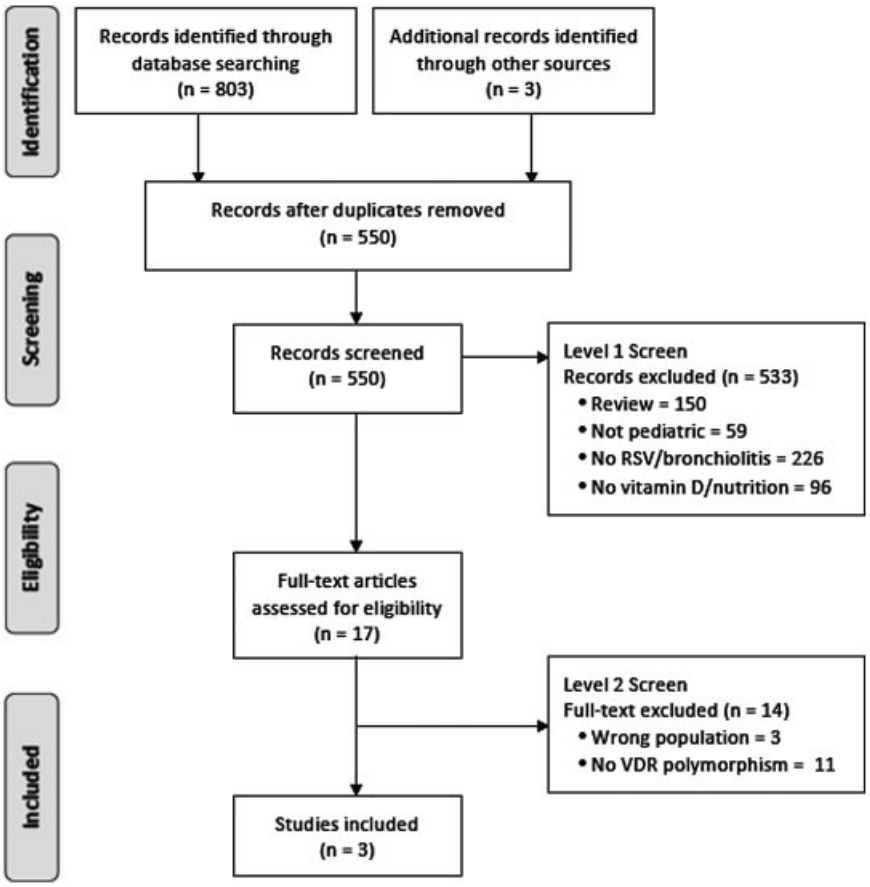

Fig. 1. Flow chart of study selection based on inclusion and exclusion criteria. The stages of a systematic selection scheme include: identification, screening, eligibility, and final included studies.

TABLE 1—Characteristics of Genetic Association Studies of Vitamin D Receptor Polymorphisms and Hospitalized RSV Bronchiolitis Meeting Criteria for Inclusion in the Current Analysis

Study and year	Geography	Study design	No. of[1] cases	No. of[1] controls	Case definition	RSV %	Exclusions	Control source	Molecular technique	HWE testing
Janssen, 2007	Netherlands	C-C[2]	480 (470)	1030 (1,008)	Bronchiolitis[3] CXR (yes), no age restriction	100	Airway abnormality or wheeze history	General population	PCR—Beadarray	Yes
Roth, 2008	Canada	C-C	56	64	ALRI[4], CXR (no) 1–24 months	82	Underlying conditions (CHD, airway)	Children, elective surgery	PCR–RFLP	Yes
Kresfelder, 2011	South Africa	C-C	296	113	Bronchiolitis[3] CXR (yes), under 2 years	100	None described	Infants, no ALRI	PCR–probe	Yes

C-C, case control; CHD, congenital heart disease; CXR, chest roentography; ALRI, acute lower respiratory infection; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; HWE, Hardy–Weinberg equilibrium.
[1]The value in brackets indicates the number of participants with available biological specimen.
[2]This study also collected biological specimen for case—parent transmission disequilibrium study.
[3]Case/control definitions were not provided.
[4]Four of the 56 cases had pneumonia.

3 SNPs on the *VDR*. Important methodological features placing this study at risk included the absence of a defined disease phenotype, no data on case or control refusal rates, and missing quality control data on *VDR* SNP measurements. From the description provided by Roth et al. it was evident that the control group was dissimilar from cases for a number of important covariates; an attempt was made to adjust for this imbalance through stratification and regression analysis. Kresfelder and coworkers may have obtained a more appropriate control population, but did not evaluate for differences in relevant covariates or complete an adjusted analysis.

