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## The early development of wheeze. environmental determinants and genetic susceptibility at 17q21

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**Abstract:** RATIONALE: Growing up on a farm protects from childhood asthma and early wheeze. Virus-triggered wheeze in infancy predicts asthma in individuals with a genetic asthma risk associated with chromosome 17q21. OBJECTIVES: To test environmental determinants of infections and wheeze in the first year of life, potential modifications of these associations by 17q21, and the implications for different trajectories of wheeze. METHODS: We followed 983 children in rural areas of Europe from birth until age 6 years. Symptoms of wheeze, rhinitis, fever, and environmental exposures were documented with weekly diaries during year 1. Asthma at age 6 was defined as ever having a reported doctor's diagnosis. Single-nucleotide polymorphisms related to ORMDL3 (rs8076131) and GSDMB (rs7216389, rs2290400) at 17q21 were genotyped. MEASUREMENTS AND MAIN RESULTS: Early wheeze was positively associated with presence of older siblings among carriers of known asthma risk alleles at 17q21 (e.g., rs8076131) (adjusted odds ratio [aOR], 1.53; 95% confidence interval [CI], 1.16-2.01). Exposure to farm animal sheds was inversely related to wheeze (aOR, 0.44; 95% CI, 0.33-0.60). Both effects were similarly observed in children with transient wheeze up to age 3 years without subsequent development of asthma (aOR, 1.71 [95% CI, 1.09-2.67]; and aOR, 0.48 [95% CI, 0.30-0.76], respectively). CONCLUSIONS: These findings suggest that the chromosome 17q21 locus relates to episodes of acute airway obstruction common to both transient wheeze and asthma. The previously identified asthma risk alleles are the ones susceptible to environmental influences. Thus, this gene-environment interaction reveals two faces of 17q21: The same genotype constitutes genetic risk and allows for environmental protection, thereby providing options for prospective prevention strategies.

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# The Early Development of Wheeze

## Environmental Determinants and Genetic Susceptibility at 17q21

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### Abstract

**Rationale:** Growing up on a farm protects from childhood asthma and early wheeze. Virus-triggered wheeze in infancy predicts asthma in individuals with a genetic asthma risk associated with chromosome 17q21.

**Objectives:** To test environmental determinants of infections and wheeze in the first year of life, potential modifications of these associations by 17q21, and the implications for different trajectories of wheeze.

**Methods:** We followed 983 children in rural areas of Europe from birth until age 6 years. Symptoms of wheeze, rhinitis, fever, and environmental exposures were documented with weekly diaries during year 1. Asthma at age 6 was defined as ever having a reported doctor's diagnosis. Single-nucleotide polymorphisms related to *ORMDL3* (rs8076131) and *GSDMB* (rs7216389, rs2290400) at 17q21 were genotyped.

**Measurements and Main Results:** Early wheeze was positively associated with presence of older siblings among carriers of known asthma risk alleles at 17q21 (e.g., rs8076131) (adjusted odds ratio [aOR], 1.53; 95% confidence interval [CI], 1.16–2.01). Exposure to farm animal sheds was inversely related to wheeze (aOR, 0.44; 95% CI, 0.33–0.60). Both effects were similarly observed in children with transient wheeze up to age 3 years without subsequent development of asthma (aOR, 1.71 [95% CI, 1.09–2.67]; and aOR, 0.48 [95% CI, 0.30–0.76], respectively).

**Conclusions:** These findings suggest that the chromosome 17q21 locus relates to episodes of acute airway obstruction common to both transient wheeze and asthma. The previously identified asthma risk alleles are the ones susceptible to environmental influences. Thus, this gene–environment interaction reveals two faces of 17q21: The same genotype constitutes genetic risk and allows for environmental protection, thereby providing options for prospective prevention strategies.

**Keywords:** childhood asthma; epidemiology; farming; infant wheeze; 17q21 locus

Among the environmental determinants of asthma and allergies, protective effects of growing up on a farm have been found with exceptionally high consistency (1). It has often

been suggested that this exposure operates already very early in life; however, this hypothesis was based only on retrospective analyses of cross-sectional studies (1). The

PASTURE (Protection against Allergy Study in Rural Environments) birth cohort provides a unique opportunity to study this hypothesis in a longitudinal setting (2).

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\*A complete list of members may be found before the beginning of the REFERENCES.

This article has an online supplement, which is accessible from this issue's table of contents at [www.atsjournals.org](http://www.atsjournals.org)

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## At a Glance Commentary

### Scientific Knowledge on the

**Subject:** Exposure to farming protects from asthma and transient wheeze. Polymorphisms at the 17q21 locus are associated with childhood-onset asthma, particularly in children experiencing virus-triggered wheeze episodes in early infancy.

### What This Study Adds to the

**Field:** Exposure to animal sheds reduces the risk of wheeze episodes in the first year of life independently from asthma at school age. This effect was restricted exactly to a genotype at 17q21 that has previously been shown to increase the risk of asthma. Individuals with this susceptible genotype might profit from protective environmental exposures in the face of their genetic risk of wheeze and asthma.

Environmental exposures and respiratory symptoms such as wheeze, rhinitis, and fever have been registered meticulously with weekly diaries throughout the first year of life. In this population, we have previously found protective effects of consumption of unprocessed cow's milk on infections of the upper but not the lower respiratory tract in the first year of life (3). In the present study, we focused on the other major farm-related exposure: regular stays in animal sheds (4). This implies exposure to inhalable dust particles of plant, animal, and microbial origin, which might affect particularly the lower airways.

Farming exposure has also been found to protect from early transient wheeze (5, 6). This wheeze phenotype is triggered by

viral infections and mostly disappears by age 3–4 years with the maturation of adaptive immunity. Viral infections have been implicated in promoting childhood asthma (7, 8), possibly by repetitively damaging the airway epithelium (9). However, early episodes of viral wheeze were shown to predispose to asthma only in individuals with a genetic asthma risk associated with the chromosome 17q21 locus (10). This locus harboring the coexpressed genes *ORMDL3* and *GSDMB* is well recognized as a major genetic risk locus for childhood-onset asthma, but its physiologic function remains elusive (11). Some clues, however, may be gained by studying how environmental factors impact the association of the 17q21 locus with asthma. The aim of the present analyses was to test environmental determinants of infections and wheeze in the first year of life, potential modifications of these associations by 17q21, and the implications for different trajectories of wheeze.

