Maternal 17q21 genotype influences prenatal vitamin D effects on offspring asthma/recurrent wheeze

Hanna M. Knihtilä[1], Rachel S. Kelly[1], Nicklas Brustad[1,2], Mengna Huang[1], Priyadarshini Kachroo[1], Bo L. Chawes[2], Jakob Stokholm[2], Klaus Bønnelykke[2], Casper-Emil T. Pedersen[2], Hans Bisgaard[2], Augusto A. Litonjua[3], Jessica A. Lasky-Su[1] and Scott T. Weiss[1]

[1]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. [2]COPSAC (Copenhagen Prospective Studies on Asthma in Childhood), Herlev and Gentofte Hospital, University of Copenhagen, Gentofte, Denmark. [3]Division of Pediatric Pulmonary Medicine, Golisano Children's Hospital, University of Rochester Medical Center, Rochester, NY, USA.

Corresponding author: Scott T. Weiss (scott.weiss@channing.harvard.edu)

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This study demonstrates that maternal 17q21 genotype influences the protective effect of prenatal vitamin D_3 supplementation against early-life asthma/recurrent wheeze and this effect appears to be independent of the child's 17q21 genotype http://bit.ly/2ZcvwzH

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Abstract

Background Prenatal vitamin D_3 supplementation has been linked to reduced risk of early-life asthma/recurrent wheeze. This protective effect appears to be influenced by variations in the 17q21 functional single nucleotide polymorphism rs12936231 of the child, which regulates the expression of *ORMDL3* (ORM1-like 3) and for which the high-risk CC genotype is associated with early-onset asthma. However, this does not fully explain the differential effects of supplementation. We investigated the influence of maternal rs12936231 genotype variation on the protective effect of prenatal vitamin D_3 supplementation against offspring asthma/recurrent wheeze.

Methods We determined the rs12936231 genotype of mother–child pairs from two randomised controlled trials: the Vitamin D Antenatal Asthma Reduction Trial (VDAART, n=613) and the Copenhagen Prospective Studies on Asthma in Childhood 2010 ($COPSAC_{2010}$, n=563), to examine the effect of maternal genotype variation on offspring asthma/recurrent wheeze at age 0–3 years between groups who received high-dose prenatal vitamin D_3 supplementation *versus* placebo.

Results Offspring of mothers with the low-risk GG or GC genotype who received high-dose vitamin D_3 supplementation had a significantly reduced risk of asthma/recurrent wheeze when compared with the placebo group (hazard ratio (HR) 0.54, 95% CI 0.37–0.77; p<0.001 for VDAART and HR 0.56, 95% CI 0.35–0.92; p=0.021 for $COPSAC_{2010}$), whereas no difference was observed among the offspring of mothers with the high-risk CC genotype (HR 1.05, 95% CI 0.61–1.84; p=0.853 for VDAART and HR 1.11, 95% CI 0.54–2.28; p=0.785 for $COPSAC_{2010}$).

Conclusion Maternal 17q21 genotype has an important influence on the protective effects of prenatal vitamin D_3 supplementation against offspring asthma/recurrent wheeze.

Introduction

Asthma represents a globally significant disease burden affecting over 300 million people worldwide [1], and results in substantial childhood morbidity as measured by school absenteeism, emergency department visits and hospitalisations [2]. The origins of asthma have been linked to fetal development and prenatal exposures are thought to play a key role in disease pathogenesis [3]. In particular, exposure to vitamin D has been linked to fetal lung and immune system development [4]. Therefore, we conducted two independent randomised controlled trials that evaluated the potential of high-dose prenatal vitamin D_3 supplementation to reduce offspring asthma/recurrent wheeze: the Vitamin D Antenatal Asthma Reduction Trial (VDAART) [5] and the Copenhagen Prospective Studies on Asthma in Childhood 2010

(COPSAC$_{2010}$) [6]. A meta-analysis of these two trials showed that high-dose prenatal vitamin D_3 supplementation reduced early-life asthma/recurrent wheeze among offspring by 26% [7].

17q12–21 is the most replicated risk locus for childhood asthma, and overexpression of *ORMDL3* (ORM1-like 3) and *GSDMB* (gasdermin-B) at this locus has been linked to increased risk of childhood-onset asthma and recurrent wheeze [8, 9]. One of the key regulators of *ORMDL3* expression is a functional single nucleotide polymorphism (SNP) rs12936231 located at the *ZPBP2* (zona pellucida binding protein 2) intronic region in the 17q21 locus. A G-to-C change at this SNP independently alters *ORMDL3* expression by switching the binding site of the CCCTC-binding factor (CTCF) from the *ZPBP2* to the *ORMDL3* intronic region in T-cells [10, 11]. We previously showed that the protective effect of prenatal vitamin D_3 supplementation against early-life asthma/recurrent wheeze in VDAART and COPSAC$_{2010}$ seemed to be influenced by the child's rs12936231 genotype [12]. However, as the supplementation is given to the mothers and genetic factors may explain interindividual variability of metabolic response to nutrient intake [13], it is possible that maternal genotype may also influence the effects of the supplementation. Furthermore, a supplementation that is dependent on the maternal genotype could be utilised for precision prevention purposes.

