



## Review article

## Association of vitamin D gene polymorphisms in children with asthma - A systematic review

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## ABSTRACT

**Introduction:** The association of Vitamin D and children with asthma is known and there are several individual studies on Vitamin D polymorphisms. However, systematic reviews on all vitamin D associated gene polymorphisms have not been done in children with asthma.

**Objective:** To investigate the association of Vitamin D associated gene polymorphisms and asthma in children (0–18 years) by systematic review and meta-analytic approach.

**Methods:** Our search included 20 full text articles of which 15 were case control studies and 5 used family based linkage disequilibrium method. Total of 2491 cases and 3682 controls were included in case control studies, with mean age of 9.58 years and 10.16 years respectively. Quantitative and qualitative analysis were done.

**Results:** Quantitative analysis revealed significant association with protective effect of Apa1 polymorphism in allele (OR 0.81 (0.71,0.91) and homozygous major form (OR 0.83 (0.70,0.98) and Taq 1 minor allele in homozygous form (OR 0.73 (0.58,0.92) in children with asthma. However, the minor allele of Apa1 (OR 1.21 (1.07,1.37), Bsm 1 in heterozygous (OR 1.35 (1.07,1.71) and homozygous minor form (OR 1.95 (1.59,2.39), major allele of Fok1 (OR 1.34 (1.17,1.52) and Taq1 (OR 1.22 (1.08,1.38) were found to be increasing the odds of asthma. Ethnic variations were noted in subgroup analysis. Qualitative analysis of the polymorphisms of the Vitamin D associated metabolic genes also showed significant associations.

**Conclusion:** Our review shows significant associations with VDR polymorphisms - Apa1, Bsm1, Fok 1, Taq 1, polymorphisms of Vitamin D metabolic genes – CYP27A1, CYP 2R1, CYP 24A1, GC and genes related to Vitamin D response element (VDRE) in children with asthma. Conducting large studies involving various ethnic regions will strengthen our knowledge on the association and aid in targeted interventions for control of asthma in children.

## 1. Introduction

With 325 million people suffering worldwide, asthma is the most common non communicable disease in children with increasing prevalence of 0.02 and 0.06% respectively in the age group of 13–14 years and 6–7 years respectively [1, 2]. Although it is well known that positive atopic status, exposure and sensitization to environmental allergens, and/or familial allergic diseases are significant risk factors associated with the development of asthma, recent evidence suggest that vitamin D deficiency may predispose to allergic phenotype [3, 4]. Vitamin D through its action on the immune system regulation seems to play a pivotal role in allergic diseases [5].

Genes associated with Vitamin D in metabolic pathway, *CYP27B1*, which increases the expression of 1,25(OH)<sub>2</sub> D<sub>3</sub> in cell types, *CYP27A1* and *CYP2R1* which influence levels of active Vitamin D, *GC* transport gene and Vitamin D receptor (*VDR*), one of the super family of nuclear receptors found in respiratory epithelial and bronchial smooth muscle that increases the expression of various cytokines and interleukins all contribute to inflammatory role of Vitamin D [6].

Number of studies have looked into the association of vitamin D levels, genotypic polymorphisms in Vitamin D associated genes with asthma as well as spirometric parameters. Based on all the studies, 5 systematic reviews have also been done. The reviews have looked into the association of *VDR* polymorphism with asthma in all age groups. Among them only one meta analysis done by Zhao et al had looked

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specifically for association in children [7, 8, 9, 10, 11]. Age can be a significant factor in analysing the association of the single nucleotide polymorphism (SNP) when all parameters are similar as shown by studies done by Poon et al and Raby et al. [12, 13] Apart from these studies, several others have looked into the association of polymorphism of Vitamin D metabolic genes with asthma and have found them also to be contributing to the disease [14, 15, 16, 17]. Hence, there is a need to look into the strength of the association of all the genes associated with Vitamin D pathway and asthma in children independently. Our systematic review is an attempt to look into the association of all the Vitamin D related genes in children.

## 2. Methods

### 2.1. Protocol and registration

We registered the systematic review protocol with PROSPERO prior to starting the review process (CRD42017058266) [18].

### 2.2. Eligibility criteria for considering studies for this review

The eligibility criteria of the studies included were - [1] study should have been an original study in human population [2] case control design or family based analysis [3] Study population should comprise of children less than 18 years [4] study should have been done on association of Vitamin D associated genes and their polymorphism with asthma in children. Only studies reported in English language were included and there was no cut off date of publication or geographical barrier for exclusion.

### 2.3. Search strategy

We designed the search strategy based on the key words included in the study. The main search strategy consisted of searching the following electronic databases from inception till January 2020. The search combined terms for asthma, vitamin D associated gene polymorphism and pediatrics. The search terms included were (asthma OR reactive airway OR wheeze OR Asthma OR Respiratory Hypersensitivity OR Bronchial Hyperreactivity), (child OR pediatrics OR pediatric OR infan\$ OR toddler\$ OR boy\$ OR girl\$ OR kid\$ OR school\$ OR juvenile\$ OR teen\$

OR pubescen\$ OR adolescen\$), (“Genetic Polymorphism” OR “Genetic polymorphisms” OR “single nucleotide polymorphism” OR SNPs OR “Single nucleotide polymorphisms”), (Vitamin D, hydrocholecalciferols, 25, hydroxyvitamin D2, cholecalciferol, Vitamin D2 or Vitamin D3).

The planning of the study and protocol development was done in accordance with Cochrane handbook for systematic reviews of interventions v.5.1.0 (updated 2011) [19]. The reporting format of the study was as per preferred reporting items for systematic review and meta analysis (PRISMA) statement [20].

### 2.4. Search methodology

We conducted a literature search in the National Library of Medicine (NLM) MEDLINE, Google Scholar and EMBASE database for studies using the search strategy. The search filters were applied to restrict studies done in human subjects and those in English language. The search was done by two authors independently. Since, there was a time lag between the search date and completion of the review we did an initial search till March 2017 and have updated the search again in various databases until January 2020. The conflict, if any, between the authors was resolved by discussion. A total of 69 studies were obtained by searching all the listed database by each author independently. After removing duplicates there were 47 references identified and screened for eligibility criteria for inclusions. After exclusion of 27 studies due to reasons cited in diagram, 20 studies were taken for final analysis (Figure 1).

### 2.5. Data collection and extraction

Data was extracted using a pre-tested standard data extraction form. Details of cases, controls, ethnicity, race, sex, genotypic methods were extracted for each study. Quality Scores were assessed using a modified scoring based on the quality Scoring used by Srivastava and Srivastava 2001 [21, 22].

