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RESEARCH LETTER

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Vitamin D receptor genetic variant associated with asthma in Swedish school-children

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To the Editor,

Preschool wheeze is a potential precursor of asthma as 50% of individuals with asthma report wheeze during the first 6 years of life.[1] Although several risk factors such as atopy and respiratory infections have been identified, predicting persistent asthma at school age is challenging.[1] Emerging evidence on the inverse relationship between vitamin D levels and the risk of childhood wheezing and asthma exists, but causality is not proven.[2,3] The main source for vitamin D in humans is its synthesis in the skin which requires sunlight. Reduced exposure to sunlight in northern compared to southern Europe might be related to higher asthma prevalence in children in the Nordic countries.

Variations in genes encoding the Vitamin D receptor (VDR), Vitamin D-Binding Protein (VDBP), and Vitamin D 25-hydroxylase (CYP2R1), key actors in Vitamin D pathway, might be associated with asthma but discordant findings exist.[4] The *VDR* gene is located on chromosome 12, where multiple genetic loci linked to asthma and atopy are located. VDR is the principal receptor for the active form of vitamin D (1,25(OH)2D) and is expressed in airway epithelium and immune cells. Through its action as a transcription factor, VDR modulates immunological processes that are associated with asthma.[5,6] VDBP binds 25-hydroxyvitamin D (25(OH)D), transports it and its metabolites to target tissues.[4] The CYP2R1 enzyme catalyses the synthesis of the active form of vitamin D.[4] We aimed to assess the risk of asthma at 7 years in relation to vitamin D levels and genetic variants in *VDR*, *VDBP* and *CYP2R1* in a cohort of preschool wheezers.

'Gene Expression in Wheezing and Asthmatic Children' (GEWAC) is a cohort of 156 preschool wheezers (cases, age; 6–48 months), enrolled at the emergency ward at Astrid Lindgren's Children's Hospital, Stockholm, Sweden, when seeking care for acute wheeze, and 102 age-matched healthy controls. In the current study a subset (110 cases and 94 healthy controls) with available genotypes in *VDR* (rs2228570, rs7975232, rs731236, rs11568820), *VDBP* (rs7041, rs4588), and *CYP2R1* (rs10766197), and vitamin D levels at preschool age and 7 years were included (Figure 1). Seventy-five cases, followed until the age of 7 years, were assessed regarding the effect of vitamin D levels (25(OH)D) and genetic variants on asthma at 7 years (Figure 1A).

Asthma diagnosis at age 7 was based on the global initiative for asthma (GINA) guidelines, as previously described (Figure 1A). Vitamin D insufficiency was defined as 25(OH)D 50–75 nmol/L and deficiency as <50 nmol/L. The 25(OH)D was assessed using competitive immunoassay (Roche Diagnostics Vitamin D total assay).

Allergic sensitization was defined as IgE ≥0.35 kU_A/L against Phadiatop or Fx5. DNA was extracted from whole blood and seven

genetic variants were analysed (Figure 1) with TaqMan allelic discrimination (Thermo Fisher Scientific). SPSS version 26 (IBM) was used for statistical analyses. The statistical methods used are described in figures' legends. Additional information about study methods and results are available at https://zenodo.org/record/7827554#.ZDkIr-xBxDV.

Cases with ($N=54/75$) and without ($N=21/75$) asthma at 7 years did not differ significantly regarding ethnic origin, allergic sensitization or vitamin D levels, neither at the first follow-up nor at 7 years. Vitamin D insufficiency at preschool age tended to be more common in cases with asthma at 7 years (42.6%, $N=23/54$) compared to those without asthma (19%, $N=4/21$, $p=.056$, Figure 2A). At the age of 7, vitamin D insufficiency was noticed for the majority of children (89.3%, 67/75), being similarly frequent among those with (90.7%, $N=49/54$) and without asthma (85.7%, $N=18/21$, $p=.679$, Figure 2A).

