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Study design

The GABRIEL Advanced Surveys: study design, participation and evaluation of bias

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Summary

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Exposure to farming environments has been shown to protect substantially against asthma and atopic disease across Europe and in other parts of the world. The GABRIEL Advanced Surveys (GABRIELA) were conducted to determine factors in farming environments which are fundamental to protecting against asthma and atopic disease.

The GABRIEL Advanced Surveys have a multi-phase stratified design. In a first-screening phase, a comprehensive population-based survey was conducted to assess the prevalence of exposure to farming environments and of asthma and atopic diseases ($n = 103\,219$). The second phase was designed to ascertain detailed exposure to farming environments and to collect biomaterial and environmental samples in a stratified random sample of phase 1 participants ($n = 15\,255$). A third phase was carried out in a further stratified sample only in Bavaria, southern Germany, aiming at in-depth respiratory disease and exposure assessment including extensive environmental sampling ($n = 895$).

Participation rates in phase 1 were around 60% but only about half of the participating study population consented to further study modules in phase 2. We found that consenting behaviour was related to familial allergies, high parental education, wheeze, doctor diagnosed asthma and rhinoconjunctivitis, and to a lesser extent to exposure to farming environments. The association of exposure to farm environments with asthma or rhinoconjunctivitis was not biased by participation or consenting behaviour.

The GABRIEL Advanced Surveys are one of the largest studies to shed light on the protective 'farm effect' on asthma and atopic disease. Bias with regard to the main study question was able to be ruled out by representativeness and high participation rates in phases 2 and 3. The GABRIEL Advanced Surveys have created extensive collections of questionnaire data, biomaterial and environmental samples promising new insights into this area of research.

Keywords: *GABRIEL Advanced Surveys*, *study design*, *asthma*, *atopy*.

Introduction

European research teams using epidemiological studies were the first to demonstrate that exposure to farming environments confers significant protection from the development of asthma, hay fever and atopy.[1–3] Since then, this protective effect has been confirmed in numerous studies in other parts of the world.[4–7] It is remarkably consistent and results in a reduction in risk of about 40% making the exposure to farming environments one of the strongest environmental influences on asthma and atopic disease.

The mechanisms and pathways of this protective effect are still unclear. Early-life contact with livestock and their fodder, and consumption of unprocessed cows' milk have been identified as the most effective protective exposures.[7] This has led to a working model in which diverse and strong microbial exposure as in farming environments and possibly xenogeneic pressure during pregnancy lead to a non-allergic state at birth by up-regulating regulatory T cells and interferon γ. Further presence of these exposures during early life then consolidates the protection against asthma and atopic disease at school age.[7]

The multi-national cross-sectional GABRIEL Advanced Surveys systematically study farming and rural environments in order to identify factors which are fundamental in protecting against asthma and atopic disease in childhood. In this paper, we report the study design and methods of the GABRIEL Advanced Surveys and discuss issues related to coverage, participation and bias.

Methods

The GABRIEL Advanced Surveys were conducted in rural areas of southern Germany (Bavaria and Baden-Württemberg), Switzerland, Austria and Poland. Data from Germany, Switzerland and Austria will often be combined to increase sample size and statistical power and will be referred to as data from German-speaking centres.

Catchment area and population

The catchment population were elementary school children (over 98% aged 6 to 12 years) in the areas described below. Elementary schools were excluded if they were private, international, Montessori or so-called Rudolf-Steiner (anthroposophic) schools. Special school classes for handicapped children were also excluded.

In Bavaria, Switzerland and Poland, districts were excluded if they had been surveyed in earlier studies investigating the protective effect of farming environments on allergic disease.[3,6,8,9] None of these earlier studies had been conducted in Baden-Württemberg or the Tyrol. Different strategies were employed to ensure a rural setting. For Austria (Tyrol), the district of Innsbruck city, and for Poland (lower Silesia), communities with >15 000 inhabitants were excluded as they were judged to be urban areas. In Germany and Switzerland, communities with at least one to two farms per 100 inhabitants were included depending on the study centre.

Approval and data protection

Ethical approval was obtained from ethics committees of the following institutions: Bavarian Medical Association (for Bavaria), Ulm University (for Baden-Württemberg), the cantons Luzern, Zurich, and Thurgau (for Switzerland), Medical University of Innsbruck (for Austria) and Medical University of Wroclaw (for Poland). Furthermore, approval was sought and obtained from the Ministries of Education in the study areas as well as local school authorities and heads of the elementary schools contacted.

The data protection concept was approved by the data protection officer of Baden-Württemberg and evaluated and approved thereafter by the respective authorities of the other study areas.

Phase 1 – General population survey

A population-based survey was conducted to assess the prevalence of farm characteristics, exposure to farming environments and of asthma and allergic diseases. The aim was to identify at least 75 farm children with reports of doctor diagnosed asthma or wheezy bronchitis per centre. This is 2–3 times the sample size of this group that was available in previous studies documenting the farm effect in our study regions.[1–3,8] To achieve this, 40 000 school children per centre had to be sampled based on estimates of response rates and of prevalence of asthma, atopy and farming in rural populations from earlier studies.[3,8]

Headmasters of elementary schools were contacted for assent to the study. Headmasters of participating schools gave numbers of children per class before the

school year started in September 2006; however, these changed due to late registration of some children. Starting in November 2006, relevant information and questionnaires were distributed via the class teachers to the families. The questionnaires assessed demographic characteristics, contact with a farming environment, and parental smoking and education. Standardised questions on symptoms and diagnoses of asthma, hay fever and atopic eczema from the International Study of Asthma and Allergies in Childhood (ISAAC) were used.[10] Furthermore, informed consent for phase 1 and preliminary informed consent for phase 2 were obtained.

