Prevention of Early Childhood Asthma and Recurrent Wheeze by Vitamin D and the Role of the 17q21 Genotype: Secondary Analysis of two Randomized Clinical Trials

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Study Populations

VDAART: Pregnant non-smoking women between 10 to 18 weeks of gestation with a history of asthma, eczema, or allergic rhinitis, or women who conceived the child with a man with a history of such diseases were recruited from three sites across the USA between October 2009 and July 2011; Boston, San Diego and St Louis.[1] Women were randomized 1:1 to a daily dose of 4,000 IU vitamin D_3 or a placebo tablet until delivery. All women additionally received a daily multivitamin containing 400 IU vitamin D_3. Randomization was performed using an automated system taking study-site and race into account, and the study was double-blinded. The families were followed post-delivery by quarterly telephone interviews and yearly in-clinic visits. VDAART was approved by the Institutional Review Boards (IRB) of the participating Clinical Centers and the Data Coordinating Center, with pregnant women signing informed consent at the enrollment visit covering both primary and secondary data analyses.

COPSAC$_{2010}$: Pregnant women between 22 to 26 weeks of gestation were recruited from across Zealand, Denmark between March 2009 and November 2010, and were randomized 1:1 to a daily dose of 2,400 IU vitamin D_3 or a placebo until 1 week postpartum.[2] All women also received a multivitamin containing 400 IU vitamin D_3. Randomization was performed using a computer-generated list of random numbers, supplied by an external investigator, and the study was double-blinded. The children were followed at the COPSAC research unit with nine scheduled clinic visits between the ages of 0 to 3 years, *ad hoc* visits for respiratory symptoms, and by daily diary-cards. The COPSAC$_{2010}$ study was approved by the Local Ethics Committee (H-B-2008-093; H-B-2009-014), the Danish Data Protection Agency (2008-41-2599), and the Danish Health and Medicines Authority (2612-3959). Written and oral informed consent for primary and secondary analyses was obtained at enrollment.

Asthma/Recurrent Wheeze Diagnosis from 0 to 3 Years:

VDAART: the children were diagnosed with asthma/recurrent wheeze using parental reporting of a physician diagnosis which was based on the following: (1) wheeze after the child's second birthday, preceded by at least one report of wheeze prior to the second birthday; (2) use of asthma controller medication after the second birthday, preceded by wheeze before the second birthday; (3) two or more reports of wheeze after the second birthday; (4) at least one report of wheeze and use of asthma controller medications at distinct visits after the second birthday; or (5) two distinct reports of use of asthma controller medications after the second birthday.[1]

COPSAC$_{2010}$: the children were diagnosed with asthma/wheeze by the COPSAC pediatricians based on the clinic visits and the diary cards, according to a previously validated quantitative symptom algorithm requiring occurrence of the following: (1) five episodes of troublesome lung symptoms within six months, each lasting at least three consecutive days; (2) typical asthma symptomatology, including exercise induced symptoms, prolonged nocturnal cough, and persistent cough outside common cold; (3) need for intermittent use of inhaled β2-agonist; and (4) response to a 3-month course of inhaled corticosteroids and relapse upon the termination of treatment. [3; 4]

Genotyping:

VDAART: Genotyping was performed using the Illumina Infinium HumanOmniExpressExome Bead chip at Illumina, San Diego, CA. Genotypes were called with Illumina Genome Studio software. The 17q21 rs12936231 SNP was genotyped on this array. We excluded individuals with an individual genotyping call rate < 0.95 or a sex mismatch.

COPSAC$_{2010}$: Genotyping was performed using the Illumina Infinium HumanOmniExpressExome Bead chip at the AROS Applied Biotechnology AS center, Aarhus, Denmark. Genotypes were called with Illumina Genome Studio software and rs12936231 was genotyped on this array. We excluded individuals with individual genotyping call rate < 0.95, sex mismatch, genetic duplicates, outlying heterozygosity > 0.27 and < 0.037, and those individuals not clustering with the CEU individuals (Utah residents with ancestry from northern and Western Europe) through a multi-dimensional clustering analyses (MDS) seeded with individuals from the International Hap Map Phase 3.