Some of the results of these studies have been reported previously in the form of a late-breaking abstract (12).

## Methods

The methods of this study are described in detail in the online supplement. The PASTURE birth cohort was derived from rural areas of Austria, Finland, France, Germany, and Switzerland (2). Pregnant women were recruited during their third trimester, and their children were followed until the age of 6 years. Half of these women lived and worked on livestock farms. The study was approved by the local research ethics committees in each country, and written informed consent was obtained from all parents.

**Table 1.** Study Population Characteristics

Characteristic	n (%)
Total	983 (100.0)
Female sex	482 (49.0)
Presence of older siblings	623 (63.4)
Exposure to dogs in first year	308 (31.3)
Living on a farm	474 (48.2)
Birth by cesarean section	173 (17.6)
Parental history of atopic disease	396 (40.3)
ETS exposure in first year	82 (8.3)
Maternal age, yr	
<25	94 (9.6)
≥25 and <30	336 (34.2)
≥30 and <35	361 (36.7)
≥35	192 (19.5)
Maternal education	
Low	159 (16.2)
Medium	436 (44.4)
High	388 (39.5)
Birth weight, kg	
≥4.0	128 (13.0)
≥3.5 and <4.0	352 (35.8)
≥3.0 and <3.5	391 (39.8)
≥2.5 and <3.0	98 (10.0)
<2.5	14 (1.4)

*Definition of abbreviation:* ETS = environmental tobacco smoke.

Questionnaire information on lifestyle and parental background was obtained within the third trimester of pregnancy, when the child was 2 months of age, and every year from age 1 to 6 (6). Additional information on occurrence of infections and farm-related exposures such as animal sheds, as well as on feeding practices, was collected from weekly and 4-weekly diaries between 8 and 53 weeks of life.

In total, 983 individuals (i.e., 87% of the originally included 1,133 children) (6) were included in this analysis with 37,306 person-weeks of observation in the first year of life. In a subsample of 791 children (80.5%), single-nucleotide polymorphisms

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**Author Contributions:** G.J.L. and M.J.E. had responsibility for the statistical analysis and the interpretation of results, completed the background literature search, drafted and revised the manuscript, and collated comments from the other authors. E.v.M., J.P., J.R., C.B.-F., and R.L. obtained funds, codesigned the study, had responsibility for data collection and management of the study, and contributed to the interpretation of results and to the manuscript. J.G., A.M.K., A.H., J.-C.D., and P.I.P. had responsibility for data collection and managing the study and contributed to the manuscript. M.D., A.J.H., and C.R. were involved in statistical analyses and interpretation of results and contributed to the manuscript. M.K. was responsible for genetic analyses, data collection, and managing the study. The PASTURE Study Group had responsibility for study design, data collection, and management of the study in Austria, Finland, France, Germany, and Switzerland. All authors approved the final version of the submitted manuscript. The corresponding author had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

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**Table 2.** Mutually Adjusted Environmental Determinants of Disease during First Year of Life

Environmental Exposures	Wheeze	Rhinitis	Fever
Presence of older siblings	<b>1.42 (1.14–1.77), P = 0.002</b>	<b>1.52 (1.38–1.67), P &lt; 0.001</b>	<b>1.38 (1.20–1.58), P &lt; 0.001</b>
Exposure to animal sheds	<b>0.58 (0.47–0.73), P &lt; 0.001</b>	<b>0.66 (0.60–0.73), P &lt; 0.001</b>	<b>0.56 (0.47–0.65), P &lt; 0.001</b>
Exposure to dogs	<b>0.76 (0.60–0.95), P = 0.018</b>	<b>0.89 (0.81–0.98), P = 0.020</b>	0.94 (0.82–1.09), P = 0.425
Exposure to cats	0.96 (0.78–1.18), P = 0.720	<b>0.86 (0.78–0.94), P = 0.001</b>	1.08 (0.95–1.22), P = 0.264

Odds ratios and 95% confidence intervals are based on generalized estimating equations using data between 8 and 53 weeks of life. Models include animal shed exposure; presence of older siblings; exposure to dogs; exposure to cats; country; sex; parental history of asthma, hay fever, or atopic dermatitis; maternal education; birth mode; birth weight; environmental tobacco smoke; duration of breastfeeding; introduction of complementary foods; type of cow's milk consumed; seasons of sampling; and age (squared) plus interaction terms of age with country. Boldface indicates *P* values < 0.05.

(SNPs) at the 17q21 asthma susceptibility locus, specifically SNP rs8076131 in the *ORMDL3* gene and SNPs rs2290400 and rs7216389 in the *GSDMB* gene, were genotyped in cord blood as described before (6). At the 6-year follow-up, data on asthma among 863 children (87.8%) were obtained.

Early wheeze, rhinitis (a runny nose or common cold), and fever (measured temperature,  $\geq 38.5^{\circ}\text{C}$ ) were defined as any occurrence during the previous 7 days as registered in the weekly diaries. Asthma at age 6 years was defined as a parent-reported physician's diagnosis of asthma at least once per lifetime or spastic, obstructive, or asthmatic bronchitis at least twice. Transient wheeze was defined as any wheeze in the first 3 years of life but not beyond.

### Statistical Analyses

The analyses were restricted to participants with complete information in more than

20 weekly diaries corresponding to half of the average number of completed weekly diaries during the first year. The median number of completed weekly diaries was 42 weeks (interquartile range, 40–44 wk). We did not find synchronized patterns between comparable variables in weekly or 4-weekly diaries, indicating good compliance with weekly documentation.

Repeated measurements of exposures and outcomes from weekly diaries were aggregated, and the resulting summary statistics of presence or absence during the first year of life as well as the genetic data were related to occurrence of asthma at age 6 years using logistic regression modeling adjusted for siblings; farming; country; sex; parental history of asthma, hay fever, or atopic dermatitis; maternal education; and environmental tobacco smoke (ETS).