### 2.6. Statistical analysis

Meta analysis was performed using the Odds ratio (OR) and the 95 % confidence intervals (CI) calculated by pooling the results from each individual study when there were similarity across the reported SNPs. Random effect model with inverse variance method of pooling data was

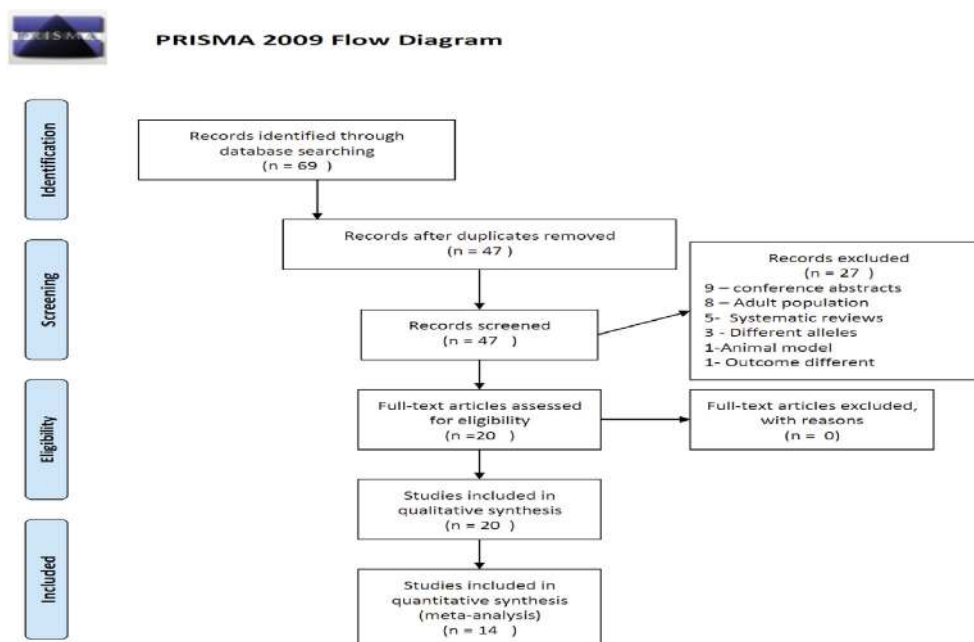


Figure 1. PRISMA diagram of the search done [20].

**Table 1.** Demographic details extracted from the studies included in the analysis.

S	No Studies	Year of pub	Country & Ethnicity	Population	Type of study	Method	Unit analyzed	HWE tested	No of cases	Mean age	No of controls	Mean age of controls	Matching bet case & Controls
1	Einisman et al [25]	2015	Chile American	Hospital based	Case control	PCR	Genotype	Not specified	75	9.1yr (6 -15y)	227	10.3 (2–12 yr)	Yes
2	Fawzy et al [26]	2018	Egypt Egyptian	Hospital	Case control	RT PCR analysis	Genotype	Yes	96	10	96	7–14 yrs	yes
3	Hou et al [27]	2017	China Asians	Hospital	Case control	PCR- RFLP	SNP	Not specified	70	8.84 +/- 3.21	70	8.04+/- 3.01	yes
4	Hutchinson et al [28]	2018	Ireland European	Hospital	Case control	RFLP	Genotype	Yes	44	8.7	55	Mean age not mentioned	no
5	Iordanidou et al [29]	2014	Greece European	Hospital	Case control	PCR	Gene	Yes	127	8.4+/- 0.2	91	9.6+/- 1.04	Yes
6	Ismail et al [30]	2013	Egypt Egyptian	Hospital Based	Case control	PCR RFLP	SNP	No	51	8.6	33	7.8	Yes
7	Leung et al [17]	2015	Hongkong Asians	Hospital	Case control	Taqmann (PCR amplification)	SNP	Yes	914	11+/- 0.4	1231	13.7 +/- 4.5	Yes
8	Kilic et al [31]	2019	Turkish European	Hospital	Case control	RFLP	SNP	YES	100	9.5+/- 2.8	80	9.5+/-2.5	YES
9	Maalmi et al [32]	2013	Tunisia African	Population	Case control	PCR RFLP	Genotype	Yes	155	9.1 (4 16yrs)	225	9.5 (2–16yrs)	Yes
10	Munkhbayarlahk et al - Taiwan [33]	2019	Taiwan Asian	Hospital	Case control	PCR amplification (MassARRAY)	SNP	Yes	94	7.77+/- 4.09	129	28.11+/- 9.67	no
11	Munkhbayarlahk et al - Mongolian [33]	2019	Mongolian Asian	Hospital	Case control	PCR Amplification (MassARRAY)	SNP	Yes	115	11.94+/- 10.28	256	27.75+/- 13.16	No
12	Nabih et al [34]	2014	Egypt Egyptian	Community	Case control	PCR	Genotype	Yes	180	7.98+/-3.22	180	8.5+/- 2.22	YES
13	NavasNazario et al [35]	2015	Yale European	Community	Case control	PCR	Genotype	Yes	81	5.8	247	5.6	Yes
14	Papadopoulou et al [36]	2015	Cypriot European	Community	Case control	PCR	Genotype allele	Yes	190	16.9 (16–18year)	671	17 (16–18yrs)	yes
15	Pillai et al [37]	2011	America African American	Hospital	Case control	PCR	SNP	Yes	139	11.2 (6-17y)	74	11.8 (6-17y)	Yes
16	Santos et al [38]	2018	Brazil	Hospital	Observational, Case control	PCR	Promoter region	Not specified	60	8.7+/-2.5	17	10.8 = /- 2.2	yes
Studies based on family based analysis of transmission													
1	Bosse et al [15]	2009	Canada Canadian	Family Based	FBAT	PCR	SNP	Yes	1064 probands 210	17.6	Not specified	48.3	No
2	Poon et al [13]	2004	Quebec Canadian	Community	FBAT	FBAT	Genes & SNP	Yes	570 subjects 223 probands	16 (2–46yrs)	569	48 (3-96y)	Not matched
3	Raby et al [12]	2004	America Americans	Community	FBAT	FBAT	Genes	Yes	1041 children 582 complete family	Girls 8.2 y boys 8.1 y	Not mentioned	30 -55y	NO
4	Wjst et al [16]	2006	Germany European	Population	Family based	PCR RFLP	Genotype	Yes	210 families	13.6	131	Among families	No
5	Volmert et al [39]	2004	Germany European	Community	FBAT	PCR	SNP	Not specified	176 families with 352 parents 411 children	10.7+/- 3.8		11.5+/- 4.3	Yes

(Abbreviation foot note - HWE – Hardy-Weinberg Equilibrium, PCR – Polymerase chain reaction, RFLP – Restriction Fragment Length Polymorphism, FBAT – Family Based Analysis of transmission, SNP- Single nucleotide polymorphism, RT – Reverse transcriptase).

used in consideration with the diverse nature of the studies included in this review. All data analysis was done using STATA 14 [23]. Heterogeneity in each analysis was assessed based on the  $I^2$  statistics following the guidance provided in the Cochrane handbook for classifying the heterogeneity as moderate ( $I^2$ : 30–50 %), substantial ( $I^2$ : 50–75 %) and considerable ( $I^2$ : 75–100 %) [24]. Subgroup analyses were conducted to assess the association based on the ethnicity with major or minor allele for the SNPs as well as for individual alleles. Forest plots were employed to evaluate the weighted average of the study results. If the number of studies was not adequate enough to calculate the forest plot the results were qualitatively analysed. Publication bias was assessed based on the symmetry observed in the funnel plot.

### 3. Results

#### 3.1. Demographic details of the study included

A total of 20 studies were taken for analysis. 15 studies used case control method to analyse the significance of polymorphisms and the rest had used family based linkage disequilibrium method of analysis. Among the 15 studies, study done by Munkhbayalakh et al had included Taiwan and Mongolese population individually and hence they have been included separately making it as data available from 16 studies. A total of 2491 individual cases were included apart from those involved in family-based studies with a mean age of 9.58 years. The total number of controls included in the study were 3682, with a mean age of 10.16 years. One of the studies did not have age matched controls and in another age of the controls was not mentioned. Hardy Weinberg equilibrium had been calculated in almost all studies. The following table gives detail demographic characters of the studies involved (Table 1).

