Journal of Steroid Biochemistry and Molecular Biology 201 (2020) 105692

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Vitamin D receptor polymorphisms are associated with severity of wheezing illnesses and asthma exacerbations in children

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ARTICLE INFO

Keywords:
Acute asthma
Wheeze
Children
Single nucleotide polymorphism
VDR

ABSTRACT

Single nucleotide polymorphisms (SNPs) of the vitamin D receptor (*VDR*) gene have shown linkage and association with asthma development in multiple cohort studies. However, the majority of investigations have focused on asthma phenotypes in cohorts with stable disease. We investigated the relationship between VDR SNPs and the frequency and severity of acute episodes of wheeze/asthma in a cohort of Australian children, as the ability to identify children at risk of more severe exacerbations could lead to personalized and improved genotype-specific treatment pathways. We successfully genotyped five SNPs of the *VDR* gene (rs2525046, rs9729, rs1544410 (*Bsm*I), rs22239179, and rs2228570 (*Fok*I)) in 657 children presenting to a tertiary children's hospital with acute asthma, bronchiolitis, or a wheezing illness. The relationships between *VDR* SNPs and exacerbation severity scores, β_2-agonist use, and frequency of respiratory exacerbations were analysed using multiple regression. The rs2525046 (*Fok*I) CT genotype was associated with higher VDR mRNA intensity levels ($p = 0.007$) compared to the CC genotype. A trend towards significance ($p = 0.056$) was identified between the rs2525046 TT genotype and higher VDR mRNA intensity levels compared to the CC genotype. Children with rs2228570 AA genotype had higher exacerbation severity scores ($p = 0.001$) and poorer β_2-agonist treatment response (doses at 6 h: $p = 0.009$ and 12 h: $p = 0.033$) compared to those with the GG genotype. Children with rs1544410 (*Bsm*I) TT genotype had lower exacerbation severity scores ($p = 0.005$) compared to those with the CC genotype. Children with rs2228570 GA genotype presented to and/or were admitted to hospital more times since birth with respiratory ($p = 0.011$) and wheezing ($p = 0.021$) illnesses than children with the GG genotype. No associations were identified between rs9729, rs2525046 and r2239179 polymorphisms and acute wheezing/asthma variables. These findings suggest that genetic variants at the *VDR* locus may play a role in acute wheeze/asthma severity in children.

1. Introduction

Wheezing and asthma exacerbations are a leading cause of hospitalisations for children in developed countries [1]. Asthma is a multifactorial disease caused by a complex interaction of genetic predisposition and environmental exposures. A significant proportion of the inter-individual risk for developing asthma is due to genetic differences [2–4]. Numerous studies have identified loci, candidate genes, and single nucleotide polymorphisms (SNPs) which show linkage and association with asthma development [5–8]. However, limited studies have evaluated the association between these isolated genetic variations and mechanisms of acute wheeze and asthma.

There has been increasing interest in the role of vitamin D in wheezing and asthma. Vitamin D, a fat-soluble nutrient widely recognised for its role in bone health, also plays a role in immune regulation [9]. Its biological effects, including regulation of helper T-cell development and subsequent cytokine secretion profiles, are achieved through the regulation of gene expression, which is mediated by the vitamin D receptor (VDR) [8]. The *VDR* gene is located on chromosome 12, region q13 − 23: a region commonly linked to asthma in genome-wide linkage analyses [10].

Family-based cohort studies have identified *VDR* genetic variants that are strongly associated with asthma traits in particular populations [7,8]. Such findings suggest that the *VDR* locus harbours variants that

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https://doi.org/10.1016/j.jsbmb.2020.105692
Received 1 September 2019; Received in revised form 28 April 2020; Accepted 29 April 2020
Available online 05 May 2020

contribute to asthma risk. However, the majority of investigations have focused on asthma phenotypes in cohorts with stable disease. Our study aimed to identify *VDR* polymorphisms associated with the frequency and/or severity of wheezing illnesses and asthma exacerbations in a cohort of Australian children. We hypothesised that genetic variations at the *VDR* locus would be associated with an increased frequency of hospital visits and more severe exacerbations. To test this hypothesis, the relationships between 5 *VDR* SNPs and exacerbation severity scores, treatment response, and frequency of hospital presentations/admissions for acute wheezing illnesses and asthma exacerbations were examined in a cohort of 657 Australian children.