Generalized estimating equations (GEEs) with an autoregressive correlation structure were used to compute associations

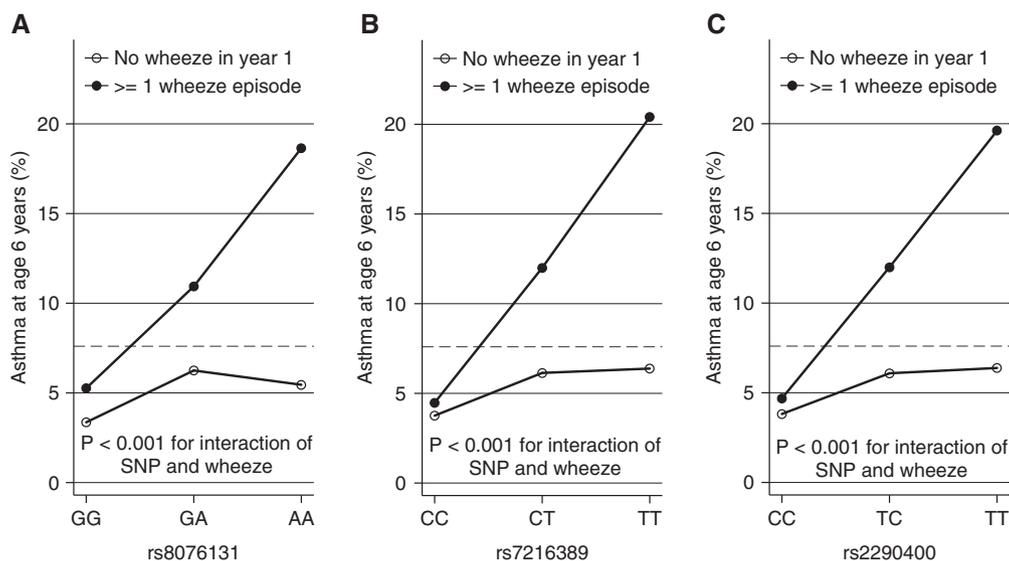
of weekly repeated measurements of animal shed exposure with wheeze, rhinitis, and fever during the first year of life. Exposure to animal sheds was defined as the child's having been taken during the previous 7 days to a cow, pig, or horse stable; a poultry shed; or a barn. For each of these items, the time in hours per week was also reported. Covariables were selected *a priori* and included siblings; country; sex; parental history of asthma, hay fever, or atopic dermatitis; maternal education; birth mode; birth weight; exposure to dogs and cats, ETS; duration of breastfeeding; introduction of complementary foods during the first year of life (13); type of cow's milk consumed (3); and seasons of sampling. Age was entered as a continuous variable in weeks plus a multiplicative interaction term with country to account for time-dependent variations in wheeze prevalence across countries. Day care attendance and consumption of infant

**Table 3.** Association of Wheeze in First Year with 17q21, Stratified for Asthma at Age 6 Years

SNP	All Children	Stratum Asthma, Age 6 yr	Stratum No Asthma, Age 6 yr	<i>P</i> Value for Interaction
rs8076131				
GG	1.00	1.00	1.00	0.008
GA	1.20 (0.92–1.57)	1.06 (0.41–2.75)	1.04 (0.78–1.39)	
AA	1.31 (0.98–1.75)	2.78 (1.09–7.14)	1.03 (0.74–1.43)	
rs2290400				0.078
CC	1.00	1.00	1.00	
TC	1.06 (0.83–1.36)	1.33 (0.52–3.40)	1.01 (0.76–1.34)	
TT	1.32 (0.99–1.75)	2.84 (1.10–7.35)	1.13 (0.81–1.59)	
rs7216389				0.048
CC	1.00	1.00	1.00	
CT	1.04 (0.81–1.33)	1.33 (0.52–3.40)	0.98 (0.75–1.30)	
TT	1.24 (0.93–1.64)	2.83 (1.09–7.32)	1.03 (0.73–1.45)	

*Definition of abbreviation:* SNP = single-nucleotide polymorphism.

Odds ratios and 95% confidence intervals are based on generalized estimating equations using data between 8 and 53 weeks of life. Models adjusted for animal shed exposure; presence of older siblings; exposure to dogs; exposure to cats; country; sex; parental history of asthma, hay fever, or atopic dermatitis; maternal education; birth mode; birth weight; environmental tobacco smoke; duration of breastfeeding; introduction of complementary foods; type of cow's milk consumed; seasons of sampling; and age (squared) plus interaction terms of age with country.



**Figure 1.** Interactions of the 17q21 single-nucleotide polymorphisms (SNPs) with early wheeze for school-age asthma. (A) rs8076131. (B) rs7216389. (C) rs2290400. The dashed horizontal lines show the overall prevalence of asthma among children (7.6%).

formula during the first year of life did not confound the presented analyses.

Results from logistic and GEE models were expressed as adjusted odds ratios (aORs). Plotted probabilities of infections over the first year of life were derived from GEE models. A sensitivity analysis performed to calculate relative risk ratios by GEE using Poisson instead of logistic models led to the same conclusions. Meta-analytical techniques were used to test heterogeneity of effects between countries in the first year of life. Interactions were tested using multiplicative interaction terms in the respective models. All estimates ( $\beta$ , aOR, risk ratio) are given with 95% confidence intervals (CIs). All statistical analyses were performed using STATA 13.1 software (StataCorp, College Station, TX).

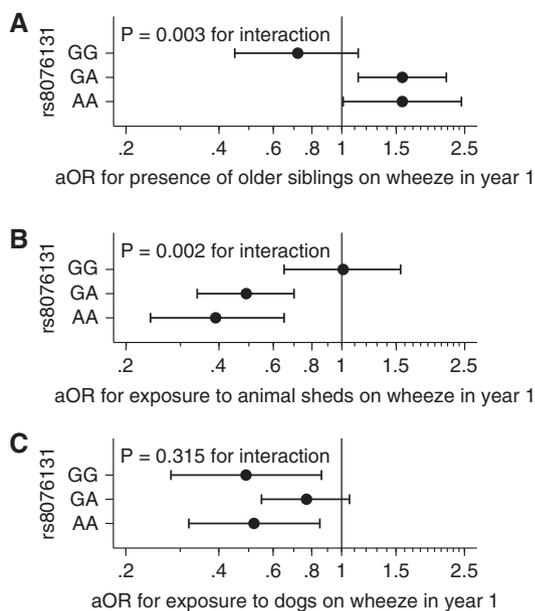
## Results

Sociodemographic factors of the study population of 983 children are given in Table 1. Children with assessment of asthma at age 6 years were characterized by higher maternal age and more cesarean sections compared with children with no information on asthma (see Table E1 in the online supplement). The subsample with genetic data was enriched for female sex and spontaneous mode of delivery (Table E2). Farming was associated with more older siblings, more pets, higher birth weight, less parental atopic disease,

and lower maternal education (data not shown).

