

Letter to the Editor

Vitamin D binding protein and asthma severity in children

To the Editor:

Pulmonary disorders including asthma have been linked to the vitamin D axis.^{1,2} This axis includes vitamin D, vitamin D binding protein (VDBP), and the vitamin D receptor. VDBP is a serum protein that binds circulating vitamin D with high affinity and also possesses immunomodulatory functions that may be of particular relevance in pulmonary infections and inflammation.³⁻⁵

A recent study identified that circulating VDBP levels inversely correlate with lung function in an adult cohort with chronic obstructive pulmonary disease and that sputum VDBP contributes to macrophage activation.⁴ Little is known about the role of VDBP in children with asthma. We hypothesized that children with severe therapy-resistant asthma (STRA) have higher levels of VDBP in the airway—measured in the bronchoalveolar lavage (BAL) fluid—than do nonasthmatic controls, which is associated with worse clinical parameters of asthma control including lung function, symptoms, and pathophysiology.

Fifteen children with STRA, 7 with moderate asthma, and 6 nonasthmatic controls were recruited from the Royal Brompton Hospital, London. STRA was defined as previously.⁶ Briefly, children with STRA had persistent symptoms despite treatment with high-dose inhaled corticosteroids (ICS) (at least 800 µg/d of beclomethasone equivalent) and trials of add-on drugs (long-acting β₂-agonists, leukotriene receptor antagonists, and oral theophylline in a low, anti-inflammatory dose), recurrent severe asthma exacerbations, and/or persistent airflow obstruction (post-oral steroid, postbronchodilator Z score < -1.96 for FEV₁ despite above therapy); all children had been through a detailed protocol to optimize adherence and other aspects of basic management, as far as is possible. Approval for the study of pediatric patients was given by the Royal Brompton Hospital Ethics Committee, and written informed consent from parents and age-appropriate assent from children was obtained in each case. Children with moderate asthma were well controlled on a lower dose (<800 µg/d) of ICS. The nonasthmatic controls comprised children with no lower respiratory tract disease undergoing a clinically indicated bronchoscopy for upper airway problems such as stridor.

The VDBP level in BAL, serum, and cell culture supernatant was measured by using ELISA (Immunodiagnostik, Bensheim, Germany), and serum 25-hydroxyvitamin D₃ level was measured by using a 2-dimensional high performance liquid chromatography system tandem mass spectrometry. Total filtered BAL cells (1×10^6 per mL in RPMI/10% FCS) were cultured for 72 hours at 37°C/5% CO₂ in the presence of LPS (50 ng/mL, Sigma-Aldrich, Poole, United Kingdom) with or without dexamethasone (10^{-7} mol/L, Sigma-Aldrich).

Children with STRA had significantly higher levels of VDBP in BAL but not in serum as compared with those in moderate asthma ($P < .05$) and control individuals ($P < .01$). There was a negative association between the concentration of VDBP in BAL, but not in serum, and asthma control (assessed by using the asthma control test; BAL VDBP Spearman $r = -0.5$, $P = .01$; serum VDBP Spearman $r = 0.2$, $P = .3$) as well as spirometry

(FEV₁% [BAL VDBP Spearman $r = -0.4$, $P = .01$; serum VDBP Spearman $r = -0.3$, $P = .2$]) and a positive association with ICS usage (BAL VDBP Spearman $r = 0.6$, $P = .002$; serum VDBP Spearman $r = -0.3$, $P = .2$). There was no correlation between BAL VDBP and serum VDBP (Spearman $r = -0.199$; $P = .3$) (Fig 1). *In vitro*, the corticosteroid dexamethasone did not promote VDBP synthesis by total BAL cells ($n = 7$, paired t test $P = .8$; data not shown). There was no association between circulating 25(OH)vitamin D₃ level and either BAL or serum VDBP concentration (BAL VDBP Spearman $r = -0.3$, $P = .1$; serum VDBP Spearman $r = 0.1$, $P = .6$) (Fig 2).