All questionnaire data were entered at the data centre at Ulm University by an automatic data capture system (Teleform Desktop V9.1 and Teleform Workgroup V10.1; Autonomy Cardiff, Vista, CA, USA). This system was set to issue an automatic alert upon detection of invalid tick marks. In addition, specific items were re-evaluated by trained data entry clerks.

Eligibility for phase 2, inclusion and exclusion criteria

The GABRIEL Advanced Surveys have a multi-phase stratified design. The phase 1 study population eligible for phase 2 were children whose parents had expressed preliminary consent to dust sampling, blood withdrawal and genetic analyses.

Only children with the nationality of their study country, both of whose parents were born in the study country, were included to achieve a more homogenous population in subsequent phases, especially with regard to the genetic study modules. In rural Poland, it was assumed that the prevalence of migrants was negligible and these items were neither assessed nor used as inclusion criteria. For logistical reasons, the Polish phase 2 had to be conducted in the subsequent school year. Thus, all children in the final grade (grade 6, $n = 3759$) were excluded. For Switzerland, children whose phase 1 questionnaires arrived at the data centre after 5 April 2007 ($n = 1302$) were excluded because phase 2 fieldwork had to be started in order to be completed by the summer holidays.

Stratified sampling for phase 2

The phase 1 study population eligible for phase 2 was stratified into three exclusive strata of contact to farming environments: (i) farm children, that is, children who lived on a farm that was currently run by the family, (ii) exposed non-farm children, that is, children who did not qualify as farm children but had regularly (at least once a week over a period of 6 months) visited stables or barns or had regularly consumed farm milk ever in life, and (iii) unexposed non-farm children, that is, the remaining children. For Poland, with more traditional rural communities and different exposure patterns, the definition of farm children was altered: farm children were children from families who reported breeding any kind of farm animals or conducting any sort of cultivation. The definitions of exposed and unexposed non-farm children remained unchanged. Using these three exposure strata, disproportionate stratified random samples were drawn per centre for phase 2 (Table 2) to decrease the number of unexposed children in the phase 2 study population due to economic constraints. The numbers to be sampled were based on the ascertained asthma prevalence and estimates of the prevalence of atopic sensitisation from previous studies in order to achieve at least 75 asthmatic and 200 atopic farm children with the same reasoning as for phase 1 sampling.

In Poland, a pilot was conducted among 379 children to evaluate response rates and acceptance of fieldwork. Of those, 267 were later randomly sampled as phase 2 participants.

Phase 2 – Main survey

Phase 2 was designed to ascertain detailed information on exposure to farming environments and to sample bio-specimens. School visits at which children were examined and biomaterial and environmental samples were obtained were performed by trained fieldworkers between April and July 2007 (November 2007 to April 2008 for Poland). In Austria, these visits were conducted at local centres of the emergency medical service. Parents received information material, a questionnaire and dust sampling equipment 2 weeks prior to the school visit and informed consent for all study modules was sought. The questionnaires and dust samples were collected at the school visit or – if forgotten – mailed to the study centre by the parents.

Questionnaire

A detailed parental questionnaire was used to assess the child's contact to farm animals and presence during farming activities throughout life. For this

purpose, 43 questions were designed based on farming activities previously investigated and on qualitative interviews with farmers to single out the most important aspects of daily farm life. The questions included activities like feeding animals, littering or removing dung, harvesting hay or ensiling grass. They were posed in a matrix format with rows assessing five different age periods (pregnancy, first year of life, second and third year of life, fourth and fifth year of life, and in the past 12 months) and columns assessing the intensity of exposure in five categories (never/almost never, about once a month, about once a week, up to 15 min once a day and daily >15 min). Furthermore, information on health and well-being, living conditions, nutritional aspects (especially farm milk) and early life was obtained.

To evaluate the parental information on the child's exposure to farming environments, the fieldworkers performed a standardised interview with the child at the school visit inquiring about habits of playing on the farm and contact with farm animals. This was not performed in Poland where the children were asked to participate in another interview on their opinions about participation in the GABRIEL Advanced Surveys.[11]

Objective measurements and bio-specimens

Body weight, body height, and waist and hip circumference were measured according to WHO guidelines (using Seca 862, 214 and 200; Seca, Hamburg, Germany).

One nasal swab per child was collected from either nostril inserting the swab about 1 cm deep and sampling the full circumference of the anterior nasal cavity. Swabs were sterile before use and adequate for polymerase chain reaction of samples (MD 559; Mast Diagnostica, Reinfeld, Germany). The swabs were stored at –80°C.

Blood was withdrawn by study physicians or study nurses and transported to the laboratory of the study centre where serum and ethylenediaminetetraacetic acid (EDTA) plasma was extracted and aliquoted on the same day. In Baden-Württemberg, an automatic whole blood count was performed with EDTA whole blood (Coulter® LH750; Beckman Coulter Inc., Fullerton, CA, USA; $n = 1920$). Genomic DNA was later purified from stored EDTA whole blood using the Puregene chemistry (QIAGEN, Hilden, Germany) on an Autopure LS instrument (QIAGEN, Hilden, Germany). In Bavaria, in addition, a PAXGene™ tube (BD, Heidelberg, Germany) was collected for RNA extraction ($n = 2111$). Extracted DNA was stored at 4°C. All other blood samples and their products were stored at least at –20°C; most samples were stored at –80°C.

Blood serum immunoglobulin E (IgE) antibody measurements were performed in one central laboratory at the Robert-Koch Institute, Berlin, Germany, using the UNICAP 1000 (Phadia AB, Uppsala, Sweden). We determined levels of total serum IgE antibodies and specific serum IgE antibodies against *Dermatophagoides pteronyssinus* (d1), cat dander (e1), timothy grass (g6), cultivated rye (g12), common silver birch (t3), mugwort (w6), a food mix (fx5: egg white, milk, fish, wheat, peanut and soybean) and a grass mix (gx3: sweet vernal grass, rye grass, timothy grass, cultivated rye and velvet grass).

