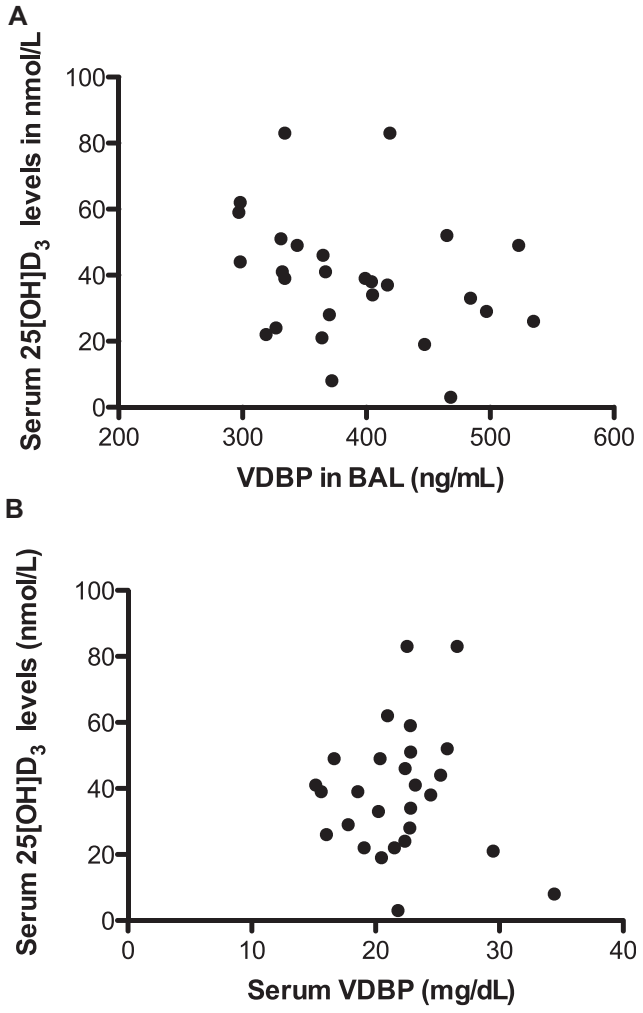

FIG 2. No association between serum 25-hydroxyvitamin D_3 levels (25 [OH] D_3) and BAL VDBP (**A**; $r = -0.3$; $P = .1$) and serum VDBP (**B**; $r = 0.1$; $P = .6$). Correlation was determined by using the Spearman rank correlation coefficient.

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A.G. received research support (James Trust Fellowship) from the British Medical Association. C.H. gratefully acknowledges institutional support through the Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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Available online March 28, 2012.
doi:10.1016/j.jaci.2012.02.017

Amish children living in northern Indiana have a very low prevalence of allergic sensitization

To the Editor:

The prevalence of allergic sensitization has increased in most developed counties over the past century. In the United States, the third National Health and Nutrition Examination Survey[1] found 54.3% of the study population to have evidence of allergic sensitization. European studies[2] have shown similar findings. In contrast to these studies of increasing prevalence, there are now a number of studies demonstrating that certain populations have a significantly lower prevalence of allergic sensitization and a lower prevalence of asthma.[3] Children who reside on traditional Swiss farms are among those with a low prevalence of allergic sensitization.[4]

We sought to compare the prevalence of allergic sensitization in a population of Amish children aged 6 to 12 years with both children living on farms in Switzerland and nonfarm Swiss children. The Amish dispersed from Switzerland approximately 200 years ago seeking freedom from religious persecution. Although the Amish can be found in a number of countries and various communities in America, the largest concentrations are found in Pennsylvania, Ohio, and Indiana. In Indiana, there are approximately 25,000 members in their community. They live primarily an agrarian lifestyle. Many families live on working farms. All Amish families have horses that are used for transportation. A significant percentage drinks raw milk. They do not use electricity in their homes. They have large families.