Analysis of Relationship Between Polymorphism and Disease

A relationship between disease and *Fok*I polymorphism was investigated in all three eligible studies. The allele frequency in the controls ranged from 13% to 38%. All three documented a statistically significant relationship between the *Fok*I f allele and disease; the reported ORs ranged from 1.25 to 1.90 (Table 2). Heterogeneity assessment using the I^2 value returned a moderate score of 52%. Meta-analysis calculated significantly increased odds of RSV/bronchiolitis (OR 1.52, CI: 1.12, 2.05) for the minor allele using a random effect model (Forest plot shown in Fig. 2). At the genotype level, when compared with FF, all three studies reported ORs above 1 for both fF and ff genotypes, with approximately half of the studies reporting the difference to be statistically significant (Table 2). Again, combined ORs for the fF and ff genotypes, relative to FF, were calculated using random effects (Forest plot shown in Fig. 3). Although the results did not achieve statistical significance, the summary estimate and corresponding CI suggest increased odds (OR 1.73, CI: 0.92-3.36, $P = 0.10$) for the fF genotype. Similarly, for the ff genotype the combined odds ratio (OR 2.24, CI: 0.98–5.14, $P = 0.06$) did not quite achieve statistical significance.

Neither of the two studies evaluating the *Taq*I polymorphism demonstrated a different minor allele frequency for cases; unadjusted OR point estimates were 1.04 and 1.20 and both CIs crossed one (Table 3). Furthermore, evaluation of the genotype distributions failed to suggest any association with RSV-bronchiolitis. To control for race imbalances between groups, Roth et al. repeated the *Taq*I genotype using a subsample of children with two Caucasian parents. Although the OR estimates for both tT and tt exceeded 2, neither achieved statistical significance. Roth et al. also attempted to control for differences in known RSV risk factors using logistic regression. In this analysis, the OR for the tT versus TT genotype was calculated as 3.86 (CI: 1.31–11.39, $P = 0.014$). As only one of the two *Taq*I studies performed an adjusted analysis, no risk adjusted meta-analysis was possible.

TABLE 2— *Fok*I Genotype and Allele Distributions Reported for the Included Studies

*Fok*I (rs10735810)	Geno	Cases, n (%)	Control, n (%)	OR (95% CI)	*P*-Value
Roth (2007)					
Overall					
Genotype	FF	14 (25)	24 (38)		
	Ff	29 (52)	37 (58)	1.34 (0.59, 3.05)	0.480
	ff	13 (23)	3 (5)	7.43 (1.80, 30.67)	0.006
Allele[1]	f, (%)*	55 (49)	43 (34)	1.91 (1.13, 3.20)	
Subsample[2]					
Genotype	FF	5 (20)	22 (39)		
	Ff	14 (56)	33 (59)	1.87 (0.59, 5.92)	0.289
	ff	6 (24)	1 (2)	26.4 (2.58, 271.09)	0.006
Allele[1]	f, (%)*	26 (52)	35 (31)	2.38 (1.20, 4.7)	
Janssen (2007)					
Genotype	FF	NR	NR (38)	1.00	0.035
	Ff	NR	NR (48)	1.22 (0.93, 1.60)	
	ff	NR	NR (14)	1.6 (1.12, 2.29)	
Allele	f, (%)	NR[3] (44)	NR (38)	1.25 (1.06, 1.50)	0.01
Kresfelder (2011)					
Genotype	FF	177 (60)	86 (76)	1.00	0.008[4]
	Ff	109 (37)	24 (21)	3.18 (1.90, 5.34)	
	ff	10 (3)	3 (3)	1.62 (0.44, 6.01)	
Allele[1]	f, (%)	129 (22)	30 (13)	1.82 (1.18, 2.80)	0.006[4]

Geno, genotype; OR, odds ratio; CI, confidence interval; NR, not reported.
[1]Allele frequencies and odds ratios calculated using raw data provided in original article.
[2]Represents the genotype and allele counts and frequencies for the study participants with two white/Caucasian parents.
[3]Not reported, percentage calculated from the minor allele frequency.
[4]Bonferroni adjusted *P* values for multiple comparison were 0.032 (genotype) and 0.024 (allele).

DISCUSSION

Our systematic review identified three case–control studies of moderate methodological quality evaluating at least one *VDR* polymorphism. The main finding was a statistically significant association at the allele level between the *Fok*I *VDR* polymorphism and hospitalization for RSV-bronchiolitis. To our knowledge, this genetic association meta-analysis is the first on severe RSV-bronchiolitis.

