may be misclassified. Such misclassification could affect the background rate of asthma and ultimately the attributable risk proportion. However, because most atopics were sensitive to multiple allergens (mean = 4.3), the probability of an atopic participant being positive to any untested allergen and not to at least 1 of the tested allergens is likely low, as is the probability of a transiently negative IgE panel. Finally, it would seem unlikely that the use of subject-reported, doctor-diagnosed asthma as opposed to a clinical assessment or medical chart review would change the main finding of this study, which is that a large proportion of asthma patients with atopy do not have atopic asthma.

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Skin barrier abnormality caused by filaggrin (*FLG*) mutations is associated with increased serum 25-hydroxyvitamin D concentrations

To the Editor:

Epidermal exposure to solar UVB radiation results in synthesis of vitamin D_3, which is converted to 25-hydroxy (25-OH) vitamin D in the liver.[1] In addition to skin pigmentation, epidermal protection against UVB radiation is provided by using trans-urocanic acid (UCA),[2] which is generated when histidase metabolizes histidine in filaggrin.[3] Loss-of-function mutations in the filaggrin gene (*FLG*) affect 10% of Europeans and their descendants[4] and reduce filaggrin protein levels in the skin, as well as its metabolites, including UCA.[5] Because experimental knockdown of filaggrin markedly increases the UV sensitivity of keratinocytes,[6] we hypothesized that the concentration of serum 25-OH vitamin D would be increased in carriers of common *FLG* mutations. We included 5 general population cohorts from Denmark and Germany: 2 children's cohorts, Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) and German Infant Study on the Influence of Nutrition Intervention (GINI)/Influence of Lifestyle Factors on the Development of the Immune System and Allergies in East and Wes Germany (LISA), and 3 cross-sectional adult cohorts, Health2006, Monitoring of Trends and Determinants in Cardiovascular Disease Healthy Survey (Monica), and Cooperative Health Research in the Region of Augsburg (KORA) F4. Atopic dermatitis (AD), dietary vitamin D intake, and vitamin D status were assessed as described in the Methods section and Table E1 in this article's Online Repository at www.jacionline.org.

Subjects were dichotomized into those not carrying an *FLG* mutation or carriers of at least 1 of the R501X or 2282del4 *FLG* mutations. Serum 25-OH vitamin D concentrations were log transformed to approximate a normal distribution. One-way ANOVA was applied to detect differences in log-transformed values of serum measurements of 25-OH vitamin D between study groups (Table I). Multiple linear regression models were used to adjust for potential confounding and known risk factors, including sex, age, body mass index, dietary vitamin supplements, AD, and season of vitamin D testing.[1] Results were computed as percentage differences compared with the reference group corresponding to the back-transformed β-coefficients from the linear regression analyses on log-transformed outcomes multiplied by 100. This was done to compensate for the heterogeneous 25-OH vitamin D measurements in the 5 cohorts. Between-study heterogeneity was tested by using the χ^2-based Q-statistic and quantified by using the I^2 value as a measure of the proportion of variance between the study-specific estimates that are attributable to between-study difference rather than random variation (I^2 describes the percentage of total variation across studies that is due to heterogeneity rather than chance). Because there was no evidence for between-study heterogeneity, the study-specific estimates were combined according to the inverse-variance weighted fixed-effects model. The forest plot was prepared by using Review Manager Computer program Version 5.1 (2011; the Nordic Cochrane Centre, the Cochrane Collaboration, Copenhagen, Denmark). All *P* values were 2-tailed, and statistical significance was defined as a *P* value of less than .05, except for the heterogeneity test, which was considered to be significant at the 10% level. SAS version 9.2 software (SAS Institute, Cary, NC) was used for the COPSAC, Health2006, Monica and KORA F4 study data analyses, and R version 2.13.1 software (http://www.R-project.org) was used for the GINI/LISA study.