Sphingolipid Metabolism:

VDAART: Blood was sampled at ages 1 and 3 years at each clinical site and shipped to the Data Coordinating Center in Boston, where processing and aliquoting was done. Plasma was separated and immediately stored at -80°C until global metabolomic profiling at Metabolon, Inc., NC.
COPSAC$_{2010}$: Blood was sampled at 6 months at the clinical research unit, where plasma was separated, aliquoted and then immediately stored at -80°C at the Danish National Biobank until global metabolomic profiling at Metabolon, Inc., NC.

For both the VDAART and the COPSAC$_{2010}$ sample profiling was performed using ultrahigh performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS) and four platforms covering a broad range of the metabolome: (1) The first aliquot was analyzed using positive ion mode, chromatographically optimized for more hydrophilic compounds. The extract was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1x100 mm, 1.7 µm) using water and methanol, containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA); (2) The second aliquot was also analyzed using positive ion mode, however it was chromatographically optimized for more hydrophobic compounds. The extract was gradient eluted from the same C18 column as above using methanol, acetonitrile, water, 0.05% PFPA and 0.01% FA operated at an overall higher organic content; (3) The third aliquot was analyzed using negative ion mode with a separate dedicated C18 column. The basic extracts were gradient eluted from the column using methanol and water with 6.5mM ammonium bicarbonate at pH 8; (4) The fourth aliquot was analyzed via negative ionization following elution from HILIC column (Waters UPLC BEH Amide 2.1x150 mm, 1.7 µm) using a gradient consisting of water and acetonitrile with 10mM ammonium formate, pH 10.8. Metabolites were analyzed as measured LC-MS peak areas. In VDAART, data were processed in two batches sent six months apart (batch one n=245; batch two n=688) sent six months apart then merged and scaled together based on equivalence of the control groups. If a metabolite had a missingness of 50% or greater in either dataset it was excluded from further analysis. The COPSAC$_{2010}$ data underwent the standard metabolomic QC pipeline[3,66-68]; metabolites with a signal-to-noise ratio <10 were considered unquantifiable and excluded, as were metabolites with undetectable/missing levels for >10% of the samples. All remaining missing values were imputed with the half the minimum peak intensity for that metabolite across the whole population, then data were pareto scaled to account for the differences in the scales of measurements across the metabolome. In both datasets metabolites were log-transformed to create approximately Gaussian distributions and to stabilize variance.

Metabolites were identified by their mass-to-charge ratio (m/z), retention time (rt), and through a comparison to library entries of purified known standards. From the metabolomic profiles, we extracted five metabolites from the sphingolipid metabolism pathway which were measured and passed QC in our metabolomic dataset. Sphingosine-1-phosphate (HMDB00277) has been shown to be one of the most important sphingolipid metabolites for airway hyperresponsiveness, mast cell activation and inflammation in mechanistic asthma models.[5-7] To further characterize the sphingolipid metabolism pathway downstream of the *ORMDL3*-regulated rate limiting SPT enzyme, we also analyzed levels of Sphinganine (HMDB00269), Sphinganine-1-phosphate (HMDB01383), Phosphoethanolamine (HMDB00224) and Sphingosine (HMDB00252).

Establishment of ORMDL3-overexpressing stable line

Experiments were conducted in 16HBE cells purchased from ATCC. *ORMDL3* was stably overexpressed in 16HBE cells using a lenti-viral system. An *ORMDL3* Human Tagged open reading frame (ORF) clone in lentiviral particles was obtained from Origene (RC202279L3V). For lenti-viral transduction, 5×10^4 cells were seeded in 48-well plates, and lenti-virus was added to the cells in the presence of 8 µg/ml polybrene (Millipore, TR-1003-G) overnight. After puromycin selection, the overexpression of *ORMDL3* was determined by qPCR and Western blot analysis (**eFigure 1**).

Measurement of Sphinosine-1-phosphate levels in a ORMDL3 overexpressing cell line:

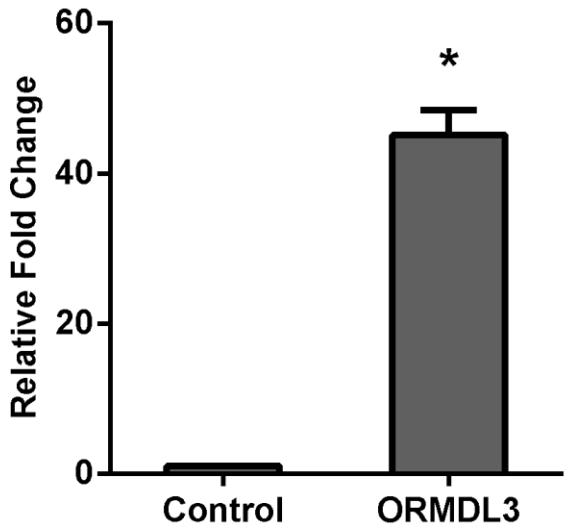
In order to determine whether there are functional implications of vitamin D treatment and *ORMDL3* expression on the levels of Sphinosine-1-phosphate, we treated 16HBE cells with or without overexpression of *ORMDL3*.