Early wheeze occurred at least once in 35% of study participants, with a prevalence peak in winter. Two-thirds of wheeze

episodes were accompanied by reports of a common cold, and 23% were accompanied by fever. Wheeze, rhinitis, and fever in the first year of life were positively related to the presence of older siblings and



**Figure 2.** Association of environmental exposures with wheeze in year 1 in relation to the *ORMDL3* single-nucleotide polymorphism rs8076131 at 17q21. (A) Presence of older siblings. (B) Exposure to animal sheds. (C) Exposure to dogs. Adjusted odds ratios (aORs) and 95% confidence intervals are given for associations of environmental exposures with wheeze between 8 and 53 weeks of life. Generalized estimating equations adjusted for siblings, exposure to animal sheds, or exposure to dogs, respectively; exposure to cats; country; sex; parental history of asthma, hay fever, or atopic dermatitis; maternal education; birth mode; birth weight; environmental tobacco smoke; duration of breastfeeding; introduction of complementary foods; type of cow's milk consumed; seasons of sampling; and age (squared) plus interaction terms of age with country. The interaction *P* values refer to a dominant model of the AA/GA versus GG genotypes.

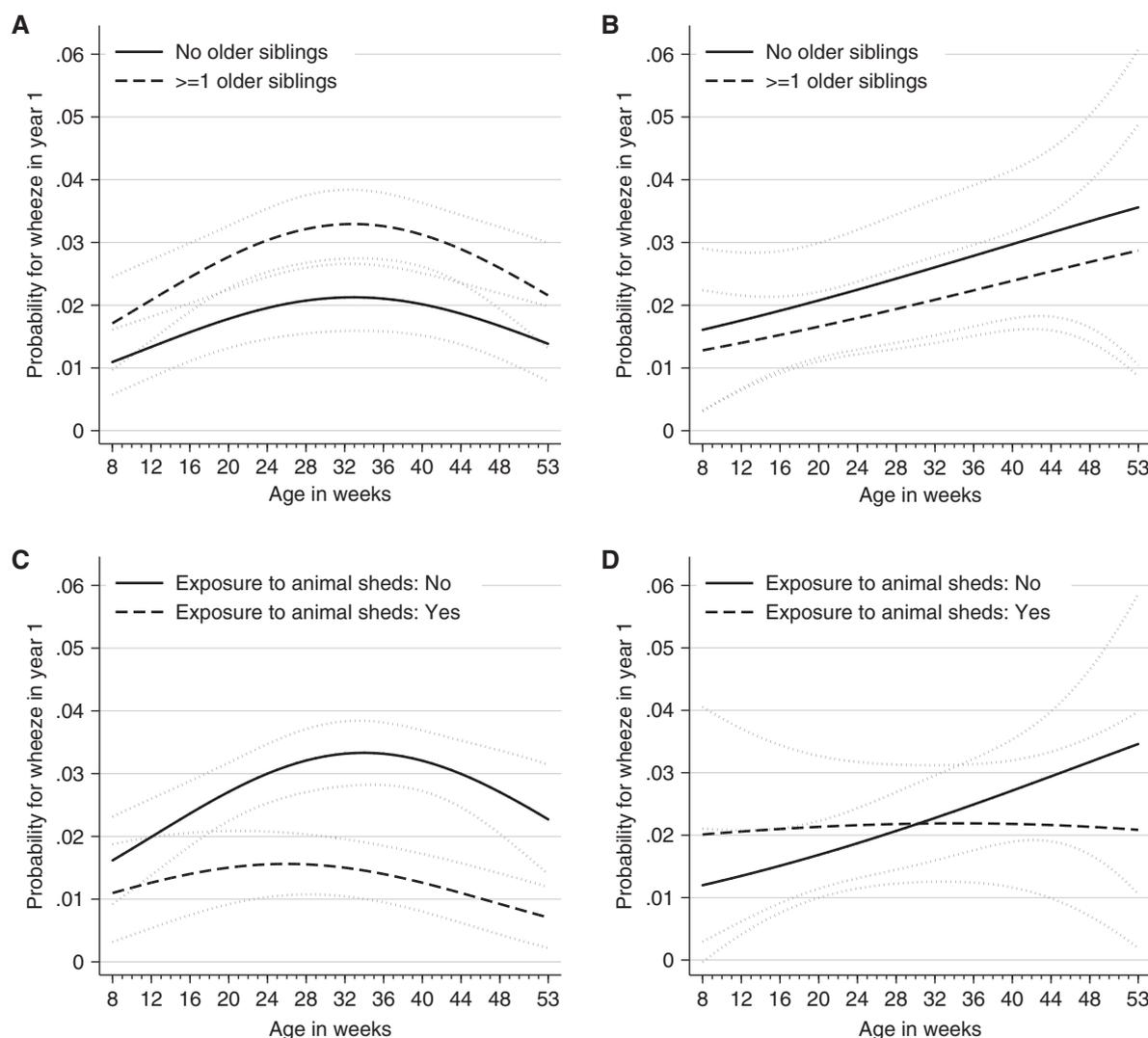
inversely associated with farm exposure, particularly exposure to animal sheds. Exposure to pets was inversely related to rhinitis and, in the case of dogs, also to wheeze (Table 2). The effects of animal shed exposure on early wheeze, rhinitis, and fever were strongest when occurring in the same week; time lags between exposure and outcome of 1 or more weeks in both directions weakened the associations. The information on animal shed exposure was available for three subcategories: cow, pig, or horse stables; poultry sheds; and barns. A sensitivity analysis mutually adjusting

these items revealed a consistent effect of cow, pig, or horse stables on wheeze, rhinitis, and fever, whereas exposure to barns did not exert an effect on wheeze (Table E3).