#### 3.2. Quality score and assessment

Criteria was selected based on the reference by Srivastava and Srivastava [21]. Scores were categorized as good quality if score was > eight, fair > five and poor if score was < five. HWE had been analyzed in most of the studies except in four of the studies. Three of the studies were

of good quality and three had score < five and fourteen of the studies had scoring between five to eight (Table 2).

#### 3.3. Funnel plot analysis for publication bias

The studies included for Taq 1 and Fok 1 were within the 95% confidence limit and there was no evidence of publication bias. However, the studies included in Bsm 1 and Apa 1 had number of studies outside confidence limits and hence may have had publication bias. Heterogeneity was observed for all polymorphisms in all genetic models and results tabulated (Figure 2).

#### 3.4. Analysis of the polymorphisms and asthma

The included studies were analysed quantitatively and qualitatively for association with Vitamin D related genes and asthma in children. Significance of association was found using forest plot for 15 of the case control studies for each of the polymorphisms of individual alleles and genotype association in homozygous and heterozygous state. The studies which had found significance of association by family based analysis technique (FBAT) or linkage disequilibrium (LD) were included for qualitative analysis.

#### 3.5. Association of SNPs associated with VDR gene

The genetic variants of the VDR located in chromosome 12q13.11 have been implicated in asthmatic populations. The most studied single nucleotide polymorphisms (SNP) associated with this gene are located in last intron Apa1 (rs7975232), Bsm 1 (rs1554410) and last exon Taq1 (rs73126) near the 3' end of the gene. The Fok1 (rs2228570) located in exon 2 sequence has also been implicated in number of studies [29, 36].

#### 3.6. Association of Apa 1 (rs7975232) polymorphism with asthma

Apa 1 polymorphism association with asthma in children was analysed in 11 case control studies and by 2 studies using FBAT techniques. A total of 2150 cases and 1878 controls were analysed in case control

Table 2. Quality Score of the studies included.

Criteria/Studies	Representativeness of cases	Source of controls	Ascertainment of asthma	Sample size	Quality control of genotyping methods	Hardy-Weinberg equilibrium (HWE)	Total score
Einisman et al [25]	1	0.5	1	1	0	0	3.5
Fawzy et al [26]	1	1.5	2	0	1	1	6.5
Hou et al [27]	1	1.5	2	0	0	0	3.5
Hutchinson et al [28]	1	1.5	2	0	0	1	5.5
Iordanidou et al [29]	1	1.5	2	1	0	1	7
Ismail et al [30]	1	1	2	0	1	0	5
Leung et al [17]	1	1.5	2	2	0	1	7.5
Kilic et al [31]	1	1.5	2	0	0	1	5.5
Maalmi et al [32]	1	1.5	2	1	0	1	6.5
Munkhbayarlakh et al [33]	1	1.5	2	1	0	1	6.5
Nabih et al [34]	1	2	2	0	0.5	1	6.5
NavasNazario et al [35]	1	2	1	1	0	1	6
Papadopoulou et al [36]	2	2	2	1	0.5	1	8.5
Pillai et al [37]	1	1.5	2	0	0.5	1	6
Santos et al [38]	1	1.5	2	0	0	0	4.5
Studies based on family based analysis of transmission							
Bosse et al [15]	2	2	2	2	1	1	9
Poon et al [13]	2	0	2	2	0.5	1	7.5
Raby et al [12]	2	2	2	2	1	1	10
Wjst et al [16]	1	2	2	1	0.5	1	7.5
Vollmert et al [39]	2	2	2	1	1	0.5	8

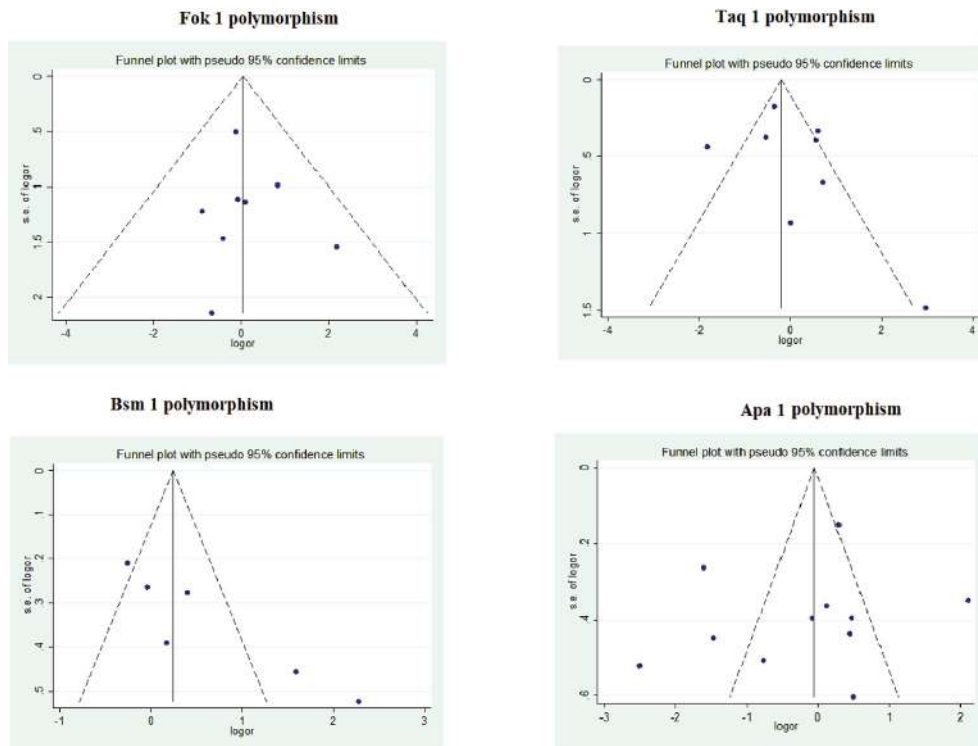


Figure 2. Funnel plot analysis for publication bias.