To quanify sphingosine-1-phosphate the cells were plated into 12-well plates at 4×10^5 cells/well. After 24 hours, the cells were starved overnight, then treated with 1α,25-vitamin D3 (Sigma-Aldrich) at three diffferent concentrations (0nM, 0.1nM and 1nM) for 10 hours. The levels of sphingosine-1-phosphate were subsequently quantitated by ELISA (MyBioSource). Independent ELISA were performed three times. The concentrations in the *ORMDL3* overexpressed cell line and the controls were compared to determine whether overexpression of *ORMDL3* reduced the ability of vitamin D to generate increased sphingolipid levels. An unpaired Students t-test was used to compare levels of Sphingosine-1-phosphate between the control and the ORMDL3 overespressed lines, at the three different vitamin d treament levels 0nM, 0.1nM and 1nM.

eFigures:

eFigure1: ORMDL3 overexpression level detection. ORMDL3 overexpression level was deteced by (A) mRNA level (RT-PCR) (Mean and SD was shown), and (B) protein level (western blot). * indicates $p < 0.05$, according to the unpaired Student's *t* test.

A



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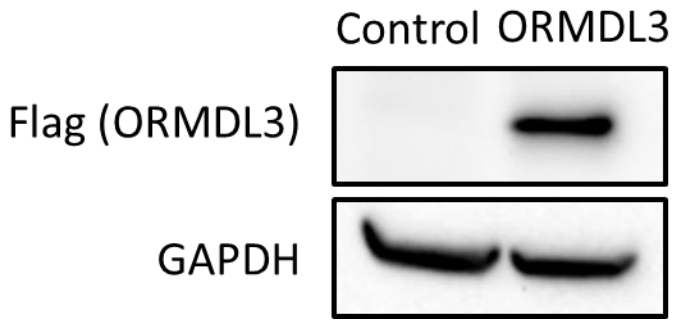
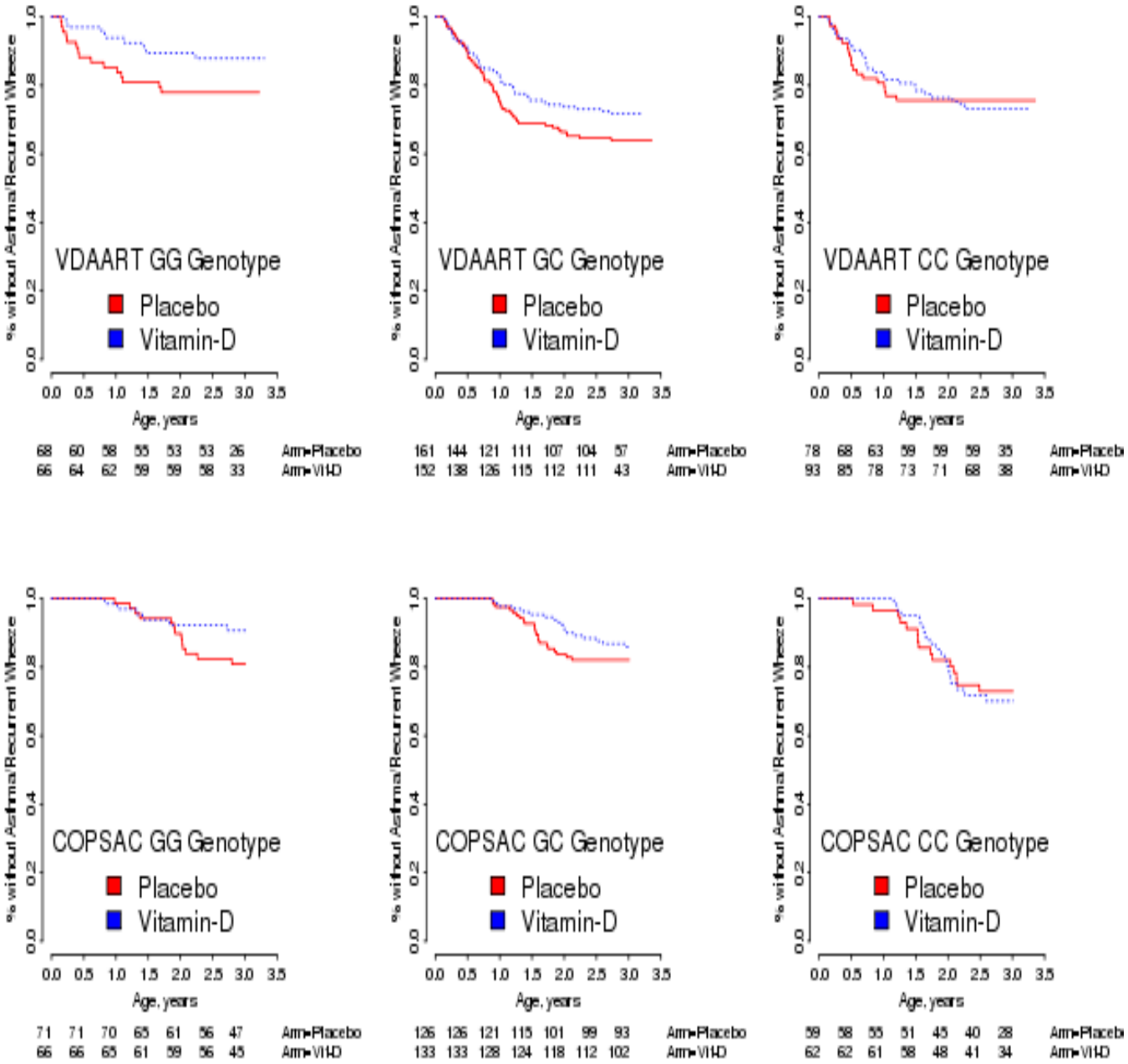