In Poland, only *D. pteronyssinus*, cat dander, common silver birch and the grass mix were measured. In addition, skin prick tests with extracts of *D. pteronyssinus*, *Dermatophagoides farinae*, mixed grass pollen, betula verru, cat epithelia, egg white, cows' milk, and saline and histamine as negative and positive controls, respectively, were performed ($n = 2524$ among those randomly selected; all from ALK-Abelló, Hungerford, Berkshire, UK).

A dust sample of the child's mattress was provided by the parents using a standardised dust collection protocol previously applied.[12] The parents were sent nylon sampling socks and instructed to vacuum the whole area of the mattress equably for 2 min according to detailed photo instructions. The socks were stored preferably at –80°C but at least at –20°C upon arrival at the study centre.

Data entry was performed at the data centre in Ulm, Germany, and in Wroclaw, Poland, via a secure Citrix connection to the Ulm server using the same automatic data capture system and standards as in phase 1.

Eligibility for phase 3 and inclusion criteria

Phase 3 was only conducted in Bavaria for both logistical reasons and because a strong protective effect on asthma by farm milk consumption had been observed in the Bavarian study population (data not shown). Eligible were phase 2 participants who had given consent to all phase 2 modules. Inclusion criteria were the availability of the following samples obtained in phase 2: serum with valid IgE measurements, DNA, RNA, a nasal swab and dust.

Stratified sampling for phase 3

The phase 2 study population eligible for phase 3 was stratified into disease categories within each previously defined exposure stratum of contact to farming. We defined asthma inclusively as either reported wheeze in the past 12 months or ever inhaler use for asthma reported or a reported doctor's diagnosis of asthma at least once or wheezy bronchitis at least twice throughout the lifetime. Atopy was defined as specific IgE antibodies of at least 0.35 kU/L against *D. pteronyssinus*, cat dander or common silver birch, or a positive reaction to the grass mix. We defined the following three disease strata: (i) asthmatic children, (ii) non-asthmatic atopic children, and (iii) non-asthmatic, non-atopic children. Using these newly formed nine exposure and disease strata, disproportionate stratified random samples were drawn for phase 3 (Table 4). A total sample size of 900 children was fixed by economic constraints. Disproportionate sampling was used to increase power by creation of equally sized samples within each of the nine strata defined by the main characteristics under investigation: exposure to farming environments, asthma and atopy.

Phase 3 – In-depth survey

Phase 3 aimed at objective in-depth exposure and disease characterisation. The study modules comprised repeated collection of milk and mattress dust in late autumn 2007 and in spring 2008. In addition, settled dust was collected in the child's bedroom in late autumn 2007. Among the farm children, scooped and settled dust from stables and barns were also collected in late autumn 2007. All dust collection was performed using standardised collection protocols.[12–14] For these purposes, the children's homes were visited by trained fieldworkers. Environmental samples are being used for microbial analysis, by measuring microbial markers (endotoxin, muramic acid and glucan levels) as well as microorganisms with molecular techniques (denaturing gradient gel electrophoresis, quantitative polymerase chain reaction).