In the present study, we evaluated the influence of variation in the maternal rs12936231 genotype on the protective effect of high-dose prenatal vitamin D_3 supplementation against offspring asthma and recurrent wheeze in VDAART and COPSAC$_{2010}$. The primary end-point was asthma/recurrent wheeze in the child's first 3 years of life.

Material and methods

Study subjects

VDAART (ClinicalTrials.gov: NCT00920621) and COPSAC$_{2010}$ (ClinicalTrials.gov: NCT00856947) trial designs and populations have been described in detail previously [5, 6]. Both trials randomised pregnant women (at 10–18 gestational weeks in VDAART and at 22–26 gestational weeks in COPSAC$_{2010}$) to receive either high-dose vitamin D_3 supplementation (4000 IU·day^{-1} in VDAART and 2400 IU·day^{-1} in COPSAC$_{2010}$) or placebo in addition to regular prenatal vitamin D_3 supplementation (400 IU·day^{-1}). A subset of the mothers in COPSAC$_{2010}$ were additionally randomised to receive prenatal fish oil supplementation in a factorial 2×2 design [14]. VDAART recruited US mothers who had a history of asthma, eczema or allergic rhinitis, or whose partners had a history of any of these diseases. COPSAC$_{2010}$ is a Danish population-based mother–child cohort.

Clinical end-point

Asthma/recurrent wheeze in the child's first 3 years of life was assessed based on the predefined criteria of both trials. For VDAART, the definition was based on parental report of physician-diagnosed asthma at age 0–3 years or parental report of recurrent wheeze satisfying at least one of the following five conditions ascertained from quarterly questionnaires since birth: 1) wheeze after the child's second birthday, preceded by wheeze before the second birthday; 2) asthma control medication use after the second birthday, preceded by wheeze before the second birthday; 3) at least two episodes of wheeze after the second birthday; 4) at least one episode of wheeze and asthma control medication use at distinct visits after the second birthday; or 5) two distinct reports of asthma control medication use after the second birthday [5]. For COPSAC$_{2010}$, asthma/recurrent wheeze was defined as meeting all of the following four criteria captured in daily symptom diaries from birth: 1) at least five episodes of troublesome lung symptoms within 6 months, each lasting at least 3 consecutive days; 2) typical symptoms of asthma; 3) intermittent bronchodilator use; and 4) response to a 3-month inhaled corticosteroid trial and relapse upon cessation [6].

Genotype

The Infinium HumanOmniExpressExome Bead chip (Illumina, San Diego, CA, USA) was used to determine the 17q21 genotype of SNP rs12936231 for the mothers and children in both cohorts. Only mother–child pairs with maternal genotype data were included in the present study. The G allele was considered the dominant low-risk allele and the C allele was considered the recessive high-risk allele [12].

Sphingolipid metabolites

In a subset of children in VDAART, five metabolites from the sphingolipid pathway (sphingosine-1-phosphate, sphingosine, sphinganine-1-phosphate, sphinganine and phosphoethanolamine) were measured using untargeted metabolomic profiling (Metabolon, Durham, NC, USA) from plasma samples that were drawn at ages 1 and 3 years as described previously [12].

Statistical analyses

The Chi-squared test or one-way ANOVA was used to evaluate differences in baseline characteristics by maternal rs12936231 genotype. Cox proportional hazards regression and Kaplan–Meier survival curves were used to evaluate the effect of prenatal vitamin D_3 supplementation on asthma/recurrent wheeze at age 0–3 years among subgroups according to maternal rs12936231 genotype. Multivariable logistic regression models were used to analyse interactions between maternal rs12936231 genotype and vitamin D_3 supplementation on the risk of asthma/recurrent wheeze at age 0–3 years. The interaction models for $COPSAC_{2010}$ were adjusted for fish oil supplementation, as a subset of the mothers were additionally randomised to fish oil supplementation. Comparisons between different rs12936231 genotypes were performed using 1) an additive model comparing GG genotype, GC genotype and CC genotype, and 2) a dominant model comparing GG/GC genotype and CC genotype. R version 3.6.0 (www.r-project.org) was used for all statistical analyses and two-tailed tests with a confidence level of 95% were applied.

Results

Baseline characteristics

Table 1 illustrates the baseline characteristics of the study subjects. In total, 613 mother–child pairs from VDAART and 563 mother–child pairs from $COPSAC_{2010}$ were included in the present study. All subjects had information on maternal rs12936231 genotype. Child rs12936231 genotype information was available for 565 subjects in VDAART and 502 subjects in $COPSAC_{2010}$. The serum 25-hydroxyvitamin D levels at randomisation did not differ between mothers with different rs12936231 genotypes in either of the cohorts, but mothers in VDAART had significantly lower 25-hydroxyvitamin D levels at randomisation than those in $COPSAC_{2010}$ (mean 23.2 $ng \cdot mL^{-1}$ for VDAART and 30.5 $ng \cdot mL^{-1}$ for $COPSAC_{2010}$; p<0.001). Vitamin D insufficiency (25-hydroxyvitamin D <30 $ng \cdot mL^{-1}$) was observed in 471 mothers (77%) in VDAART and in 269 mothers (48%) in $COPSAC_{2010}$ at randomisation. Insufficiency was especially prevalent among African American subjects in VDAART (238 out of 260 (92%)). There was no difference in the number of subjects in the vitamin D_3 intervention arms by genotype in either of the cohorts. Within $COPSAC_{2010}$, which additionally supplemented a subset of women with fish oil, no difference in genotype frequency was observed between any of the intervention arms (supplementary table E1).