2. Materials and methods

2.1. Cohort description

This study utilised data and samples obtained from participants of the Mechanisms of Acute Viral Respiratory Infections in Children (MAVRIC) study [11–13]. A sample of 657 children, aged 0–18 years, who presented to the emergency department (ED) at Princess Margaret Hospital (PMH) for Children, Perth, Western Australia (WA) with an acute wheezing illness diagnosed as either acute asthma, bronchiolitis or a wheezing illness by the attending ED physician, were recruited from 2002 to 2015. We have previously established that most MAVRIC children recruited with these diagnoses had a concurrent acute respiratory infection with rhinovirus, which confers an increased risk of a later diagnosis of asthma [12,13]. Children born pre-term (< 37 weeks) or with an underlying chronic illness other than asthma were excluded from the study.

An exacerbation severity score was assigned to each participant using age-specific validated scoring systems. Scores were determined by research personnel based on clinical parameters recorded in the participant's medical record at the time of presentation. For children < 2 years of age, exacerbations were categorised as mild (< 5), moderate (5–10), or severe (> 10) based on Bentur Modification [14]. For children 2 − 18 years old, exacerbations scores ranged from 5 to 15: mild (5–7), moderate [8–11], and severe [12–15].(15) All participants received treatment per standard hospital protocol [16]. The number of inhaled β_2-agonist doses administered in 6, 12 and 24 h after presentation were recorded. A larger number of doses indicated a poorer treatment response. The PMH Human Ethics Committee approved the study (#1761 EP). Parental/guardian written informed consent was obtained prior to participation.

2.2. Data and sample collection

All samples were obtained from participants at time of recruitment. A study questionnaire was used to obtain participants' demographics and medical history. Peripheral blood samples were collected for genetic analyses and DNA was extracted by standard techniques [17]. For each participant, the number of presentations and/or admissions to WA public hospitals for an acute respiratory illness from birth to time of database access was determined using the public hospitals database.

2.3. Single nucleotide polymorphism selection and genotyping

We analysed five SNPs of the *VDR* gene. An initial panel of SNPs was chosen based on findings of published literature, including expression quantitative trait loci [18], and VDR polymorphisms previously linked to asthma susceptibility [8,19,20]. SNPs were selected preferentially for 1) likelihood or known effect on gene expression or function, 2) previous association with asthma or atopy, 3) location in the gene, and 4) minor allele frequency (MAF) ≥5% in Caucasians. SNP linkage was assessed using linkage disequilibrium data derived from the dbSNP/HapMap CEU dataset [21,22]. One of each pair of highly linked SNPs and synonymous-coding SNPs were excluded. Table 1 summarises the SNPs selected for genetic analysis. Genotyping was performed by the Australian Genome Research Facility using iPLEX assay on a MALDI-TOF MassARRAY platform (Sequenom, San Diego, CA, USA).

Table 1
VDR single nucleotide polymorphisms selected for genetic analysis.

SNP	Chromosome position	MAF	Alleles
rs2525046	12:47838964	0.406	A/G
rs9729	12:47842840	0.490	A/C/G
rs1544410 (*Bsm*I)	12:47846052	0.296	A/G
rs2239179	12:47863983	0.361	A/G
rs2228570 (*Fok*I)	12:47879112	0.329	A/C/G/T

2.4. Gene expression microarray analysis

Nasal epithelial samples were collected using flocked swabs [23]. Total RNA was extracted, processed, and hybridised to genome-wide gene expression microarrays (Human Gene ST2.1; Affymetrix, Santa Clara, California). A custom chip description file (hugene21sthsentrezg; version 20) was used to custom map microarray probe-sets to the genome [23]. Microarray intensity levels, as a measurement of gene expression, were reported on a log scale [24]. These analyses were adjusted for batch effects associated with the microarray analysis.