In Switzerland, 28,686 questionnaires were distributed to families of children aged 6 to 12 years in phase 1 of the GABRIEL study (2006-2007). The GABRIEL questionnaire assessed demographic characteristics, contact to farming environment, symptoms and reported diagnoses of asthma, hay fever, and atopic dermatitis as well as parental smoking (Table I).[5] A stratified random sample

FEV_1 ($r = -0.4$; $P = .01$). **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 ($r = 0.2$; $P = .3$), FEV_1 ($r = -0.3$; $P = .2$), 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; $*P < .05$, $**P < .01$.

TABLE I. Characteristics and farm exposures of the study population

	n (%)		
	Amish (n = 157)	Swiss farmer (n = 3,006)	Swiss nonfarmer (n = 10,912)
Female sex	61 (39.9)	1,542 (51.3)	5,263 (48.3)
Age (y), mean ± SD	10.1 ± 1.70	9.9 ± 1.89	9.9 ± 1.88
No. of children, mean ± SD*	5.9 ± 2.25	3.3 ± 1.27	2.4 ± 0.94
Maternal smoking†			
Never	64 (92.8)	1,567 (79.3)	5,171 (62.6)
Ex-smoker	0	240 (12.1)	1685 (20.4)
Current	5 (7.2)	170 (8.6)	1,398 (16.9)
Parents or siblings affected by			
Asthma	8 (5.2)	451 (15.2)	2,064 (19.1)
Hay fever	24 (15.4)	559 (18.8)	4,127 (38.3)
Atopic dermatitis	10 (6.4)	482 (16.3)	2,078 (19.3)
Family lives on a farm†	61 (84.7)	2,024 (100.0)	
Child regularly spent time in a barn	143 (91.7)	2,735 (91.8)	
Child regularly drank milk directly from a farm	124 (79.5)	2,614 (87.2)	
Type of animal farming†			
Dairy	17 (23.6)	1,322 (66.9)	
Cow/cattle	34 (47.2)	1,158 (58.6)	
Pig	10 (13.9)	640 (32.4)	
Sheep	7 (9.7)	328 (16.6)	
Poultry	34 (47.2)	690 (34.9)	
Horse	55 (76.4)	373 (18.9)	
Goat	9 (12.5)	358 (18.1)	
Bunny/rabbit	17 (23.6)	718 (36.3)	

*Derived as the number of siblings plus 1 from the child's questionnaire and analyzed per family.
†Per family (n = 72 families among the Amish, n = 2,024 families among the Swiss farmer, and n = 8,493 families among the Swiss nonfarmer).

TABLE II. Asthma and allergic disease

	n (%)		
	Amish (n = 157)	Swiss farmer (n = 3,006)	Swiss nonfarmer (n = 10,912)
Wheeze (past 12 mo)	6 (3.9)	156 (5.3)	1,020 (9.5)
Doctor diagnosis of asthma*	8 (5.2)	202 (6.8)	1,218 (11.2)
Doctor diagnosis of bronchitis†	22 (14.3)	504 (17.7)	2,445 (24.1)
Doctor diagnosis of croup†	22 (14.1)	320 (11.4)	1,545 (15.6)
Inhaler (ever)	8 (5.1)	265 (8.9)	1,494 (13.8)
Rhinoconjunctivitis (past 12 mo)	8 (5.2)	143 (4.8)	1,412 (13.0)
Doctor diagnosis of hay fever (ever)	1 (0.6)	94 (3.1)	1,259 (11.6)
Doctor diagnosis of atopic eczema (ever)	2 (1.3)	226 (7.6)	1,310 (12.1)
Atopy‡	10 (7.2)	223 (25.2)	281 (44.2)
Dematophagoides pteronyssinus§‖	8 (5.8)	82 (9.3)	102 (16.4)
Cat§‖	1 (0.7)	32 (3.6)	66 (10.6)
Birch‖		71 (8.0)	122 (19.6)
Mixed trees§	2 (1.4)		
Mixed grasses§¶	4 (2.9)	178 (20.1)	248 (39.8)