The prevalence of maternal asthma was higher in the maternal high-risk CC genotype relative to the GC genotype and GG genotype in $COPSAC_{2010}$ (35% *versus* 22% *versus* 26%, respectively; p=0.025), with a similar but nonsignificant trend in VDAART (47% *versus* 38% *versus* 37%, respectively; p=0.121). However, we observed no significant differences in offspring asthma/recurrent wheeze between different maternal rs12936231 genotypes in either cohort.

TABLE 1 Baseline characteristics in the Vitamin D Antenatal Asthma Reduction Trial (VDAART) and Copenhagen Prospective Studies on Asthma in Childhood 2010 ($COPSAC_{2010}$) trials

	VDAART				$COPSAC_{2010}$			
	Maternal rs12936231 genotype			p-value	Maternal rs12936231 genotype			p-value
	GG	GC	CC		GG	GC	CC	
Subjects	132	299	182		152	263	148	
Vitamin D_3 intervention	60 (46)	158 (53)	84 (46)	0.223	71 (47)	136 (52)	77 (52)	0.559
Maternal race				0.009*				NA
African American	67 (51)	115 (39)	80 (44)		0	0	0	
Caucasian	55 (42)	131 (44)	65 (36)		152 (100)	263 (100)	148 (100)	
Other	10 (8)	53 (18)	37 (20)		0	0	0	
Maternal asthma	49 (37)	114 (38)	85 (47)	0.121	39 (26)	59 (22)	51 (35)	0.025*
Maternal baseline# serum 25-hydroxyvitamin D level $ng \cdot mL^{-1}$	23.0±9.6	23.7±10.8	22.5±10.6	0.457	30.7±10.5	29.7±9.3	31.7±10.7	0.167
Child rs12936231 genotype				<0.001*				<0.001*
GG	68 (51)	55 (21)	0		70 (51)	62 (26)	0	
GC	64 (49)	137 (52)	82 (49)		68 (49)	116 (49)	67 (52)	
CC	0	74 (28)	85 (51)		0	57 (24)	62 (48)	
Child sex (male)	63 (48)	160 (54)	101 (55)	0.377	74 (49)	135 (51)	78 (53)	0.775
Child asthma/recurrent wheezing at 0–3 years	34 (26)	93 (31)	50 (28)	0.467	25 (16)	45 (17)	30 (20)	0.640

Data are presented as n, n (%) or mean±SD, unless otherwise stated. NA: not applicable. #: before intervention, *i.e.* at 10–18 gestational weeks for VDAART and at 22–26 gestational weeks for $COPSAC_{2010}$. *: p<0.05.

Maternal 17q21 genotype and vitamin D_3 intervention

In both VDAART and $COPSAC_{2010}$, high-dose prenatal vitamin D_3 supplementation resulted in a significantly reduced risk of asthma/recurrent wheeze in the children of mothers with the low-risk GG or GC genotype, but not in the high-risk CC genotype (VDAART: hazard ratio (HR) 0.54, 95% CI 0.37–0.77; p<0.001 for GG/GC compared with HR 1.05, 95% CI 0.61–1.84; p=0.853 for CC and $COPSAC_{2010}$: HR 0.56, 95% CI 0.35–0.92; p=0.021 for GG/GC compared with HR 1.11, 95% CI 0.54–2.28; p=0.785 for CC) (table 2 and figure 1). Additional sensitivity analyses in $COPSAC_{2010}$ excluding subjects receiving fish oil supplementation also demonstrated a significant protective effect of vitamin D_3 supplementation among the offspring of mothers with the GG or GC genotype, but not in mothers with the CC genotype (supplementary table E2). In VDAART, a clear genotype-specific protective effect of the supplementation was observed among mothers with insufficient vitamin D levels at randomisation, but not among those with sufficient levels (supplementary table E3). However, there was no clear pattern in $COPSAC_{2010}$.

Table 3 illustrates the results from multivariable logistic regression models for an interaction between maternal rs12936231 genotype and high-dose prenatal vitamin D_3 supplementation on offspring risk of asthma/recurrent wheeze. There was a significant interaction in the additive model of VDAART (p=0.048), and a borderline significant interaction in the additive model of $COPSAC_{2010}$ (p=0.070) and the dominant models of both VDAART (p=0.059) and $COPSAC_{2010}$ (p=0.053). In contrast, no interaction between fish oil supplementation and maternal rs12936231 genotype on offspring risk of asthma/recurrent wheeze was observed in $COPSAC_{2010}$ (supplementary table E4).

Maternal and offspring 17q21 genotype combinations and vitamin D_3 intervention

There was inherently a high correlation between maternal and child rs12936231 genotype (table 1). Comparison of mother and child rs12936231 genotype combinations demonstrated a protective effect of high-dose prenatal vitamin D_3 supplementation when both mother and child had the low-risk GG or GC genotype (HR 0.54, 95% CI 0.35–0.83; p=0.005 for VDAART and HR 0.57, 95% CI 0.31–1.02; p=0.060 for $COPSAC_{2010}$) (table 4 and figure 2). However, no protective effect was seen if the mother had the high-risk CC genotype, regardless of child genotype. Race-stratified analyses in VDAART demonstrated a clear allele-additive modifying effect among African Americans based on maternal genotype: HR 0.24 (95% CI 0.09–0.65; p=0.005) for the GG genotype, HR 0.82 (95% CI 0.45–1.50; p=0.520) for the GC genotype and HR 1.12 (95% CI 0.52–2.39; p=0.770) for the CC genotype (table 5).