The concentrations of 25-OH vitamin D in cord blood were independent of *FLG* mutation status ($P = .65$), indicating no difference at birth. A joint analysis including 9950 subjects aged 4 to 81 years using the raw model showed a strong effect of *FLG* mutations, with 10.7% (95% CI, 7.0% to 14.6%; $P < .0001$) higher 25-OH vitamin D concentrations in *FLG* mutation carriers. An adjusted joint analysis showed a 10.1% (95% CI, 6.7% to 13.6%; $P < .0001$) difference, suggesting that the association was not confounded (Fig 1). An analysis excluding subjects with AD (n = 8849) produced a similar result of 9.1% (95% CI, 5.2% to 13.1%; $P < .0001$). The I^2 measure indicated no evidence for significant between-study heterogeneity ($I^2 = 0\%$; 95% CI, 0% to 79.2%; Q-test $P = .51$). No interaction was detected between the *FLG* genotype and age ($P = .22$) or sex ($P = .56$). The *FLG* effect was stronger in the summer half year (interaction $P = .12$; April-September: 13.1% [95% CI, 8.3% to 18.1%], Q-test $P = .6$, n = 4398) than in the winter half year (October-March: 7.2% [95% CI, 2.5% to 12.2%], Q-test $P = .7$, n = 5552). Only 20 subjects were compound heterozygous/homozygous *FLG* mutation carriers in the 5 cohorts. No overall dose-response effect of *FLG* mutation status on 25-OH vitamin D concentrations was detected. The 9 compound heterozygous/homozygous carriers from the Health2006 study had 29.6% (95% CI, −9.0% to 84.6%) higher 25-OH vitamin D concentrations compared with 9.6% (95% CI, 2.3% to 17.4%) in heterozygous carriers.

Although rather different measures were used, we showed significantly higher concentrations of serum 25-OH vitamin D in carriers of *FLG* mutations with homogeneity across the studies. Although not significant, the effect of *FLG* mutations increased over the summer half year, supporting the hypothesis that the difference in vitamin D status was caused by UV exposure. Filaggrin is hydrolyzed in a humidity-sensitive fashion to its constituent amino acids and their deiminated carboxylic acid derivates, including UCA.[3] This process typically accelerates during the winter in temperate

TABLE I. Descriptive statistics for selected variables from the 5 study populations and unadjusted geometric mean differences of serum 25-OH vitamin D concentrations between categories of covariables*

