Ascaris exposure and its association with lung function, asthma, and DNA methylation in Northern Europe

Nils O. Jõgi, MD,[a,d] Negusse Kitaba, PhD,[e] Torgeir Storaas, MD, PhD,[d] Vivi Schlünssen, MD, PhD,[f,g] Kai Triebner, PhD,[a,b] John W. Holloway, PhD,[e] William G. C. Horsnell, PhD,[h,i,j] Cecilie Svanes, MD, PhD,[d,c] and Randi J. Bertelsen, PhD[a,k]

Bergen, Norway; Southampton and Birmingham, United Kingdom; Aarhus and Copenhagen, Denmark; Cape Town, South Africa; and Orléans, France

Background: *Ascaris* infections, with a worldwide prevalence above 10%, can cause respiratory pathology. However, long-term effects on lung function in humans are largely unknown.
Objective: We investigated the associations of *Ascaris* exposure with lung function, asthma, and DNA methylation.
Methods: Serum *Ascaris* IgG antibodies were measured in 671 adults aged 18 to 47 years (46% women) from Aarhus, Bergen, and Tartu RHINESSA study centers. Seropositivity was defined as IgG above the 90th percentile. Linear and logistic regressions were used to analyze *Ascaris* seropositivity as associated with lung function and asthma, adjusted for age, height, and smoking and clustered by center. DNA methylation in blood was profiled by a commercial methylation assay.
Results: *Ascaris* seropositivity was associated with lower FEV_1 (−247 mL; 95% CI, −460, −34) and higher odds for asthma (adjusted odds ratio, 5.84; 95% CI, 1.67, 20.37) among men but not women, also after further adjusting for house dust mite sensitivity, consistent across study centers. At a genome-wide level, *Ascaris* exposure was associated with 23 differentially methylated sites in men and 3 in women. We identified hypermethylation of the *MYBPC1* gene, which can regulate airway muscle contraction. We also identified genes linked to asthma pathogenesis such as *CRHR1* and *GRK1*, as well as a differentially methylated region in the *PRSS22* gene linked to nematode infection.
Conclusion: *Ascaris* exposure was associated with substantially lower lung function and increased asthma risk among men. Seropositive participants had sex-specific differences in DNA methylation compared to the unexposed, thus suggesting that exposure may lead to sex-specific epigenetic changes associated with lung pathology. (J Allergy Clin Immunol 2021;■■■:■■■-■■■.)

Key words: Ascaris*, helminth, lung function, asthma, EWAS, DNA methylation, RHINESSA*

Ascaris lumbricoides infection is estimated to affect at least 800 million people worldwide.[1] Seropositivity to *Ascaris* is high in low-income countries, but it is believed to have decreased over time in affluent countries as a result of improved sanitary conditions.[2] Reports of *Ascaris* seroprevalence and infection rates are scarce in Europe. However, studies suggest unexpectedly high seroprevalence, with 42%[3] reported in the Netherlands, 29% reported in our analysis of a Norwegian cohort (parents of the current study population),[4] and 13% reported in a general population in Estonia.[5] There are no standardized assays for assessing *Ascaris* exposure; mostly in-house end-point titration ELISAs are used. Stool sampling is the reference standard for determination of current *Ascaris* infections but is unsuitable for assessing previous exposure to *Ascaris.* Evidence for infective *Ascaris* life cycles in Europe have been presented in a study of sewage treatment plants, where 95% of sewage sludge contained viable *Ascaris* eggs.[6] Thus, the potential risk for acquiring helminth infections in affluent countries could be higher than previously recognized.