Supplementary table E5 shows the association between child sphingolipid levels and prenatal vitamin D_3 supplementation stratified by maternal and child rs12936231 genotype. Consistent increases in the measured sphingolipid levels were seen when both the mother and child had the low-risk GG or GC genotype. However, the sample sizes were small for these stratified analyses, and we were unable to distinguish the effects of maternal and child genotypes on the changes in child sphingolipid levels.

Discussion

The present study provides evidence that the protective effect of high-dose prenatal vitamin D_3 supplementation on early-life asthma/recurrent wheeze is dependent on variation in maternal 17q21 functional SNP rs12936231 genotype. Vitamin D_3 supplementation significantly reduced the risk of asthma/recurrent wheeze among the offspring of mothers with the low-risk GG or GC genotype, whereas no protective effect was seen in the offspring of mothers with the high-risk CC genotype. The significant effects of maternal rs12936231 genotype variation were observed in two independent cohorts, VDAART

TABLE 2 Effect of prenatal vitamin D_3 supplementation on the development of asthma/recurrent wheeze by age 0–3 years stratified by maternal 17q21 genotype

Maternal rs12936231 genotype	VDAART			$COPSAC_{2010}$		
	Cases/total n/N	HR (95% CI)	p-value	Cases/total n/N	HR (95% CI)	p-value
GG/GC#	127/431	0.54 (0.37–0.77)	<0.001*	70/415	0.56 (0.35–0.92)	0.021*
GG	34/132	0.39 (0.18–0.83)	0.015*	25/152	0.51 (0.22–1.18)	0.117
GC	93/299	0.58 (0.39–0.88)	0.011*	45/263	0.59 (0.33–1.08)	0.086
CC	50/182	1.05 (0.61–1.84)	0.853	30/148	1.11 (0.54–2.28)	0.785

VDAART: Vitamin D Antenatal Asthma Reduction Trial; $COPSAC_{2010}$: Copenhagen Prospective Studies on Asthma in Childhood 2010; HR: hazard ratio. #: combined maternal GG and GC genotype. Analyses were performed using Cox proportional hazard regression. G is considered the dominant low-risk allele and C is considered the recessive high-risk allele. *: p<0.05.