2.5. Statistical analysis

Analysis of the dataset was restricted to children discharged from PMH with a diagnosis of acute asthma, bronchiolitis, or a wheezing illness. Participants' characteristics were compared using independent sample *t*-test for numerical data and Pearson χ^2 for categorical variables. Each genotype was assigned a binary value (0,1). Variables for the heterozygote and the less common homozygote were entered into each model to determine the potential association with each genotype and the likely genetic model of each SNP (either recessive, co-dominant or dominant).The associations between SNPs and binary outcome variables were analysed by logistic regression with adjustment for age and gender. A p value < 0.05 was considered statistically significant. Results that were significant, or demonstrated a trend towards significance (p < 0.1) were also adjusted for ethnicity to determine the effect of ethnicity on the model. Odds ratios, 95 % confidence intervals (CI) and p-values were determined using the common genotype as the baseline variable. Continuous variables were tested for normality and natural log transformations were completed for skewed distributions. Exacerbation severity scores of children under 2 years of age and ≥2 years were standardised (Z-score) and then combined for analysis. The associations between SNPs and continuous variables were analysed by linear regression with adjustment for age and gender. Geometric means, 95 % CI, and *p* values were determined using the common genotype as the baseline variable. Analyses of number of exacerbations since birth were adjusted for both age and observation time (birth to time of database access), as the participants' ages ranged from 0–18 years of age. All statistical analyses were performed using SPSS version 24 (SPSS Inc. Chicago, IL, USA). We expected to detect an increase or decrease in 2 of the number of ED presentations/admissions between the genotype groups. Our study had over 80 % power to detect this difference between the genotypes with a minor allele frequency > 30 %, at a level of 0.05 significance.

3. Results

3.1. Population demographics

Characteristics of the children included in the study are shown in Table 2. Of the 736 children recruited, 657 (89.3 %) consented to both participation in the study and to the collection of peripheral blood

Table 2
Population demographics of the study group (n = 736).

Characteristic	Demographics
Age at recruitment (years), mean ± SD	5.18 ± 3.69
Males, n (%)	440 (59.9)
Ethnicity (% Caucasian*)	64.2
Virus isolated, n† (%)	506 (81.6)
Exacerbation severity score‡	
< 2 years old§, mean ± SD	8.06 ± 2.00
2−18 years old‖, mean ± SD	10.55 ± 2.33
Atopy, n¶ (%)	402 (66.9)
Total respiratory exacerbations**, mean ± SD	5.87 ± 6.24
Total wheezing exacerbations**, mean ± SD	4.48 ± 5.53
β2-agonist doses in 6 h††, mean ± SD	7.12 ± 3.68
β2-agonist doses in 12 h††, mean ± SD	10.57 ± 5.94
β2-agonist doses in 24 h††, mean ± SD	15.02 ± 9.79

*Information available for 590 subjects.
†Assessed in 620 subjects.
‡Exacerbation severity score:
< 2 years old: < 5 (mild), 5–10 (moderate), and > 10 (severe).
2−18 years old: 5–7 (mild), 8–11 (moderate), and 12–15 (severe).
§Information available for 163 subjects.
‖Information available for 554 subjects.
¶Information available for 601 subjects.
**Based on ED presentations and inpatient admissions.
††Information available for 588 subjects.

Table 3
Genotyped versus non-genotyped subject characteristics.

Variable	Genotyped	Not genotyped	*P* value
n (% of total)	657 (89.3)	79 (10.7)	
Age (mean ± SD)	**5.31 ± 3.7**	**4.07 ± 3.5**	**0.005**
Sex (M:F)	397/260	43/34	0.438
Virus positive (%)	90.5	9.49	0.734
Exacerbation severity score (Z-score) (mean ± SD)	0.145 ± 0.98	−0.064 ± 1.06	0.102
Total respiratory exacerbations (mean ± SD)	5.95 ± 6.40	5.14 ± 4.92	0.394
Total wheezing exacerbations (mean ± SD)	4.56 ± 5.60	3.73 ± 4.81	0.319

samples for genotyping. Children genotyped were significantly older than those not genotyped (5.31 vs. 4.07 years, $p = 0.005$) (Table 3). There was no significant difference between the genotyped and non-genotyped children in terms of gender ($p = 0.438$), mean severity score ($p = 0.102$), or total number of respiratory ($p = 0.394$) or wheezing presentations/admissions ($p = 0.319$).

