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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Protective effects of breastfeeding on respiratory symptoms in infants with 17q21 asthma risk variants

To the Editor,

Genetic polymorphisms at the 17q21 locus have been associated with the subsequent onset of childhood asthma and appear to strengthen the association between childhood asthma and early episodes of wheezing.[1,2] A recent study of Loss et al[2] showed that 17q21 alleles modified the effect of exposure to older siblings and animal shed on episodes of wheeze in infancy. As environmental factors seem to play a role with respect to the effect modification by the 17q21 polymorphism, our aim was to assess whether the association between asthma-associated 17q21 variants, and lower respiratory symptoms during the 1st year of life, may be modified by breastfeeding. In addition, we investigated whether the described interactions with other environmental exposures, such as older siblings[2] and tobacco exposure,[3,4] were reproducible.

We tested our hypothesis within the prospective Basel-Bern Infant Lung Development (BILD) birth cohort of healthy unselected infants (n = 368) living in urban environments.[5] Parental written informed consent was obtained, and the study was approved by the Ethics Committees of Basel and Bern. Respiratory symptoms, such as occurrence of cough, wheeze, or difficulty breathing during the night and day—and their severity—were assessed by weekly telephone interviews using a standardized symptom score (each on a scale of 0-4, with 0 indicating no symptoms and ≥3 severe symptoms).[5] As a primary outcome, *the respiratory symptom score* was calculated as a sum total of daytime and nighttime symptom scores (on a scale of 0-8).[5] The secondary outcome was episodes of *wheeze* in the 1st year of life that were defined as a whistling sound in the chest audible to the parents, or doctor-diagnosed wheeze. Wheeze episodes have been recorded since 2004 on a weekly basis (based on a "yes or no" question); therefore, we restricted our sample to those infants with complete information on wheeze (n = 252).

Genomewide genotyping was performed using Illumina HumanOmniExpress Bead Chips (Illumina Inc., San Diego, CA, USA). Five major tagging SNPs at the locus 17q21: rs7216389, rs4795405, rs8079416, rs8065126, and rs3902025 were included in the analysis. These variants were selected as representative of the five highest asthma-associated tagging bins based on unpublished 17q21 fine mapping data (1446 children, 763 asthmatics, from the German MAGIC and ISAAC II studies), presented at the 11th Meeting of the European Human Genetics Societies. For the purposes of our study, either the major tagging SNP from the respective bin (rs3902025) or a proxy in high linkage disequilibrium was analyzed.

Generalized additive mixed model with quasi-Poisson and binomial distribution for count and binary outcomes was used to investigate weekly measured respiratory symptom scores and any breastfeeding ("yes or no" for each week under observation). We applied autoregressive AR(1) modeling to account for interchild variation. Each SNP was coded as 0/1/2 for the number of risk alleles and analyzed separately under the additive model. The interaction was tested by adding to the adjusted model the multiplicative interaction term between breastfeeding and SNP.

Next, we attempted a replication of top SNPs within the Protection against Allergy Study in Rural Environments (PASTURE) birth cohort study (n = 799) that was conducted in rural areas. Information on respiratory symptoms (defined as the presence of wheeze or cough) and any breastfeeding was collected from weekly and 4-weekly diaries. We used a stringent Bonferroni P-value correction threshold of 0.01 (0.05/5) and 0.025 (0.05/2) for discovery and replication analysis, respectively. Further information on demographic (eTable 1) and genotype characteristics, methods, and meta-analyses of both cohorts is provided in the Supporting Information.

The 17q21 SNPs were not associated with respiratory symptom score during the 1st year of life. When we stratified infants by breastfeeding status, we found that, during those weeks when infants were breastfed, the carriers of asthma risk alleles of the

[#]Members of the BILD and PASTURE study groups are listed in the Appendix.

TABLE 1 Association[a] of 17q21 genotype (additive effect for risk allele) with respiratory symptoms and wheeze by breastfeeding