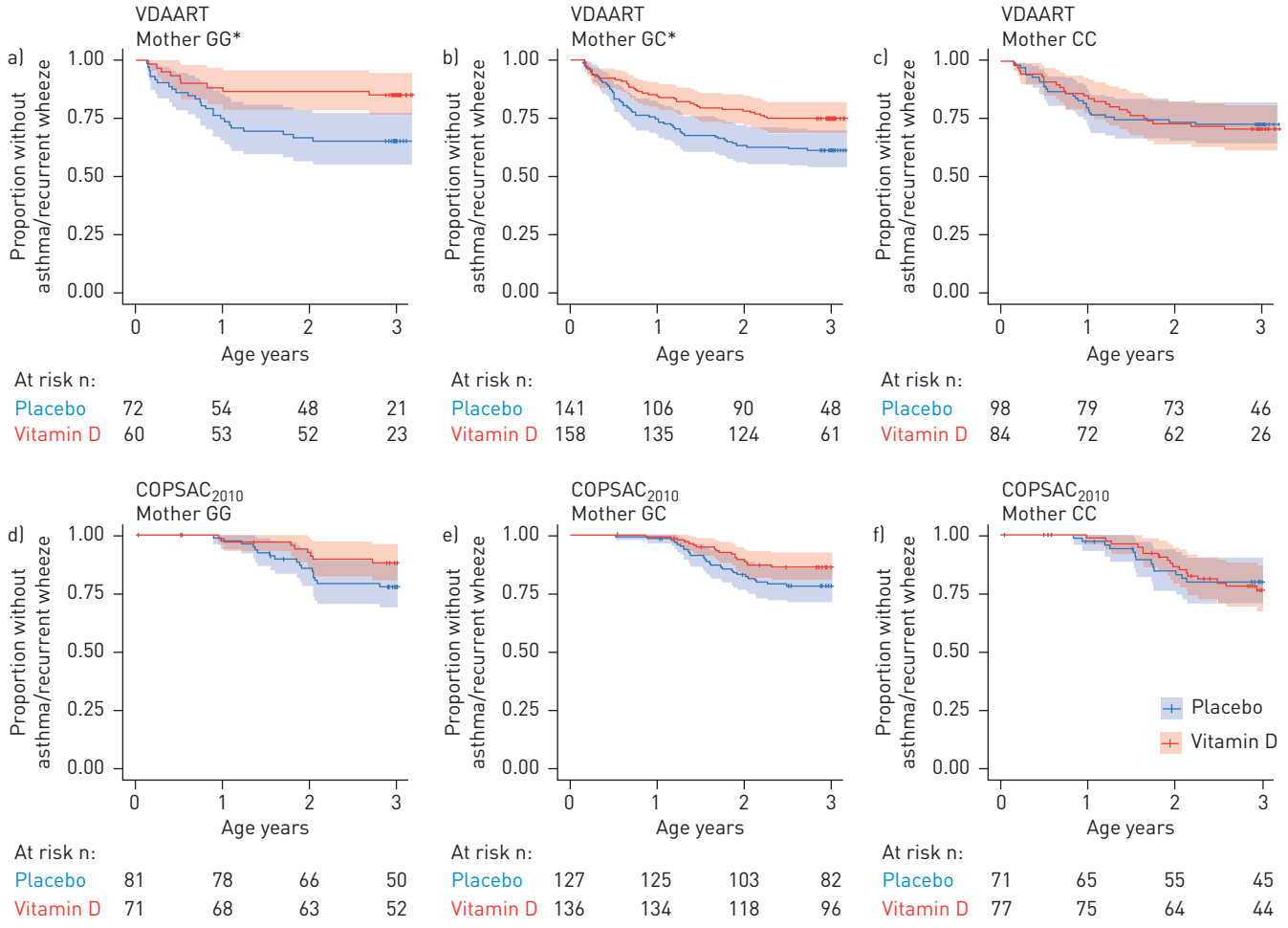

FIGURE 1 Kaplan–Meier survival curves for the effect of high-dose prenatal vitamin D_3 supplementation on the development of asthma/recurrent wheeze at age 0–3 years in the a–c) Vitamin D Antenatal Asthma Reduction Trial (VDAART) and d–f) Copenhagen Prospective Studies on Asthma in Childhood 2010 ($COPSAC_{2010}$) trials stratified by maternal 17q21 functional single nucleotide polymorphism rs12936231 genotype: a, d) GG, b, e) GC and c, f) CC. *: p<0.05 in the Cox proportional hazard regression model.

TABLE 3 Multivariable models[#] for the interaction between maternal 17q21 genotype and prenatal vitamin D_3 supplementation on the risk of offspring asthma/recurrent wheeze at 0–3 years

	VDAART[¶]		$COPSAC^{+}_{2010}$	
	Estimate	p-value	Estimate	p-value
Additive model				
Maternal rs12936231 genotype	−0.03	0.321	−0.02	0.514
Vitamin D_3 intervention	−0.20	0.002*	−0.13	0.015*
Maternal rs12936231 genotype×vitamin D_3 intervention	0.10	0.048	0.08	0.070
Dominant model				
Maternal rs12936231 genotype	−0.09	0.112	−0.04	0.445
Vitamin D_3 intervention	−0.14	0.001*	−0.09	0.016*
Maternal rs12936231 genotype×vitamin D_3 intervention	0.15	0.059	0.14	0.053

The additive model compares maternal genotypes GG *versus* GC *versus* CC and the dominant model compares maternal genotypes GG/GC *versus* CC. VDAART: Vitamin D Antenatal Asthma Reduction Trial; $COPSAC_{2010}$: Copenhagen Prospective Studies on Asthma in Childhood 2010. [#]: for VDAART, the model was: asthma/recurrent wheeze~mother genotype×vitamin D_3 intervention+child sex+child race+study site; for $COPSAC_{2010}$, the model was: asthma/recurrent wheeze~mother genotype×vitamin D_3 intervention+child sex+fish oil intervention; [¶]: n=613; [+]: n=563. *: p<0.05.

TABLE 4 Effect of prenatal vitamin D_3 supplementation on the development of early-life asthma/recurrent wheeze stratified by maternal and offspring 17q21 genotype combinations

Maternal rs12936231 genotype	Child rs12936231 genotype	VDAART			$COPSAC_{2010}$		
		Cases/ total n/N	HR (95% CI)	p-value	Cases/ total n/N	HR (95% CI)	p-value
GG/GC#	GG/GC#	91/324	0.54 (0.35–0.83)	0.005*	47/316	0.57 (0.31–1.02)	0.060
GG	GG	11/68	0.26 (0.06–1.20)	0.083	11/70	0.43 (0.11–1.62)	0.212
GG	GC	23/64	0.44 (0.18–1.06)	0.068	10/68	0.68 (0.19–2.42)	0.554
GC	GG	11/55	0.54 (0.17–1.77)	0.311	7/62	0.41 (0.08–2.10)	0.282
GC	GC	46/137	0.69 (0.39–1.25)	0.222	19/116	0.64 (0.26–1.59)	0.334
GC	CC	23/74	0.60 (0.26–1.36)	0.218	16/57	0.65 (0.24–1.74)	0.391
CC	GC	23/82	1.02 (0.45–2.32)	0.966	10/67	0.80 (0.23–2.76)	0.723
CC	CC	22/85	1.16 (0.50–2.68)	0.731	16/62	1.60 (0.58–4.41)	0.362

VDAART: Vitamin D Antenatal Asthma Reduction Trial; $COPSAC_{2010}$: Copenhagen Prospective Studies on Asthma in Childhood 2010; HR: hazard ratio. #: combined GG and GC genotype. Analyses were performed using Cox proportional hazard regression. G is considered the dominant low-risk allele and C is considered the recessive high-risk allele. *: p<0.05.

and $COPSAC_{2010}$. Interestingly, the influence of maternal rs12936231 genotype on the vitamin D_3–asthma relationship appeared to be even greater than that of the child's genotype which we reported previously [12]. This is among the first studies to demonstrate the importance of maternal genotype in prenatal interventions, which may have important implications for precision prevention.