All cases ($N=110$) carried the rs11568820-C allele compared to 95.7% ($N=90/94$) of the controls, $p=.044$. Among cases, a significant difference between asthmatics ($N=53/74$) and non-asthmatics at 7 years ($N=21/74$) was found in rs2228570 genotype distribution ($p=.003$, Figure 2B). When homozygotes rs2228570-GG and heterozygotes AG were pooled together, they appeared to have higher risk of asthma compared to homozygotes AA ($p=.009$, OR = 6.13 (95% CI: 1.57–23.97). Cases heterozygous (AG) for rs2228570 were five times more likely to have asthma at 7 years compared to any of the homozygotes ($p=.004$, OR = 5.28, 95% CI: 1.68–16.63). The association between rs2228570 and asthma at 7 years remained significant after adjustment for vitamin D, allergic sensitization and rhinovirus infection at preschool age. No significant differences between the cases with and without asthma at 7 years were found for any of the other genetic variants.

Children heterozygous (AG) for rs2228570 with asthma diagnosis at 7 years had significantly more days with asthma symptoms (median 22.5 (IQR: 10.0–38.5), $p<0.01$) and exacerbations (3 (IQR: 0–4), $p=.023$) the year preceding the 7 years' follow-up compared to any of the homozygotes AA and GG (7 (IQR: 0.0–15.8), 0 (IQR: 0.0–2.0)) (Figure 2C–F).

We found that the vitamin D levels were not related to asthma at 7 years in the GEWAC cohort, neither at preschool age nor at 7 years. Our findings are in line with an Australian high-risk cohort where no association between vitamin D levels and asthma was found.[2] However, in the same cohort, the number of Vitamin D-deficient (<50 nmol/L) follow-ups per child, from birth up to 10 years of age, was positively related to asthma.[2] Several observational studies report an inverse relationship between vitamin D levels and childhood wheezing as well as asthma.[2,3] Randomized controlled trials of vitamin D supplementation to reduce asthma exacerbations report discordant results.[6] The strongest protective effect of vitamin D supplementation against respiratory infections and asthma exacerbations was noticed in subjects with vitamin D levels <25 nmol/L at baseline.[6] Thus, the impact of vitamin D insufficiency on asthma might be evident only when the vitamin D level is below a certain threshold. Children in Sweden are recommended supplements of 10 μg (400 IU) of vitamin D daily from birth until the age of 2 years, and children with dark skin until the age of 5 years. An increased prenatal vitamin D intake may protect from childhood asthma,[3] as vitamin D is implicated in several pathways leading to airway inflammation and remodelling.[6] Interestingly, these biological effects might be modified by genetic variants involved in Vitamin D signalling.[4]

Key messages

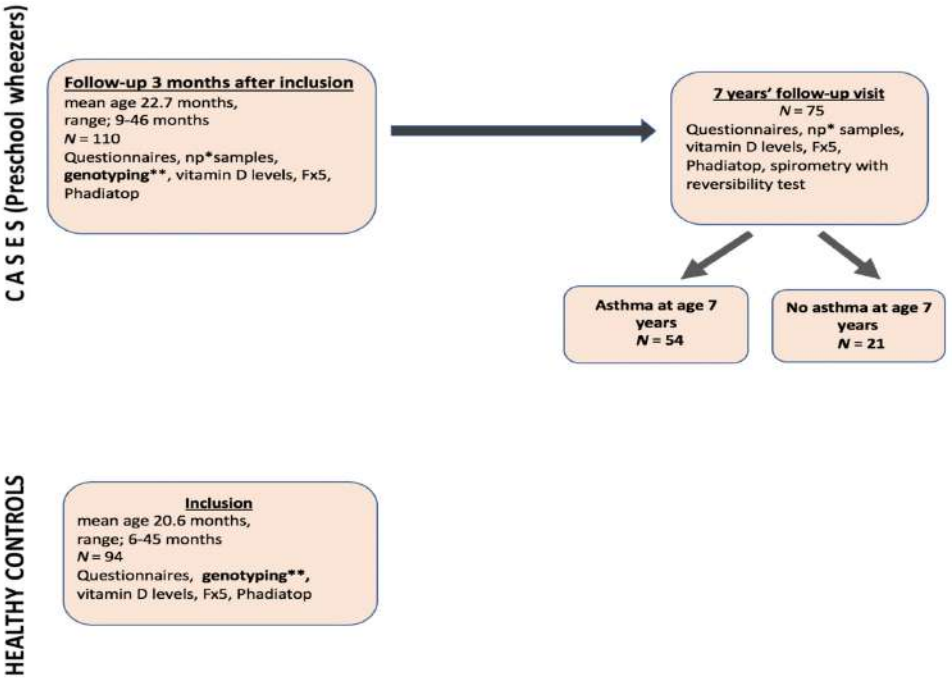
- Rs11568820, a genetic variant in Vitamin D Receptor (*VDR*) is associated with preschool wheeze.
- Rs2228570 (*VDR*) is associated with asthma at 7 years in children with early-life wheeze.
- Rs2228570 contributes to asthma susceptibility at 7 years irrespective of longitudinal vitamin D levels.