3.2. Gene expression

VDR genotype distributions did not deviate from Hardy-Weinberg equilibrium ($p > 0.987$). The rs2525046 CT genotype was associated with higher VDR mRNA intensity levels in nasal epithelial cells compared to the CC genotype (6.379 vs. 5.872, 95 % CI −0.870 to −0.143, $p = 0.007$) (Table 4). There was a trend towards significance between the rs2525046 TT genotype and VDR mRNA intensity levels when compared to the CC genotype (6.369 vs. 5.872, 95 % CI −1.008 to 0.014, $p = 0.056$). No other significant associations were identified between genotypes and gene expression levels.

3.3. Exacerbation severity

Children with rs2228570 (*Fok*I) AA genotype had higher mean exacerbation severity Z-scores (GM = 0.522, 95 % CI 0.170 to 0.665, $p = 0.001$) compared to children with the GG genotype (GM = 0.105, 95 % CI -0.046 to 0.256; N = 595) (Table 5, Fig. 1). Children with rs1544410 (*Bsm*I) TT genotype had lower mean severity Z-scores (GM = -0.081, 95 % CI -0.607 to -0.110, $p = 0.005$) compared to children with the CC genotype (GM = 0.278, 95 % CI 0.123 to 0.433; N = 595). The relationship between the rs22239179 CC genotype and mean exacerbation severity Z-scores approached significance when compared to children with the TT genotype ($p = 0.058$). No further associations were identified between genotypes and exacerbation severity Z-scores.

Table 4
Association between VDR genotypes and VDR mRNA intensity levels.

		VDR mRNA intensity level			
SNP	Genotype	Mean	95 % CI		P value
rs2525046	CC	5.872	reference		
	CT	**6.379**	**−0.870**	**−0.143**	**0.007**
	TT	**6.369**	**−1.008**	**0.014**	**0.056**
rs9729	TT	5.915	reference		
	GT	6.305	−0.527	0.397	0.091
	GG	6.24	−0.241	0.892	0.249
rs1544410 (*Bsm*I)	CC	6.216	reference		
	CT	6.158	−0.296	0.412	0.743
	TT	6.274	−0.955	0.840	0.885
rs2239179	TT	6.275	reference		
	TC	6.232	−0.388	0.475	0.838
	CC	5.978	−0.332	0.927	0.338
rs2228570 (*Fok*I)	GG	6.317	reference		
	GA	6.113	−0.168	0.577	0.276
	AA	6.093	−0.533	0.981	0.512

Table 5
Association between VDR genotypes and exacerbation severity Z-scores.

			Severity Z-Score			
SNP	Genotype	N	Geometric mean	95 % CI		*P* value
rs2525046	CC	196	0.117	−0.044	0.279	reference
N = 595	CT	289	0.225	−0.072	0.286	0.239
	TT	110	0.181	−0.166	0.294	0.586
rs9729	TT	157	0.105	−0.072	0.282	reference
N = 590	GT	300	0.188	−0.107	0.273	0.392
	GG	133	0.227	−0.106	0.350	0.294
rs1544410 (*Bsm*I)	CC	225	0.278	0.123	0.433	reference
N = 595	CT	289	0.177	−0.271	0.071	0.250
	TT	81	**−0.081**	**−0.607**	**−0.110**	**0.005**
rs2239179	TT	205	0.239	0.079	0.399	reference
N = 594	TC	281	0.207	−0.209	0.145	0.723
	CC	108	**0.017**	**−0.451**	**0.008**	**0.058**
rs2228570 (*Fok*I)	GG	242	0.105	−0.046	0.256	reference
N = 595	GA	273	0.149	−0.125	0.213	0.610
	AA	80	**0.522**	**0.170**	**0.665**	**0.001**