Although the molecular mechanisms underlying prenatal vitamin D_3 supplementation and asthma are incompletely understood, vitamin D metabolites are known to exert several effects on lung development and immune system functions that could potentially explain this association. Vitamin D influences fetal lung development [15, 16] and results from VDAART demonstrated that prenatal vitamin D_3 supplementation might have beneficial effects on offspring lung function [17]. Vitamin D also influences several key immune system functions and vitamin D receptors are expressed on a variety of immune cells [4]. Specifically, vitamin D can activate regulatory T-cells [18] and increase steroid responsiveness in asthmatic subjects through stimulation of interleukin-10 production by regulatory T-cells [19]. Furthermore, vitamin D can influence the balance between type 1 and 2 T-helper cells [4], which is typically altered towards a type 2 T-cell predominance in asthma [20].

Several genome-wide association studies have demonstrated a link between the genetic variants in the 17q21 locus and childhood-onset asthma and recurrent wheeze [8, 9]. These risk variants result in cell-specific increases in the expression of *ORMDL3* which regulates *de novo* sphingolipid synthesis by inhibiting the rate-limiting enzyme serine palmitoyl transferase [21] and decreasing levels of sphingolipids [22]. In mice, decreased sphingolipid metabolism has been linked to increased airway hyperreactivity, suggesting that the functional link between the 17q21 locus and asthma susceptibility may be mediated through altered sphingolipid metabolism [23, 24]. However, the causality and exact molecular mechanisms between altered sphingolipid metabolism and asthma have not been verified [25]. Vitamin D metabolites have several modulatory effects on the sphingolipid pathway [26]. Furthermore, vitamin D can alter CTCF recruitment [27]. Therefore, we hypothesised that variation in rs12936231, which alters *ORMDL3* expression *via* changes in the CTCF binding site, may in part explain the differing effects of prenatal vitamin D_3 supplementation on offspring asthma/recurrent wheeze. We previously demonstrated that overexpression of *ORMDL3* in bronchial epithelial cells inhibits the production of sphingosine-1-phosphate by vitamin D_3 and that prenatal vitamin D_3 supplementation resulted in increased levels of key sphingolipids in children with the rs12936231 GG or GC genotype, but not in those with the CC genotype [12]. This supports the hypothesis that the protective effects of vitamin D_3 may be mediated through the sphingolipid pathway and explain the lack of effect when *ORMDL3* is overexpressed [11]. In the present study, we found that the protective effect of prenatal vitamin D_3 supplementation decreased with increasing numbers of maternal rs12936231 risk alleles, suggesting that the risk allele alters key pathways that mediate the effects of vitamin D_3 or that vitamin D_3 intervention does not affect genetically high-risk subjects. However, we were unable to distinguish the effects of maternal and child genotype on the changes in child sphingolipid levels in response to prenatal vitamin D_3 supplementation, possibly because of the small sample sizes in the stratified analyses. Therefore, further research is needed to confirm the exact molecular mechanisms underlying the association between vitamin D and asthma, and to clarify whether the genotype-specific effects of vitamin D_3 supplementation are mediated *via* altered maternal sphingolipid metabolism.

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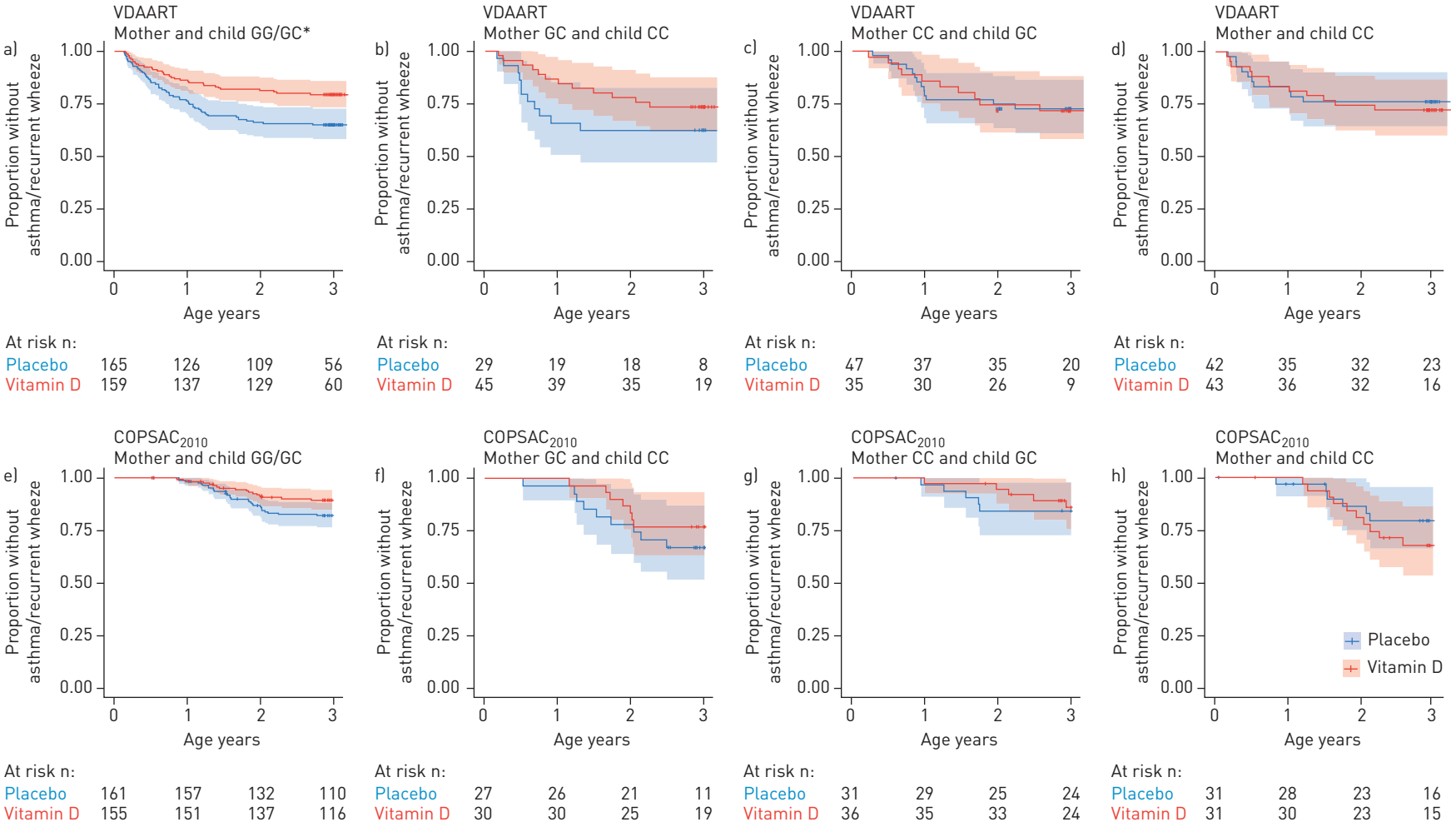