In this cross-sectional study, we identify that BAL, but not serum, levels of VDBP are significantly higher in STRA and that BAL VDBP levels inversely correlate with asthma severity, as assessed by the asthma control test, spirometry, and ICS usage. Serum VDBP status did not influence any of these parameters of asthma control and did not correlate with BAL VDBP level. Dexamethasone is reported to increase the VDBP level in the airway in mice and in human hepatoma cells,^{7,8} suggesting a potential iatrogenic cause for our observations; however, our findings indicate no modulation of VDBP expression by total BAL cells exposed to dexamethasone *in vitro*. These data highlight the potential importance of the vitamin D axis, beyond vitamin D itself, in children with asthma.

The vast majority of circulating vitamin D, both 25(OH)D₃ and 1,25(OH)D₃, is bound to VDBP, which circulates at levels that far exceed those of its cargo. The cellular uptake of vitamin D can occur via both VDBP-independent and -dependent pathways, although we do not yet fully comprehend the implication of signaling via these different routes. *In vitro* addition of VDBP to monocyte cultures impairs vitamin D responsiveness⁹ presumably by limiting the free diffusion of unbound vitamin into the cells. This may represent one mechanism by which excessive VDBP in the airway may limit vitamin D bioactivity with potential immunological consequences.

An alternative explanation may relate to the effects of VDBP on innate cell function. VDBP augments the chemotactic functions of monocytes and neutrophils and together with the macrophage activating factor drives macrophage activation *in vitro* toward a highly phagocytic phenotype with increased superoxide generation.^{3,4} Alveolar macrophages are the most abundant cell type in the airway and exhibit phenotypic changes in asthma. In health, local signals are likely to restrain these cells, making them relatively unresponsive to inflammatory stimuli, which is believed to be important for the prevention of airway hyperreactivity.¹⁰ It is plausible that in asthma, excessive airway VDBP drives alveolar macrophages into a more inflammatory state, diverting them from their tolerogenic role.

The final possible interpretation of these data could simply be that raised airway VDBP level is reflective of a chronic state of inflammation in the airway. Inflammatory cytokines are known to stimulate hepatic transcription of VDBP,⁷ and this may also occur locally in the lung. This cross-sectional study cannot imply causality, but it strongly suggests that further study of VDBP as a potential biological mechanism or disease marker in asthma is warranted.