3.4. β_2-agonist treatment response

Children with rs2228570 (*Fok*I) AA genotype required more doses of β_2-agonists in the first 6 h (GM = 8.349, 95 % CI 0.345–2.381, $p = 0.009$) and 12 h (GM = 12.316, 95 % CI 0.147–3.438, $p = 0.033$) after presentation compared to children with the GG genotype (6 h: GM = 6.986, 95 % CI 6.379–7.594; 12 h: GM = 10.523, 95 % CI 9.540–11.507; N = 534) (Table 6, Fig. 2). The association between the rs2228570 AA and number of β2-agonist doses used in the 24 h following admission approached significance ($p = 0.052$) when compared with the GG genotype. No further associations were identified between

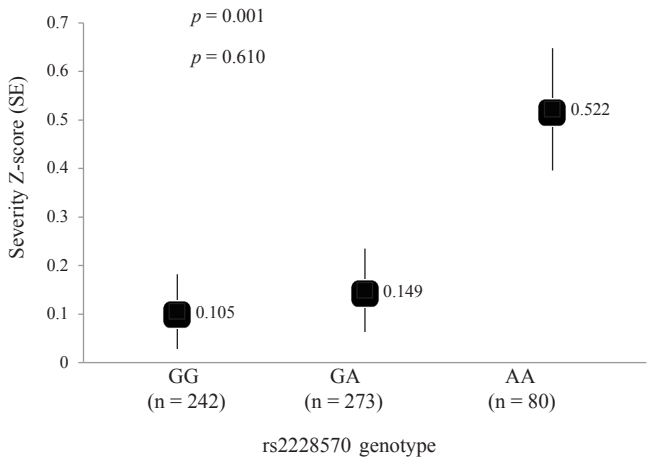

Fig. 1. Children with rs2228570 (*Fok*I) AA genotype had higher mean exacerbation severity Z-scores compared to children with the GG genotype.

genotypes and β_2-agonist use.

Children with the least common homozygote (AA) genotype of rs2228570 required significantly more doses of β_2-agonists in the first 6 h and 12 h following presentation compared to children with the most common (GG) homozygote.

3.5. Number of exacerbations

Children with rs2228570 (*Fok*I) GA genotype presented and/or were admitted to PMH more times since birth for both respiratory (GM = 1.417, 95 % CI 0.056 to 0.424, $p = 0.011$) (Table 7) and wheezing illnesses (GM = 1.195, 95 % CI 0.034 to 0.415, $p = 0.021$) (Table 8) than children with the GG genotype (respiratory presentations: GM = 1.117, 95 % CI 0.829–1.530; N = 468; wheezing presentations: GM = 0.971, 95 % CI 0.618–1.332; N = 436). No further associations were identified between genotypes and the number of respiratory/wheezing presentations/admissions to PMH.

3.6. Ethnicity

Sixty-four percent of participants were of Caucasian ethnicity. Additional statistical analyses with adjustment for ethnicity were undertaken for all results that demonstrated a trend towards significance or a statistically significant result between VDR genotype and acute wheeze/asthma outcomes. We found that Caucasian ethnicity did not significantly contribute to any of these models. Therefore, our results and conclusion remain the same.

4. Discussion

Previously published literature has identified VDR genetic variants strongly associated with asthma traits in particular populations. However, the majority of these studies have focused on asthma phenotypes in cohorts with stable disease. The MAVRIC cohort is the largest cohort of children in the world that contains data and samples collected from children presenting to a tertiary hospital with an acute wheezing illness, as well as longitudinal data pertaining to the frequency of exacerbations during each child's lifetime. As such, the MAVRIC cohort represents a unique opportunity to study the role of host genetics on the severity, treatment response and the persistence of wheezing illnesses in children.