Our meta-analysis demonstrated that the presence of the *Fok*I minor allele significantly increased the odds for RSV-bronchiolitis. This finding has biological plausibility, as vitamin D signaling is known to have important effects on development of the respiratory system and immune-inflammatory responses. For example, vitamin D modulates white blood cell proliferation, maturation

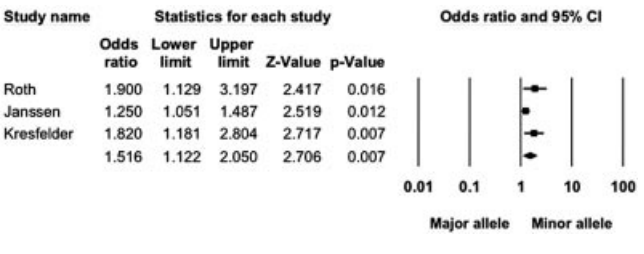

Fig. 2. Vitamin D receptor polymorphism *Fok1* allele meta-analysis. All three studies documented a statistically significant relationship between the *Fok*I f allele and disease. Meta-analysis calculated significantly increased odds of RSV/bronchiolitis for the minor allele using a random effect model.

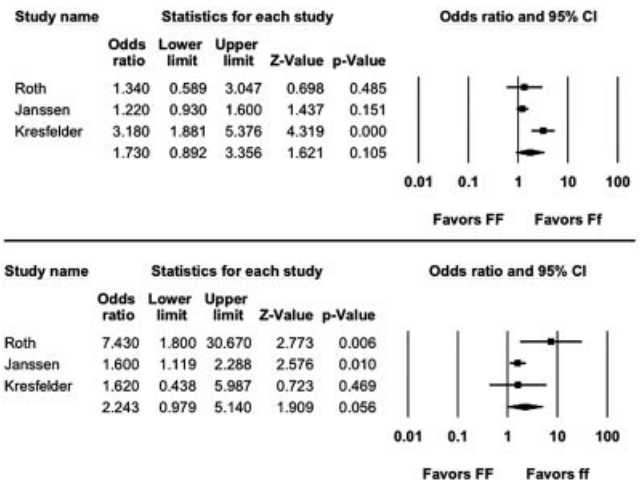

Fig. 3. Vitamin D receptor polymorphism *Fok1* genotype meta-analysis. The combined odds ratios for the fF and ff genotypes, relative to FF, are presented for association with RSV-bronchiolitis using a random effects meta-analysis model. The summary estimate and corresponding confidence interval suggest increased odds for both the ff and fF genotypes.

TABLE 3— *Taq*I Genotype and Allele Distributions Reported for the Included Studies

*Taq*I (rs7312‘36)	Geno	Cases, n (%)	Control, n (%)	OR (95% CI)	*P*-Value
Roth (2007)					
Overall					
Genotype	TT	24 (43)	32 (50)		
	Tt	28 (50)	28 (44)	1.34 (0.63, 2.81)	0.449
	tt	4 (7)	4 (6)	1.33 (0.30, 5.88)	0.704
Allele[1]	t, (%)*	36 (32)	36 (28)	1.20 (0.7, 2.1)	
Subsample[2]					
Genotype	TT	6 (24)	30 (54)		
	Tt	17 (68)	22 (39)	3.86 (1.31, 11.39)	0.014
	tt	2 (8)	4 (7)	2.50 (0.37, 16.89)	0.347
Allele[1]	t, (%)*	21 (42)	30 (27)	2.22 (1.11, 4.46)	
Janssen (2007)					
Genotype	TT	NR	NR	1.00	0.26
	Tt	NR	NR	1.23 (0.94, 1.61)	
	Tt	NR	NR	1.00 (0.68, 1.45)	
Allele	T	NR	NR	1.04 (0.87, 1.24)	0.66

Geno, genotype; OR, odds ratio; CI, confidence interval; NR, not reported.
[1]Allele frequencies and odds ratios calculated using raw data provided in original article.
[2]Represents the genotype and allele counts and frequencies for the study participants with two white/Caucasian parents.

and cytokine expression through VDR receptors on lymphocytes and macrophages.[61–63] In addition, VDR signaling also contributes to the expression of antimicrobial peptides, molecules important for innate defense again both viruses and bacteria.[64,65] These mechanisms, and others, might partly explain how a congenital or acquired vitamin D deficient state could contribute to RSV bronchiolitis and other forms of ALRI during early life.