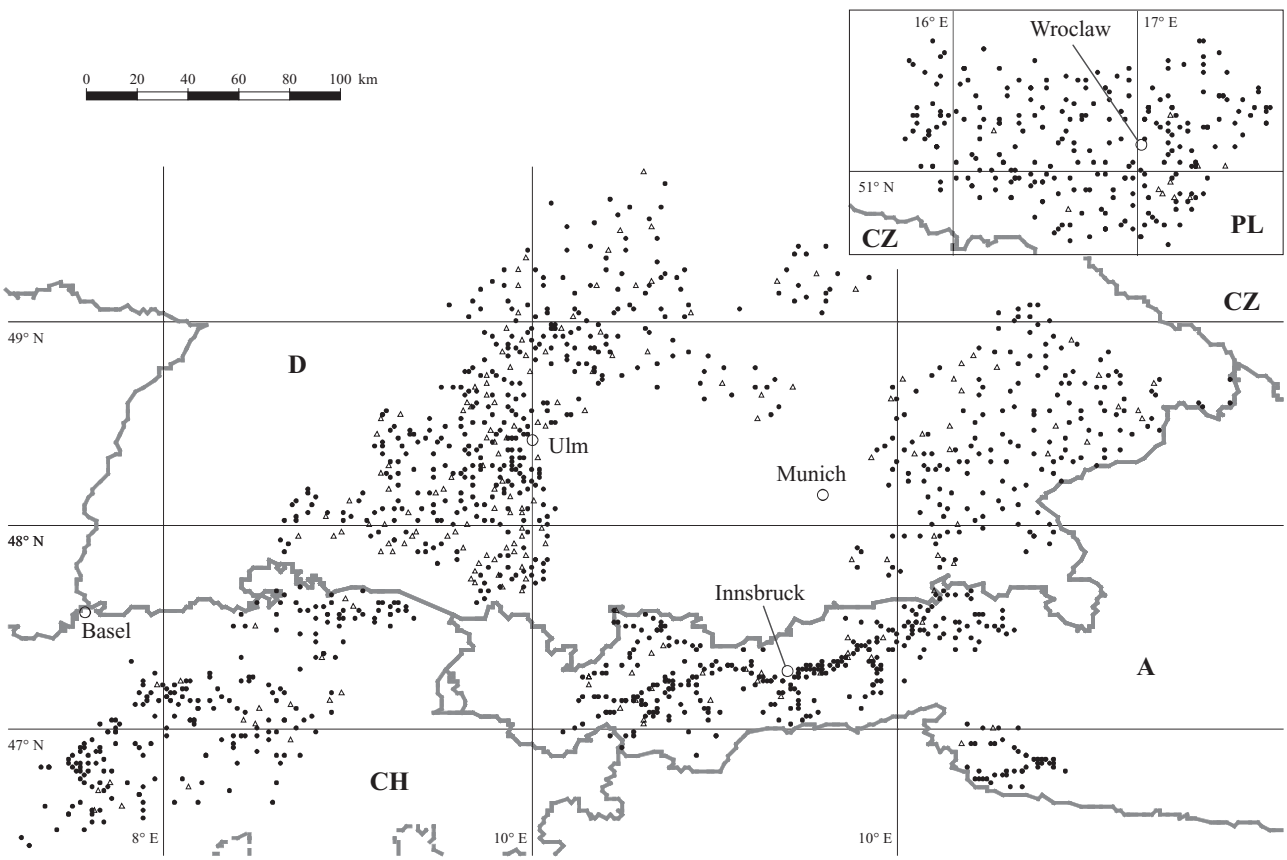

Figure 1. Geographical distribution of participating (dots) and non-participation (triangles) school and the study centres (circles). A, Austria; CH, Switzerland; CZ, Czech Republic; D, Germany; PL, Poland.

Furthermore, between April and July 2008 the children were visited at home by trained physicians who performed spirometry including a bronchodilator test and sampled exhaled air for measurement of exhaled nitric oxide [all according to current ERS/ATS (European Respiratory Society/American Thoracic Society) guidelines][15,16] and collected a throat swab. Detailed procedures will be described in subsequent publications.

Results

The geographical coverage of the GABRIEL Advanced Surveys is depicted in Figure 1. In Switzerland, within the 208 communities there were 399 schools of which only four schools did not consent to the study. Some of these schools comprised very small and individual school units, for example, a teacher visiting remote mountain settlements on a regular basis. Due to this structure and the high response rates of schools at the community level, we display Swiss data based on communities rather than schools.

Participation and sociodemographic characteristics of the study population are displayed in Table 1. The main reason for non-participation of schools was too great a workload of the headmasters and other currently running studies or projects. In total, 103 219 (57.7%) children participated in phase 1. In the German-speaking centres alone, there were 79 888 (60.4%) children. The response rates ranged from 50.2% to 64.9% across the study centres.

Table 2 displays numbers of excluded and eligible subjects and rates of preliminary consent to phase 2. A total of 44 166 (52.0% of those included) children were eligible and had preliminary consent for phase 2: 34 491 (52.7%) children in the German-speaking countries, and 9675 (49.4%) children in Poland. In Switzerland, the consent rate was lower compared with Austria and Germany, which was explained by the exclusion of migrants. Contrary to findings in Austria and Germany, migrants in Switzerland had higher consent rates than children from families with Swiss nationality (data not shown).

The numbers of eligible and sampled subjects and participation rates in phase 3 are shown in Table 3. Throughout the modules of this phase, there were high participation rates. In particular, valid spirometry was achieved for a large portion of the study population in this non-clinical setting.

To investigate participation bias, we compared characteristics of the populations with and without preliminary consent to phase 2 (Table 4). Statistical significance was detected for small prevalence ratios due to the large sample size. However, meaningfully higher prevalences of familial allergies, high parental education, wheeze, doctor diagnosed asthma and rhinoconjunctivitis were noted for consenting compared with non-consenting subjects. To a lesser extent, expo-

Table 1. Participation and sociodemographic characteristics of the phase 1 study population, *n* (%)

	Austria	Baden-Württemberg	Bavaria	Switzerland	Poland
Schools approached[a]	369	394	293	246	277
Schools participating[a]	358	303	247	208	276
Questionnaires distributed[b]	24 978	38 701	40 001	28 686	46 488
Phase 1 participants	15 731 (63.0)	23 040 (59.5)	22 507 (56.3)	18 610 (64.9)	23 331 (50.2)
Sociodemographic characteristics of the participants					
Female sex	7754 (49.9)	11 478 (49.9)	11 114 (49.4)	9230 (49.7)	11 830 (50.8)
Age [mean (SD)]	8.5 (1.21)	8.4 (1.22)	8.4 (1.22)	9.9 (1.89)	10.0 (1.80)
Families with one child assessed[c]	11 730 (85.7)	15 988 (82.3)	16 395 (84.6)	9673 (70.0)	17 593 (86.7)
Families with two children assessed[c]	1878 (13.7)	3260 (16.8)	2855 (14.7)	3544 (25.6)	2408 (11.9)
Families with three and more children assessed[c]	81 (0.6)	169 (0.9)	129 (0.7)	601 (4.4)	297 (1.4)
Nationality other than that of study centre	1010 (6.6)	688 (3.0)	287 (1.3)	1151 (6.3)	n.a.
≥1 parent not born in study country	3046 (19.7)	3518 (15.3)	1876 (8.4)	3401 (18.4)	n.a.

[a]Communities instead of schools for Switzerland.
[b]The number of questionnaires distributed by the class teachers which may have been lower than the total number of pupils due to sick leave and absence, etc.
[c]Number and percentage of total number of families assessed.
n.a., not assessed.