eFigure 2: Kaplan-Meier survival curves showing the effect of the prenatal vitamin D supplement on the offspring's risk of developing asthma/persistent wheeze at age 0 to 3 years in different genotype strata of the 17q21 functional SNP rs12936231 in the COPSAC$_{2010}$ and VDAART trials



Numbers at risk in the Placebo and Vitamin D arm are shown underneath the x-axis

eTables:

eTable 1: Total number of children in VDAART and in COPSAC$_{2010}$ and the number included in each of the analyses, together with their baseline characterstics

Characteristic	VDAART								COPSAC					
	Total Population (n=806)		Genotype Population (n=618)		Age One Yr Metabolite Population (n=413)		Age Three Yrs Metabolite Population (n=353)		Total Population (n=581)		Genotype Population (n=517)		Age Six Month Metabolite Population (n=441)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
rs12936231, N (%)														
CC	188	23.3%	171	27.7%	112	27.1%	106	30.0%	121	20.8%	121	23.4%	97	22.0%
GC	350	43.4%	313	50.6%	221	53.5%	172	48.7%	259	44.6%	259	50.1%	229	51.9%
GG	151	18.7%	134	21.7%	80	19.4%	75	21.2%	137	23.6%	137	26.5%	115	26.1%
missing	117	14.5%	0	0.0%	0	0.0%	0	0.0%	64	11.0%	0	0.0%	0	0.0%
Study Arm, N (%)														
Placebo	401	49.8%	307	49.7%	211	51.1%	174	49.3%	286	49.2%	256	49.5%	213	48.3%
Vitamin D	405	50.2%	311	50.3%	202	48.9%	179	50.7%	295	50.8%	261	50.5%	228	51.7%
Asthma/wheeze, 0-3yrs, N (%)														
No	530	65.8%	450	72.8%	287	69.5%	264	74.8%	477	82.1%	425	82.2%	358	81.2%
Yes	218	27.0%	168	27.2%	126	30.5%	89	25.2%	104	17.9%	92	17.8%	83	18.8%
missing	58	7.2%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Sex, N (%)														
Male	421	52.2%	321	51.9%	218	52.8%	187	53.0%	298	51.3%	269	52.0%	227	51.5%
Female	385	47.8%	297	48.1%	195	47.2%	166	47.0%	283	48.7%	248	48.0%	214	48.5%
Race, N (%)														
Black	390	48.4%	290	46.9%	195	47.2%	168	47.6%	0	0.0%	0	0.0%	0	0.0%
Caucasian	265	32.9%	200	32.4%	134	32.4%	113	32.0%	581	100.0%	517	100.0%	441	100.0%
Other	151	18.7%	128	20.7%	84	20.3%	72	20.4%	0	0.0%	0	0.0%	0	0.0%

eTable 2: Baseline characteristics of the participants from COPSAC$_{2010}$ stratified by vitamin D and fish-oil intervention