The described association between *VDR* polymorphisms and disease is also consistent with what has been reported in related clinical research. First, there have been numerous pediatric case–control and cohort studies investigating the relationship between 25OHD levels and ALRI. Of the seven studies published to date, five have reported a statistically significant association with 25OHD concentrations, one identified a relationship with very severe disease only, and one was a negative study.[22–27,66] Of particular relevance are the two prospective newborn cohort studies identifying low cord blood vitamin D levels as an independent predictor of ALRI at 3 months and RSV infection during the first year.[26,27] Second, a growing body of individual genetic association studies and meta-analysis have identified associations with *VDR* polymorphisms and both the progression and treatment response for chronic infection.[42,43] Although investigations of acute infectious diseases are limited, a very recent study demonstrated a relationship between *Fok*I and urinary tract infection in children.[44]

The development of a clinically efficacious parenterally administered RSV antibody has helped decrease RSV-bronchiolitis related hospital admissions in certain high risk populations. Unfortunately, prediction tools using clinically or environmentally apparent characteristics on children without heart disease and prematurity have had unworkable discriminatory value. The results of recent twin studies imply that 20–25% of susceptibility to RSV-bronchiolitis could be genetic, and this meta-analysis suggests that some of this susceptibility may be due to polymorphisms in the *VDR* gene. We speculate that incorporation of specific genetic knowledge, such as the *VDR Fok*I polymorphism status, into a clinical predictor tool would improve discriminatory ability. However, a limitation of the current meta-analysis is that the combined OR for the *Fok*I polymorphism could not be adjusted for patient characteristics (age, gestation, heart disease) and environmental factors (older sibling, daycare attendance) known to impact RSV-bronchiolitis predisposition. Given this deficit further research in this area will be required to determine whether the *Fok*I *VDR* polymorphism remains an independent predictor after appropriate confounder adjustment. Any further work in this area should also be comprehensive enough to consider interaction between risk factors. For example, similar to what has been observed for other diseases we might expect additive and potentially multiplicative interactions between 25OHD levels and the *VDR* polymorphism state in the relationship with severe RSV disease.[67–69] In addition, the *VDR* polymorphisms might be influenced by polymorphisms in other vitamin D related genes (vitamin D binding protein, cytochrome P450 enzymes) or genes that produce proteins similarly involved in innate immunity. Without appropriate attention to these considerations any further study on the relationship between *VDR* polymorphisms and RSV-bronchiolitis cannot sufficiently advance the field.

There are a number of limitations to the conclusions of this systematic review. First, a relatively small number of studies investigated *VDR* polymorphisms and RSV-bronchiolitis. Consequentially we can only comment on the relationship between disease and these specific polymorphisms. In particular, the small number of studies and individual study sizes significantly reduced the analytical power at the genotype level. For example, given the small number of patients and low allele frequency we were unable to draw a definitive conclusion on whether *Fok*I demonstrates a recessive or incomplete pattern. Better understanding of whether this different risk associated with the fF and ff genotypes will be important if *VDR* is to be considered for inclusion into a clinical-metabolite-genetic prediction model.

The second potential limitation relates to the methodological quality of the studies. Interpreting our assessment of methodological quality (Supplementary data files) using the interim guidelines for the assessment of cumulative evidence on genetic associations, we consider the studies assessed here to have moderate credibility overall. The component analysis demonstrated that all three studies used an appropriate selection of case subjects, adequate ascertainment of exposure and incorporated population stratification into their analysis. However, as is common among genetic association studies, all three reports failed to provide sufficient information around controls or used a non-representative sample of cases introducing some risk for bias. Furthermore, two of the studies did not provide details on the study participation rate and the adequacy of biological sample. Despite the above limitations leading to potential bias, the included studies demonstrated associations in the same direction, with the overall result strengthened by the present meta analysis.

In conclusion, RSV is the major pathogen responsible for ALRI in children. Attempts to use clinically apparent characteristics to predict which late preterm or healthy newborns will develop severe RSV have only been moderately successful. Genetic studies have suggested that some of this unmeasured susceptibility is due to genetic variability. Our meta-analysis of three studies showed a significant association between the *Fok*I *VDR* polymorphism and severe RSV-bronchiolitis. This finding is consistent with multiple other publications reporting lower 25OHD levels in children who have or go on to develop RSV or ALRI. It is possible that knowledge of *VDR* polymorphism status may improve the discriminatory ability of prediction tools.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

E-Fig. 1. Vitamin D receptor gene: established linkage disequilibrium blocks and common polymorphisms. Vitamin D receptor (VDR) gene structure, linkage disequilibrium (LD) blocks and commonly studied single nucleotide polymorphisms (SNPs). Three distinct LD blocks have been identified in the gene and are termed A, B and C. Block A contains the 3′ untranslated region (UTR) and exon 9. Block B contains exons 3–9, which include *Bsm1* and *Taq1* SNPs. Block C is split into three smaller LD blocks, termed C1, C2, and C3. The commonly studied *Fok1* SNP is situated in exon 2 in an LD breaking spot and is unique since it does not display evidence of LD with other SNPs in the VDR. (Gene structure adapted from material presented in Rukin et al.[30])

Table A—Final Medline Search Strategy

Table B—Level 1 Screening Criteria

Table C—Level 2 Screening Criteria

Table D—Newcastle-Ottawa Assessment of Methodological Study Quality

Table E—Component Assessment of Study Methodological Quality