SNP	Risk allele	Total RR (95% CI)/OR (95% CI)	Stratum by exposure		*P*-value interaction[b]
			Weeks with breastfeeding RR (95% CI)/OR (95% CI)	Weeks without breastfeeding RR (95% CI)/OR (95% CI)	
Discovery: BILD (*n* = 368 and 252 for respiratory symptoms and wheeze, respectively)					
Respiratory symptoms[c]		No. of weeks = 19 252	No. of weeks = 12 511	No. of weeks = 6741	
rs7216389	T	0.98 (0.90-1.08)	**0.82 (0.72-0.93)**	1.09 (0.96-1.24)	**0.0006**
rs4795405	C	1.03 (0.94-1.12)	0.85 (0.74-0.97)	1.10 (0.97-1.24)	**0.0041**
rs8079416	C	1.07 (0.98-1.16)	0.97 (0.85-1.11)	1.07 (0.94-1.21)	0.217
rs8065126	C	1.10 (1.01-1.21)	1.01 (0.88-1.15)	1.12 (0.98-1.26)	0.125
rs3902025	T	1.10 (1.00-1.10)	1.01 (0.88-1.16)	1.12 (0.98-1.27)	0.204
Wheeze[d]		No. of weeks = 13 101	No. of weeks = 8564	No. of weeks = 4537	
rs7216389	T	0.91 (0.67-1.22)	0.65 (0.39-1.09)	1.12 (0.76-1.67)	0.052
rs4795405	C	0.90 (0.67-1.22)	0.59 (0.34-1.02)	1.17 (0.79-1.73)	0.020
rs8079416	C	1.15 (0.85-1.57)	1.05 (0.62-1.76)	1.25 (0.84-1.88)	0.718
rs8065126	C	1.08 (0.77-1.51)	0.69 (0.40-1.17)	1.46 (0.93-2.28)	0.037
rs3902025	T	1.16 (0.84-1.61)	0.89 (0.50-1.57)	1.37 (090-2.08)	0.253
Replication: PASTURE (*n* = 799)					
Respiratory symptoms[d]		No. of weeks = 31 691	No. of weeks = 14 734	No. of weeks = 16 957	
rs7216389	T	1.10 (1.02-1.19)	1.11 (0.98-1.27)	1.11 (1.00-1.22)	0.689
rs8076131	A	1.06 (0.98-1.14)	0.99 (0.88-1.33)	1.11 (1.01-1.22)	0.370
Wheeze[d]					
rs7216389	T	1.10 (0.95-1.26)	1.03 (0.81-1.31)	1.15 (0.97-1.36)	0.799
rs8076131	A	1.12 (0.97-1.29)	0.95 (0.74-1.20)	**1.24 (1.04-1.46)**	0.174

[a]Adjusted for sex, week of age, the presence of older siblings, birthweight, gestational age, mode of delivery, child care, maternal education, maternal/parental atopy, maternal smoking in pregnancy, week of study, and study centers. In the replication analysis, the association was additionally adjusted for farm exposure.
[b]Interaction was tested by adding the product between breastfeeding and corresponding SNP in the adjusted model.
[c]Per-allele RR and 95% CI derived from generalized additive mixed model with quasi-Poisson distribution.
[d]Per-allele OR and 95% CI derived from generalized additive mixed model with binomial distribution.
Significant associations after Bonferroni correction are in boldface.
BILD, Basel-Bern Infant Lung Development birth cohort; PASTURE, Protection against Allergy Study in Rural Environments birth cohort; OR, odds ratio; RR, risk ratio; CI, confidence interval.

most strongly associated SNPs (rs7216389-T and rs4795405-C, Table 1) were more responsive to the protective effect of breastfeeding on respiratory symptoms. In contrast, during those weeks when infants were not breastfed, the same genotype showed a trend toward an increased risk of respiratory symptoms, resulting in a significant interaction effect for both SNPs (*P* for interaction 0.0006 and 0.0041, respectively, Table 1). Although the direction of the association in the entire wheeze subset of infants, and across strata by breastfeeding, was the same as in the main analysis, no significant interaction was observed between the 17q21 locus and breastfeeding in relation to wheeze that may be explained by limited power and conservative correction for multiple comparisons.

In the PASTURE cohort, the protective effect of breastfeeding on wheeze was present only in carriers of asthma risk alleles of rs8076131 (the closest proxy of rs4795405, $r^2 = 0.92$; r^2-value is based on a study by Toncheva et al[6]) (Figure 1). Similar effects were observed in carriers of risk alleles of rs4795405 in relation to wheeze in the BILD cohort. However, we found no evidence for an interaction. The meta-analysis of interaction effects in the BILD and PASTURE data yields a borderline significant effect for rs4795405 ($P = 0.028$, eFigure 1). Factors that may weaken the breastfeeding interaction in the PASTURE cohort were population-specific genetic and environmental factors, such as high farm exposure and an interaction of breastfeeding status with farming exposure in relation to respiratory symptoms (data not shown). We hypothesize that the influence of the 17q21 locus on respiratory symptoms may be modified by multiple environmental factors, and their relative small size impact may depend on the environmental context.