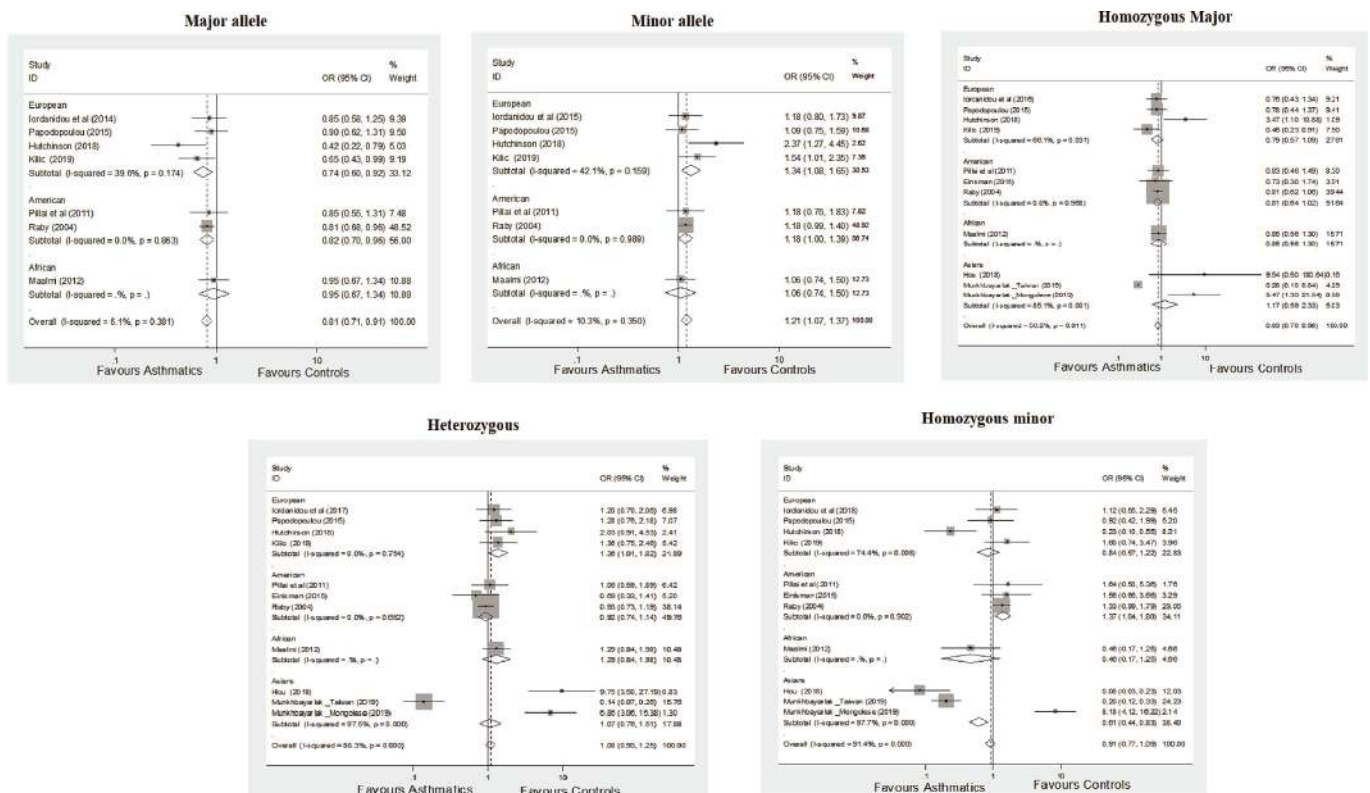


Figure 3. Forest Plot analysis of Apa 1 polymorphism.

**Table 3.** Results of the tests of association for the VDR gene polymorphisms.

SNP	TYPE	NO. OF STUDIES	TEST OF ASSOCIATION	TEST OF HETEROGENEITY	
			OR (95% CI)	P	I <sup>2</sup>
Apa (overall)	Allele Major	7	0.81 (0.71,0.91)	0.381	6.1%
	Allele minor	7	1.21 (1.07,1.37)	0.350	10.3%
	Homozygous major	11	0.83 (0.70,0.98)	0.011	56.2%
	Heterozygous	11	1.08 (0.93,1.25)	0.000	88.3%
	Homozygous minor	11	0.91 (0.77,1.09)	0.000	91.4%
Apa (European)	Allele Major	4	0.74 (0.60,0.92)	0.174	39.6%
	Allele minor	4	1.34 (1.08,1.65)	0.159	42.1%
	Homozygous major	4	0.79 (0.57,1.09)	0.031	56.1%
	Heterozygous	4	1.36 (1.01,1.82)	0.754	0.0%
	Homozygous minor	4	0.84 (0.57,1.22)	0.008	74.4%
Apa (American)	Allele Major	2	0.82 (0.70,0.96)	0.0%	0.863
	Allele minor	2	1.18 (1.00,1.39)	0.0%	0.989
	Homozygous major	3	0.81 (0.64,1.02)	0.0%	0.968
	Heterozygous	3	0.92 (0.74,1.14)	0.0%	0.652
	Homozygous minor	3	1.37 (1.04,1.80)	0.0%	0.902
Apa (Africans)	Allele Major	1	0.95 (0.67,1.34)		
	Allele minor	1	1.06 (0.74,1.50)		
	Homozygous major	1	0.85 (0.56,1.30)		
	Heterozygous	1	1.29 (0.84,1.98)		
	Homozygous minor	1	0.46 (0.17,1.25)		
Apa (Asians)	Allele Major	0			
	Allele minor	0			
	Homozygous major	3	1.17 (0.59,2.33)	0.001	85.1%
	Heterozygous	3	1.07 (0.76,1.51)	0.000	97.5%
	Homozygous minor	3	0.61 (0.44,0.83)	0.000	97.7%
Bsm1 (overall)	Allele (major)	3	1.10 (0.90,1.34)	0.177	42.3%
	Allele (minor)	3	0.91 (0.75,1.11)	0.177	42.3%
	Homozygous major	6	1.25 (0.87,1.74)	0.038	60.7%
	Heterozygous	6	1.35 (1.07,1.71)	0.000	84%
	Homozygous minor	3	1.35 (1.07,1.71)	0.000	
Bsm1 (European)	Allele (major)	2	0.93 (0.71,1.22)	0.656	0.0%
	Allele (minor)	2	1.07 (0.82,1.40)	0.656	0.0%
	Homozygous major	2	0.77 (0.48,1.26)	0.732	0.0%
	Heterozygous	2	1.18 (0.81,1.72)	0.251	24.2%
	Homozygous minor	2	0.98 (0.06,1.47)	0.338	0.0%
Bsm1 (African)	Allele (major)	1	1.34 (1.00,1.80)		
	Allele (minor)	1	0.74 (0.55,1.00)		
	Homozygous major	1	2.15 (1.23,3.76)		
	Heterozygous	1	0.77 (0.51,1.16)		
	Homozygous minor	1	0.84 (0.54,1.29)		
Bsm1 (Asians)	Allele (major)	0			
	Allele (minor)	0			
	Homozygous major	3	2.07 (0.46,9.21)	0.038	60.7%
	Heterozygous	3	3.36 (2.12,5.32)	0.003	83.3%
	Homozygous minor	3	4.40 (3.18,6.09)	0.000	98%
Taq 1 (overall)	Allele (major)	7	1.22 (1.08,1.38)	0.000	76.1%
	Allele (minor)	7	1.13 (1.01,1.27)	0.001	72.7%
	Homozygous major	10	0.99 (0.86,1.14)	0.000	95%
	Heterozygous	10	1.08 (0.93,1.26)	0.000	75.1%
	Homozygous minor	10	0.73 (0.58,0.92)	0.001	70.1%
Taq 1 (European)	Allele (major)	4	0.99 (0.80,1.23)	0.031	66.2%
	Allele (minor)	4	1.01 (0.82,1.25)	0.031	66.2%
	Homozygous major	4	0.92 (0.68,1.24)	0.060	59.4%
	Heterozygous	4	1.17 (0.87,1.56)	0.918	0.0%
	Homozygous minor	4	0.87 (0.58,1.31)	0.001	53%

(continued on next page)

Table 3 (continued)