Respiratory pathology caused by *Ascaris* infections is well known. The natural life cycle of *Ascaris* includes migration

From [a]the Department of Clinical Science, [b]the Core Facility for Metabolomics, and [c]the Centre for International Health, University of Bergen, Bergen; [d]the Department of Occupational Medicine, Haukeland University Hospital, Bergen; [e]the Human Development and Health, Faculty of Medicine, University of Southampton, Southampton; [f]the Department of Public Health, Environmental Occupation, and Health, Danish Ramazzini Centre, Aarhus University, Aarhus; [g]the National Research Centre for the Working Environment, Copenhagen; [h]the Institute of Infectious Disease and Molecular Medicine/Division of Immunology, University of Cape Town, Cape Town; [i]the Institute of Microbiology and Infection, University of Birmingham, Birmingham; [j]the Laboratory of Molecular and Experimental Immunology and Neurogenetics, CNRS–University of Orléans and Le Studium Institute for Advanced Studies, Orléans; and [k]the Oral Health Centre of Expertise in Western Norway, Bergen.

The last 3 authors contributed equally to this article, and all should be considered senior author.

The RHINESSA study received funding from the Research Council of Norway (grants 274767, 214123, 228174, 230827, and 273838), European Research Council Starting Grant project BRuSH 804199, the European Union's Horizon 2020 research and innovation program (grant 633212, Ageing Lungs in European Cohorts Study WP2), the Bergen Medical Research Foundation, and the Western Norwegian Regional Health Authorities (grants 912011, 911892, and 911631). In addition, study centers received local funding from the following: for Bergen, the above grants for study establishment and coordination, as well as the World University Network (Research Development Fund and Sustainability Fund grants), the Norwegian Labour Inspection, and the Norwegian Asthma and Allergy Association; for Tartu, the Estonian Research Council (grant PUT562); and for Aarhus, the Danish Wood Foundation (grant 444508795) and the Danish Working Environment Authority (grant 20150067134). N. O. Jõgi was funded by a Department of Clinical Science PhD grant from the University of Bergen.

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

Received for publication May 27, 2021; revised October 29, 2021; accepted for publication November 5, 2021.

Corresponding author: Nils Oskar Jõgi, Department of Clinical Science, University of Bergen, PO Box 7804, N-5020 Bergen, Norway. E-mail: Nils.Jogi@uib.no. Or: William Horsnell, Department of Pathology, Division of Immunology, Institute of Infectious Disease and Molecular Medicine & Division of Immunology, UCT Faculty of Health Sciences, Room 3.20, Falmouth Building, Observatory 7925, Cape Town, South Africa. E-mail: wghorsnell@gmail.com.

0091-6749

https://doi.org/10.1016/j.jaci.2021.11.013

Abbreviations used
aOR: Adjusted odds ratio
CpG: 5′-C-phosphate-G-3′
dmCpG: Differentially methylated CpG
DMR: Differentially methylated region
EWAS: Epigenome-wide association study
HDM: House dust mite
NTU: NovaTec units
RHINESSA: Respiratory Health in Northern Europe, Spain, and Australia
SPT: Skin prick test

through the lungs,[7] which has been demonstrated to cause eosinophilic pneumonitis and related alterations on computed tomography.[8-11] *Ascaris* infection is also associated with increased risk of asthma symptoms,[12,13] especially among children in endemic regions.[12,14,15] Importantly, detection of anti–*A lumbricoides* IgE antibodies, rather than current *A lumbricoides* infection, is associated with wheezing in atopic children.[14] Elevated IgE levels toward *Ascaris* have been associated with current infection,[13] and higher levels are in particular observed in children with chronic infections.[16] One study suggests that increased levels of *Ascaris*-specific IgE reflects protection from infection rather than exposure.[17] Measuring *Ascaris*-specific IgE as marker for ever exposure can be therefore questionable. On the other hand, *Ascaris*-specific IgG may overestimate the prevalence of infection as a result of the persistence of antibodies long after patients undergo deworming therapy.[18] Thus, IgG is assumed to be more suitable for assessing previous exposure.