Characteristic	Vitamin D Only (n=128)	Fish Oil Only (n=123)	Vitamin D + Fish Oil (n=133)	Placebo Only (n=133)	p-value
rs12936231, N (%)					
CC	24 (18.8)	26 (21.1)	38 (28.6)	33 (24.8)	0.411
GC	71 (55.5)	58 (47.2)	62 (46.6)	68 (51.1)	
GG	33 (25.8)	39 (31.7)	33 (24.8)	32 (24.1)	
Sex, N (%)					
Male	74 (57.8)	62 (50.4)	67 (50.4)	66 (49.6)	0.513
Female	54 (42.2)	61 (49.6)	66 (49.6)	67 (50.4)	

eTable 3: Effect of prenatal vitamin D supplementation on development of asthma/persistent wheeze from 0 to 3 years in the VDAART and COPSAC$_{2010}$ trials stratified by genotype of the 17q21 functional SNP rs12936231; assuming dominance of the G allele

Vitamin D vs· placebo	VDAART N=618		COPSAC$_{2010}$ N=517		Combined analysis
rs12936231	total/ cases	HR (95% CI), p-value	total/ cases	HR (95% CI), p-value	HR (95% CI), p-value
GG/GC	447/124	0·69 (0·48-0·98), p=0·041	396/59	0·65 (0·39-1·09), p=0·103	0·68 (0·50-0·91), p=0·009
CC	171/44	1·07 (0·59-1·95), p=0·821	121/33	1·08 (0·55-2·15), p=0·822	1·08 (0·69-1·69), p=0·751

eTable 4: Mean of blood peak intensity of five key sphingolipid metabolites measured at age six months in 441 children from COPSAC$_{2010}$ in the placebo and vitamin D groups stratified by 17q21 genotype. C is the risk allele

	Six Month Samples		
	Coefficient	**95% CI**	**p value**
sphinganine-1-phosphate			
CC	0.031	(-0.117,0.180)	0.679
GC	-0.115	(-0.224,-0.006)	0.039*
GG	0.005	(-0.164,0.173)	0.958
sphinganine			
CC	0.056	(-0.173,0.286)	0.630
GC	0.009	(-0.123,0.142)	0.893
GG	0.07	(-0.137,0.278)	0.508
sphingosine-1-phosphate			
CC	0.023	(-0.118,0.165)	0.746
GC	-0.104	(-0.204,-0.004)	0.043*
GG	-0.04	(-0.19,0.11)	0.601
sphingosine			
CC	-0.017	(-0.239,0.205)	0.882
GC	-0.012	(-0.165,0.141)	0.873
GG	0.018	(-0.191,0.226)	0.869
phosphoethanolamine			
CC	0.105	(-0.053,0.264)	0.196
GC	-0.04	(-0.151,0.07)	0.476
GG	0.058	(-0.099,0.215)	0.471

CC n=97 (22%); GC n=229(52%); GG n=115 (26%)
**Significant at the 95% Confidence interval*

eTable 5: Results of multivariable interaction models exploring the relationship between a diagnosis of asthma/recurrent wheeze by age 3 years, the vitamin D intervention, 17q21 genotype and five key sphingolipids in VDAART· The table shows Beta estimate (p-values) for the individual variables/terms for a generic model: *asthma/recurrent wheeze~sphingolipid metabolite* vitamin D intervention* genotype*