In accordance with Loss et al,[2] we were able to replicate the interaction between the 17q21 locus and the presence of older siblings. Consistent with other studies,[2,3] we did not find interaction with maternal smoking during pregnancy (eTable 2).

There are several interpretations we can consider on the interaction between 17q21 SNPs and breastfeeding in relation to

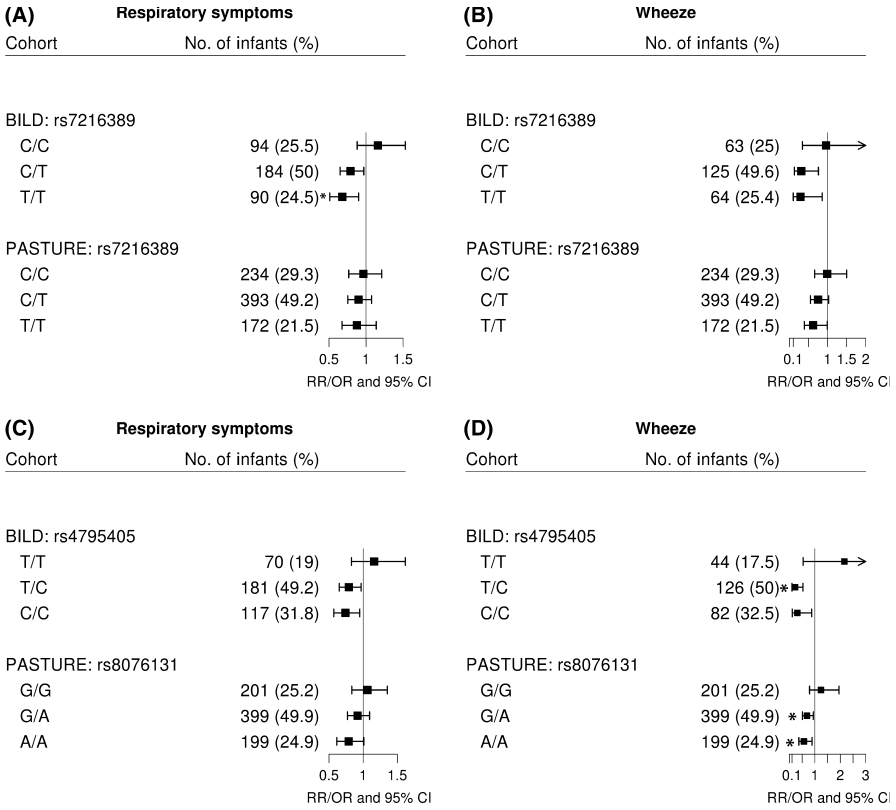

FIGURE 1 Associations of breastfeeding with respiratory symptoms and wheeze in the BILD discovery cohort and in PASTURE replication cohort according to rs7216389 and rs4795405 (the proxy is rs8076131): (A) respiratory symptoms and rs7216389; (B) wheeze and rs7216389; (C) respiratory symptoms and rs4795405 (the proxy is rs8076131); (D) wheeze and rs4795405 (the proxy is rs8076131). Associations (*Bonferroni-significance) were adjusted for sex, week of age, the presence of older siblings, birthweight, gestational age, mode of delivery, child care, maternal education, maternal/parental atopy, maternal smoking in pregnancy, week of study, and study centers. In the replication cohort, the association was additionally adjusted for farm exposure. Results were expressed as a risk ratio (RR) for the association between respiratory symptom score in the BILD cohort and as an odds ratio (OR) for other associations. All estimates are given with 95% confidence interval (95% CI)

respiratory symptoms. First, breastmilk is rich in immune components inhibiting virus replication, regulating mucosal immunity,[7] and shifting the gut microbiota toward species which strengthen the immune response.[8] Second, the 17q21 locus may increase susceptibility to viral infection.[1] Third, DNA methylation in CpG cites of rs7216389 and rs4795405 was associated with mRNA expression of orosomucoid-like 3 (*ORMDL3*) gene.[9] This would make carriers of the asthma risk genotype potentially more responsive to the protective effect of breastfeeding. Finally, epigenetic phenomena are known to be related to 17q21.[10]

In conclusion, our findings demonstrated evidence suggestive of interaction between 17q21 variants and breastfeeding in relation to respiratory symptoms in the 1st year of life. Infants with the asthma risk allele might particularly profit from the protective effect of breastfeeding on early-life respiratory infection, which is an important target for secondary asthma prevention. As multiple exposures seem to affect 17q21 in a complex manner, observed gene-environment interactions may be specific for a given environment (eg, rural versus urban context).