*Either doctor diagnosis of asthma at least once in lifetime or doctor diagnosis of wheezy bronchitis at least twice in lifetime (ISAAC Phase 2 reported doctor diagnosed asthma definition).
†At least once in lifetime.
‡The results are derived from 138 Amish participants in the skin prick test and a stratified random sample of the Swiss (n = 884 farmer, n = 628 nonfarmer). For the Amish, atopy was defined as a skin prick test wheal of 3 mm or more after subtraction of the negative control against *D pteronyssinus*, cat, mixed trees, or mixed grasses. For the Swiss, atopy was defined as a specific IgE level of 0.7 kU/L or more against either *D pteronyssinus*, cat, or birch or a specific IgE level of 0.35 kU/L or more against mixed grasses.
§Skin prick test wheal of 3 mm or more after subtraction of the negative control among the Amish.
‖Specific IgE (single test) level of 0.7 kU/L or more among the Swiss.
¶Specific IgE (group test) level of 0.35 kU/L or more among the Swiss.

of consenting children was invited to assess serum-specific IgE to inhalant and food allergens. Atopy was defined as any positive IgE level of 0.7 kU/L or more. For the Amish study, permission was obtained from the Amish School Board to distribute questionnaires in 7 schools from different church districts for an unbiased sample selection of children aged 6 to 12 years. A total of 185 questionnaires were distributed, of which 157 were returned. Of these, 138 children participated in skin prick testing after parental consent. Among the Amish, a slightly modified GABRIEL phase 1 questionnaire was distributed in December 2010. Consenting children were invited for a skin prick test session in March 2011. Skin prick testing was done by using a Duotip-Test (Lincoln Diagnostics, Decatur, Ill) to similar although not identical inhalant allergens (ALK-Abelló, Round Rock, Tex). A positive skin prick test result was a wheal greater than or equal to 3 mm after subtraction of the negative control.

In Switzerland, 65% returned questionnaires; 89% of the subsample had IgE level measurements. Among the Amish, 85% returned questionnaires and 75% participated in skin prick testing. Based on parental reporting, the prevalence of ever having asthma was 5.2%, 6.8%, and 11.3% among Amish, Swiss farm, and Swiss nonfarm children, respectively. The corresponding figures for allergic sensitization were 7.2%, 25.2%, and 44.2%. Dust mite sensitization figures were 5.8%, 9.3%, and 16.4% respectively. Sensitization to mixed grasses was 2.9%, 20.1%, and 39.8% among Amish, Swiss farm, and Swiss nonfarm children.

Sex and age distribution was similar in all study groups. Amish children and those living on a Swiss farm drink milk directly from the farm (79.5% and 87.2%, respectively). More than 90% of the Amish and Swiss farm children regularly spent time in barns. As part of the definition, none of the Swiss nonfarm children drank unprocessed milk directly from the farm or had regular contact with farm animals. The mean number of children per family was 5.9 among the Amish, 3.3 among the Swiss farm families, and 2.4 among the Swiss nonfarm families (Table II).

The results of this study demonstrate a very low prevalence of allergic sensitization in Amish children living in northern Indiana. We acknowledge that atopy was assessed by 2 different methods in this study and discrepancies between skin prick test reactivity and specific IgE assays have been reported.[2] However, farm studies in an alpine area of Austria have shown similar differences in atopic sensitization (18.8% vs 32.7%) in farm versus nonfarm children when using skin prick testing.[4]

It has been demonstrated previously that early exposures associated with Swiss farm life are protective against the development of atopic disease[4]; however, the Amish in our study had a

significantly lower prevalence than the Swiss farm children. The parental reporting of wheezing was similar in both the Amish and Swiss farm children. A study of children living in rural areas of Crete demonstrated a lower prevalence of atopy based on skin testing than in children living in a city.[6] The prevalence of allergic sensitization was 24%, again very similar to that in the Swiss farm children. This level of sensitization was found in 4 rural areas with no difference in those living on farms. This study again suggests a protective effect of rural life. Significant differences in allergic sensitization in children were also found in a study of 3 Chinese cities.[7] Children living in the Westernized city of Hong Kong had twice the prevalence of atopy as children living in Beijing. There was also a significantly higher prevalence of wheeze in the children living in Hong Kong. In this study, exposure to cotton quilts and raw fruits and vegetables were protective factors.