In our study, we found that the rs2525046 CT genotype was associated with higher VDR mRNA intensity levels in nasal epithelial cells compared to the CC genotype, and that there was a trend towards significance between the TT genotype when compared to the CC genotype. These findings suggest a likely relationship between the rs2525046 genotype and VDR mRNA intensity. While the measured differences between genotypes are not very large numerically, they are on a log scale, and thus represent larger differences. Furthermore, small differences in genes that affect the immune response can have larger impacts downstream. Nevertheless, further study of VDR protein levels are needed to determine the impact of gene variants on VDR protein expression levels.

The *VDR* SNP rs2228570 (*Fok*I) has been previously associated with increased asthma susceptibility [20,25,26], and poorer asthma control [25]. We found that children with the rs2228570 AA genotype had both higher exacerbation severity scores and poorer β_2-agonist treatment response. While it is possible that the rs2228570 polymorphism is not responsible for the observed association and is simply linked to another causative allele, data for Caucasians from HapMap shows that rs2228570 is not in linkage disequilibrium with the other well-known candidate genes for asthma in the 12q13 region [22]. The rs2228570 polymorphism is a known restriction site, and has been implicated in several diseases related to immune and hormonal regulation [27–29]. Such findings suggest that the rs2228570 polymorphism conveys an immunoregulatory effect mediated by the vitamin D receptor.

The *VDR* genetic variant rs1544410 (*Bsm*I) has also been identified as a genetic risk factor for asthma [8]. Interestingly, our study found that children with rs1544410 TT genotype had roughly a 3-fold decrease in exacerbation severity scores compared to children with the CC

Table 6
VDR genotypes and treatment response to inhaled β_2-agonists.

			β_2-agonist doses in 6 h				β_2-agonist doses in 12 h				β_2-agonist doses in 24 h			
SNP	Genotype	N	Geometric mean	95 % CI		*P* value	Geometric mean	95 % CI		*P* value	Geometric mean	95 % CI		*P* value
rs2525046	CC	183	7.096	6.455	7.737	reference	10.682	9.648	11.715	reference	15.341	13.662	17.019	reference
N = 534	CT	260	7.317	−0.493	0.934	0.544	10.995	−0.837	1.464	0.593	15.662	−1.547	2.190	0.735
	TT	91	7.046	−0.998	0.897	0.917	10.47	−1.739	1.317	0.786	14.894	−2.928	2.034	0.724
rs9729	TT	147	7.177	6.478	7.876	reference	10.815	9.688	11.943	reference	15.663	13.825	17.501	reference
N = 531	GT	274	7.173	−0.756	0.747	0.991	10.693	−1.334	1.089	0.843	15.199	−2.439	1.511	0.644
	GG	110	7.072	−1.03	0.82	0.823	10.717	−1.590	1.394	0.897	15.181	−2.914	1.950	0.697
rs1544410	CC	190	6.979	6.342	7.616	reference	10.484	9.457	11.511	reference	14.966	13.298	16.634	reference
N = 534	CT	271	7.411	−0.267	1.131	0.226	11.177	−0.434	1.821	0.228	15.963	−0.834	2.829	0.285
	TT	73	6.949	−1.045	0.985	0.954	10.203	−1.917	1.355	0.736	14.596	−3.028	2.288	0.785
rs2239179	TT	174	6.865	6.217	7.514	reference	10.476	9.428	11.523	reference	14.817	13.117	16.516	reference
N = 534	TC	266	**7.469**	**-0.115**	**1.322**	**0.099**	11.177	−0.458	1.861	0.235	16.181	−0.516	3.246	0.155
	CC	94	7.117	−0.692	1.195	0.601	10.468	−1.530	1.516	0.993	14.682	−2.606	2.336	0.915
rs2228570	GG	219	6.986	6.378	7.594	reference	10.523	9.540	11.507	reference	14.918	13.319	16.516	reference
N =534	GA	247	7.077	−0.591	0.773	0.794	10.647	−0.979	1.227	0.826	15.304	−1.407	2.179	0.672
	AA	68	**8.349**	**0.345**	**2.381**	**0.009**	**12.316**	**0.147**	**3.438**	**0.033**	**17.567**	**−0.025**	**5.325**	**0.052**