Table 2. Eligibility and selection for phase 2 and participation rates, *n* (%)

	Austria *n* = 15 731	Baden-Württemberg *n* = 23 040	Bavaria *n* = 22 507	Switzerland *n* = 18 610	German-speaking centres *n* = 79 888	Poland *n* = 23 331
Exclusion due to foreign nationality[a]	3440 (21.9)	3807 (16.5)	2158 (9.6)	3793 (20.4)	13 198 (16.5)	n.a.
Further exclusion[b]	n.a.	n.a.	n.a.	1302 (7.0)	1302 (1.6)	3759 (16.1)
Preliminary consent and thus eligible for phase 2 (% of included)	6775 (55.1)	11 169 (58.1)	11 183 (55.0)	5364 (39.7)	34 491 (52.7)	9675 (49.4)
Farm children	753 (11.1)	956 (8.6)	1797 (16.1)	1027 (19.2)	4533 (13.1)	2686 (27.8)
Exposed non-farm children	1755 (25.9)	2758 (24.7)	2708 (24.2)	1445 (26.9)	8666 (25.1)	1953 (20.2)
Unexposed non-farm children	4267 (63.0)	7455 (66.8)	6678 (59.7)	2892 (53.9)	21 292 (61.7)	5036 (52.1)
Selected for phase 2 (% of included)[c]	2098 (17.1)	2497 (13.0)	2573 (12.6)	2500 (18.5)	9668 (28.0)	3951 (20.2)
Farm children	539 (25.7)	938 (37.6)	1014 (39.4)	986 (39.4)	3477 (36.0)	1806 (45.7)
Exposed non-farm children	814 (38.8)	814 (32.6)	814 (31.6)	794 (31.8)	3236 (33.5)	852 (21.6)
Unexposed non-farm children	745 (35.5)	745 (29.8)	745 (29.0)	720 (28.8)	2955 (30.6)	1293 (32.7)
Participation in phase 2 (% of selected)						
Parental questionnaire	1672 (79.7)	2248 (90.0)	2297 (89.3)	2202 (88.1)	8419 (87.1)	2541 (64.3)
Children's interview	1702 (81.1)	2220 (88.9)	2267 (88.1)	2289 (91.6)	8478 (87.7)	n.a.
Anthropometrics	1717 (81.8)	2239 (89.7)	2304 (89.5)	2301 (92.0)	8561 (88.5)	2529 (64.0)
Nasal swab	1707 (81.4)	2219 (88.9)	2316 (90.0)	2203 (88.1)	8445 (87.4)	2350 (59.5)
Dust sample	1587 (75.6)	2052 (82.2)	2146 (83.4)	2231 (89.2)	8016 (82.9)	2329 (58.9)
Serum sample and IgE measurement	1660 (79.1)	1988 (79.6)	2153 (83.7)	2222 (88.9)	8023 (83.0)	2435 (61.6)
Available parental questionnaire and IgE measurement	1597 (76.1)	1950 (78.1)	2104 (81.8)	2031 (81.2)	7682 (79.5)	2391 (60.5)
DNA extracted	1652 (78.7)	2166 (86.7)	2134 (82.9)	1946 (77.8)	7898 (81.7)	2475 (62.6)

[a]Child without nationality of the study country or at least one parent not born in the study country (including missing values).
[b]Not in the sixth grade at phase 1 for Poland; submission of questionnaire to the data centre after 5 April 2007 for Switzerland.
[c]For Poland, numbers are given for those randomly selected and for whom a visit at the schools was attempted.
n.a., not applicable.

Table 3. Stratified random sampling and participation rates for phase 3 in Bavaria [all numbers, *n* (%)]

	Exposed		Unexposed
	Farm children	Non-farm children	Non-farm children
Eligible for phase 3 ($n = 1903$)[a]	98/159/504	113/163/335	97/175/259
Selected for phase 3 ($n = 895$)[a]	98/100/100	100/100/100	97/100/100
Participation in phase 3 (% of selected)	*n* (%)	*n* (%)	*n* (%)
Late autumn 2007			
Milk sample	278 (93.3)	270 (90.0)	273 (91.9)
Mattress dust	290 (97.3)	284 (94.7)	283 (95.3)
Settled dust bedroom	286 (96.0)	281 (93.7)	282 (94.9)
Settled dust stable	248 (83.2)	n.a.	n.a.
Settled dust barn	268 (90.0)	n.a.	n.a.
Scooped dust stable	260 (87.2)	n.a.	n.a.
Scooped dust barn	272 (91.3)	n.a.	n.a.
Spring 2008 (% of contacted)[b]			
Milk sample	269 (92.6)	269 (94.7)	269 (94.7)
Mattress dust	285 (98.3)	280 (98.6)	282 (99.3)
Throat swab	281 (96.9)	279 (98.2)	275 (96.8)
Spirometry[c]	233 (80.3)	242 (85.2)	236 (83.1)
Exhaled NO	280 (96.6)	274 (96.5)	272 (95.8)

[a]Asthmatic/non-asthmatic/non-asthmatic non-atopic.
[b]Only the $n = 858$ children who participated in any module in late autumn 2007 were re-contacted in spring 2008 (290 farm, 284 exposed non-farm and 284 unexposed, non-farm children).
[c]Valid spirometry measurements judged by one paediatric pulmonologist expert based on European Respiratory Society/American Thoracic Society guidelines.
n.a., not assessed.

sure to farming environments was associated with preliminary consent in Poland: farm children were less likely to consent whereas exposed non-farm children were more likely to consent. For the associations between being a farm child or an exposed non-farm child (both vs. unexposed non-farm children) with disease, there were no meaningful discrepancies between the whole phase 1 population and the subset consenting to phase 2 (Table 5).

Non-random selection occurred in Poland where fieldwork ended prematurely for logistical reasons caused by implementation of a video questionnaire for validation of the assessment of wheeze during an additional school visit among 1747 children who had provided phase 2 questionnaires and blood samples. Originally, 5587 children were randomly sampled for phase 2 (2574 farm children, 1218 exposed non-farm children and 1795 unexposed non-farm children). The Polish stratified random phase 2 sample was reduced to $n = 3951$ (Table 2) by exclusion of 93 of the 267 schools. Their geographical distribution is shown in Figure 2. There was no difference in prevalence of the characteristics displayed in Table 4 between the whole Polish phase 1 population and the population in the schools contacted in the Polish phase 2 (data not shown).