	sphinganine-1-phosphate	**sphinganine**	**phosphoethanolamine**	**sphingosine**	**sphingosine-1-phosphate**
AGE ONE SAMPLES					
Sphingolipid Metabolite	-9·26 (0·009*)	0·16 (0·893)	-4·04 (0·082)	0·04 (0·976)	-14·02 (0·019*)
Vitamin D Intervention	-3·92 (0·005*)	-0·77 (0·314)	-3·20 (0·005*)	-0·79 (0·369)	-5·52 (0·017*)
rs12936231 genotype	-1·65 (0·040*)	0·10 (0·803)	-0·36 (0·554)	0·13 (0·773)	-2·62 (0·076·)
Vitamin D Intervention*sphingolipid metabolite	11·43 (0·008*)	1·12 (0·541)	8·40 (0·008*)	1·15 (0·581)	16·64 (0·023*)
Vitamin D Intervention*rs12936231 genotype	2·70 (0·013*)	0·56 (0·377)	2·03 (0·024*)	0·40 (0·581)	3·97 (0·034*)
rs12936231 genotype*Sphingolipid metabolite	5·66 (0·020*)	0·02 (0·987)	1·60 (0·364)	-0·05 (0·960)	8·75 (0·059·)
Vitamin D Intervention*rs12936231 genotype*sphingolipid	**-8·15 (0·011*)**	**-1·27 (0·425)**	**-5·72 (0·022*)**	**-0·74 (0·675)**	**-12·35 (0·035*)**
AGE THREE SAMPLES					
Sphingolipid Metabolite	-4·57 (0·133)	-1·13 (0·501)	0·06 (0·986)	-0·24 (0·898)	-3·16 (0·483)
Vitamin D Intervention	-2·89 (0·049*)	-1·74 (0·092)	-1·81 (0·247)	-1·47 (0·185)	-3·79 (0·078)
rs12936231 genotype	-0·63 (0·359)	-0·29 (0·563)	-0·05 (0·948)	-0·12 (0·830)	-0·34 (0·721)
Vitamin D Intervention*sphingolipid metabolite	6·55 (0·126)	2·37 (0·304)	2·79 (0·565)	1·64 (0·522)	9·68 (0·153)
Vitamin D Intervention*rs12936231 genotype	2·33 (0·031*)	1·73 (0·024*)	2·51 (0·039*)	1·55 (0·060)	3·06 (0·050*)
rs12936231 genotype*sphingolipid metabolite	2·58 (0·216)	1·20 (0·320)	0·68 (0·789)	0·72 (0·589)	1·73 (0·586)
Vitamin D Intervention*rs12936231 genotype*sphingolipid	**-6·12 (0·050*)**	**-3·66 (0·040*)**	**-6·84 (0·074)**	**-3·19 (0·106)**	**-8·84 (0·076)**

**Significant at the 95% Confidence interval*

eTable 6: Results of multivariable interaction models exploring the relationship between a diagnosis of asthma/recurrent wheeze by age 3 years, the vitamin D intervention, 17q21 genotype and five key sphingolipids measured at six months in 441 children from COPSAC$_{2010}$. The table shows Beta estimate (p-values) for the individual variables/terms for a generic model: *asthma/recurrent wheeze~sphingolipid metabolite* vitamin D intervention* genotype*

	Sphinganine-1-phosphate	Sphinganine	Phosphoethanolamine	Sphingosine	Sphingosine-1-phosphate
Sphingolipid Metabolite	0·12 (0·867)	-0·27 (0·573)	0·62 (0·419)	0·44 (0·372)	0·48 (0·539)
Vitamin D Intervention	-1·13 (0·019*)	-1·14 (0·020*)	-1·14 (0·018*)	-1·14 (0·018*)	-1·12 (0·021*)
rs12936231 genotype	0·10 (0·684)	0·09 (0·716)	0·11 (0·659)	0·06 (0·793)	0·10 (0·674)
Vitamin D Intervention*Sphingolipid metabolite	0·03 (0·981)	-0·03 (0·973)	-0·22 (0·852)	-0·27 (0·758)	-0·07 (0·955)
Vitamin D Intervention*rs12936231 genotype	0·72 (0·051)	0·74 (0·047*)	0·72 (0·052)	0·75 (0·044*)	0·72 (0·053)
rs12936231 genotype*Sphingolipid metabolite	0·09 (0·878)	0·57 (0·171)	-0·28 (0·670)	0·07 (0·860)	-0·10 (0·873)
Vitamin D Intervention*rs12936231 genotype*Sphingolipid metabolite	-0·33 (0·717)	-0·76 (0·244)	-0·30 (0·750)	-0·29 (0·660)	-0·31 (0·756)