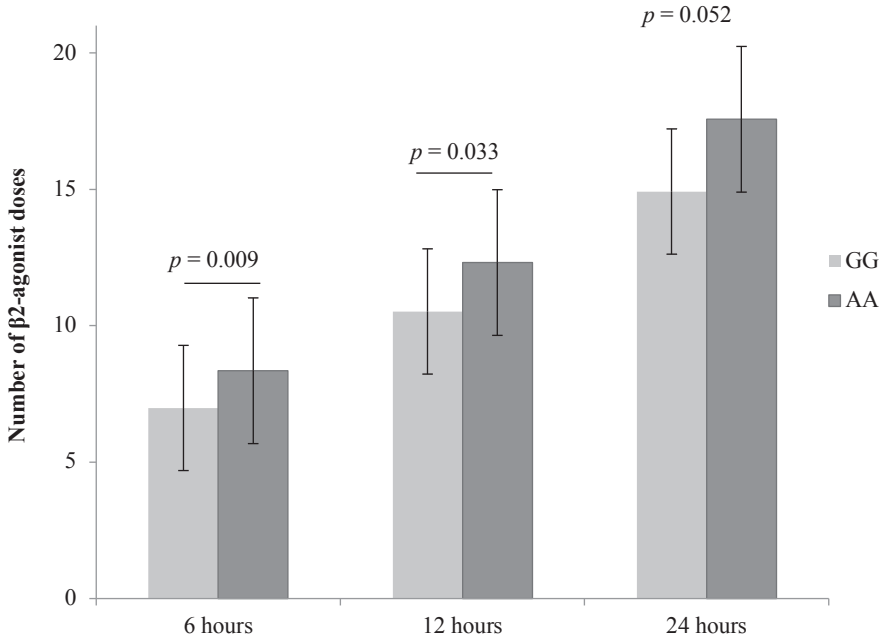

Fig. 2. Children with rs2228570 (*Fok*I) AA genotype had poorer β_2-agonist treatment response compared to children with the GG genotype.

Table 7
Association between VDR genotypes and the total number of respiratory presentations and/or admissions for acute respiratory illness.

			Respiratory exacerbations*,†			
SNP	Genotype	N	Geometric mean	95 % CI		*P* value
rs2525046	CC	162	1.325	0.968	1.688	reference
N = 468	CT	221	1.301	−0.219	0.170	0.804
	TT	85	1.347	−0.230	0.274	0.864
rs9729	TT	128	1.327	0.961	1.700	reference
N = 466	GT	233	1.343	−0.192	0.223	0.885
	GG	105	1.314	−0.261	0.234	0.915
rs1544410 (*Bsm*I)	CC	183	1.318	0.976	1.666	reference
N = 468	CT	219	1.315	−0.192	0.186	0.974
	TT	66	1.317	−0.270	0.269	0.997
rs2239179	TT	170	1.280	0.931	1.635	reference
N = 467	TC	216	1.348	−0.124	0.260	0.486
	CC	81	1.192	−0.341	0.165	0.495
rs2228570 (*Fok*I)	GG	195	1.177	0.829	1.530	reference
N = 468	GA	215	**1.417**	**0.056**	**0.424**	**0.011**
	AA	58	1.200	−0.257	0.303	0.872

* Total number of respiratory presentations including ED presentations and inpatient admissions.
† Natural log transformed.

Table 8
Association between VDR genotypes and the total number of wheezing presentations and/or admissions for acute respiratory illness.