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CONFLICTS OF INTEREST

Dr. von Mutius reports holding grants from the European Commission, the European Research Council, and the German Research Foundation, during the conduct of the study. Dr. von Mutius has also received personal fees from the following organizations for her contribution outside the context of the submitted work: the American Academy of Allergy, Asthma & Immunology, the Ökosoziales Forum Oberösterreich, Mundipharma, HAL Allergie GmbH, from DOC Congress SRL, American Thoracic Society, University of Tampere; GBS RE HEFCE, Novartis Pharma, OM Pharma SA, AbbVie Deutschland GmbH & Co. KG, medUpdate GmbH, and System Analytic Ltd.

Dr. Latzin reports personal fees from OM Pharma SA, Roche, Vertex and Gilead, all outside of the submitted work.

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AUTHOR CONTRIBUTIONS

Drs Gorlanova and Illi had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Urs Frey, Olga Gorlanova, Sabina Illi, Michael Kabesch, Philipp Latzin, Erika von Mutius, and Antoaneta A. Toncheva. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: Olga Gorlanova, Urs Frey. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Olga Gorlanova, Sabina Illi. Obtained funding: Urs Frey. Study supervision: Urs Frey, Olga Gorlanova.

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O. Gorlanova[1]
S. Illi[2,3,4]
A. A. Toncheva[5]
J. Usemann[1,6]
P. Latzin[6]
M. Kabesch[5]
J.-C. Dalphin[7]
R. Lauener[8,9]
J. R. Pekkanen[10,11]
E. Von Mutius[2,3,4]
J. Riedler[12]
C. E. Kuehni[13]
M. Röösli[14]
U. Frey[1]
on behalf of the BILD and PASTURE study groups[#]

[1]*University Children's Hospital (UKBB), University of Basel, Basel, Switzerland*
[2]*Dr von Hauner Children's Hospital, Ludwig Maximilian University, Munich, Germany*
[3]*Comprehensive Pneumology Center Munich (CPC-M), Munich, Germany*
[4]*Member of the German Center for Lung Research, Munich, Germany*
[5]*Department of Pediatric Pneumology and Allergy, University Children's Hospital Regensburg (KUNO), Regensburg, Germany*
[6]*Division of Respiratory Medicine, Department of Paediatrics, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland*
[7]*Department of Respiratory Disease, UMR/CNRS 6249 Chrono-environment, University Hospital, University of Besançon, Besançon, France*
[8]*Children's Hospital of Eastern Switzerland, St. Gallen, Switzerland*
[9]*Christine Kühne Center for Allergy Research and Education (CK-CARE), Davos, Switzerland*
[10]*National Institute for Health and Welfare, Kuopio, Finland*
[11]*Department of Public Health, University of Helsinki, Helsinki, Finland*
[12]*Children's Hospital Schwarzach, Teaching Hospital Paracelsus Private Medical University Salzburg, Salzburg, Austria*
[13]*Institute for Social and Preventive Medicine, University of Bern, Bern, Switzerland*
[14]*Swiss Tropical and Public Health Institute Basel, Basel, Switzerland*

Correspondence: Olga Gorlanova, MD, University Children's Hospital (UKBB), Spitalstrasse 33, 4056 Basel, Switzerland (*olga.gorlanova@ukbb.ch*).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

APPENDIX

GROUP INFORMATION

The Basel-Bern Infant Lung Development (BILD) cohort was part of the collaboration responsible for this work. Its current members are

as follows (in alphabetical order): Pinelopi Anagnostopoulou, MD (Bern University Hospital, Bern); Urs Frey, PhD (University of Basel Children's Hospital, Basel); Oliver Fuchs, PhD (Bern University Hospital, Bern); Olga Gorlanova, MD (University of Basel Children's Hospital, Basel); Insa Korten, PhD (Bern University Hospital, Bern); Philipp Latzin, PhD (Bern University Hospital); Loretta Müller, PhD (University of Basel Children's Hospital, Basel); Elena Proietti, PhD (University of Zurich Children's Hospital, Zurich); Anne Schmidt, PhD ((University of Basel Children's Hospital, Basel); Jakob Usemann, PhD (University of Basel Children's Hospital, Basel). The Protection against Allergy Study in Rural Environments (PASTURE) cohort, current study group (in alphabetical order by study center):

Finland: Anne Hyvärinen, PhD (National Institute for Health and Welfare, Kuopio); Anne Karvonen, PhD, (National Institute for Health and Welfare, Kuopio); Pirkka Kirjavainen, PhD, (National Institute for Health and Welfare, Kuopio); Sami Remes, MSc, (National Institute for Health and Welfare, Kuopio); Marjut Roponen, PhD, (National Institute for Health and Welfare, Kuopio).