	COPSAC: data collected 1998-2005	GINI/LISA: data collected 2005-2009	Health2006: data collected 2006-2008	Monica: data collected 1993-1994	KORA F4: data collected 2006-2008
	Danish children born of mothers with asthma (4 y), n = 277	Unselected German children (10 y), n = 1238	Unselected Danish adults (18-69 y), n = 3112	Unselected Danish adults (40-70 y), n = 2500	Unselected German adults (32-81 y), n = 2823
25-OH vitamin D (nmol/L), mean (95% CI)	72.2 (69.3 to 75.3)	71.2 (69.8 to 72.6)	38.9 (38.2 to 39.7)	59.2 (58.3 to 60.3)	32.5 (31.8 to 33.2)
Supplementary dietary vitamin† (% [n/n_{total}])	96.4 (267/277)	3.3 (41/1238)	21.7 (675/3112)	57.5 (1438/2500)	8.0 (226/2823)
Mean difference (95% CI)†	35.68 (8.80 to 69.21)	14.58 (2.79 to 27.73)	3.97 (−0.67 to 8.83)	12.65 (8.90 to 16.54)	34.42 (24.47 to 45.17)
FLG mutations‡ (% [n/n_{total}])	11.9 (33/277)	6.6 (82/1238)	8.1 (251/3112)	7.6 (189/2500)	7.9 (222/2823)
Mean difference (95% CI)	−0.16 (−12.23 to 13.58)	16.80 (8.05 to 26.26)	10.73 (3.34 to 18.65)	9.35 (2.58 to 16.57)	10.96 (2.61 to 19.99)
AD,§ (% [n/n_{total}])	46.2 (128/277)	33.0 (408/1238)	10.0 (311/3112)	2.5 (63/2500)	6.8 (191/2823)
Mean difference (95% CI)	1.03 (−7.08 to 9.85)	4.20 (−0.03 to 8.60)	−4.06 (−9.90 to 2.15)	−6.32 (−15.90 to 4.36)	−1.63 (−9.55 to 6.98)
Female sex (% [n/n_{total}])	50.9 (141/277)	48.5 (600/1238)	55.0 (1713/3112)	49.8 (1246/2500)	51.0 (1441/2823)
Mean difference (95% CI)	−11.49 (−18.47 to −3.90)	−3.12 (−6.82 to 0.73)	3.98 (0.12 to 7.99)	−2.35 (−5.60 to 1.01)	−3.51 (−7.49 to 0.65)
Age mean (y)	4.0	10.2	49.3	53.4	56.0
Age ≥55 y, mean difference (95% CI)	—	—	10.18 (6.01 to 14.50)	−3.38 (−6.62 to −0.03)	0.15 (−3.99 to 4.46)
Body mass index (kg/m^2),‖ mean (±2 SD), mean difference (95% CI [reference = low])	15.7 (13.4 to 18.0)	17.3 (12.4 to 22.3)	25.9 (16.6 to 35.2)	25.9 (17.6 to 34.3)	27.6 (18.0 to 37.2)
Low-medium	1.98 (−9.29 to 14.65)	5.52 (−0.21 to 11.58)	3.09 (−2.21 to 8.68)	3.44 (−1.37 to 8.47)	−3.62 (−9.13 to 2.22)
Medium-high	−8.15 (−18.27 to 3.22)	3.21 (−2.43 to 9.17)	−3.93 (−8.89 to 1.27)	1.21 (−3.49 to 6.14)	−6.90 (−12.22 to −1.26)
High	−0.66 (−11.71 to 11.78)	−1.82 (−7.13 to 3.79)	−14.43 (−18.83 to −9.78)	−9.20 (−13.41 to −4.78)	−21.84 (−26.30 to −17.10)
Time of year of vitamin D testing (reference = January-March)					
April-June	5.30 (−6.39 to 18.46)	11.59 (6.16 to 17.29)	21.56 (15.32 to 28.13)	15.70 (9.84 to 21.87)	41.87 (34.35 to 49.81)
July-September	32.38 (18.32 to 48.10)	61.22 (53.57 to 69.25)	48.13 (40.94 to 55.69)	63.30 (55.67 to 71.31)	80.69 (69.53 to 92.58)
October-December	4.59 (−6.61 to 17.13)	35.57 (28.89 to 42.60)	35.66 (29.02 to 42.65)	20.50 (15.25 to 25.98)	33.56 (27.19 to 40.26)

*Unadjusted difference in 25-OH vitamin D concentrations in percentages (with 95% CIs) from reference category calculated from the back-transformed (anti-log) β-estimate obtained in univariate linear regression models.

†Supplementary oral intake of vitamin D (Health2006 and KORA F4) or multivitamin (COPSAC, GINI/LISA, and Monica) supplements because separate vitamin D supplement use was not available.

‡*FLG* null mutation defined as at least 1 of the *FLG* mutations R501X or 2282del4.

§AD definition differed between the cohorts. Details are reported in the Methods section in this article's Online Repository.