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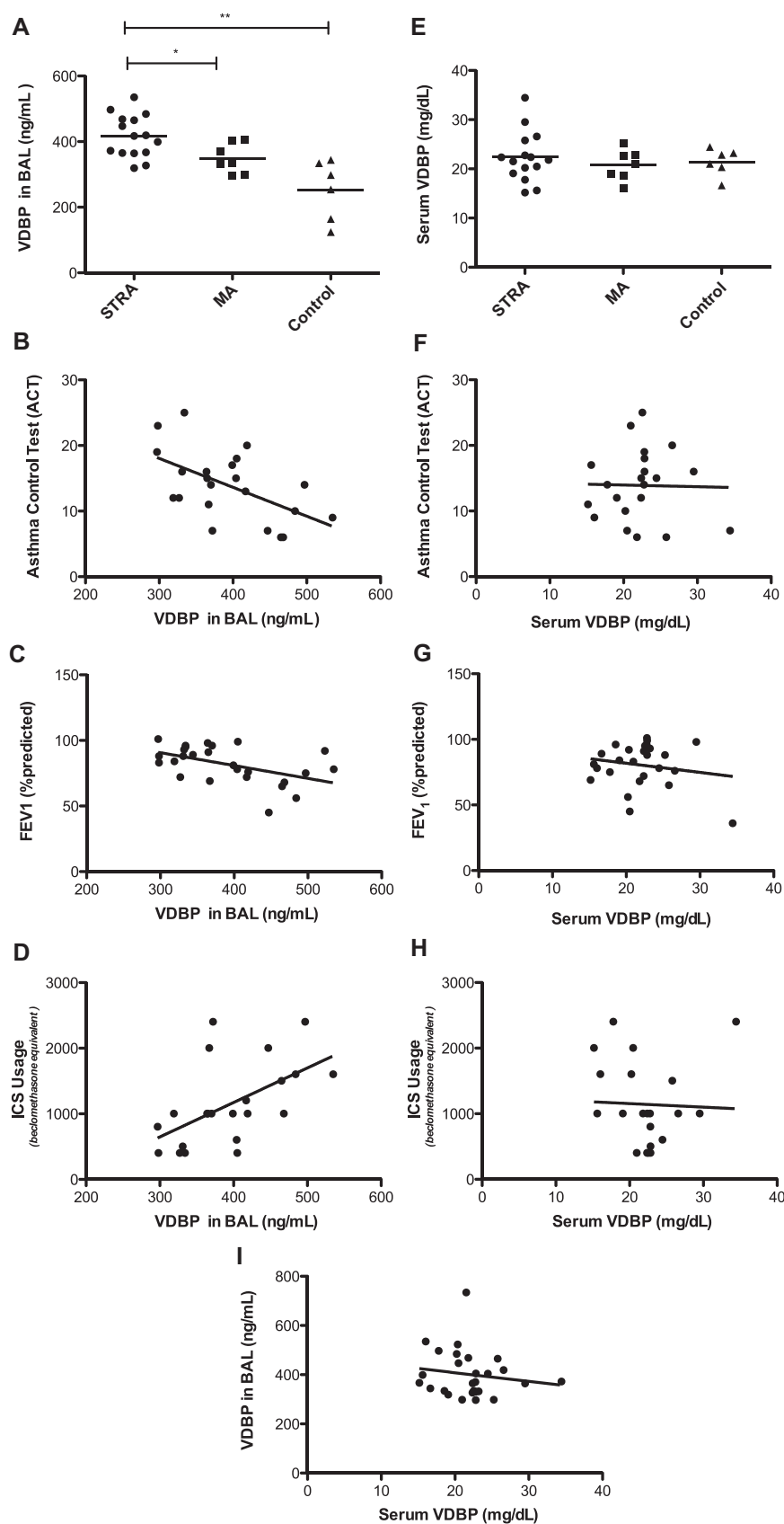


FIG 1. A, BAL VDBP in pediatric STRA, MA, and controls. BAL VDBP levels were higher in STRA than in MA and controls (Kruskal-Wallis test $P = .002$). B, Negative association between BAL VDBP levels and asthma control (assessed by using the ACT; $r = -0.5$; $P = .01$). C, Inverse association between BAL VDBP and

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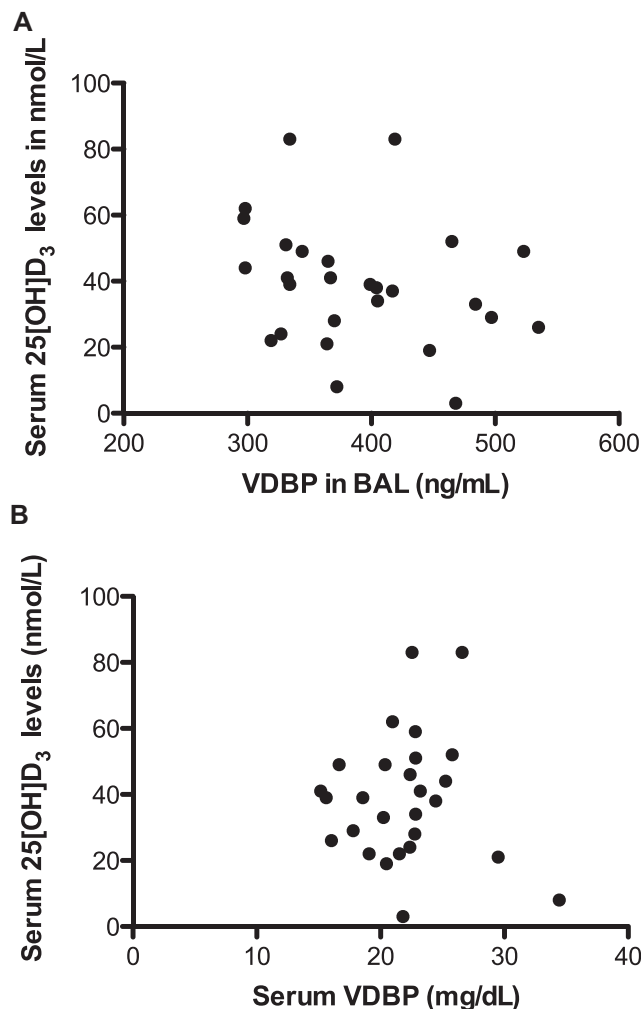