The potential for long-term effects on lung function have been highlighted in murine studies. Recurrent infection with *Ascaris suum* increases lung cytokine responses, promoting severe impairment of respiratory function and a polarized systemic T_H2/T_H17 immune response.[19] Furthermore, infection with the murine parasitic nematode *Nippostrongylus brasiliensis,* which, like *Ascaris* in humans, transits the lungs of mice, has demonstrated long-term effects on lung cellular and physiologic characteristics.[20] The infection has also been associated with development of fibrosis and emphysema-like changes.[21] Infection with gut-restricted helminths can also result in immunologic and structural changes in the lung.[22] Sex differences in parasite infections are described in animal models,[23] with a different response to infection between male and female mice.[24] In humans, infection rates have been reported to be higher among women,[25,26] and one of our previous studies revealed sex-specific patterns in the associations of parental helminth exposure with the allergic outcomes of their offspring.[4] This body of work suggests that parasite infection could be an important predictor of long-term respiratory health in humans and that there might be substantial sex differences.

Further, viral, bacterial, and parasitic infections in humans have been shown to cause long-lasting changes in DNA methylation,[27-30] some of which have been linked to altered host immunity.[31] In addition to the potential direct damage and subsequent changes in the airways, epigenetic changes due to infection might contribute to the pathogenesis of allergic diseases related to helminth infections.

The role of exposure to *Ascaris* in determining lung function in humans has not yet been addressed, but *Ascaris* infection has previously been associated with asthma severity.[13] The aim of our study was to explore the association between *Ascaris* exposure and lung function, the potential sex differences in such associations, and whether *Ascaris* exposure was associated with differentially methylated DNA.

METHODS

Study population

The study population included 671 adults from Aarhus (Denmark) (n = 53), Bergen (Norway) (n = 474), and Tartu (Estonia) (n = 144), investigated in 2014-15 as part of the RHINESSA study (Respiratory Health In Northern Europe, Spain and Australia study; www.rhinessa.net). The RHINESSA clinical study included all adult offspring of the participants of the population-based European Community Respiratory Health Survey study in 10 study centers. Helminth serology was performed in Danish, Estonian, and Norwegian study centers. The present analysis included RHINESSA study participants from these study centers with available lung function and serum samples (86% of the total population). Protocols for questionnaires and clinical examination were standardized across study centers.

Approval was obtained from the local ethics committees for each center. On the basis of cross-sectional data, the main objective of the study was to investigate the association of *Ascaris* exposure with lung function and genome-wide DNA methylation profiles in blood.

Lung function, asthma symptoms, and sensitization

Lung function was measured using a standard spirometry method with a NDD EasyOne spirometer following American Thoracic Society/European Respiratory Society criteria.[32] Interview data were used to define ever having asthma, wheeze, and ≥3 asthma symptoms (wheeze, nocturnal chest tightness, nocturnal breathlessness, nocturnal cough, any asthma attack, or use of asthma medication) during the last 12 months before the study. Further details are provided in the Online Repository available at www.jacionline.org, and questionnaire forms are available at www.rhinessa.net. In order to account for lung function in a wide age range, we calculated the percentage of predicted values for FVC and FEV_1 using the Global Lung Initiative reference values[33] using the online calculator. Bronchodilator reversibility was defined according to 2019 Global Initiative for Asthma guidelines: increase in FEV_1 of >12% and >200 mL from baseline 15 minutes after inhalation of 200 μg salbutamol. Allergic sensitization was determined by skin prick test (SPT) to 12 allergens (ALK-Abelló, Hørsholm, Denmark) including *Dermatophagoides pteronyssinus and Dermatophagoides farinae;* 0.9% saline and 10 mg/mL histamine solution were used for negative and positive controls. The house dust mite (HDM) allergens used were Der p 1 (9.8 μg/100,000 Standardized Quality Units [SQ-U]) 19.6 μg/mL in 10 HEP Soluprick) and Der p 2 (0.7 μg/100,000 SQ-U 1.4 μg/mL in Soluprick). Reactions to the allergens were read after 15 minutes. Reactivity was considered positive if the mean wheal size was 3 mm greater than the negative control. The mean diameter of positive histamine wheals was 4.3 mm. "Any positive HDM SPT" was defined as positive SPT reactivity toward *D pteronyssinus* and/or *D farinae*. SPTs were carried out by trained nurses following a standardized protocol, similar in all study centers.