			Wheezing exacerbations*			
SNP	Genotype	N	Geometric mean	95 % CI		*P* value
rs2525046	CC	151	1.060	0.968	1.688	reference
N = 436	CT	206	1.127	−0.134	0.267	0.514
	TT	79	1.101	−0.219	0.301	0.757
rs9729	TT	120	1.044	0.674	1.422	reference
N = 434	GT	217	1.155	−0.101	0.325	0.303
	GG	97	1.100	−0.199	0.312	0.663
rs1544410 (*Bsm*I)	CC	169	1.132	0.786	1.487	reference
N = 436	CT	206	1.081	−0.245	0.143	0.605
	TT	61	1.054	−0.357	0.201	0.582
rs2239179	TT	158	1.111	0.758	1.473	reference
N = 435	TC	201	1.132	−0.177	0.218	0.836
	CC	76	0.896	−0.475	0.045	0.104
rs2228570 (*Fok*I)	GG	180	0.971	0.618	1.332	reference
N = 436	GA	201	**1.195**	**0.034**	**0.415**	**0.021**
	AA	55	1.013	−0.244	0.329	0.771

* Total number of wheezing presentations including ED presentations and inpatient admissions.

genotype. These results suggest that the rs1544410 T allele possibly plays a protective role in wheezing/asthma exacerbations. Due to these conflicting results, further studies are needed to examine the role of rs1544410 polymorphisms in acute wheeze/asthma.

In this study, no associations were identified between rs9729, rs2525046 and r2239179 polymorphisms and acute wheeze/asthma. It is possible that a significant association existed that was not identified, but the likelihood of this occurring is small due to the large size of our cohort. Further, we acknowledge that the statistical significance (p-value > 0.05) of some of our findings were marginal, which raises the possibility that we obtained these results by chance. Replication in another independent cohort is warranted. When studying genetic associations, consideration must also be given to the presence of functional haplotypes. Subsequent studies on similar cohorts should therefore be extended to include both a larger panel of SNPs and haplotype patterns.

Other potential limitations of our study must be acknowledged. Firstly, to account for the clinical heterogeneity of asthma diagnoses and the fact that an asthma diagnosis may not be reliably made before the age of 5 years, children with discharge diagnoses of bronchiolitis or wheezing illness were included in our cohort. While we acknowledge that not all children who wheeze in their younger years develop asthma, all diagnoses included in the study are wheezing illnesses which are associated with viral respiratory tract infections and confer an increased risk of a later diagnosis of asthma [12,30]. Further, our cohort was standardised by the use of age-specific severity scoring systems and a validated treatment protocol.

Secondly, older children were overrepresented in the genotyped group. The age of the participant also affected the number of total presentations/admissions since birth. To address this, our statistical analyses were adjusted for both age and observation time. We also acknowledge that this is a single centre study, and while PMH was the main tertiary public children's hospital in WA, children who presented

to either their general practitioner or a private hospital were not included in the study. Further, participant recruitment was limited by both investigator presence and parental consent. Therefore, our cohort represents a convenience sample of children presenting to a single centre with a wheezing illness.

Nevertheless, this is the first manuscript to investigate the interplay between vitamin D receptor genetic variants, VDR mRNA intensity levels, and severity and recurrence of acute exacerbations of wheeze/asthma. We identified genetic variants at the *VDR* locus associated with acute wheezing/asthma exacerbation severity and treatment response in a largely Caucasian cohort of children. We also identified a likely relationship between the rs2525046 genotype and VDR mRNA intensity levels, as well as an association between the heterozygote genotype of rs2228570 and higher numbers of wheezing exacerbations. In conclusion, these findings suggest that *VDR* polymorphisms may play a role in the severity and perhaps even the development of acute wheeze/asthma. Further studies are needed to validate these findings and elucidate the underlying mechanisms linking *VDR* polymorphisms to acute wheeze/asthma. The ability to identify children at risk of more severe exacerbations could lead to personalized and improved genotype-specific treatment pathways in the future.

CRediT authorship contribution statement

Katharine Leiter: Formal analysis, Investigation, Writing - original draft. **Kimberley Franks:** Data curation, Writing - review & editing. **Meredith L Borland:** Resources, Writing - review & editing. **Laura Coleman:** Formal analysis, Writing - review & editing. **Leesa Harris:** Formal analysis, Writing - review & editing. **Peter N. Le Souëf:** Funding acquisition, Project administration. **Ingrid A. Laing:** Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The author reports no conflicts of interest.

Acknowledgements

University of Western Australia. Grant numbers: NHMRC APP#1087700.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jsbmb.2020.105692.

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