‖Body mass index: participants were divided into quartiles in each study.

climates because of forced indoor heating. Moreover, skin inflammation, as observed in subjects with AD, reduces filaggrin levels and is often most severe in the winter.[7] Hence reduced solar radiation in the winter in combination with humidity and inflammatory driven hydrolization in mutation and nonmutation carriers might explain the reduced effect of *FLG* mutations over the winter. Overall, AD did not influence the effect of *FLG* mutations on serum 25-OH vitamin D concentrations. Remember that AD is typically mild in subjects from the general population and that most subjects outgrow the disease. Hence in the adult studies AD would only have a

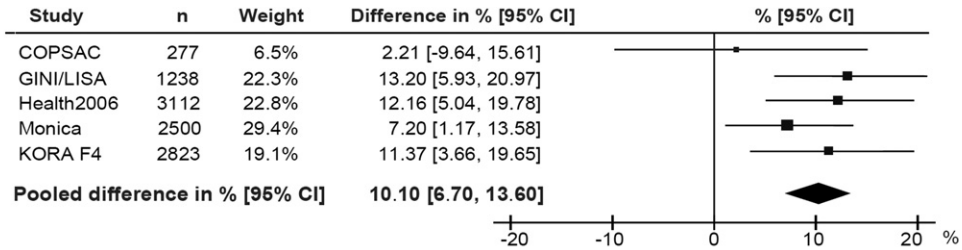

FIG 1. Forest plot illustrating the study-specific and combined differences in geometric means of serum 25-OH vitamin D concentrations in percentages between *FLG* mutation (R501X, 2282del4, or both) and non-mutation carriers adjusted for age, sex, body mass index, AD, supplementary vitamin D intake, and time of year of vitamin D testing.

small effect on vitamin D status. However, in the studies involving children, AD was more likely to be a current disorder, potentially masking the effect of *FLG* mutations on 25-OH vitamin D concentrations. The null effect in COPSAC by age 4 years might also be due to the extensive use of vitamin D supplements (96% of participants) and the small sample size. Potential misclassification because of limited genotyping and methodology-based differences in the measurements of vitamin D_3 concentrations is assumed to result in slightly less precise association estimates.

Despite not knowing whether our findings are geographically restricted, they challenge the hypothesis that the increasing levels of atopic disease have been caused by vitamin D insufficiency because of reduced solar UVB exposure.[8] Moreover, they offer an alternative explanation for the evolutionary heterozygous advantage that gave rise to the independent emergence and persistence of several haplotypically independent *FLG* null alleles and marked regional differences in frequency.[9] The possible effect of 10% higher vitamin D concentrations on morbidity remains to be elucidated.

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Limitations of reliance on specific IgE for epidemiologic surveillance of food allergy

To the Editor:

It is extraordinarily difficult to estimate the true prevalence of food allergy. Questionnaire-based estimates tend to overestimate the real rate of food allergy, defined here as evidence of both IgE sensitization and clinical reactivity, whereas the "gold standard," food challenge, is logistically difficult. In fact, in the extensive literature review conducted for development of the National Institute of Allergy and Infectious Diseases Guidelines for the Diagnosis and Management of Food Allergy, the authors could only conclude that food allergy affects more than 1% to 2% but <10% of the population.[1]

Allergen-specific IgE offers a potential surrogate. Recently, for example, a nationally representative survey, NHANES (National Health and Nutrition Examination Survey) 2005-2006, collected data on food-specific IgE. However, it is not clear how to extrapolate food-specific IgE levels to estimate prevalence of food allergy in the absence of clinical data, given that most people with low-level sensitization to foods are not clinically allergic.[1] One option that has been widely used is to apply positive predictive values (PPVs) for higher levels of food-specific IgE generated from previous work.[2-5] For example, with the use of previously reported 50% and 95% PPVs and NHANES data, one group estimated the US prevalence of food allergy to be 2.5%,[3] whereas others applied PPVs to inner city patients with asthmatics.[5] However, the only PPVs available were generated from referral pediatric allergy populations,[6-8] which are unlikely to be valid for the general population. Here, we incorporate dietary data from NHANES 2005-2006 to relate patterns of reported food consumption to food-specific IgE levels. In doing so, we found the marked limitations of using IgE data alone to estimate prevalence of food allergy.