Ascaris IgG antibodies

Ascaris IgG antibodies levels were measured by ImmunoCAP (Thermo Fisher Scientific, Waltham, Mass). Additionally, in a subsample of the Bergen cohort, *Ascaris* IgG_4 antibodies were measured using an indirect end-point titration (in 4 dilutions) ELISA (n = 265) with *Ascaris lumbricoides* crude worm antigen preincubated with *Toxocara* somatic antigen to reduce cross-reactivity.[4] On the basis of previous findings from Europe as well as from our previous study,[4] we assume IgG seropositivity toward *Ascaris* to be around

10%, and therefore seropositivity was defined as values above the 90th percentile (cutoff, 4.53 mg/L).

Epigenome-wide association studies

Methylation data were profiled from peripheral blood using HumanMethylationEPIC (Illumina, San Diego, Calif). Methylation input data (IDAT files) (n = 551) were processed using the R/Bioconductor package minfi.[34] The batch effect was adjusted using COMBAT from SVA.[35] Normalization was carried out using BMIQ.[36] Probes with a detection *P* value above .01 in 1 or more samples, probes with a bead count of <3 in at least 5% of samples, non-CG probes, cross-reactive probes, and probes on the X or Y chromosome were excluded from the analysis, resulting in a total of 747,053 probes used for downstream analysis. To identify differentially methylated 5′-C-phosphate-G-3′ (CpG), a robust multiple regression model was applied using limma[37] on methylation beta value stratified by sex, adjusting for age, personal smoking status, and estimated cell type proportions (B, CD4T, CD8T, natural killer, monocytes, and granulocytes).[38] Multiple test correction was applied using the Benjamini-Hochberg false discovery rate at a significance level of .05. Manhattan plots were generated using the R package qqman.[39] Differential methylated regions were predicted using DMRcate.[40] Functional enrichment was identified using Enrichr in R.[41] Transcription factor enrichment was carried using eFORGE.[42]

Potential cross-reactivity with other helminths

In order to account for potential cross-reactivity with helminths, 446 Bergen center samples were tested using NovaLisa IgG ELISA (NovaTec Immunodiagnostica, Dietzenbach, Germany) for the presence of IgG antibodies against *Ascaris lumbricoides, Echinococcus* spp, *Taenia solium, Toxocara canis,* and *Trichinella spiralis* according to the manufacturer's instructions. A result of >11 NovaTec units (NTU) was considered positive. A result of 9 to 11 NTU was considered a gray zone and <9 NTU negative.

Sensitivity analyses

For sensitivity analyses, we applied a separate 90th percentile cutoff for men and women that was additionally adjusted for pet ownership, occupation, body mass index, and education. To account for differences in lung function in different age groups, a percentage of predicted spirometry values was used. In a subsample of *Ascaris* IgG measurements obtained via 3 different methods, we selected the samples that were negative according to both NovaTec ELISA and in-house ELISA, then used the mean concentration of these negative samples ±3.3 SD[43] as an alternative cutoff for the ImmunoCAP results. In a subsample of 98 participants with the highest IgG values, we also measured *Ascaris*-specific IgE serum concentrations (ImmunoCAP) and its relation to lung function and asthma.

Statistical analyses

Descriptive statistics for the study population are reported as means and SDs for normally distributed data and otherwise as median and interquartile range.

Logistic regression was used to model the association between *Ascaris* seropositivity and asthma status, wheeze, and ≥3 asthma symptoms (dichotomous) 12 months before the study. FVC and FEV_1 were included as continuous outcomes. Linear regression was used to model the association between anti-*Ascaris* IgG concentration and FVC and FEV_1 values.