FIGURE 2 Kaplan–Meier survival curves for the effect of high-dose prenatal vitamin D_3 supplementation on the development of asthma/recurrent wheeze at age 0–3 years in the a–d) Vitamin D Antenatal Asthma Reduction Trial (VDAART) and e–h) Copenhagen Prospective Studies on Asthma in Childhood 2010 ($COPSAC_{2010}$) trials stratified by mother and child 17q21 functional single nucleotide polymorphism rs12936231 genotype combinations: a, e) mother and child GG/GC (combined genotype), b, f) mother GC and child CC, c, g) mother CC and child GC, and d, h) mother and child CC. G is considered as the dominant low-risk allele and C is considered as the recessive high-risk allele. *: p=0.005 in the Cox proportional hazard regression model.

TABLE 5 Effect of prenatal vitamin D_3 supplementation on the development of early-life asthma/recurrent wheeze stratified by maternal and child 17q21 genotype in African Americans and in other races from the Vitamin D Antenatal Asthma Reduction Trial (VDAART)

Maternal rs12936231 genotype	Child rs12936231 genotype	African American[#]			Other races[¶]		
		Cases/ total n/N	HR (95% CI)	p-value	Cases/ total n/N	HR (95% CI)	p-value
Mother							
GG/GC[+]		67/182	0.55 (0.33–0.90)	0.018*	60/249	0.54 (0.33–0.91)	0.021*
GG		24/67	0.24 (0.09–0.65)	0.005*	10/65	0.82 (0.23–2.89)	0.750
GC		43/115	0.82 (0.45–1.50)	0.520	50/184	0.46 (0.26–0.82)	0.008*
CC		27/80	1.12 (0.52–2.39)	0.770	23/102	0.86 (0.37–1.98)	0.720
Mother and child							
GG/GC[+]	GG/GC[+]	54/145	0.54 (0.31–0.96)	0.035*	37/179	0.58 (0.30–1.12)	0.100
GC	CC	9/25	0.89 (0.24–3.33)	0.870	14/49	0.47 (0.17–1.35)	0.160
CC	GC	13/38	1.07 (0.36–3.18)	0.910	10/44	0.75 (0.19–2.89)	0.670
CC	CC	11/34	1.64 (0.48–5.61)	0.430	11/51	0.81 (0.25–2.66)	0.730

[#]: n=262; [¶]: n=351 (Caucasian n=251, Asian n=26, American Indian or Alaska Native n=8, Native Hawaiian or Other Pacific Islander n=8 and other n=58); [+]: combined GG and GC genotype. Analyses were performed using Cox proportional hazard regression. G is considered the dominant low-risk allele and C is considered the recessive high-risk allele. *: p<0.05.

Because African Americans have increased risk of asthma and differ in 17q21-associated risk effects and allele frequencies compared with Caucasians [9], we investigated the effects of maternal genotype separately among African Americans in VDAART. *GSDMB* has been proposed as the leading candidate gene at the 17q21 locus for childhood-onset asthma in African Americans [28]. Our study demonstrates that the protective effects of prenatal vitamin D_3 supplementation against asthma/recurrent wheeze seem to be strongly dependent on maternal rs12936231 genotype, suggesting that in addition to *GSDMB*, *ORMDL3* might play an important role in the gene–environment interactions of asthma at the 17q21 locus among African Americans. In African Americans, rs12936231 is in low linkage disequilibrium with rs2305480 and rs11078927, which have shown the strongest associations with childhood-onset asthma in this population [28], indicating that the effects of the rs12936231 genotype are independent from those seen with rs2305480 or rs11078927. Vitamin D deficiency is extremely common among African Americans [4, 29] and therefore the genotype-specific protective effects of prenatal vitamin D_3 supplementation provide an especially important aspect for precision prevention of asthma among this population.