**Significant at the 95% Confidence interval*

eTable 7: rs12936231 genotype and asthma status by race in VDAART

		No Asthma/wheeze, 0-3yrs	Asthma/wheeze, 0-3yrs	Total
		N (%)	N (%)	N (%)
Black (n=290)	**CC**	51 (26·7)	22 (22·2)	73 (25·2)
	GC	81 (42·4)	64 (64·6)	145 (50·0)
	GG	59 (30·9)	13 (13·1)	72 (24·8)
Caucasian (n=200)	**CC**	35 (22·9)	14 (29·8)	49 (24·5)
	GC	82 (53·6)	24 (51·1)	106 (53·0)
	GG	36 (23·5)	9 (19·1)	45 (22·5)
Other (n=128)	**CC**	41 (38·7)	8 (36·4)	49 (38·3)
	GC	49 (46·2)	13 (59·1)	62 (48·4)
	GG	16 (15·1)	1 (4·5)	17 (13·3)

eTable 8: Combined analysis of effect of prenatal vitamin D supplementation on development of asthma/persistent wheeze from 0 to 3 years stratified by genotype of the 17q21 functional SNP rs12936231 in Black and in Caucasian/Other subjects from VDAART

Vitamin D vs· placebo	**African American N=290**		**Caucasian+Other N=328**		**Combined analysis**
rs12936231 strata	total/ cases	HR (95% CI), p-value	total/ cases	HR (95% CI), p-value	HR (95% CI), p-value
GG	72/13	0·29 (0·0·8-1·07), p=0·063	62/10	0·90 (0·26-3·14), p=0·880	0·53 (0·22-1·29), p=0·163
GC	145/64	0·83 (0·50-1·35), p=0·449	168/37	0·66 (0·34-1·28), p=0·219	0·76 (0·51-1·14), p=0·179
CC	73/22	1·29 (0·54-3·09), p=0·560	98/22	0·87 (0·38-2·00), p=0·739	1·05 (0·58-1·92), p=0·870

eTable 9: Association between blood peak intensity of five key sphingolipid metabolites and vitamin D intervention in VDAART children stratified by 17q21 genotype and race; results shown for ages one and three combined according to a mixed model

	African American, Black		Caucasian+Other	
	Coefficient	**P-value**	**Coefficient**	**P-value**
sphinganine-1-phosphate				
CC	-0.012	0.591	-0.015	0.519
GC	0.024	0.159	-0.009	0.558
GG	0.035	0.136	0.053	0.120
sphinganine				
CC	-0.012	0.791	0.012	0.718
GC	0.018	0.543	-0.006	0.798
GG	0.073	0.193	0.067	0.212
sphingosine-1-phosphate				
CC	-0.011	0.482	-0.012	0.316
GC	0.008	0.454	-0.003	0.720
GG	0.025	0.056	0.022	0.257
sphingosine				
CC	-0.006	0.889	-0.006	0.852
GC	0.007	0.821	0.006	0.779
GG	0.061	0.228	0.058	0.190
phos-phoethanolamine				
CC	-0.011	0.646	0.020	0.427
GC	-0.001	0.972	-0.040	0.037
GG	0.007	0.792	0.069	0.033

eTable 10: Results of multivariable interaction models exploring the relationship between a diagnosis of asthma/recurrent wheeze by age 3 years, the vitamin D intervention, 17q21 genotype and five key sphingolipids in VDAART· The table shows Beta estimate (p-values) for the multiinteraction term: *asthma/recurrent wheeze~sphingolipid metabolite* vitamin D intervention* genotype*; with additional adjustment for, or stratification by, race

		Vitamin D Intervention*rs12936231 genotype*sphingolipid				
		sphinganine-1-phosphate	**sphinganine**	**phospho-ethanolamine**	**sphingosine**	**sphingosine-1-phosphate**
Adjusting for Race Category	**YEAR ONE**	-8.5 (0.012)	-1.39 (0.402)	-6.33 (0.015)	-0.95 (0.605)	-12.95 (0.034)
	YEAR THREE	-6.47 (0.039)	-4.08 (0.025)	-7.31 (0.058)	-3.69 (0.065)	-9.92 (0.05)
YEAR ONE	**African American, Black**	-4.17 (0.439)	0.22 (0.926)	-4.2 (0.304)	0.67 (0.785)	-8.04 (0.377)
	Caucasian+Other	-9.56 (0.036)	-3.13 (0.258)	-5.67 (0.117)	-2.97 (0.371)	-13.8 (0.103)
YEAR THREE	**African American, Black**	-6.24 (0.165)	-5.35 (0.036)	-10.13 (0.065)	-4.22 (0.121)	-7.95 (0.247)
	Caucasian+Other	-4.58 (0.331)	-4.3 (0.152)	-2.76 (0.644)	-4.92 (0.138)	-10.14 (0.179)

eTable11: Effect of prenatal vitamin D supplementation on development of asthma/persistent wheeze from 0 to 3 years in the VDAART and COPSAC$_{2010}$ trials stratified by genotype of the 17q21 functional SNP rs12936231; Excluding the children of 256 mothers who additionally received fish-oil supplementation during pregnancy