All models were adjusted for age, sex, height (measured before spirometry), and smoking (interview data) and clustered within study center. For sensitivity analyses, we applied a separate 90th percentile cutoff for men and women, adjusted for pet ownership, occupation, body mass index, and education. To account for differences in lung function in different age groups, the percentage of predicted spirometry values were used. Stata 16.0 (StataCorp, College Station, Tex) was used for regression analyses.

RESULTS

Population characteristics

The study participants were aged 18 to 47 years (median, 28 years) (Table I). A total of 14.9% were current smokers (Bergen 12%, Aarhus and Tartu 22%). Ever asthma was higher among Aarhus participants (21%) compared to Bergen and Tartu (15% and 7%, respectively), while 3 or more asthma symptoms during the last 12 months showed a similar prevalence in all study centers (6%). Sensitization to *D pteronyssinus* and/or *D farinae* by SPTs was lower in Tartu (11%) compared to Aarhus and Bergen (21% and 24%, respectively).

Ascaris seropositivity was present in 6.9% of participants from Tartu and in 11% of participants from Aarhus and Bergen (Table I). Women were more often seropositive than men (15.5% vs 5.5%, respectively). We compared the characteristics of *Ascaris*-seropositive and -seronegative participants and found that women were more often seropositive (Table II).

In a Norwegian subpopulation (n = 446), 5 helminths were measured using a NovaLisa ELISA with *Ascaris* seroprevalence of 5.6% (additionally 9.1% including gray zone) *Echinococcus* 0.7% (+3.6% including gray zone), *Taenia* 2.5% (+4.7% including gray zone), *Toxocara* 0.7% (+1.6% including gray zone), and *Trichinella* 1.3% (+0.9% including gray zone) (see Fig E1, *A,* in the Online Repository available at www.jacionline.org). When stratified by sex, women accounted for most of the seropositive samples (Fig E1, *B*).

Ascaris IgG antibody levels and HDM SPT sensitivity

There was a statistically significant difference in *Ascaris* IgG antibody level between HDM SPT-positive and -negative study participants (Wilcoxon Mann-Whitney *U* test, $P = .04$) with a median difference of 0.33 mg/L higher IgG antibody levels among the HDM SPT positive results (see Fig E2 in the Online Repository available at www.jacionline.org). Overall, 129 participants (19%) were sensitized to any HDM, and 68 (10%) were, according to our definition, *Ascaris* IgG positive, with 14 (2%) positive toward both (see Fig E3 in the Online Repository).

Ascaris IgG antibody serum level, lung function, and asthma symptoms

Among men, *Ascaris* IgG seropositivity (according to ImmunoCAP results) was associated with lower FEV_1 (−247 mL; 95% CI, −460, −34) (Table III) with an effect size larger than that of current smoking (−151 mL; 95% CI, −501, 199). There was a clear dose–response pattern, with subsequently decreasing FEV_1 with increasing *Ascaris* IgG antibody levels (see Fig E4 in the Online Repository available at www.jacionline.org). In a linear regression model, a 1 mg/L rise in *Ascaris* IgG concentration was associated with 40 mL lower FEV_1 among men (95% CI, −60, −21). Seropositive men had 5% lower predicted FEV_1 and 4% lower FEV_1/FVC ratio compared to seronegative men; this pattern was not seen for women (see Fig E5, *A* and *B*, in the Online Repository). These associations remained after adjusting for allergic sensitization to HDM (Table III) and when using an in-house ELISA method for detection anti-*Ascaris* IgG_4 antibodies (see Fig E6 in the Online Repository). No association was seen for FVC among men. For women, no clear pattern was seen for FEV_1 or FVC (Fig E4), apart from a slightly lower FVC for