NHANES is a complex population-based survey of the noninstitutionalized civilian US population (http://www.cdc.gov/nchs/nhanes.htm). In the 2005-2006 survey, specific IgE by ImmunoCap to milk, peanut, egg, and shrimp was obtained on a random subsample. Here, subjects 2 years and older were included (except for shrimp, whereby subjects 6 years and older were included) in analyses of shrimp consumption over the past 30 days, and milk, egg, and peanut consumption over the past year. Subjects were considered to consume cow's milk if they reported drinking whole, 2%, 1%, nonfat, or unpasteurized milk as a beverage, in their cereal, or in coffee or tea, excluding rice, soy, or "other" milk. Subjects were considered to eat hen's eggs if they reported eating egg, including eggs, egg whites, egg substitutes, egg salads, quiche, and soufflés. Although most egg substitutes contain egg whites, sensitivity analyses were done excluding egg substitutes. Lobster, crayfish, and crab were grouped with shrimp as "crustaceans." Peanut consumption could not be defined with as much certainty and was included only as an exploratory analysis; subjects were asked if they "ate peanut butter or other nut butter," and if they ate "peanuts, walnuts, seeds, or other nuts" in the past year. History of asthma attack, itchy rash, allergic reaction, and problems with sneezing in the past year were by self-report (for details, see this article's Methods section in the Online Repository at www.jacionline.org). No questionnaire data specifically related to food allergy.

According to the cutoffs used by others,[3] for each food we divided subjects into groups defined by food-specific IgE: "unlikely food allergy" (food-specific IgE of 0.35-2 kU/L), "probable food allergy" (food-specific IgE between 2 and 7 kU/L for egg, 15 kU/L for milk, 14 kU/L for peanut, and 5 kU/L for shrimp), and "likely food allergy" (food-specific IgE above those levels). The percentage of subjects in each IgE category consuming the relevant food was calculated. Within each category of IgE, the percentage of subjects with specific medical symptoms was compared by category of food consumption. Survey weights, strata, and sampling units were incorporated into the analyses, except for analyses of symptoms by consumption status because the numbers of people in the higher IgE categories were too small.

Subjects (n = 5459) had complete data for at least one of the food frequency questions and food-specific IgE; 7106 subjects had data on shrimp recall and IgE. Subjects who had dietary data were older ($P < .001$) and were more likely to be female ($P < .001$), to have a higher income ($P < .001$), and to be white ($P < .001$). No differences were observed by history of

METHODS

Study populations

COPSAC. A total of 288 children born to mothers with asthma were enrolled in the COPSAC prospective birth cohort.[E1] Children were born between August 1998 and December 2001 and were enrolled at 1 month of age and visited the COPSAC clinic at scheduled visits every 6 months and thereafter, as well as for any acute complaints from the skin or airways. Only COPSAC participants with full data (n = 277) were included in all COPSAC analyses. COPSAC was conducted in accordance with the guiding principles of the Declaration of Helsinki, as approved by the Ethics Committee of Copenhagen (KF 01-289/96) and the Danish Data Protection Agency (2002-41-2434). Informed written consent was obtained from the parent or parents. Data validity was ensured by using quality control procedures. The prevalence of AD and *FLG* mutations was 46.2% (128/277) and 11.9% (33/277), respectively. The crude association between AD and *FLG* mutations was an odds ratio (OR) of 2.24 and a 95% CI of 1.06 to 4.76.

GINI/LISA. The influence of the Lifestyle Factors on the Development of the Immune System and Allergies in East and West Germany PLUS the Influence of Traffic Emissions and Genetics (LISAplus) study is a population-based birth cohort study.[E2] A total of 3097 healthy, full-term neonates were recruited between 1997 and 1999 in Munich, Leipzig, Wesel, and Bad Honnef, Germany. The participants were not preselected based on family history of allergic diseases. A total of 5991 mothers and their newborns were recruited into the German Infant Study on the Influence of Nutrition Intervention PLUS Environmental and Genetic Influences on Allergy Development (GINIplus) between September 1995 and June 1998 in Munich and Wesel, Germany.[E3] Infants with at least 1 allergic parent, sibling, or both were allocated to the interventional study arm, investigating the effect of different hydrolyzed formulas for allergy prevention in the first year of life. All children without a family history of allergic diseases and children whose parents did not provide consent for the intervention were allocated to the nonintervention arm. In both studies only subjects of white German descent were included, and approval by the local ethics committees and written consent from participant's families were obtained. The prevalence of AD and *FLG* mutations was 33.0% (408/1238) and 6.6% (82/1238), respectively. The crude association between AD and *FLG* mutations was an OR of 1.65 and a 95% CI of 1.05 to 2.60.