When studying the individual responses to prenatal interventions, it is essential to distinguish the influence of maternal and offspring genetic characteristics on the studied effect. However, relatedness results in a strong correlation between maternal and offspring genotypes and complicates this separation. Nevertheless, animal studies have demonstrated that maternal genetic effects can influence complex traits such as early-life obesity even more than the direct genetic effects of the offspring [30]. We found that vitamin D_3 supplementation had a significant protective effect against asthma/recurrent wheeze in the offspring of mothers with the low-risk rs12936231 genotype in both VDAART and $COPSAC_{2010}$. This effect was even stronger than we previously observed with the child genotype: a risk reduction of 46% was seen in VDAART and 44% in $COPSAC_{2010}$ among the offspring of mothers with the low-risk genotype who received high-dose vitamin D_3, whereas a smaller risk reduction of 31% in VDAART and 35% in $COPSAC_{2010}$ was previously observed among children with the low-risk genotype [12]. The hypothesis of maternal genotype imparting a stronger influence on the prenatal vitamin D_3 effects than child genotype was also supported by our findings on the maternal and offspring genotype combinations, although it should be noted that the combination analyses were restricted by relatively small sample sizes. No protective effect was seen if the mother had a high-risk genotype regardless of the genotype of the child. These findings suggest that maternal genotype has an independent influence on child responses to prenatal vitamin D_3 supplementation and highlight the need for further research to thoroughly elucidate the role of maternal genetic effects in prenatal exposures and offspring asthma to enable more targeted preventive actions for the disease.

We chose asthma/recurrent wheeze at age 0–3 years as the primary outcome of our study because prenatal supplementation seems to have the strongest influence in early life [17, 31]. However, it should be acknowledged that the diagnosis of asthma before school age is challenging and only a fraction of children with wheezing in early life will continue to have symptoms later in life [32]. As none of the available tests

can definitively diagnose asthma in young children, the diagnosis is based on a multifactorial evaluation of symptoms and risk factors [2]. However, even in the absence of asthma diagnosis, wheezing during early life can have long-term effects on lung function and quality of life [33, 34], and results in a substantial economic burden [35].

This study has several limitations. First, the analysis of interaction between maternal genotype and vitamin D, which remained only borderline significant in most models, is limited by the relatively small sample sizes of both cohorts. Second, VDAART recruited only parents with asthma or allergies, whereas $COPSAC_{2010}$ is a population-based cohort. The higher incidence of asthma/recurrent wheeze in VDAART might in part explain the stronger genotype-dependent protective effects of vitamin D_3 observed in VDAART. Another possible explanation is racial differences, as VDAART consists of a multiethnic population with predominantly African Americans, whereas $COPSAC_{2010}$ consists of a more homogeneous Caucasian population. This was further supported by our race-stratified analyses in VDAART, which demonstrated strongest genotype-specific protective effects of vitamin D_3 supplementation among African Americans. Furthermore, $COPSAC_{2010}$ used a lower vitamin D_3 dose (2400 *versus* 4000 $IU \cdot day^{-1}$) that was started later than in VDAART (22–26 *versus* 10–18 gestational weeks), which might in part explain the weaker protective effects seen in $COPSAC_{2010}$. As lung development begins in the first trimester of pregnancy, it is possible that both trials missed a crucial time window for influencing lung development. Furthermore, recent evidence suggests that alveolarisation can continue up to adolescence [36] and therefore postnatal vitamin D sufficiency might be required for maximal effects on lung function. Another limitation of the study is that the interaction between vitamin D_3 supplementation and maternal genotype remained only borderline significant in most of the models, which might be due to low statistical power. However, the fact that a significant maternal genotype-dependent protective effect was observed in the two independent trials with substantially different populations and designs increases the confidence in our findings, and can also be seen as a strength in terms of generalisability of the findings to other populations.

In conclusion, maternal rs12936231 genotype variation seems to have an important influence on the protective effects of prenatal vitamin D_3 supplementation against early-life asthma/recurrent wheeze. A significant protective effect was observed in the offspring of mothers with the low-risk GG or GC genotype, but no protective effect was seen in the offspring of mothers with the high-risk CC genotype. These findings imply that maternal genotype may play an important role in prenatal precision prevention strategies aimed at influencing offspring health.

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Author contributions: H.M. Knihtilä, R.S. Kelly and N. Brustad performed the statistical analyses. H.M. Knihtilä wrote the first draft of the manuscript. S.T. Weiss, A.A. Litonjua, J.A. Lasky-Su, R.S. Kelly and H. Bisgaard contributed to the study concept, design, supervision and manuscript revision. All authors contributed to data acquisition and interpretation of the data, and revised the manuscript for important intellectual content and provided their approval for its submission. S.T. Weiss had full access to the all the data in the study, takes full responsibility for the integrity of the data and the accuracy of the data analysis, and had authority over manuscript preparations and the decision to submit the manuscript for publication.

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