This Amish population of children aged 6 to 12 years has a very low prevalence of allergic sensitization. Because of the small number of children in the study, we were not able to determine specific differences in lifestyle, diet, exposures, or family history between those Amish children who were sensitized and those who were not sensitized. Among the factors thought to be associated with this low prevalence may be large family size.[8] The Amish families have a mean of 5.9 children per family as compared with 2.4 for the Swiss nonfarm families. All the Amish children and the Swiss farm children had exposure to large animals and a significant percentage consumed milk directly from the farm. Recent studies have implicated a protective effect from farm milk. In one study, certain whey proteins in farm milk were inversely associated with asthma.[9] The Amish are of Swiss descent and therefore have a genetically similar background to the comparative Swiss children. However, the nonfarm Swiss children have a prevalence of allergic sensitization similar to the National Health and Nutrition Examination Survey data of randomly selected Americans, suggesting no protective genetic effect of Swiss decent. The prevalence of asthma and allergic sensitization continues to rise in the Westernized populations. Although we have not determined specific mechanisms, this study continues to support the effect of early farm exposures and their impact in significantly reducing the prevalence of asthma and allergic sensitization. Previous studies in Switzerland and Crete show a protective effect of farm life or rural living, with a 50% reduction in the prevalence of allergic sensitization as compared with nonfarm or urban living. Therefore, given the exceedingly low level of sensitization of 7.2% among Amish children, we feel that there may be additional protective factors in this population.

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This study was supported by the St Vincent Foundation, Indianapolis, Ind; an AAAAI ART mini-grant; and the European Commission as part of GABRIEL (contract no. 018996 under the Integrated Program LSH-2004-1.2.5-1).

Disclosure of potential conflict of interest: J. Weber received research support from the European Commission. E. von Mutius has consulted for Novartis, GlaxoSmithKline, ALK, and ProtectImmun; has received a speaker's fee from InfectoPharm; has received research support from Airsonnett AB; and has been a member of the expert panel for UK Research Excellence Framework. The rest of the authors declare that they have no relevant conflicts of interest.

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Available online April 17, 2012.
doi:10.1016/j.jaci.2012.03.016

STAT6 and *LRP1* polymorphisms are associated with food allergen sensitization in Mexican children

To the Editor:

Food allergy, affecting up to 8% of children in the United States,[1] is most often mediated by allergen-specific IgE antibodies in the blood. Food allergen sensitization is an intermediate phenotype measured by specific IgE blood tests or skin prick tests (SPTs) and used in the diagnosis of food allergy. Despite family history being a risk factor, no genetic variants have been conclusively identified for food sensitization or clinical food allergy.[2] By using the Mexico City Childhood Asthma Study, we examined associations between food sensitization (based on SPTs) and single nucleotide polymorphisms (SNPs) spanning 5 autosomal candidate genes reviewed by Hong et al[2] (*CD14* [cluster of differentiation 14], *IL10*, *IL13*, *SPINK5* [serine peptidase inhibitor, Kazal type 5 isoform], and *STAT6* [signal transducer and activator of transcription 6]).

We previously conducted a genome-wide association study of asthma in the Mexico City Childhood Asthma Study among 492 children with physician-diagnosed asthma (aged 5-17 years) and their parents, who were recruited from a pediatric allergy clinic in Mexico City.[3] The children's clinical evaluation included SPTs to 6 major food allergens that are common in the Mexican diet (milk, egg, wheat, soy, peanuts, and tree nuts). An SPT result was declared positive if the largest diameter of the wheal exceeded 4 mm. Testing was considered valid if the reaction to the positive control (histamine) exceeded 6 mm and also exceeded 4 mm above the negative control (glycerin).[4] There were 162 trios having an asthmatic child with a positive SPT result to at least 1 food allergen. We examined SNP associations with food sensitization in the 5 candidate gene regions in these 162 trios.[2] As all probands were asthmatic patients, it was not possible to adjust for asthma.