Our results indicated an association between rs11568820 and preschool wheeze, while the rs2228570-AG genotype was associated with asthma at 7 years, regardless of vitamin D status. Previous evidence on a potential link between rs11568820 (Figure 1B) and preschool wheeze is lacking. The C allele has been shown to decrease VDR transcriptional activity compared to the T allele (Figure 1B). Genotype rs11568820-AA has been significantly associated with asthma, in children aged 7–14 years.[7] Rs2228570 is located in exon 2, the G allele codes for a shorter protein that has higher activity in vitro (Figure 1B,[8]), and is associated with increased susceptibility to asthma as well as a more severe phenotype.[5] However, these associations could not be confirmed in American or Chinese populations.[9]

In our study, homozygotes rs2228570-GG and heterozygotes AG, when pooled together, had a higher risk of asthma than homozygotes AA and similar results have been reported in a meta-analysis by Zhao et al.[5] Furthermore, we found that heterozygous children (AG) were five times more likely to have asthma at 7 years and had more often asthma symptoms than homozygotes (AA or GG). Whitfield et al.[8] have demonstrated that the transcriptional activity of VDR was lower for AG genotype compared to AA and GG, respectively. The rs2228570-G allele has been associated with greater airway obstruction, and a higher requirement of medication to reach asthma control.[9] However, other studies found a protective effect of the G allele or GG homozygosity against asthma.[9] Genotypes may affect asthma susceptibility towards different directions depending on covariates; age, environmental or other unrecognized factors.

Our finding that different *VDR* variants are associated with preschool wheeze and asthma at 7 years in our study could be attributed to inadequate statistical power, endotype-specific, age-dependent effects or gene–environment interactions in a time-dependent manner. It is possible that other significant associations were not detected due to underpowered samples. Other limitations were potential recall bias, and restricted external validity due to the high-risk nature of our cohort.

(A)



(B)

Genetic variant	Gene	Position (GRCh38)	Location	Alleles ancestral/mutant	Restriction enzyme	Functional significance
rs731236	*VDR*	chr12:47844974	Exon 9 (Ile352Ile)	A > G	TaqI	Coding sequence-silent codon change (Poon et al), mRNA stability>translation efficiency
rs7975232	*VDR*	chr12:47845054	Intron 8	C > A	ApaI	Intron variant mRNA stability>translation efficiency
rs2228570	*VDR*	chr12:47879112	Exon 2 (Met1Thr)	A > G	FokI	Initiator Codon Variant Missense (Met1Thr). It might affect the protein structure as well as transcriptional activity
rs11568820	*VDR*	chr12:47908762	Exon 1 (promoter)	C > T	-	C allele decreases VDR transcriptional activity compared to the A allele
rs7041	*VDBP*	chr4:71752617	Exon 11	A > C	-	Intron variant, coding sequence variant, missense variant
rs4588	*VDBP*	chr4:71752606	Exon 11	G > A	-	Intron variant, coding sequence variant, missense variant
rs10766197	*CYP2R1*	chr11:14900334	Promoter	G > A	-	Upstream transcript variant