SNP	TYPE	NO. OF STUDIES	TEST OF ASSOCIATION	TEST OF HETEROGENEITY	
			OR (95% CI)	P	I <sup>2</sup>
Taq 1 (American)	Allele (major)	2	1.35 (1.14,1.59)	0.001	90.8%
	Allele (minor)	2	1.35 (1.15,1.59)	0.666	0.0%
	Homozygous major	3	1.22 (0.98,1.52)	0.002	83.4%
	Heterozygous	3	0.98 (0.79,1.22)	0.0	0.918
	Homozygous minor	3	0.77 (0.58,1.06)	0.292	18.9%
Taq 1 (Africans)	Allele (major)	1	1.32 (0.98,1.78)		
	Allele (minor)	1	0.78 (0.56,1.02)		
	Homozygous major	1	1.14 (0.74,1.74)		
	Heterozygous	1	1.349 (0.89,2.03)		
	Homozygous minor	1	0.43 (0.23,0.80)		
Taq 1 (Asians)	Allele (major)	0			
	Allele (minor)	0			
	Homozygous major	2	0.68 (0.51,0.92)	0.000	99.4%
	Heterozygous	2	1.08 (0.57,2.03)	0.000	93.8%
	Homozygous minor	2	EXCLUDED		
Fok 1 (overall)	Allele (major)	6	1.34 (1.17,1.52)	0.000	92%
	Allele (minor)	6	0.89 (0.78,1.02)	0.052	54.3%
	Homozygous major	10	0.96 (0.80,1.16)	0.000	90.7%
	Heterozygous	10	0.99 (0.85,1.14)	0.000	90.2%
	Homozygous minor	10	1.07 (0.90,1.27)	0.000	86.3%
Fok 1 (European)	Allele (major)	2	1.32 (0.96,1.81)	0.547	0.0%
	Allele (minor)	2	0.76 (0.55,1.04)	0.547	0.0%
	Homozygous major	2	1.61 (0.98,2.64)	0.782	0.0%
	Heterozygous	2	0.82 (0.55,1.23)	0.650	0.0%
	Homozygous minor	2	0.93 (0.55,1.54)	0.233	29.6%
Fok 1 (American)	Allele (major)	2	0.94 (0.80,1.11)	0.540	0.0%
	Allele (minor)	2	0.93 (0.79,1.10)	0.237	28.3%
	Homozygous major	3	0.94 (0.67,1.32)	0.622	0.0%
	Heterozygous	3	0.95 (0.76,1.18)	0.280	21.5%
	Homozygous minor	3	0.89 (0.70,1.12)	0.888	0.0%
Fok1 (Africans)	Allele (major)	2	3.54 (2.66,4.72)	0.379	0.0%
	Allele (minor)	2	0.89 (0.66,1.20)		
	Homozygous major	2	2.79 (1.90,4.09)	0.648	0.0%
	Heterozygous	2	1.46 (0.98,2.16)	0.388	0.0%
	Homozygous minor	2	0.67 (0.32,1.42)		
Fok1 (Asians)	Allele (major)	0			
	Allele (minor)	0			
	Homozygous major	2	0.44 (0.26,0.75)	0.000	96%
	Heterozygous	2	0.61 (0.42,0.88)	0.000	98.6%
	Homozygous minor	2	1.45 (0.94,2.23)	0.000	97.2%
Fok1 (Egyptian)	Allele (major)	0			
	Allele (minor)	0			
	Homozygous major	1	0.28 (0.17,0.44)		
	Heterozygous	1	1.75 (1.14,2.68)		
	Homozygous minor	1	2.32 (1.40,3.83)		

studies and the polymorphic major allele "A" was analysed in 7 studies. It was found to be significantly associated with protective effect in asthmatic children with (OR 0.81 (0.71,0.91) irrespective of ethnicity. The minor allele was noticed to be significantly associated increasing the odds of disease (OR 1.21 (1.07,1.37) and this significance was noticed in European population in subgroup analysis. 11 studies looked at association of homozygous form of A allele and found to be significantly associated with asthmatic children (OR 0.83 (0.70, 0.98)) but was not replicated when individual ethnic groups were analysed. No significant association was noticed in heterozygous and homozygous form of the reference allele. There was slight variation in subgroup analysis where in Asian population homozygous form of minor allele was found to be protective in asthmatic children (OR 0.61 (0.44,0.83).

In family based analysis, minor allele was noticed to be under transmitted to affected offspring in a study done by Raby et al [12]. In contrast, in the sister study done using same parameters by Poon et al [13] noticed over transmission of minor allele in affected cases. As both had similar ethnic population the difference in age group of the population could be attributed as the reason for this variation. Interestingly study done by Wjst et al done in Germany [16] had found no association in family transmission [12, 25, 27, 28, 29, 31, 32, 33, 36, 37] (Figure 3, Table 3).

### 3.7. Association of Bsm1 (rs1544410) with asthma in children

6 studies including 757 cases and 2022 controls analysed Bsm1 allele. There was no significant association noticed in 3 of the studies

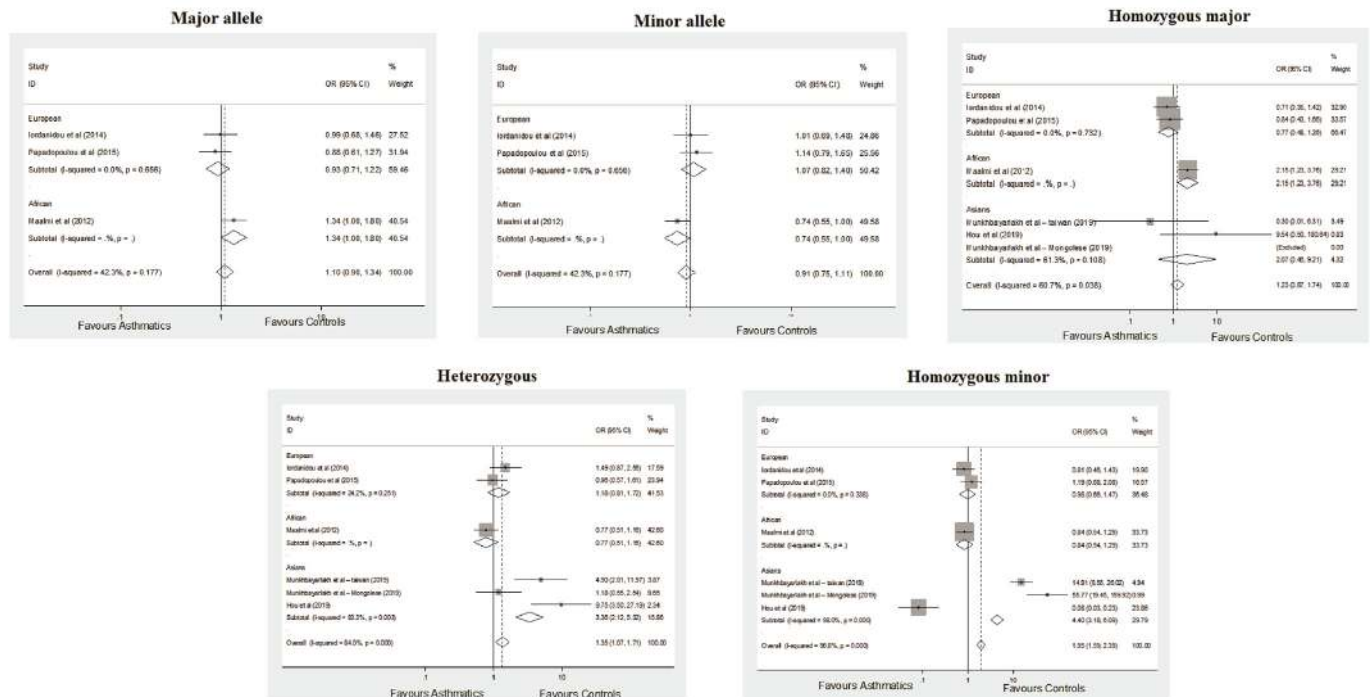


Figure 4. Forest analysis of Bsm 1 polymorphism.