Discussion

The GABRIEL Advanced Surveys are multi-centre cross-sectional surveys with a complex design to investigate the protective effect of exposure to farming environments on asthma and atopic disease. Economic constraints limited objective measurements to informative subpopulations. Sampling of these populations depended on data ascertained among the whole population.

For phase 2 and phase 3, we employed disproportionate stratified random sampling which led to an efficient conduct of the surveys with regard to the main exposure and diseases under investigation by decreasing the number of unexposed and non-diseased children substantially. Statistical analyses of phase 2 and phase 3 will account for the complex survey design appropriately, mostly by using stratified weighted regression models. The survey weights will usually be designed to extend to the phase 1 population that was eligible for phase 2 as this is the sampling frame for the first stratified random sample. Taylor Series Expansion will mainly be employed for variance estimation. Primary analyses will be cross-sectional. When repeated measurements such as repeated dust

Table 4. Prevalence ratios for characteristics of consenting vs. non-consenting subjects[a]

	Austria PR [95% CI]	Baden-Württemberg PR [95% CI]	Bavaria PR [95% CI]	Switzerland PR [95% CI]	Poland PR [95% CI]
Female vs. male sex	0.99 [0.95, 1.02]	1.00 [0.96, 1.05]	0.96 [0.94, 0.98]	0.99 [0.96, 1.01]	0.97 [0.94, 1.00]
Age (completed years)					
≤7	0.95 [0.90, 1.01]	0.89 [0.79, 1.00]	0.97 [0.93, 1.01]	0.95 [0.92, 0.99]	0.98 [0.90, 1.06]
8	0.97 [0.93, 1.02]	1.01 [0.94, 1.09]	0.97 [0.94, 1.01]	0.98 [0.95, 1.02]	0.99 [0.95, 1.04]
9	1.00 [Reference]	1.00 [Reference]	1.00 [Reference]	1.00 [Reference]	1.00 [Reference]
10	0.98 [0.93, 1.02]	1.02 [0.95, 1.10]	1.01 [0.98, 1.05]	1.03 [1.00, 1.07]	0.99 [0.94, 1.03]
>10	1.04 [0.98, 1.10]	1.01 [0.95, 1.08]	1.03 [0.98, 1.07]	0.99 [0.94, 1.03]	0.93 [0.89, 0.96]
>1 vs. ≤1 sibling	1.08 [1.05, 1.12]	1.06 [1.02, 1.11]	1.04 [1.01, 1.07]	1.05 [1.03, 1.08]	0.95 [0.93, 0.98]
Current parental smoking (yes vs. no)	1.07 [1.03, 1.10]	0.95 [0.90, 1.00]	0.98 [0.95, 1.00]	0.99 [0.96, 1.01]	0.92 [0.89, 0.95]
Familial allergies (yes vs. no)[b]	1.20 [1.16, 1.24]	1.36 [1.30, 1.42]	1.24 [1.21, 1.27]	1.16 [1.13, 1.18]	1.22 [1.18, 1.26]
High vs. low parental education[c]	1.02 [0.99, 1.06]	1.44 [1.36, 1.52]	1.20 [1.17, 1.23]	1.12 [1.09, 1.15]	1.23 [1.19, 1.26]
Wheeze in the past 12 months (yes vs. no)	1.32 [1.26, 1.37]	1.44 [1.35, 1.52]	1.21 [1.17, 1.25]	1.21 [1.18, 1.25]	1.24 [1.21, 1.29]
Doctor diagnosed asthma (yes vs. no)[d]	1.28 [1.22, 1.34]	1.28 [1.21, 1.36]	1.19 [1.15, 1.24]	1.18 [1.14, 1.22]	1.22 [1.18, 1.26]
Rhinoconjunctivitis in the past 12 months (yes vs. no)	1.30 [1.25, 1.35]	1.38 [1.32, 1.45]	1.26 [1.22, 1.29]	1.17 [1.14, 1.20]	1.24 [1.21, 1.28]
Farm vs. unexposed non-farm children	1.01 [0.96, 1.07]	1.02 [0.97, 1.08]	0.99 [0.95, 1.02]	1.02 [0.98, 1.07]	0.94 [0.91, 0.98]
Exposed vs. unexposed non-farm children	1.09 [1.05, 1.13]	1.11 [1.06, 1.16]	1.05 [1.02, 1.08]	1.08 [1.05, 1.11]	1.10 [1.06, 1.14]

[a]Subjects are from the phase 1 study population eligible for phase 2.
[b]Either asthma or hay fever or atopic eczema for at least one parent or sibling.
[c]At least one parent qualified for university entrance.
[d]A reported doctor diagnosis of asthma at least once or wheezy bronchitis at least twice ever in life.
PR, prevalence ratio; 95% CI, 95% confidence interval.

samples in phase 3 are analysed in a longitudinal manner, the correlation between the items will be accounted for by appropriate techniques, including generalised estimating equations.

Roughly half of the phase 1 participants were not eligible for phase 2 due to lack of consent. The consenting population had, in general, a higher prevalence of familial allergies, high parental education, doctor diagnosed asthma, wheeze and rhinoconjunctivitis than the non-consenting group as well as to the whole population. To a lesser extent, the consenting population consisted of more exposed non-farm children and – for Poland – of less farm children. Familial allergies and high parental education showed the strongest associations with consent to phase 2 and will generally be evaluated as confounders in subsequent analyses. This self-selection of participants may lead to biased prevalence estimates of characteristics ascertained in phase 2 and phase 3. However, this bias can partially be accounted for by adjustment of the survey weights.