FIGURE 1 (A) Flow-chart of the GEWAC population included in the current study. GEWAC cohort was enrolled at the paediatric emergency ward at Astrid Lindgren's Children's Hospital, Stockholm, Sweden, between 2008 and 2012 when seeking care for acute wheeze. Age-matched controls were enrolled at the surgical day-care ward. 110/156 cases and 94/102 controls had genotypes and vitamin D levels (25(OH)D) available at 3 month follow-up and at inclusion, respectively. Seventy-five cases with vitamin D levels (25(OH)D) available at 3-months and 7 years were included in a longitudinal analysis of genetic variants in *VDR*, *VDBP* and *CYP2R1*. Vitamin D insufficiency was defined as 25(OH)D 50–75 nmol/L and deficiency as <50 nmol/L. The definition of asthma at 7 years of age was based on Global Initiative for Asthma (GINA) guidelines and included one mandatory criterion (a diagnosis of asthma by a paediatric allergist) and three additional criteria: lower respiratory symptoms, medication for treatment of wheeze the preceding 12 months or airway reversibility >12% after the use of bronchodilator with salbutamol. All children that fulfilled the compulsory and at least one of the optional criteria (symptoms, medication or reversibility) were classified as having asthma. *Nasopharyngeal samples, **Genotypes available for all children for rs2228570, rs731236, rs11568820, rs7041, rs4588 and rs10766197 but the genotype for rs7975232 was undetermined in 6/110 cases and 3/94 controls. (B) Overview of all seven genetic variants studied in GEWAC cohort, and their functional significance. Rs2228570 A > G codes for a missense mutation (Met1Thr) at the transcription start site in *VDR* which results in a 3-aminoacid shorter protein from the G-allele compared to the A-allele. The shorter form of VDR, shown in vitro to have a 1.7-fold higher activity and homozygosity for rs2228570-G, leads to the production of a *VDR* receptor with greater affinity for the active form of vitamin D and thereby a higher downstream transcriptional activity. Rs11568820 is binding site for the intestine-specific transcription factor CDX-2 (Caudal-type homeobox protein 2).