TABLE I. Characteristics of study populations

Characteristic	Total (n = 671)	Aarhus (n = 53)	Bergen (n = 474)	Tartu (n = 144)
Female (%)	46.2	50.9	46	45.1
Age (years), mean (range; SD)	28 (18-47; 6.6)	28 (19-47; 7.7)	28 (18-47; 10)	29 (18-42; 5.6)
Height (cm), median (range; IQR)	175 (146-200; 14)	176 (159-191; 15)	175 (146-200; 13)	177 (149-197; 16)
FVC (mL), median (range; IQR)	4.77 (1.52-8.28; 1.61)	4.64 (3.13-6.61; 1.07)	4.75 (1.52-8.28; 1.59)	4.86 (2.84-7.62; 1.72)
FEV_1 (mL), median (range; IQR)	3.86 (1.18-6.79; 1.2)	3.79 (2.36-5.25; 0.99)	3.82 (1.18-6.79; 1.23)	3.94 (2.25-5.86; 1.22)
FEV_1/FVC ratio, median (range; IQR)	0.82 (0.49-1.26; 0.08)	0.81 (0.68-0.93; 0.07)	0.82 (0.58-0.98; 0.07)	0.83 (0.49-1.26; 0.08)
Smoking				
Never smokers (%)	63.4	66.7	63.7	61
Ex-smokers (at least 1 year) (%)	21.7	11.1	24.1	17.1
Current smoking (%)	14.9	22.2	12.2	22
Asthma, ever (%)	13.9	20.5	15.3	6.5
Wheeze, ever (%)	13.1	20	12.8	11.8
Asthma symptoms, last 12 months (%)	6.2	6.7	6.1	6.4
D pteronyssinus–specific IgE positive (%)	24.3	35.9	25.1	17.5
Any HDM SPT positivity (%)*	20.9	20.8	23.8	11.1
Atopic (any SPT or sIgE positive) (%)	45.1	44.2	49.3	31.5
Anti-*Ascaris* IgG (mg/L) median (range; IQR)	2.21 (0.03-23.3; 1.62)	2.31 (0.73-6.6; 1.78)	2.18 (0.03-23.3; 1.73)	2.24 (0.69-6.66; 1.32)
Ascaris seropositive at 90th percentile (cutoff, 4.53 mg/L) (%)	10	11.3	11	6.9
Ascaris seropositive (cutoff at mean ± 3.3 SD of negative samples at 6.1 mg/L) (%)	3.9	3.8	4.4	2.1
Anti-*Ascaris* IgG levels (%)				
<2 mg/L	42.2	35.9	43.9	39.2
2-4 mg/L	43.4	47.2	41.1	49.0
4-6 mg/L	10.3	13.2	10.3	9.2
6-8 mg/L	2.1	3.8	1.7	2.6
>8 mg/L	2.1	0	3.0	0

*SPT positive to *Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae*.

TABLE II. Characteristics of study population stratified by *Ascaris* seropositivity

	Ascaris seropositive (≥90th percentile, 4.53 mg/L)	
Variable	Seronegative	Seropositive
Sex*		
Female (%)	84.5	15.5
Male (%)	94.5	5.5
Age (years), mean (range; SD)	28 (18-47; 6.6)	29 (18-43; 6.9)
Height (cm), median (range; IQR)	176 (146-200; 20)	170 (152-195; 12)
Smoking (%)		
Never smokers	64.2	56.1
Ex-smokers (at least 1 year)	21.3	25.8
Current smoking	14.5	18.2
Asthma, ever (%)	13.4	18.2
Wheeze, ever (%)	12.7	16.7
Asthma symptoms, last 12 months (%)	6.2	6.1
Mother's education (%)		
Primary	9.5	18.2
Secondary	38.4	34.9
University or college	52.1	47
Father's education (%)		
Primary	8.6	10.8
Secondary	38.6	41.5
University or college	52.8	47.7

*Difference between groups $P < .05$.

Ascaris IgG antibody level of 6-8 mg/L compared to *Ascaris* IgG level <2 mg/L.