In contrast to rhinitis and fever, early wheeze was also associated with three SNPs on the 17q21 locus. The associations were much stronger in individuals who eventually developed asthma up to age 6 years, but they were absent in children without subsequent asthma (Table 3). When we looked at this interaction of 17q21, wheeze, and asthma from a different

angle, we found that a genetic risk for asthma at the 17q21 locus was present only in children who experienced wheeze during the first year of life (Figure 1).

We next tested whether this genotype interacted for wheeze in year 1 with its environmental determinants. We found that presence of older siblings and exposure to animal sheds were associated with wheeze only in carriers of the asthma risk genotype rs8076131-AA/GA, whereas exposure to dogs did not interact with this genotype (Figure 2). In the susceptible genotype, the risk effect of



**Figure 3.** Association of presence of siblings or animal shed exposure with wheeze over the first year by rs8076131 genotypes. (A) Presence of older siblings, rs8076131-AA/GA. (B) Presence of older siblings, rs8076131-GG. (C) Exposure to animal sheds, rs8076131-AA/GA. (D) Exposure to animal sheds, rs8076131-GG. Odds ratios and 95% confidence intervals (*dotted lines*) are given for associations of contact to animal sheds or siblings with wheeze between 8 and 53 weeks of life. Generalized estimating equations adjusted for siblings or animal sheds; exposure to dogs; exposure to cats; country; sex; parental history of asthma, hay fever, or atopic dermatitis; maternal education; birth mode; birth weight; environmental tobacco smoke; duration of breastfeeding; introduction of complementary foods; type of cow’s milk consumed; seasons of sampling; and age (squared) plus interaction terms of age with country and age with animal sheds.

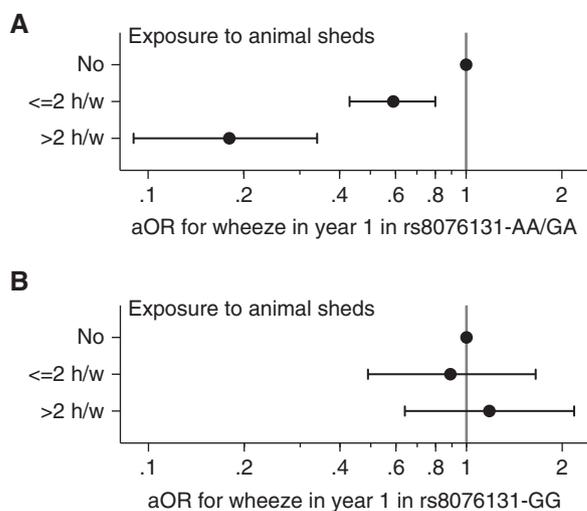
presence of older siblings and the protective effect of exposure to animal sheds were more pronounced between ages 30 and 43 weeks (i.e., 7 and 10 months, respectively); in the insusceptible genotype, however, no effect was noted, as shown by continuously overlapping confidence bands (Figure 3).

Presence of siblings made a qualitative difference, and the effect was not related to the sibship size in the susceptible genotype (data not shown). The animal shed exposure, however, showed a dose–response relationship with the time spent weekly in animal sheds in the susceptible genotype; an exposure time of 2 or more hours per week, corresponding to about 20 minutes per day on average, reduced the risk of wheeze attacks in the susceptible genotype by more than 80%, whereas in the insusceptible genotype even the most time-intensive exposure did not exert an effect (Figure 4). The associations of wheeze with presence of older siblings and exposure to animal sheds in the susceptible genotype were homogeneous across countries, as shown by low  $I^2$  values (Figure 5). The environmental determinants interacted with each other in the susceptible genotype; in children with animal shed exposure, the effect of older siblings was absent

(aOR, 0.95; 95% CI, 0.62–1.49;  $P = 0.049$  for interaction).

Finally, we tested whether the environmental effects on wheeze in the first year of life in the group with the susceptible genotype also operated in children who lost wheeze symptoms after their third birthday (i.e., in children with the transient wheeze phenotype). As shown in Table 4, the effects of both presence of older siblings and exposure to animal sheds were also present in the stratum of children who stopped wheezing by age 3 years; in the case of presence of older siblings the effect was somewhat stronger than among children who continued wheezing until age 6 years. As expected on the basis of the high linkage disequilibrium of the three 17q21 SNPs (Table E4), the interactions of the environmental exposures with the two *GSMDL* SNPs were similar to those of the *ORMDL3* SNP rs8076131 (Tables E5 and E6).

In this rural population, ETS exposure was quite rare (8.3%), only moderately associated with early wheeze (aOR, 1.42; 95% CI, 1.04–1.92), and did not interact with 17q21 ( $P = 0.731$ ). ETS was not associated with asthma and did not affect other associations tested, and numbers of smokers were too low to test interactions with genotype (data not shown).



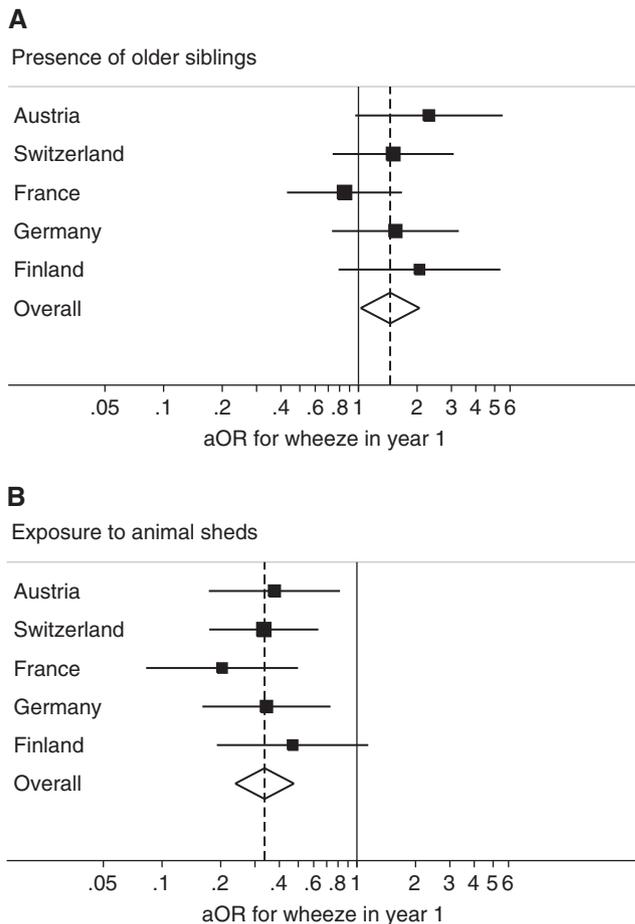
**Figure 4.** Dose–response (weekly duration of animal shed visits in hours) relationship of animal shed exposure and wheeze in year 1. (A) rs8076131-AA/GA. (B) rs8076131-GG. Adjusted odds ratios (aORs) and 95% confidence intervals are given for associations of contact to animal sheds with wheeze between 8 and 53 weeks of life. Generalized estimating equations adjusted for presence of older siblings; exposure to dogs; exposure to cats; country; sex; parental history of asthma, hay fever, or atopic dermatitis; maternal education; birth mode; birth weight; environmental tobacco smoke; duration of breastfeeding; introduction of complementary foods; type of cow’s milk consumed; seasons of sampling; and age (squared) plus interaction terms of age with country.