Health2006. A total of 3471 adults aged 18 to 69 years (of 7931 invited) from the general population in Copenhagen,[E4,E5] all with Danish citizenship, underwent a 2-hour general health examination between June 2006 and May 2008 in Glostrup, Denmark. The survey had a broad health focus and did not focus on skin diseases. Only Health2006 participants with full data (n = 3112) were included in all Health2006 analyses. The Ethics Committee of the Copenhagen County approved the study (KA-20060011). Written and verbal consent was given by the participants to be enrolled in the study and for their information to be stored in the hospital database and used for research. The prevalence of AD and *FLG* mutations was 10.0% (311/3112) and 8.1% (251/3112), respectively. The crude association between AD and *FLG* mutations was an OR of 2.77 and a 95% CI of 2.00 to 3.85.

Monica. A total of 2656 participants originating from the Danish part of the Monitoring of Trends and Determinants in Cardiovascular Disease (Monica) health survey performed between 1982 and 1984[E6] were selected to represent equal numbers of women and men aged 30, 40, 50, and 60 years and were invited for the MONICA10 study. This second study, including health examination and vitamin D measurement, was performed between 1993 and 1994 in Glostrup, Denmark. Monica participants (n = 2500) with full data were included in all Monica analyses. All participants completed a questionnaire about current and previous diseases, intake of medication, and lifestyle risk factors. The Monica study was approved by the local Ethics Committee system in Denmark (KA-01430). Written informed consent was obtained from all subjects. The prevalence of AD and *FLG* mutations was 2.5% (63/2500) and 7.6% (189/2500), respectively. The crude association between AD and *FLG* mutations was an OR of 1.81 and a 95% CI of 0.85 to 3.87.

KORA F4. From 2006 to 2008, 3080 participants aged 32 to 81 years were enrolled in the KORA F4 study, the 7-year follow-up of the population-representative KORA S4 survey (n = 4261 subjects, 1999-2001). A total of 2823 KORA F4 participants with full data were included in all KORA F4 analyses. All subjects of the KORA S4 and F4 studies had German nationality and lived in Southern Germany, either in the city of Augsburg or in one of the 2 adjacent counties. The study design, sampling method, and data collection have been described in detail elsewhere.[E7] The prevalence of AD and *FLG* mutations was 6.8% (191/2823) and 7.9% (222/2823), respectively. The crude association between AD and *FLG* mutations was an OR of 1.76 and a 95% CI of 1.12 to 2.77.

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TABLE E1. Selected study cohort characteristics and analytic details