Among men, *Ascaris* seropositivity was associated with increased odds of ever having asthma (adjusted odds ratio [aOR], 5.84; 95% CI, 1.67, 20.37), ever wheezing (aOR, 3.78; 95% CI, 1.85, 7.74), and having ≥3 asthma symptoms during the last 12 months (aOR, 3.59; 95% CI, 2.01, 6.47) compared to seronegative men (Table IV). Among women, *Ascaris* seropositivity was associated with decreased odds of ever having asthma (aOR, 0.42; 95% CI, 0.18, 0.96), ever wheezing (aOR, 0.63; 95% CI, 0.16, 2.44), and ≥3 asthma symptoms during the last 12 months before the study (aOR, 0.24; 95% CI, 0.15, 0.40). These associations for men and women did not change when adjusting for HDM sensitivity (Table IV) and were consistent for men across study centers (Fig 1) and with different methods for detection of antibodies against *Ascaris* among the Bergen cohort (Fig E5; Fig E6). For women, the number of *Ascaris*-seropositive participants was too low in Aarhus (n = 2) for us to perform a meta-analysis by study center. There was no statistically significant difference in FEV_1 reversibility between the seropositive and seronegative participants (3% vs 3.5%, respectively).

Sensitivity analyses

The results did not change when we used the 90th percentile cutoff separately for men and women. When adjusting for pet ownership, occupation, body mass index, smoking, and parental or own education in the models, the effect size remained significant. Analyses of postbronchodilator lung function measures (n = 352) gave comparable results as for prebronchodilator measures (association with postbronchodilator FEV_1 191 [95% CI,−434, 43] mL). For men, *Ascaris* seropositivity was associated with 6% lower predicted FEV_1 (95% CI, 11.8, −0.2) (Table III). The associations with percentages predicting FVC in men or with percentages predicting FEV_1 or FVC in

TABLE III. Associations of *Ascaris* seropositivity with lung function

Characteristic	Male sex (n = 361)		Female sex (n = 310)	
	AD (95% CI)	AD-HDM (95% CI)	AD (95% CI)	AD-HDM (95% CI)
FVC (mL)	−97 (−535, 340)	−87 (−501, 324)	−42 (−174, 89)	−42 (−179, 95)
FEV_1 (mL)	−247 (−460, −34)	−232 (−408, −56)	−29 (−80, 21)	−30 (−76, 16)
FEV_1/FVC ratio	−3.2% (−9.3, 2.9)	−3% (−9.3, 3.3)	+0.1% (−2.6, 3.5)	+0.5% (−2.4, 3.3)
FVC % predicted*	−1.9% (−13.2, 9.4)	−1.7% (−11.2, 8.6)	−0.3% (−2.9, 2.3)	−0.3% (−3.1, 2.4)
FEV_1 % predicted*	−6% (−11.8, −0.2)	−5.7% (−10.5, −0.8)	−1.7% (−2.9, 0.4)	−0.5% (−2.1, 1.1)

Shown are the adjusted differences (AD) as well as the AD with adjustment for HDM sensitivity (AD-HDM). AD are adjusted for height and age, clustered by study center; in addition to these, AD-HDM are adjusted for any HDM SPT positivity.
*Not adjusted for age or height.

TABLE IV. Adjusted odds ratio (95% CI), stratified by sex, for *Ascaris* seropositivity as associated with respiratory symptoms in a logistic regression model

Characteristic	Male sex (n = 361)		Female sex (n = 310)	
	AD	AD-HDM	AD	AD-HDM
Asthma, ever	5.84 (1.67, 20.37)	5.44 (1.71, 17.34)	0.42 (0.18, 0.96)	0.38 (0.23, 0.65)
Wheeze, ever	3.78 (1.85, 7.74)	3.4 (1.99, 6.81)	0.63 (0.16, 2.44)	0.61 (0.18, 2.07)
Combined asthma symptoms*	3.59 (2.01, 6.47)	3.8 (1.96, 7.34)	0.24 (0.15, 0.40)	0.24 (0.12, 0.47)

*More than 2 asthma symptoms (wheeze, tightness of breath, nocturnal breathlessness, nocturnal cough, asthma attack, use of asthma medication) during the last 12 months.