## Discussion

The main findings of this analysis were associations of wheeze, rhinitis, and fever in the first year with environmental determinants such as exposure to farming, exposure to pets, and presence of siblings. In addition, wheeze was determined by genetic polymorphisms at the 17q21 locus, particularly in children who developed asthma by age 6 years. Moreover, this locus interacted with presence of siblings and exposure to animal sheds in that these environmental exposures exerted their effects only in the genotype previously related to asthma risk. The latter associations were also present in children who stopped wheezing by age 3 years and thus did not develop asthma.

A major strength of this study is the health diaries, which facilitated prompt and complete recording of disease occurrence and related exposures on a weekly basis throughout the entire period of 10 months. The absence of synchronized patterns between weekly or 4-weekly diaries argues against retrospective completion of questionnaires, thereby supporting high data quality. The concomitant recording of exposures and outcomes in diaries is unlikely to constitute a source of reverse causation, for the following reasons. First, the effect of animal shed exposures on wheeze was restricted to a distinct genotype at the 17q21 locus, and children are brought to the animal shed regardless of their genotype, which is unknown to their parents. Second, the interaction with animal shed was specific; exposure to pets did not interact. Third, the association was specific for animal shed exposure; barn exposure hardly affected wheeze. Fourth, the dose dependency of the effect of animal shed exposure constitutes a strong criterion for causality.

Admittedly, viral triggers of wheeze episodes were not specified by polymerase chain reaction. However, we confirmed the interaction of wheeze with the chromosome 17q21 locus for asthma (Figure 1), which has previously been reported for wheeze episodes with polymerase chain reaction–based detection of human rhinovirus and other viruses (10). Moreover, the number of respiratory episodes rather than specific viral agents may determine the subsequent



**Figure 5.** Effect homogeneity across countries in the rs8076131-AA/GA genotype. (A) Presence of older siblings. (B) Exposure to animal sheds. Adjusted odds ratios (aORs) and 95% confidence intervals are given for associations of contact to animal sheds or siblings with wheeze between 8 and 53 weeks of life. Generalized estimating equations for each country adjusted for presence of older siblings or animal sheds; exposure to dogs; exposure to cats; sex; parental history of asthma, hay fever, or atopic dermatitis; maternal education; birth mode; birth weight; environmental tobacco smoke; duration of breastfeeding; introduction of complementary foods; type of cow's milk consumed; seasons of sampling; and age (squared). Overall aORs (95% confidence intervals),  $I^2$  values, and  $P$  values for homogeneity: (A) 1.45 (1.03–2.06), 0.6%, and 0.403; (B) 0.34 (0.24–0.48), 0.0%, and 0.769.

development of asthma (14), which relativizes the value of identifying the viral triggers of wheeze episodes.

In this study, we focused on a very early phase of wheeze development. In the first year of life, wheeze is highly prevalent (35% in our cohort). In contrast, only 7.6% of children develop asthma up to age 6 years (Figure 1), thereby rendering the transient character of early wheeze obvious for the vast majority of cases and suggesting an association with viral respiratory infections. Among the environmental determinants of early wheeze, we found the presence of siblings to increase the risk of wheeze. In the framework of the hygiene hypothesis,

the presence of siblings has been interpreted as a source of viral infections (15). The particularly strong effect of siblings in the stratum of transient wheezers (Table 4) and the interaction with the genotype in this stratum (Table E6) may support this notion. However, the presence of older siblings might also point to immunological mechanisms related to the intrauterine milieu. Previous pregnancies might drive the maternal immune system toward a more tolerant status, which might impact the early immune development of the younger children in that they are more susceptible to infections but less prone to atopy.

Alternatively, older siblings might also impact the microbial colonization of the younger child (16). This assumption, however, seems to conflict with the protective effect of pets, which might similarly affect the microbial environment and colonization of the infant (17). Exposure to microbial stimuli might foster the development of the immune system, resulting in a powerful immune response to viral infections but without clinical manifestation of symptoms.

An analogous phenomenon might be observed with farm exposure. Farm children live in larger families and more frequently have siblings; thus, viral exposure is as least as common as in non-farm children. Consequently, the farm exposure may relate to the child's ability to repress symptoms of viral respiratory infections. Playing in animal sheds, children inhale a complex mixture of microorganisms and bioactive compounds from animals as well as the animals' bedding and fodder. Small particles heavily loaded with environmental bacteria and fungi (18), pollen allergens, or other relevant compounds enter the airways and may directly impact the respiratory system by triggering receptors of the innate immunity (19, 20) or by altering the composition of the lung microbiome. This notion is supported by experimental evidence derived from animal models where protection from eosinophilic airway inflammation and airway hyperresponsiveness was achieved by exposure to bacteria and fungi (21–24) and by exposure to arabinogalactan, an oligosaccharide found in grass and hay (25).