FIG 2. No association between serum 25-hydroxyvitamin D₃ levels (25 [OH] D₃) and BAL VDBP ($r = -0.3$; $P = .1$) and serum VDBP ($r = 0.1$; $P = .6$). Correlation was determined by using the Spearman rank correlation coefficient.

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REFERENCES

1. Gupta A, Sjoukes A, Richards D, Banya W, Hawrylowicz C, Bush A, et al. Relationship between serum vitamin D, disease severity and airway remodeling in children with asthma. *Am J Respir Crit Care Med* 2011;184:1342-9.
2. Hollams EM, Hart PH, Holt BJ, Serralha M, Parsons F, de Klerk NH, et al. Vitamin D and atopy and asthma phenotypes in children: a longitudinal cohort study. *Eur Respir J* 2011;38:1320-7.
3. Chishimba L, Thickett DR, Stockley RA, Wood AM. The vitamin D axis in the lung: a key role for vitamin D-binding protein. *Thorax* 2010;65:456-62.
4. Wood AM, Bassford C, Webster D, Newby P, Rajesh P, Stockley RA, et al. Vitamin D-binding protein contributes to COPD by activation of alveolar macrophages. *Thorax* 2011;66:205-10.
5. Metcalf JP, Thompson AB, Gossman GL, Nelson KJ, Koyama S, Rennard SI, et al. Gc-globulin functions as a chemotaxin in the lower respiratory tract: a potential mechanism for lung neutrophil recruitment in cigarette smokers. *Am Rev Respir Dis* 1991;143:844-9.
6. Bush A, Saglani S. Management of severe asthma in children. *Lancet* 2010;376:814-25.
7. Guha C, Osawa M, Werner PA, Galbraith RM, Paddock GV. Regulation of human Gc (vitamin D-binding) protein levels: hormonal and cytokine control of gene expression in vitro. *Hepatology* 1995;21:1675-81.
8. Zhao J, Yeong LH, Wong WSF. Dexamethasone alters bronchoalveolar lavage fluid proteome in a mouse asthma model. *Int Arch Allergy Immunol* 2007;142:219-29.
9. Chun RF, Lauridsen AL, Suon L, Zella LA, Pike JW, Modlin RL, et al. Vitamin D-binding protein directs monocyte responses to 25-hydroxy- and 1,25-dihydroxyvitamin D. *J Clin Endocrinol Metab* 2010;95:3368-76.
10. Holt PG, Strickland DH, Wikstrom ME, Jahnsen FL. Regulation of immunological homeostasis in the respiratory tract. *Nat Rev Immunol* 2008;8:142-52.

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FEV₁ ($r = -0.4$; $P = .01$). **D**, Positive association between BAL VDBP levels and ICS usage ($r = 0.6$; $P = .002$). **E**, No difference in serum VDBP in STRA, MA, and controls (Kruskal-Wallis test $P = .8$). **F-H**, No association between serum VDBP and asthma control ($r = 0.2$; $P = .3$), FEV₁ ($r = -0.3$; $P = .2$), and ICS usage ($r = -0.3$; $P = .2$). **I**, No correlation between VDBP in BAL and serum ($r = -0.199$; $P = .3$). Correlation was determined by using the Spearman rank correlation coefficient. Differences between 3 groups were assessed by using the Kruskal-Wallis test (nonnormal distribution) and then the Mann-Whitney *U* test was used to compare differences between groups. ACT, Asthma control test; MA, moderate asthma; * $P < .05$, ** $P < .01$.