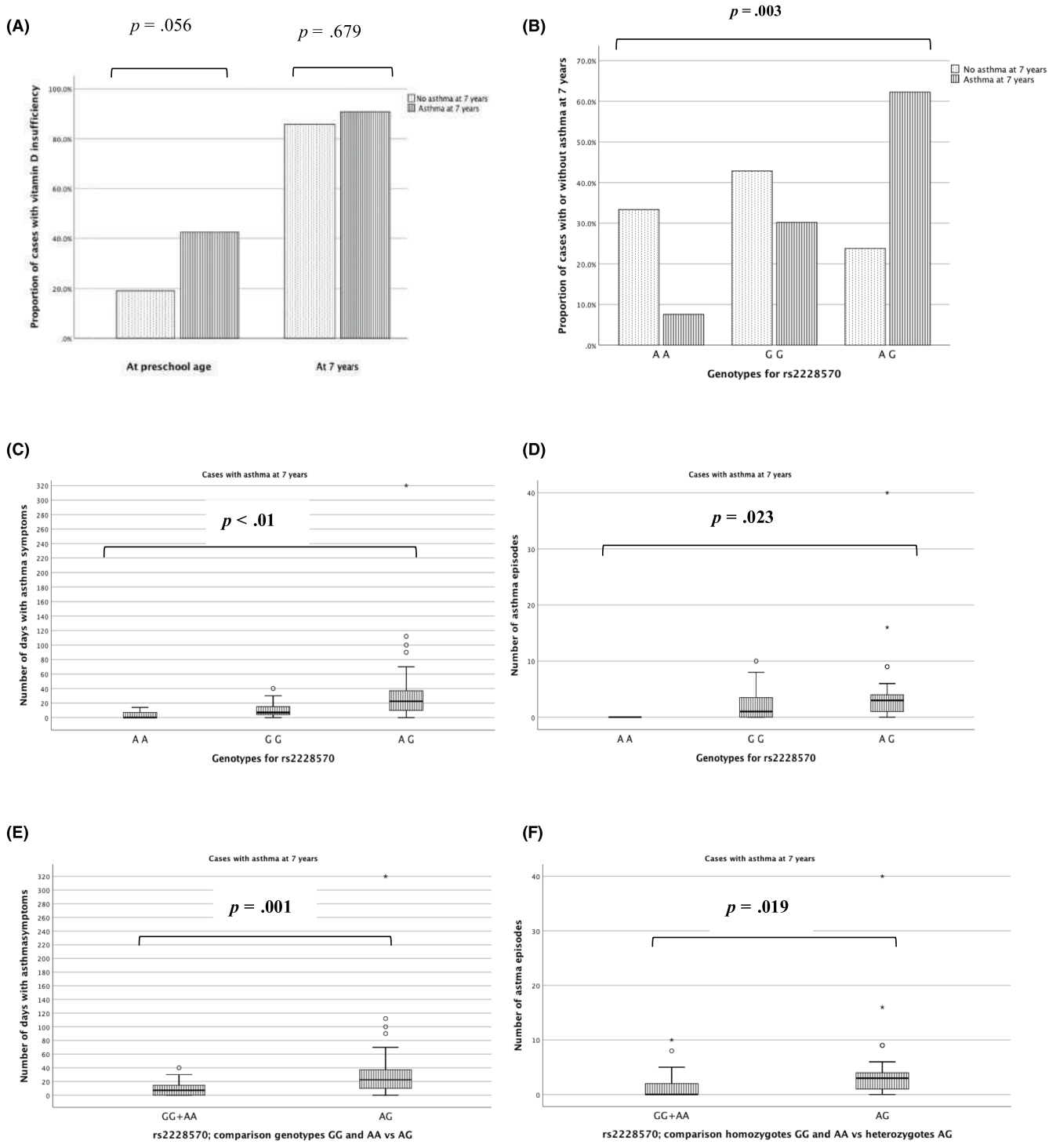


FIGURE 2 Proportion of cases with vitamin D insufficiency (25(OH)D < 75 nmol/L) at preschool age (at first revisit) and at 7 years in cases without asthma at 7 years ($N = 21$), and cases with asthma at 7 years ($N = 54$) (A). Genotype distribution for *VDR* rs2228570 in cases with and without asthma at 7 years ($N = 74$). The bars represent the frequencies of the three genotypes for *VDR*-rs2228570; AA, GG, AG, in cases with and without asthma at 7 years (B). Box plots for the number of days and asthma episodes in cases with asthma at 7 years across three genotypes for *VDR*-rs2228570 (AG; $N = 33$, AA; $N = 4$, GG; $N = 16$) (C, D). Box plots for the number of days and asthma episodes in cases with asthma at 7 years in homozygotes (GG and AA) compared to heterozygotes (AG) for *VDR*-rs2228570 (E, F). *p* Values were calculated with Chi-square and Fisher's exact tests for categorical data and Mann–Whitney or Kruskal-Wallis for continuous data.

The main strength of our study is its longitudinal design of a thoroughly described cohort of preschool wheezers with a high prevalence of asthma at 7 years. Previous studies include participants in predominantly middle childhood with the exception of a handful reports about preschool children all of whom though were of Asian origin.[9] Given the reported ethnicity- and age-dependent effects of

VDR genetic variance on asthma susceptibility,[5] our study adds significantly to the current knowledge in the field.

In conclusion, rs2228570 in *VDR*, arises as a significant determinant of persistent asthma at school age among preschool wheezers, irrespective of vitamin D levels. Additional longitudinal studies in larger paediatric populations are needed to further elucidate the role of *VDR* in childhood asthma.

AUTHOR CONTRIBUTIONS

G.H., A.F., J.R.K., and C.S. designed the study. A.F., A.H., S.C., and C.S. analysed the data and performed statistical analyses. AF drafted the manuscript. All authors participated in data interpretation, provided important intellectual input and revised the final manuscript.

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CONFLICT OF INTEREST STATEMENT

M.vH. reports personal fees from Thermo Fisher Scientific, outside the submitted work. A.F., I.H., S.C., G.H. and A.H. have no conflict of interest to report. J.R.K and C.S. have received nonfinancial support from Thermo Fisher Scientific in other research projects, but not to complete this particular project.

DATA AVAILABILITY STATEMENT

Due to the nature of this research, participants of this study did not agree for their data to be shared publicly, so supporting data is not available.

ETHICAL STATEMENT

The study protocol was approved by the Regional Ethics Committee of Stockholm (Dnr 2008/378-31/4 and Dnr 2014/399-31/3). Written and oral information about the study was provided to the parents and written consent was obtained from parents and/or legal guardians prior to the study.

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