In two independent studies, we previously found an inverse relation of farm exposure and transient wheeze (5, 6), which implies specific protection against viral respiratory infections or alleviation of the symptoms. One might argue that viral infections just unmask an underlying defect of the physical barrier or the local immunity of the asthmatic airway mucosa without causally contributing to the development of asthma (26). This would imply, however, that the associations of viral infections and early wheeze were present mainly in individuals who subsequently developed asthma. The present analysis, however, clearly showed a protective effect of animal shed exposure on wheeze in children who

**Table 4.** Homogeneity of Environmental Effects on Wheeze in First Year of Life in the rs8076131-AA/GA Genotypes, Stratified for Transient Wheeze

Stratum	Animal Shed Exposure	Presence of Siblings
All children	0.44 (0.33–0.60), $P < 0.001$	1.53 (1.16–2.01), $P = 0.002$
Stratum with wheeze only up to yr 3	0.48 (0.30–0.76), $P = 0.002$	1.71 (1.09–2.67), $P = 0.019$
Stratum with wheeze beyond yr 3	0.34 (0.21–0.53), $P < 0.001$	1.30 (0.92–1.84), $P = 0.143$

Odds ratios and 95% confidence intervals are based on generalized estimating equations using data between 8 and 53 weeks of life in the rs8076131-AA/GA genotype. Models include animal shed exposure; siblings; exposure to dogs; exposure to cats; country; sex; parental history of asthma, hay fever, or atopic dermatitis; maternal education; birth mode; birth weight; environmental tobacco smoke; duration of breastfeeding; introduction of complementary foods; type of cow's milk consumed; seasons of sampling; and age (squared) plus interaction terms of age with country.

outgrew wheezing episodes by age 3 years and did not develop asthma. This indicates that the animal shed exposure also operates in children without any asthma predisposition or atopic condition and supports a genuine effect on virus-triggered wheeze episodes.

Previously, we failed to detect any interactions of farm-related exposures with major asthma loci in a genome-wide gene–environment interaction analysis for childhood asthma in schoolchildren, despite substantial statistical power (27, 28). The recognition of gene–environment interactions with 17q21 in the present study suggests that we previously missed the appropriate time window of early infancy. Moreover, these interactions may provide a key to the physiologic function of the 17q21 locus and its role in the development of asthma (i.e., predisposing the lower airways to react with acute obstruction to common viral infections). Similarly, the specific association of 17q21 with onset of wheeze before age 4 years points to increased vulnerability in early childhood (29). During this time window, intensive maturation of the adaptive immunity occurs, in addition to substantial lung growth with a faster increase in lung volume than in airway caliber (30). The notion of hampered lung development is supported by the previously reported three-way interaction (29, 31) of genotype, viral infections, and ETS, which in itself impairs lung development. Beyond infancy and before school age, wheeze may gradually become more independent of viral triggers and may reflect a response characteristic of an airway system primed for asthma possibly promoted by atopic

sensitization (6). Additionally, the association of 17q21 with inflammatory diseases of other organ systems, such as inflammatory bowel disease and ankylosing spondylitis (32), suggests that inflammatory processes are insufficiently contained in susceptible individuals.

So far, the genetic risk constituted by the 17q21 locus has been seen as a determinant hardly modifiable by preventive or therapeutic measures. The discovery of these impressive gene–environment interactions may change this perception, however. Owing to a minor allele frequency of about 50% and a dominant effect, three-fourths of a population seems to be amenable to environmental prevention either despite or actually because of their underlying genotype.

The restriction of the animal shed effect to the susceptible 17q21 genotype implies that the genotype itself may be taken as a proxy for the environmental exposure. This situation corresponds to Mendelian randomization, a technique making use of the blinded random allocation of genotypes during meiosis and thereby mimicking the design of a randomized controlled trial in an observational setting. In this sense, the present findings about the protective potential of animal shed exposure achieve a rather high level of evidence. Nevertheless, clinical trials are necessary to confirm the effectiveness of reducing asthma risk by preventing early respiratory infections. Treatment of respiratory syncytial virus infection indeed reduced risk of wheezing (33); however, other viral infections (14) should be targeted as well. In the future, isolation of the active principle of animal shed dust may provide a means for

naturally controlling viral infections or enhancing the viral defense of children at risk of severe airway disease.

Taking all of our data together, we found environmental determinants of wheeze in infancy. The corresponding effects were restricted to a genotype encoded at the 17q21 locus that was previously related to risk of childhood-onset asthma. In the susceptible genotype, the environmental exposures of presence of siblings and exposure to animal sheds also impacted transient wheeze without subsequent development of asthma. Exposure to animal sheds may reduce genetically determined asthma risk by promoting the individual's capability of coping with early viral infections. Altogether these findings reveal the dual nature of the relationship between 17q variants and childhood asthma: The same genotype that constitutes genetic risk is amenable to environmental protection and may respond to future prevention strategies. ■