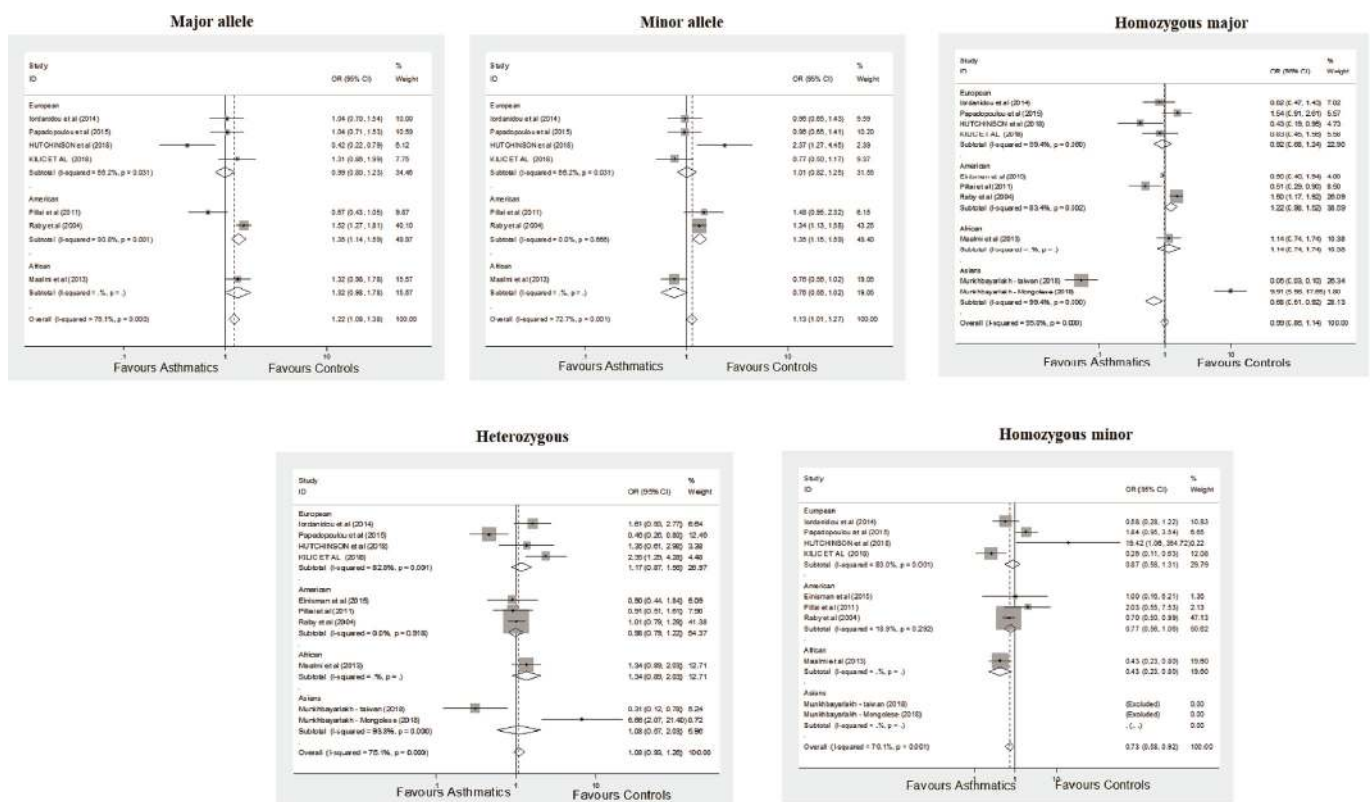


Figure 5. Forest plot analysis of Taq 1 polymorphism.

which analysed individual alleles and in 6 studies involving homozygous major form. However, significant association was noticed for heterozygous (OR 1.35 (1.07, 1.71) and homozygous minor (OR 1.95 (1.59,2.39) forms. Subgroup analysis showed significant association in Asian population in heterozygous form (OR 3.36 (2.12,5.32) and

homozygous minor form (OR 4.40 (3.18,6.09) not noticed in other ethnic groups [27, 29, 32, 33, 36]

In the family based analysis, Raby et al and Wjst et al did not find significant transmission of Bsm 1 allele in contrast to Poon et al who had noticed significant transmission among asthmatics probably due to the



difference in age group analysed. Bosse et al used two gene model of IL10 and minor allele of rs1544410 and found them to be a predictor of risk of asthma in contrast to the finding in case control studies [12, 13, 15, 16]. (Figure 4, Table3).

### 3.8. Association of Taq1 (rs731236) with asthma in children

Taq1 polymorphism was analysed in 10 studies which included 2080 cases and 1808 controls. The major allele, analysed in 7 studies was found to be significantly associated (OR 1.22 (1.08,1.38) which was also noticed in subgroup analysis in African and American children, with OR 1.32 (0.98,1.78) and 1.35 (1.14,1.59) respectively. No association was noticed for the homozygous major allele but the homozygous minor form was found to be associated protectively with asthmatic children (OR 0.73 (0.58–0.92). Difference was noticed in the subgroup analysis with Asian asthmatic children showing significant association in homozygous major form (OR 0.68 (0.51,0.92) and African children with homozygous minor allele (OR 0.43 (0.23,0.80). Thus, it can be inferred that Taq 1 polymorphism had varying association depending on ethnicity with asthmatic children [15, 25, 28, 29, 31, 32, 33, 36, 37].

In the family based analysis Wjst et al and Raby et al had not found significant transmission of the polymorphism. However, Poon et al had noticed significant over transmission of the major allele in the population again attributed to the difference in the age group analysed [12, 13, 16] (Figure 5, Table3).

### 3.9. Association of Fok 1(rs2228570) with asthma in children

Nine case control studies with 2077 cases had analysed the association of Fok1 polymorphism with asthma. The major allele analyzed in 6 studies was found to have significant association (OR 1.34 (1.17,1.52). Subgroup analysis showed significant association of major allele in both European and African population which was not seen in American population. Minor allele did not exhibit any association. Similarly no association was noticed with alleles in homozygous or heterozygous form. In

subgroup analysis, the major allele in homozygous form (OR 2.79 (1.90,4.09) and in heterozygous form (OR 1.46 (0.98,2.16) was found to increase the odds of the disease in African population. In contrast, the Egyptian population showed significant protective association of major allele in homozygous form with asthmatics (OR 0.28 (0.17,0.44). There was also significant association of minor allele in homozygous with OR 2.32 (1.40, 3.83) and in heterozygous form with OR 1.75 (1.14, 2.68) [12, 25, 29, 30, 31, 32, 33, 34, 37].

In family based analysis, Wjst et al in German population analysed Fok 1 allele but no transmission was noticed in families with asthma though significant association was found with IgE levels. Similarly none of the family based studies done by Vollmert et al in Germany. Poon et al in Canada, Nazario et al in Egypt showed association with Fok 1 allele irrespective of ethnicity [13, 16, 35, 39] (Figure 6, Table3).

### 3.10. Association of other VDR polymorphisms in family based studies

There were several other VDR polymorphisms analysed by several studies. Study done by Santos et al in the promoter region gene *CDX2* and in Exons 2 and 3 showed significant association with asthmatic children [38]. Poon et al. in Quebec, Canada analysed 12 SNPs in Canadian population with mean age of 16 years and found significant over transmission of six alleles (rs3782905C, rs1540339A, rs2239185C, Bsm1G, Taq1T (p < 0.05) to asthmatic children with substantial linkage disequilibrium among the haplotypes. Strong association was found between genetic variants of VDR locus and asthma [13].