The main focus of phase 2 and phase 3 is to explore associations between exposure to farming environments and disease. Within the GABRIEL Advanced Surveys, we found evidence for the decreased prevalence of wheeze, asthma and rhinoconjunctivitis among farm children compared with their unexposed rural peers. These associations may only be biased by self-selection into the study if it depends on both exposure and disease. We did not detect meaningful discrepancies between the whole phase 1 population and the subset consenting to phase 2 for these associations. This suggests that the consenting study population is representative of the whole population with regard to the associations that will be investigated in the context of the main study question. Furthermore, response rates for phase 2 and phase 3 were generally high, which – together with the random sampling – secured representativeness.

There is evidence that self-selection out of farming may influence associations between exposure to

Table 5. Association of exposure to farming environments with wheeze, doctor diagnosed asthma[a] and rhinoconjunctivitis among all[b] and among consenting[c] subjects

	Austria PR [95% CI]	Baden-Württemberg PR [95% CI]	Bavaria PR [95% CI]	Switzerland PR [95% CI]	Poland PR [95% CI]
Farm vs. unexposed non-farm children					
Whole phase 1 population					
Wheeze in the past 12 months (yes vs. no)	0.52 [0.42, 0.65]	0.56 [0.47, 0.67]	0.59 [0.52, 0.67]	0.64 [0.54, 0.77]	1.00 [0.93, 1.07]
Doctor diagnosed asthma (yes vs. no)	0.47 [0.36, 0.62]	0.62 [0.53, 0.72]	0.57 [0.50, 0.66]	0.72 [0.61, 0.85]	0.91 [0.85, 0.98]
Rhinoconjunctivitis (yes vs. no)	0.40 [0.31, 0.51]	0.37 [0.31, 0.44]	0.32 [0.27, 0.39]	0.30 [0.23, 0.38]	0.94 [0.88, 1.01]
Population with consent to phase 2					
Wheeze in the past 12 months (yes vs. no)	0.43 [0.33, 0.57]	0.59 [0.47, 0.75]	0.54 [0.46, 0.64]	0.69 [0.56, 0.84]	0.99 [0.90, 1.08]
Doctor diagnosed asthma (yes vs. no)	0.42 [0.30, 0.60]	0.69 [0.56, 0.86]	0.58 [0.49, 0.69]	0.72 [0.59, 0.88]	0.89 [0.81, 0.98]
Rhinoconjunctivitis (yes vs. no)	0.42 [0.31, 0.56]	0.37 [0.29, 0.48]	0.34 [0.28, 0.43]	0.31 [0.23, 0.42]	0.94 [0.86, 1.02]
Exposed vs. unexposed non-farm children					
Whole phase 1 population					
Wheeze in the past 12 months (yes vs. no)	0.81 [0.71, 0.92]	0.91 [0.80, 1.04]	0.81 [0.73, 0.89]	0.83 [0.75, 0.91]	1.17 [1.08, 1.26]
Doctor diagnosed asthma (yes vs. no)	0.82 [0.70, 0.96]	0.89 [0.79, 1.01]	0.83 [0.75, 0.92]	0.83 [0.75, 0.92]	1.09 [1.02, 1.18]
Rhinoconjunctivitis (yes vs. no)	0.70 [0.62, 0.80]	0.77 [0.69, 0.87]	0.73 [0.65, 0.81]	0.71 [0.64, 0.80]	1.06 [0.99, 1.14]
Population with consent to phase 2					
Wheeze in the past 12 months (yes vs. no)	0.71 [0.61, 0.83]	0.97 [0.82, 1.15]	0.77 [0.69, 0.87]	0.80 [0.71, 0.90]	1.14 [1.04, 1.25]
Doctor diagnosed asthma (yes vs. no)	0.78 [0.65, 0.94]	0.97 [0.82, 1.14]	0.85 [0.75, 0.96]	0.83 [0.73, 0.93]	1.09 [0.99, 1.20]
Rhinoconjunctivitis (yes vs. no)	0.70 [0.60, 0.82]	0.77 [0.66, 0.90]	0.73 [0.64, 0.83]	0.66 [0.58, 0.76]	1.08 [0.98, 1.18]

[a]A reported doctor diagnosis of asthma at least once or wheezy bronchitis at least twice ever in life.
[b]After applying exclusion criteria.
[c]Subjects from the phase 1 study population consenting to and thus eligible for phase 2.
PR, prevalence ratio; 95% CI, 95% confidence interval.

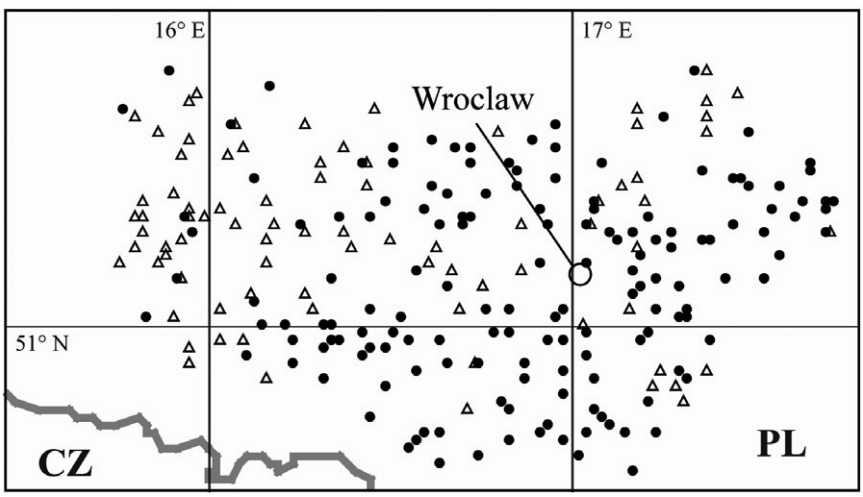

Figure 2. Polish schools that participated in phase 1 and were visited (dots) and not visited (triangles) in phase 2. CZ, Czech Republic; PL, Poland.

farming and atopic sensitisation among adults.[17] We assessed whether and when families gave up farming in all German-speaking centres. Out of those families whose child no longer lived in a farmhouse, on average 4.8% gave up farming in the year of birth of the child or thereafter. This proportion ranged from 2.7% in Switzerland to 8.8% in Bavaria. Families, whose main income is farming, are unlikely to stop farming due to asthma or atopic disease of a child. Furthermore, these figures did not differ largely for asthmatics or children with hay fever compared with the non-diseased group. Thus, avoidance of exposure to farming due to asthma or allergic disease and subsequent distortion of the association does not play a major role in our study population.

