Title: Association between Vitamin D and atopy and asthma phenotypes in children and adolescents: a longitudinal community-based study

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METHODS

Antibody measurement

Total IgE and specific IgE and IgG4 to a panel of allergens were measured from serum collected at ages 14 and 6 by ImmunoCAP (Phadia AB, Uppsala, Sweden); the panel of allergens comprised house dust mite (HDM; *Dermatophagoides pteronyssinus*), rye grass pollen (*Lolium perenne*), cat, couch grass (*Cynodon dactylon*), mould mix (*Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus, Candida albicans, Alternaria alternata and Helminthosporium halodes*), peanut and food mix (egg white, milk, fish, wheat, peanut and soybean).

Respiratory assessment

All spirometry measurements were performed in the morning (10:00–12:00 h) and conducted according to American Thoracic Society guidelines with the subjects seated [E1]. The standard variables, Forced Vital Capacity (FVC), and Forced Expiratory Volume in 1 s (FEV₁), were measured and lung function was represented in statistical analyses by the ratio FEV₁/FVC. Poor lung function was defined as FEV₁/FVC<80 at age 14 and FEV₁/FVC<85 at age 6.

BHR was assessed by methacholine (MCh) challenge testing if subjects obtained reproducible spirometry, had an FEV₁ ≥80% of predicted (age 14), had no respiratory illness in the last 14 days and had withheld from their asthma medications for the standard period [E1]. MCh challenges were performed with a Koko® digidoser (nSpire Health Inc., Longmont, CO, USA) using a modified dosimeter technique [E2]. Subjects were given an initial saline dose followed by doubling doses of MCh (Age 14: 0.0625, 0.125, 0.25, 0.5, 1, 2, 4 and 8 mg/ml; 0.06, 0.10, 0.20, 0.50, 1.0, 1.8, 3.9, & 7.8 mg/ml) delivered via DeVilbiss 646 nebulisers
At age 14 testing was stopped once a patient's FEV₁ had fallen by ≥20% or the highest dose was administered. At age 6 testing was stopped once a patient's FEV₁ had fallen by ≥15% or the highest dose was administered.

**Statistical analyses**

Continuous variables were log₁₀-transformed for use in regression analyses after antibody values below the limits of detection were ascribed a value equivalent to half the limit of detection.

As selection of variables was based on *a priori* knowledge from the literature of their contributions to asthma- or atopy-related processes, we have shown actual p values rather than correcting for multiple testing [E3]. Analyses were performed using SPSS (SPSS Inc., Chicago, Il).

**ONLINE REPOSITORY FIGURE LEGEND**

**Figure E1.** Comparison of two methods of vitamin D measurement. Vitamin D levels were measured from serum by an enzyme immunoassay for all subjects. For twelve subjects at each age group vitamin D levels were also measured by second method: liquid chromatography-tandem mass spectrometry (LC-MS) assay of 25 (OH) vitamin D₃, a routine assay performed by RMIT Drug Discovery Technologies (Melbourne, Victoria Australia) according to published methodology [E4].

- **A.** Vitamin D levels for twelve 14-year-olds measured by the two methods are shown (R² = 0.933).
- **B.** Vitamin D levels for twelve 6-year-olds measured by the two methods are shown (R² = 0.18).
ONLINE REPOSITORY REFERENCES