	Information on dietary vitamin supplements	Definition of AD	25-OH vitamin D analysis (traceability, CV%,* specificity, detection limit)	*FLG* genotyping
COPSAC	Parents were interviewed at each 6-mo visit to the clinic about dietary vitamin supplements for their child.	Hanifin and Rajka criteria	LC-MS/MS,† traceable to NIST SRM 972 CV%: 25OHD2 9.4% (level 25.3), 8.8% (level 41.2) CV%: 25OHD3 9.1% (level 21.1), 6.4% (level 66.5) Specificity: 25OHD2 100%, 25OHD3 100%, codetermines 3-epi-25OH Detection limit: 10.0 nmol/L	TaqMan-based allelic discrimination assay and fluorescently labeled PCR, Applied Biosystems 3100, 3730, and 7700 sequence detection system (Foster City, Calif)[E8] Call rates for the R501X and 2282del4 mutations = 100% and 100%, respectively
GINI/LISA	A food frequency questionnaire administered to the parents was designed to measure the children's usual food and nutrient intake at 10 y of age. Questions on regular dietary multivitamin supplement intake were included at the end of the questionnaire.	Information on ever having physician-diagnosed AD was collected by using self-administered questionnaires completed by the parents.	Ligand-binding assay Cobas E170 (Roche Diagnostics, Mannheim, Germany) traceable to NIST SRM 972 CV%: 13.1% (level 20.8), 3.4% (level 173.7) Specificity: 25OHD2 81%, 25OHD3 98%, codetermines 25,25OH$_2$D3 121% Detection limit: 10.0 nmol/L	iPLEX Mass Array system MALDI-TOF MS; (Sequenom, San Diego, Calif)[E9] Call rates for the R501X and 2282del4 mutations = 97.0% and 90.9%, respectively
Health2006	Participants were asked "within the past 12 months, have you taken dietary vitamin D supplements?"	AD was defined by the UK Working Party's diagnostic criteria for AD as a history of an itchy skin condition plus a minimum of 2 of 4 minor criteria.	Chemiluminescence immunoassay Cobas e411 (Roche Diagnostics, Mannheim, Germany) traceable to company standard LC-MS-MS CV%: 9.9% (level 63.0), 6.9% (level 163.7) Specificity: 25OHD2 <10%, 25OHD3 100%, codetermines 1,25(OH)$_2$D3 $\approx$ 100% Detection limit: 10.0 nmol/L	Allele-specific PCR (Luminex, Austin, Tex; Bio-Plex 200, Bio-Rad Laboratories, Hercules, Calif)[E5] Call rates for the R501X and 2282del4 mutations = 99.4% and 99.5%, respectively
Monica	Participants were asked "within the last 12 months, have you taken vitamin supplements?"	AD was defined by an affirmative answer to the question "have you ever had asthmatic eczema?" (This was a common term used in Denmark at the time.)	Chemiluminescence immunoassay IDS-iSYS (Immunodiagnostic Systems, Boldon Tyne & Wear, United Kingdom) traceable to company standard UV/LC-MS-MS CV%: 16.9% (level 16.7), 8.9% (level 185.2) Specificity: 25OHD2 100%, 25OH D3 100%, Codetermines 1,25(OH)$_2$D3 > 100% Detection limit: 9.0 nmol/L	Allele-specific PCR (Luminex, Austin, Tex; Bio-Plex 200, Bio-Rad Laboratories, Hercules, Calif)[E5] Call rates for the R501X and 2282del4 mutations = 99.96% and 99.96%, respectively
KORA F4	The use of dietary supplements consumed during the 7 days before the interview was assessed. Participants were asked to provide all relevant packages to the study center, where exact names and dosages were recorded for each supplement. Subjects using supplements that contained vitamin D were classified as vitamin D users.	AD was defined by affirmative answers to both of the following 2 questions: "Have you ever had neurodermatitis (atopic/endogenous eczema)?" and "Was this neurodermatitis/ atopic eczema diagnosed by a physician?"	Chemiluminescence immunoassay Liaison, (DiaSorin, Stillwater, Minn) traceable to company standard CV%: 12.9% (level 18.0), 7.3% (level 319.4) Specificity: 25OHD2 100, 25OHD3 104%, codetermines 1,25(OH)$_2$D2 40% and 1,25(OH)$_2$D3 17% Detection limit: 10.0 nmol/L	iPLEX Mass Array system MALDI-TOF MS; (Sequenom, San Diego, Calif)[E10] Call rates for the R501X and 2282del4 mutations = 93.6% and 94.4%, respectively

For details, please refer to previous articles.
*Total analytic imprecision of the method (according to the producer).
†HPLC (Dionex, Sunnyvale, Calif) API 3000 triple quadropole mass spectrometer (Applied Biosystems, Foster City, Calif).