However, no significant transmission of the same alleles was noticed by Raby et al in North America in slightly younger age group of children [12], Pillai et al (rs3782905 (OR 1.44 (0.77–2.70), rs1540339 (OR 0.79 (0.42–1.49), rs2239185 (OR 0.94 (0.4–1.81) [37]. and by Wjst et al in his family based analysis in Germany of 96 SNPs. However, the study showed significant transmission of SNPs Apa 1 (rs7975232) and rs2239186 [16]. Leung et al, did not find any association of VDR polymorphisms of SNPs Apa 1 (rs7975232) or rs 2239185 [17] similar to Vollmert et al who had studied the polymorphism 117(C/T) associated



Figure 6. Forest plot analysis of Fok 1 polymorphism.

**Table 4.** SNPs analyzed in various studies in Vitamin D associated genes that had significant association with children with asthma.

Gene analysed	No of studies	Name of the study	SNP analysed	Type of analysis	Significance (p value)
CYP27A1	2	Leung et al [17]	rs645163	Logistic regression Additive Dominant	Significant (p-0.018) Significant (0.022)
		Bosse et al [15]	rs4674338	FBAT	No association
CYP2R1	5	Bosse et al [15]	rs11023774	FBAT	Significant (p -0.017)
		Pillai et al [37]	rs10766197	Homozygous minor	Significant (p-0.044)
		Wjst et al [16]	Several SNPs	Case control	Transmission present
		Leung et al [17]	Several SNP	Case control	No association
		Nazario et al [35]	Several SNP	Details not known	No association
CYP27B1	3	Leung et al [17]	rs1048691 rs464638 rs4646536	Logistic regression & additive model	Significant (p-0.041) No significant association
		Wjst et al [16]	rs238532, rs2072052, rs1048691, rs4646537, rs4646536, rs703842	FBAT	No association
		Bosse et al [15]		FBAT	No association
CYP24A1	3	Bosse et al [15]	rs8124792 <b>rs2248359</b> rs927650 rs912505	FBAT	Significant (p - 0.030) Significant (p -0.032) No association
		Wjst et al [16]	rs2244719 <b>rs2296241</b> <b>rs2248137</b> rs2762943 <b>rs2248359</b> rs2426496	Transmission significance	No significance No significance p-0.0046 p-0.0256 p -0.0158 no significance
		Pillai et al [37]	<b>rs2296241</b> <b>rs2248137</b> <b>rs2248359</b> rs17219315	Case control	Associated with decreased asthma control
		Ismail et al [30]	rs282679	Case control Allele GG/TT GT/TT	Significant OR 2.68 (1.36,5.28) Significant (p -0.04) Significant (p -0.08)
		Leung et al [17]	rs2282679 rs4588 rs7041 rs222020 rs1155563 rs2298849	Case control	Not significant Not significant Significant Not significant Not significant Not significant
GC		Wjst et al [16]	rs222040 rs7041 rs221999	FBAT Transmission approach	0,0163 0,0249
		Nazario et al [35]	rs 7041 rs4588	Wild type allele Protein variant	Significant association (p < 0.001)
		Fawzy et al [26]	rs 7041 rs 4588	Allelic Codominant recessive Dominant Allelic Codominant Recessive Dominant	3.08 (2.03,4.69) p < 0.0001 11.4 (4.26,30.8) p < 0.0001 10.7 (4.26,26.9) p < 0.0001 2.30 (1.25,4.23) p < 0.0001 0.44 (0.27,0.72) p < 0.001 0.39 (0.21, 0.75) p -0.0047 0.42 (0.14,1.27) p - 0.120 0.37 (0.20,0.68) p - 0.041
		Bosse et al [15]	2 SNP		Was not included in analysis

Abbreviation – SNP – Single nucleotide polymorphism, FBAT – Family Based analysis of transmission. The polymorphisms given in bold has been analysed & documented by several authors whereas those not in bold have been analysed only by that author.

with VDR gene [39]. In conclusion, there is variation in association of other polymorphisms associated with VDR.

**3.11. Results of the studies that looked into SNPS associated with other vitamin D associated genes**

Eight studies had analysed association of polymorphism of other Vitamin D associated genes out of which 5 of the studies had used family based transmission and linkage disequilibrium analysis to study the significance of transmission.

**3.12. Review of association of CYP27A1 gene polymorphisms with asthma in children**

2 studies had looked into association of SNPs associated with CYP27A1 gene however, the SNPs analysed by them were different. Leung et al had analysed 4 SNP and Bosse et al had analysed several SNPs and, except for one SNP rs645163 which showed significant transmission in dominant and additive model, none of the other SNPs showed any association with asthma in children [15, 17] (Table 4).

### 3.13. Review of association of CYP2R1 gene polymorphisms with asthma in children

Association of CYP2R1 was analysed by 5 studies. Bosse et al had analysed SNP rs11023374 and had found significant transmission among asthmatic children. However, study done by Pillai et al in SNP rs10766197 did not find association directly with asthma but found that the particular SNP was associated with decreased baseline lung functions. Similarly, no association was noticed by Wjst et al, in his analysis of 3 SNPs of CYP2R1 including rs10776197. Nazario et al, in analysis of SNP rs10741657 and by Leung et al in Hongkong Chinese children who had analysed 8 SNPs including rs10776197 [15, 16, 17, 35, 37] (Table 4).

### 3.14. Review of association of CYP27B1 gene polymorphisms with asthma in children

CYP27B1 was analysed by 3 studies done by Boss et al, Wjst et al who had analysed 6 SNPs and Leung et al who analysed 3 SNPs out of which 2 were similar to that of Wjst et al had not found any association except for Leung et al who had found significant association in only on SNP rs104861 in the additive models. Hence it can be concluded that there is no significant association of the SNPs of this gene with asthma [15, 16, 17] (Table 4).

### 3.15. Review of association of CYP24A1 gene polymorphisms with asthma in children

CYP24A1 was analysed in 3 studies. Boss et al had analysed 4 SNPs and found association of rs8124792 and rs2248359 with asthma and atopy. Wjst et al had analysed 6 SNPs and Pillai et al analysed 4 SNPs. SNP rs2248359 was analysed by all the studies and found to be associated with asthma except in the study by Pillai et al where it was associated with decreased asthma control rather than directly with asthma diagnosis. SNP rs2296241 was noticed have significant transmission among asthmatic families by Wjst et al and was associated with lower lung functions by Pillai et al. Hence, there has been association of some polymorphisms of this gene with asthma [15, 16, 37] (Table 4).

### 3.16. Review of association of GC gene polymorphisms with asthma in children

GC gene was analysed in 6 studies. Bosse et al studied 2 SNPs associated with GC gene but as they failed the assay design, they could not be analysed. Wjst et al analysed 6 SNPs rs705120, rs222040, **rs7041**, rs4752, rs222011, rs221999. Among this rs 222040 and rs7041 was found to be associated in families with asthma. Leung et al analysed 6 SNPs. Only one SNP was common rs7041. The SNP was found to be associated with asthma but did not survive Bonferroni correction. However, the rest of the 5 SNPs rs **2282679**, rs4588, rs222020, rs1155563, rs2298849 did not have any association. Ismail et al in Egypt analysed the rs**2282679** and found that children carrying the SNP have 2.22 times more likely to develop asthma which was not noticed by Leung et al. Even the genotypes were significantly associated with asthmatic children. Navas- Nazario et al in a study done in Canada, analysed the SNPs **rs4588** and **rs7041** and found to be significant determinants of asthma in Hispanic children. They analysed the haplotype variants combining this SNP and found that the allelic variant was protective and wild type alleles of the SNPs were associated with increased risk of asthma. Fawzy et al in Egypt also analysed the same variants rs4588 and rs7041 with both SNPs showing significant association in allelic, dominant models, homozygous and heterozygous condition and the relationship persisting even in linkage disequilibrium analysis.