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## References

- von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nat Rev Immunol* 2010;10:861–868.
- von Mutius E, Schmid S; 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;61:407–413.
- Loss G, Depner M, Ulfman LH, van Neerven RJ, Hose AJ, Genuneit J, Karvonen AM, Hyvärinen A, Kaulek V, Roduit C, et al.; PASTURE Study Group. Consumption of unprocessed cow's milk protects infants from common respiratory infections. *J Allergy Clin Immunol* 2015;135:56–62.
- Illi S, Depner M, Genuneit J, Horak E, Loss G, Strunz-Lehner C, Büchele G, Boznanski A, Danielewicz H, Cullinan P, et al. Protection from childhood asthma and allergy in Alpine farm environments—the GABRIEL Advanced Studies. *J Allergy Clin Immunol* 2012;129:1470–1477.e6.
- Fuchs O, Genuneit J, Latzin P, Büchele G, Horak E, Loss G, Sozanska B, Weber J, Boznanski A, Heederik D, et al. Farming environments and childhood atopy, wheeze, lung function, and exhaled nitric oxide. *J Allergy Clin Immunol* 2012;130:382–388.e6.
- Depner M, Fuchs O, Genuneit J, Karvonen AM, Hyvärinen A, Kaulek V, Roduit C, Weber J, Schaub B, Lauener R, et al.; PASTURE Study Group. Clinical and epidemiologic phenotypes of childhood asthma. *Am J Respir Crit Care Med* 2014;189:129–138.
- Tsukagoshi H, Ishioka T, Noda M, Kozawa K, Kimura H. Molecular epidemiology of respiratory viruses in virus-induced asthma. *Front Microbiol* 2013;4:278.
- Gern JE. The ABCs of rhinoviruses, wheezing, and asthma. *J Virol* 2010;84:7418–7426.
- Holt PG, Sly PD. Viral infections and atopy in asthma pathogenesis: new rationales for asthma prevention and treatment. *Nat Med* 2012;18:726–735.
- Calışkan M, Bochkov YA, Kreiner-Møller E, Bønnelykke K, Stein MM, Du G, Bisgaard H, Jackson DJ, Gern JE, Lemanske RF Jr, et al. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med* 2013;368:1398–1407.
- Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. *Immunol Rev* 2011;242:10–30.
- Loss G, Depner M, Roduit C, Genuneit J, Karvonen A, Hyvärinen A, Lauener R, Pfefferle P, Kabesch M, Pekkanen J, et al.; PASTURE Study Group. Chr17q21 modifies environmental effects on respiratory infections in infancy and effects on asthma [abstract]. *Eur Respir J* 2015;46(Suppl 59); DOI: 10.1183/13993003.congress-2015.OA3481.
- Roduit C, Frei R, Depner M, Schaub B, Loss G, Genuneit J, Pfefferle P, Hyvärinen A, Karvonen AM, Riedler J, et al.; PASTURE study group. Increased food diversity in the first year of life is inversely associated with allergic diseases. *J Allergy Clin Immunol* 2014;133:1056–1064.
- Bønnelykke K, Vissing NH, Sevelsted A, Johnston SL, Bisgaard H. Association between respiratory infections in early life and later asthma is independent of virus type. *J Allergy Clin Immunol* 2015;136:81–86.e4.
- Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299:1259–1260.
- Penders J, Gerhold K, Stobberingh EE, Thijs C, Zimmermann K, Lau S, Hamelmann E. Establishment of the intestinal microbiota and its role for atopic dermatitis in early childhood. *J Allergy Clin Immunol* 2013;132:601–607.e8.
- Bergroth E, Remes S, Pekkanen J, Kaupilla T, Büchele G, Keski-Nisula L. Respiratory tract illnesses during the first year of life: effect of dog and cat contacts. *Pediatrics* 2012;130:211–220.
- Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, Heederik D, Piarroux R, von Mutius E; GABRIELA Transregio 22 Study Group. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med* 2011;364:701–709.
- Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Ublagger E, Schram-Bijkerk D, Brunekreef B, van Hage M, Scheynius A, et al.; Parsifal Study team. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *J Allergy Clin Immunol* 2006;117:817–823.
- Lauener RP, Birchler T, Adamski J, Braun-Fahrlander C, Bufe A, Herz U, von Mutius E, Nowak D, Riedler J, Waser M, et al.; ALEX study group. Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children. *Lancet* 2002;360:465–466.
- Peters M, Kauth M, Schwarze J, Körner-Rettberg C, Riedler J, Nowak D, Braun-Fahrlander C, von Mutius E, Bufe A, Holst O. Inhalation of stable dust extract prevents allergen induced airway inflammation and hyperresponsiveness. *Thorax* 2006;61:134–139.
- Debarry J, Garn H, Hanuszkiewicz A, Dickgreber N, Blümer N, von Mutius E, Bufe A, Gatermann S, Renz H, Holst O, et al. *Acinetobacter lwoffii* and *Lactococcus lactis* strains isolated from farm cowsheds possess strong allergy-protective properties. *J Allergy Clin Immunol* 2007;119:1514–1521.
- Conrad ML, Ferstl R, Teich R, Brand S, Blümer N, Yildirim AO, Patrascan CC, Hanuszkiewicz A, Akira S, Wagner H, et al. Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe *Acinetobacter lwoffii* F78. *J Exp Med* 2009;206:2869–2877.
- Vogel K, Blümer N, Korthals M, Mittelstädt J, Garn H, Ege M, von Mutius E, Gatermann S, Bufe A, Goldmann T, et al. Animal shed *Bacillus licheniformis* spores possess allergy-protective as well as inflammatory properties. *J Allergy Clin Immunol* 2008;122:307–312.e8.
- Peters M, Kauth M, Scherner O, Gehlhar K, Steffen I, Wentker P, von Mutius E, Holst O, Bufe A. Arabinogalactan isolated from cowshed dust extract protects mice from allergic airway inflammation and sensitization. *J Allergy Clin Immunol* 2010;126:648–656.e4.
- Fuchs O, von Mutius E. Prenatal and childhood infections: implications for the development and treatment of childhood asthma. *Lancet Respir Med* 2013;1:743–754.
- Ege MJ, Strachan DP, Cookson WO, Moffatt MF, Gut I, Lathrop M, Kabesch M, Genuneit J, Büchele G, Sozanska B, et al.; GABRIELA Study Group. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. *J Allergy Clin Immunol* 2011;127:138–144.e4.
- Ege MJ, Strachan DP. Comparisons of power of statistical methods for gene-environment interaction analyses. *Eur J Epidemiol* 2013;28:785–797.
- Bouzigon E, Corda E, Aschard H, Dizier MH, Boland A, Bousquet J, Chateigner N, Gormand F, Just J, Le Moual N, et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. *N Engl J Med* 2008;359:1985–1994.
- Quanjer PH, Stanojevic S, Stocks J, Hall GL, Prasad KV, Cole TJ, Rosenthal M, Perez-Padilla R, Hankinson JL, Falaschetti E, et al.; Global Lungs Initiative. Changes in the FEV<sub>1</sub>/FVC ratio during childhood and adolescence: an intercontinental study. *Eur Respir J* 2010;36:1391–1399.
- Smit LA, Bouzigon E, Pin I, Siroux V, Monier F, Aschard H, Bousquet J, Gormand F, Just J, Le Moual N, et al.; EGEA Cooperative Group. 17q21 variants modify the association between early respiratory infections and asthma. *Eur Respir J* 2010;36:57–64.
- Laukens D, Georges M, Libioule C, Sandor C, Mni M, Vander Cruyssen B, Peeters H, Elewaut D, De Vos M. Evidence for significant overlap between common risk variants for Crohn's disease and ankylosing spondylitis. *PLoS One* 2010;5:e13795.
- Simões EA, Carbonell-Estrany X, Rieger CH, Mitchell I, Fredrick L, Groothuis JR; Palivizumab Long-Term Respiratory Outcomes Study Group. The effect of respiratory syncytial virus on subsequent recurrent wheezing in atopic and nonatopic children. *J Allergy Clin Immunol* 2010;126:256–262.