Vitamin D vs· placebo	**COPSAC$_{2010}$, N=261**		**Combined analysis with VDAART**
rs12936231 strata	total/ cases	HR (95% CI), p-value	HR[a] (95% CI), p-value
GG	65/9	0·45 (0·11-1·81), p=0·262	0·49 (0·24-1.02), p=0·055
GC	139/23	0·56 (0·24-1·30), p=0·178	0·71 (0·50-1·01), p=0·060
CC	57/17	0.84 (0·31-2·20), p=0·715	1·00 (0·60-1·66), p=0·999

eTable 12: Association between blood peak intensity of five key sphingolipid metabolites and vitamin D intervention in VDAART children stratified by 17q21 genotype Excluding the children of 256 mothers who additionally received fish-oil supplementation during pregnancy; results shown for ages one and three combined according to a mixed model

	Excluding all those whose mothers received fish-oil (n=222 included participants)		
	Coefficient	**95% CI**	**p value**
sphinganine-1-phosphate			
CC	0.058	(-0.152,0.269)	0.59
GC	-0.142	(-0.292,0.008)	0.066
GG	8.8x10-5	(-0.215,0.215)	0.999
sphinganine			
CC	-0.012	(-0.4,0.376)	0.952
GC	0.004	(-0.185,0.193)	0.965
GG	0.098	(-0.239,0.434)	0.572
sphingosine-1-phosphate			
CC	0.034	(-0.183,0.251)	0.76
GC	-0.131	(-0.279,0.017)	0.086
GG	-0.062	(-0.259,0.136)	0.543
sphingosine			
CC	-0.095	(-0.416,0.226)	0.564
GC	-0.005	(-0.229,0.22)	0.968
GG	-0.082	(-0.374,0.21)	0.584
phosphoethanolamine			
CC	0.172	(-0.08,0.424)	0.188
GC	-0.04	(-0.19,0.109)	0.599
GG	0.028	(-0.206,0.262)	0.816

eTable 13: Results of multivariable interaction models exploring the relationship between a diagnosis of asthma/recurrent wheeze by age 3 years, the vitamin D intervention, 17q21 genotype and five key sphingolipids in VDAART· The table shows Beta estimate (p-values) for the multiinteraction term: *asthma/recurrent wheeze~sphingolipid metabolite* vitamin D intervention* genotype*; with additional adjustment for, or stratification by, race

	Sphinganine-1-phosphate	**Sphinganine**	**Phosphoethanolamine**	**Sphingosine**	**Sphingosine-1-phosphate**
Sphingolipid Metabolite	-0.23 (0.82)	-0.34 (0.565)	0.4 (0.76)	0.25 (0.704)	-0.08 (0.946)
Vitamin D Intervention	-1.13 (0.086)	-1.06 (0.126)	-1.16 (0.081)	-1.11 (0.096)	-1.15 (0.081)
rs12936231 genotype	0.14 (0.673)	0.16 (0.635)	0.12 (0.73)	0.1 (0.755)	0.13 (0.691)
Vitamin D Intervention*Sphingolipid metabolite	0.17 (0.92)	-0.18 (0.867)	-0.27 (0.873)	-0.16 (0.902)	0.59 (0.746)
Vitamin D Intervention*rs12936231 genotype	0.54 (0.291)	0.42 (0.453)	0.52 (0.333)	0.49 (0.356)	0.55 (0.288)
rs12936231 genotype*Sphingolipid metabolite	0.13 (0.876)	0.61 (0.235)	-0.22 (0.818)	0.09 (0.869)	0 (0.997)
Vitamin D Intervention*rs12936231 genotype*Sphingolipid metabolite	-0.15 (0.917)	-1.22 (0.151)	-0.88 (0.518)	-0.97 (0.344)	-0.85 (0.579)

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