Thus, all studies which had analysed rs7041 showed good strength of association. The other SNP rs228679 also shows association in one study and in the other no association [15, 16, 17, 26, 30, 35] (Table 4).

### 3.17. Review of association of vitamin D dependent genes associated with vitamin D dependent response element (VDRE) – IL10, CD28, IL8, IL1R1, CD86 to asthma in children

There have been 2 studies that has analysed the association of the genes that are associated with vitamin D response element (VDRE) in the nucleus of vitamin D target cells. Bosse et al has studied the SNPs associated. 5 SNPs associated with IL10 – rs1800871, rs1800872, rs1800896, rs3024490, rs4844553 were found to be associated significantly with asthma. Similarly 5 SNPs associated with IL1R1 rs950880, rs1420089, rs1946131, rs1921622, rs1861245 were analysed and 3 of them were found to be associated with asthma. One SNP rs 6435203 associated with CD28 was significantly associated with asthma. However, SNPs associated with IL8 and CD 86 did not show any association.

Wjst et al studied the transmission significance of these SNPs in German population. SNPs associated with IL10 – rs 3024498, rs151811, rs100000076, 11 10–57 ICA, rs 1800895, rs1800894 were analysed and found 2 SNPs were associated with asthma in the families. These SNPs were different from that of Bosse et al and hence cannot be compared. Thus, SNPs associated with these genes have shown association with asthma in children [15, 16] (Table 4).

## 4. Discussion

Childhood asthma has several contributing factors and attempts have been made to analyse the factors especially Vitamin D, that leads to asthma and exacerbation of the clinical features. Children with asthma have been found to have increased severity of disease with lower concentrations of Vitamin D [40]. COPSAC 2000 birth cohort study had analysed that levels of Vitamin D levels in the cord blood and found that it was associated with increased risk of recurrent troublesome lung symptoms which included asthma till age of 7 years [41]. However, WAO guideline panel did not find any evidence to recommend use of Vitamin D to prevent allergic diseases [42]. This contradiction in the reports can only be sorted if there are adequate genetic studies to prove the association of Vitamin D genes and asthma. An attempt to find the significance of genetic studies was done by 5 meta-analysis but was concentrated only on VDR gene. Zhao et al, in 2017 in children had included nine studies in children among which studies from Chinese literature was also included [10]. The other four meta analysis also on VDR polymorphism, was done by Makoui et al, Tizaoui et al, Han et al and Zhang et al but had included studies in adult population [8, 9, 11, 43]. However, we know that Vitamin D has several genes associated in its pathway and each of the genes may influence the inflammatory changes in asthma and some studies had been done in those SNPs. Hence, our study was to analyse the SNPs associated with all the genes associated with Vitamin D pathway in children.

Our findings showed significant association of all polymorphisms associated with VDR. Our forest plot analysis revealed significant protective association of Apa1 polymorphism both in allele and homozygous major form and Taq 1 minor allele in homozygous form in children with asthma. The minor allele of Apa1, minor allele of Bsm 1 in heterozygous and homozygous form, major allele of Fok1 and Taq1 was found to be associated and increasing the odds of asthma.

Interesting observation in subgroup ethnicity analysis revealed, the homozygous major allele of Apa1 to have association with asthma in Asian population in contrast to all other groups. The same observation of Apa1 polymorphism was noticed by Zhao et al where the Asians had 1.5 times association with asthma not seen in Caucasians and Africans – Americans. However, Makoui et al did not notice any association of this allele. The age group analysed and hence the studies included could explain the results [9, 10].

We noticed the same ethnic difference in Fok 1 polymorphism of the major allele in homozygous form where it is noticed to be associated with asthma in Asian population and Egyptian population in contrast to other ethnicity. This ethnic difference has also been observed by Makoui et al

in his study in Asian population and by Zhao et al who also observed Bsm1 and Fok1 to have opposite effects in different ethnic groups [7, 9, 10]. They had further noticed Fok 1 polymorphism to increase the asthma risk in American population, which is not shown by our study. Raby et al and Poon et al studied similar SNPs and found significantly varying type of transmission and attributed it to the age group studied.

In conclusion, it is clear that polymorphisms associated with VDR are significantly associated with asthma in children. It is essential that ethnicity and age to be taken into consideration while interpretation.

When we analysed the associations of SNPs associated with other Vitamin D related genes in the metabolic pathway - *CYP27A1*, *CYP2R1*, *CYP27B1*, *CYP24A1*, *GC* and the five genes related to Vitamin D response element (*VDRE*) - *IL10*, *IL1RL1*, *CD28*, *CD86*, *IL8* we had varying results. Studies done on SNPs of *IL10*, *IL1RL1*, *CD28*, *GC* gene polymorphisms rs7041 and rs4588 showed association with asthma. Overall, the studies indicate that genes associated with Vitamin D are associated with asthma in children.

SNPs associated with *CYP27A1*, *CYP27B1*, *IL8* and *CD86* did not show any association in the studies done. SNPs associated with *CYP2R1* showed conflicting results between 2 studies done probably due to ethnic difference.

More studies involving different population involving these genes are required to confirm this significance of association. As other meta analysis had not analysed the genes we could not get a comparative result. The influence of vitamin D related genes in the metabolic pathway is because the endogenous serum metabolite of Vitamin D (calcitriol 1.25(OH)D3) is considered a true steroid hormone contributing to the anti-inflammatory effect and hence may explain the association. However, large scale population studies done on the SNPs in Vitamin D metabolic pathway are essential to draw inferences for a genotypic correlation [14].

Our study has been the first to do a systematic review on all the genes associated with Vitamin D including the metabolic pathway which brings out the need for more studies including the SNPs. The previous meta analysis in children by Zhao et al had included eight studies [10]. Makoui et al had included 17 case control studies albeit done in adults [9]. We have included 15 case control studies with addition of 5 family-based studies that has also added significance to the analysis of the results. Vitamin D deficiency in children has been shown to be more in children with asthma [44]. Our study shows that apart from SNPs in VDR gene, SNPs associated in metabolic pathway can also contribute to this apparent association and also significant variations exist between ethnic populations. The studies have been concentrated in certain geographical regions and done several times in the same population. However, there is apparent lack of data from certain countries like India, especially where there is significant deficiency of Vitamin D in children [45]. Our study brings out the need to conduct studies on SNPs in such population to establish the association. As vitamin D is a modifiable factor it can greatly change the management of such children.

Our study had several limitations. First, we could not include all studies in the forest plot as they were not case control studies. Second, we could not include studies from other languages as only published articles in the sources cited were included. Hence, there will be an element of language bias. Thirdly, each of the genotypes had been given various representation and analysed differently in the studies which made the integration of the data difficult. Finally, a trial sequential analysis (TSA) would have given us added confirmation of the results but because of the variations in the polymorphism analysed and differences in the type of studies to analyse the significance of transmission we did not do the same.

## 5. Conclusions

In conclusion, our study has been able to establish relationship of vitamin D receptor polymorphisms with childhood asthma, however with significant ethnicity variations. It also shows the importance of Vitamin D associated metabolic gene polymorphisms. As vitamin D is a modifiable factor, this relationship will help us in intervention and

modification of certain factors in childhood asthma. This analysis also shows the need to do large scale studies in various ethnic population preferably with similar polymorphisms to bring up guidelines on interventions with regard to Vitamin D levels and supplementation in childhood asthma.

## Declarations

### Author contribution statement

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The authors declare no conflict of interest.

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