France: Amandine Chauveau, PhD, (University Hospital of Besançon, Besançon); Marie-Laure Dalphin, PhD, (University Hospital of Besançon, Besançon); Vincent Kaulek, PhD, (University Hospital of Besançon, Besançon).

Germany: Martin Depner, PhD, (Dr von Hauner Children's Hospital, Ludwig Maximilian University, Munich); Markus Ege, MD, (Comprehensive Pneumology Center Munich, Member of the German Center for Lung Research, Munich); Jon Genuneit, MD, (Ulm University, Ulm); Georg Loss, PhD, (Dr von Hauner Children's Hospital, Ludwig Maximilian University, Munich); Petra Pfefferle; PhD, (Institute for Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, Philipps University of Marburg, Marburg); Harald Renz, PhD, (Institute for Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, Philipps University of Marburg, Marburg); Bianca Schaub, MD, (Comprehensive Pneumology Center Munich, Member of the German Center for Lung Research, Munich).

Switzerland: Charlotte Braun-Fahrländer, MD, (Swiss Tropical and Public Health Institute, University of Basel, Basel); Remo Frei, PhD, (Christine Kühne-Center for Allergy Research and Education (CK-CARE), Davos; Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos); Caroline Roduit, MD (Children's Hospital, University of Zürich, Zürich).

The Netherlands: Gert Doekes, (Utrecht University, Institut for Risk Assessment Sciences (IRAS), Utrecht).

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Do mucosal biomarkers reveal the immunological state associated with food allergy?

To the Editor,

As food allergy is an immunological disorder that affects mucosal surfaces, the search for biomarkers may be promising for reflecting an implication of the mucosal immune system. The purpose of this letter was to report the ability of the mucosal biomarkers to identify food allergy patients. A systematic review (SR) was undertaken to evaluate the mucosal biomarkers associated with food allergy. Search strategies in the LILACS, LIVIVO, PubMed, Science Direct, Scopus, and Web of Science databases included the following terms: (allergy OR allergies OR allergic OR allergen OR allergenic OR "food allergy" OR "food allergies") AND (food OR egg OR milk OR peanut OR dairy OR nut) AND (salivary OR "saliva" OR "Saliva"[Mesh] OR nasal OR lacrimal OR gut OR fecal OR "oral fluid") AND ("Biomarkers"[Mesh] OR biomarker OR "biomarkers" OR "biological marker" OR "biological markers" OR marker OR markers OR biomolecule OR biomolecules OR IgA OR IgE OR "secretory antibodies"). The search databases and methods are detailed in Figure S1.

Twenty-two studies met the eligibility criteria and were included in this SR. The sample size was composed of 1950 subjects (1220 food allergy cases and 730 controls). Sixteen different biomarkers were assessed (Figure 1). Eosinophil cationic protein (ECP) was the most commonly reported biomarker (nine studies), followed by fecal calprotectin and IgA/SIgA (six studies) and by IgE and α1-antitrypsin (AAT) in four studies. Eosinophil protein X, tumor necrosis factor-α (TNFα), and tryptase were reported in three studies. Other biomarkers were reported twice or once such as eosinophil-derived neurotoxin (EDN), histamine, human β-defensin 2 (HBD2), metabolites, B-cell-activating factor (BAFF), 5-hydroxytryptamine, myeloperoxidase (MPO), prostaglandin D2 (PGD2), and peroxidase. (Table S1). Eighteen studies reported biomarkers that may be useful for diagnosis or treatment follow-up. A higher concentration of gut/fecal or salivary biomarkers in allergic patients or after challenge test than healthy controls or negative challenge was reported. All studies reported that the double-blind, placebo-controlled food challenge (DBPCFC) was done for diagnosis purposes. However, only few studies detailed if a challenge test was carried out before or after the collection of sample.

Four studies did not show differences in biomarker levels between patients and controls. Fecal and gut lavage fluid was the most frequently studied samples, whereas only two studies analyzed