The GABRIEL Advanced Surveys are one of the largest studies aimed at shedding light on the protective farm effect on asthma and atopic disease. They have already contributed to a large-scale genome-wide association study on asthma and provided insight into interactions between genes and farming environments in relation to asthma and atopic disease.[18,19] They have also contributed to investigations that showed transport of microorganisms from animal sheds and barns into farm dwellings and identified microbial diversity as a potential explanation of the farm effect on asthma.[20,21] The extensive questionnaire data ascertained and sampled biomaterial from a representative study population promise further new insights on the protective 'farm effect' on asthma and atopic disease by the GABRIELA Advanced Surveys.

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Appendix

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References

1 Braun-Fahrländer C, Gassner M, Grize L, Neu U, Sennhauser FH, Varonier HS, *et al*. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. *Clinical and Experimental Allergy* 1999; **29**:28–34.
2 Ehrenstein OS, von Mutius E, Illi S, Baumann L, Böhm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clinical and Experimental Allergy* 2000; **30**:187–193.
3 Riedler J, Braun-Fahrländer C, Eder W, Schreuer M, Waser M, Maisch S, *et al*. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001; **358**:1129–1133.
4 Tse K, Horner AA. Defining a role for ambient TLR ligand exposures in the genesis and prevention of allergic diseases. *Seminars in Immunopathology* 2008; **30**:53–62.
5 Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nature Reviews. Immunology* 2010; **10**:861–868.
6 Sozanska B, MacNeill SJ, Kajderowicz-Kowalik M, Danielewicz H, Wheatley M, Newman-Taylor AJ, *et al*. Atopy and asthma in rural Poland: a paradigm for the emergence of childhood respiratory allergies in Europe. *Allergy* 2007; **62**:394–400.
7 Ege MJ, Frei R, Bieli C, Schram-Bijkerk D, Waser M, Benz MR, *et al*. Not all farming environments protect against the development of asthma and wheeze in children. *Journal of Allergy and Clinical Immunology* 2007; **119**:1140–1147.
8 Alfvén T, Braun-Fahrländer C, Brunekreef B, Mutius E, Riedler J, Scheynius A, *et al*. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle – the PARSIFAL study. *Allergy* 2006; **61**:414–421.
9 Mutius E, Schmid S, the PASTURE Study Group. The PASTURE project: EU support for the improvement of knowledge about risk factors and preventive factors for atopy in Europe. *Allergy* 2006; **4**:407–413.
10 Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, *et al*. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *European Respiratory Journal* 1995; **8**:483–491.
11 van der Pal S, Sozanska B, Madden D, Kosmeda A, Debinska A, Danielewicz H, *et al*. Opinions of children about participation in medical genetic research. *Public Health Genomics* 2011; **14**:271–278.
12 Schram-Bijkerk D, Doekes G, Douwes J, Boeve M, Riedler J, Üblagger E, *et al*. Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study. *Clinical and Experimental Allergy* 2005; **35**:1272–1278.
13 Noss I, Wouters IM, Visser M, Heederik DJJ, Thorne PS, Brunekreef B, *et al*. Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. *Applied and Environmental Microbiology* 2008; **74**:5621–5627.
14 Noss I, Doekes G, Sander I, Heederik DJJ, Thorne PS, Wouters IM. Passive airborne dust sampling with the electrostatic dustfall collector: optimization of storage and extraction procedures for endotoxin and glucan measurement. *Annals of Occupational Hygiene* 2010; **54**:651–658.
15 American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *American Journal of Respiratory and Critical Care Medicine* 2005; **171**:912–930.
16 Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, *et al*. American Thoracic Society/European Respiratory Society Working Group on Infant and Young Children Pulmonary Function Testing. An Official American Thoracic Society/European Respiratory Society Statement: pulmonary Function Testing in Preschool Children. *American Journal of Respiratory and Critical Care Medicine* 2007; **175**:1304–1345.
17 Radon K, Schulze A, Nowak D. Inverse association between farm animal contact and respiratory allergies in adulthood: protection, underreporting or selection? *Allergy* 2006; **61**:443–446.
18 Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, *et al*. GABRIEL Consortium. A large-scale, consortium-based genomewide association study of asthma. *The New England Journal of Medicine* 2010; **363**:1211–1221.
19 Ege MJ, Strachan D, Cookson WOCM, Moffatt MF, Gut I, Lathrop M, *et al*. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. *Journal of Allergy and Clinical Immunology* 2011; **127**:138–144.
20 Normand AC, Sudre B, Vacheyrou M, Depner M, Wouters IM, Noss I, *et al*. Airborne cultivable microflora and microbial transfer in farm buildings and rural dwellings. *Occupational and Environmental Medicine* 2011. Published online 24 March 2011. DOI: 10.1136/oem.2010.061879.
21 Ege MJ, Mayer M, Normand A-C, Genuneit J, Cookson WOC, Braun-Fahrländer C, *et al*. Exposure to environmental microorganisms and childhood asthma. *The New England Journal of Medicine* 2011; **364**:701–709.