women were not significant. Serum concentrations of *Ascaris*-specific IgG and IgE were not correlated. Elevated *Ascaris*-specific IgE levels were not associated with increased odds of having asthma, asthma symptoms, or lower spirometry measurements (among the 98 participants with IgE measurements). Seven subjects had a serum IgE concentration of >0.35 kU/L. Only 2 participants had an IgE response in radioallergosorbent test (RAST) class 3 or higher, and these participants also had a highly elevated IgG response (7.5 and 9.3 mg/L), which could be indicative of current or chronic infection.

DNA methylation as related to *Ascaris* exposure

We identified 5 differentially methylated CpGs (dmCpGs) associated with *Ascaris* exposure that achieved genome-wide significance (at adjusted *P* value of .05 and inflation of 1.17) when analyzing men and women together. Two of these dmCpGs were mapped to known genes: *MYBPC2* (myosin-binding protein C) and *NAV3* (neuron navigator 3). Three dmCpGs were mapped to intergenic regions. CpG cg20041612 showed association at adjusted $P = .056$ (borderline) mapped to *EGFR* (epidermal growth factor receptor). The dmCpG cg04671734 (adjusted $P = .046$) was significantly enriched in proximity to the binding site of transcription factor DEAF1 (deformed epidermal autoregulatory factor 1 homolog) at a *q* value of 0.003.

By analysis of sex-stratified epigenome-wide association study (EWAS) results, 23 dmCpGs were identified as genome-wide significantly associated with *Ascaris* exposure in men that were mapped to 19 known genes (see Table E1 in this article's Online Repository at www.jacionline.org; Fig 2, *A*). Three dmCpGs (close to *RADIL, NAV3,* and *ACSL5*) were significantly associated in women (false discovery rate, <0.05) (Fig 2, *B*). *RADIL* and *NAV3* were hypomethylated while *ACSL5* was hypermethylated in the *Ascaris*-exposed group. The effect size and direction of association between exposed and unexposed groups for the top dmCpGs are shown in Fig 3. The regression coefficient of *NAV3* (−0.06) shows the strongest association in female subjects, while *CRHR1* (0.097) and cg04671734 (−0.079) show the strongest association in male subjects. Sex differences in relation to *Ascaris* exposure are illustrated in Fig 4.

Ascaris exposure and its association with differentially methylated regions

Five differentially methylated regions (DMRs) significantly associated with *Ascaris* seropositivity were identified in male subjects and 4 DMRs in female subjects. One DMR was identified in subjects of both sexes in the locus of *PRSS22* (brain-specific serine protease 4) (17 CpGs within 2016 bp) at Stouffer = 0.01; this was hypomethylated in the *Ascaris*-exposed group.

EWAS Atlas enrichment showing genes linked to lung function and asthma pathogenesis

To gain pathophysiologic insight into these CpGs, the significant genes were compared against the EWAS Atlas repository (https://ngdc.cncb.ac.cn/ewas/index). The methylation status of the genes *GOT1, TPD52L2, RAPGEF4, GRK1, DLEU7, CRHR1, DCAF17, SUMF1, PROCR, CLEC16A,* and *MYBPC1* was found to be linked to asthma pathogenesis[44] (see Table E2 in this article's Online Repository at www.jacionline.org).

Functional enrichment

To gain further biological insight, the significant genes (19 from men and 3 from women) were used for functional enrichment with Enrichr. In men, the top enriched biological processes include fatty acid homeostasis (GO:0055089) (*GOT1, GPAM*), amide transport (GO:0042886) (*SLC38A7, CRHR1, RAPGEF4*), peptide hormone secretion (GO:0030072) (*CRHR1, RAPGEF4*), and lipid homeostasis (GO:0055088; *GOT1, GPAM*). In women, the top enriched biological pathway was triglyceride biosynthesis (GO:0019432; *ACSL5, AGPAT3*) (Table E2).

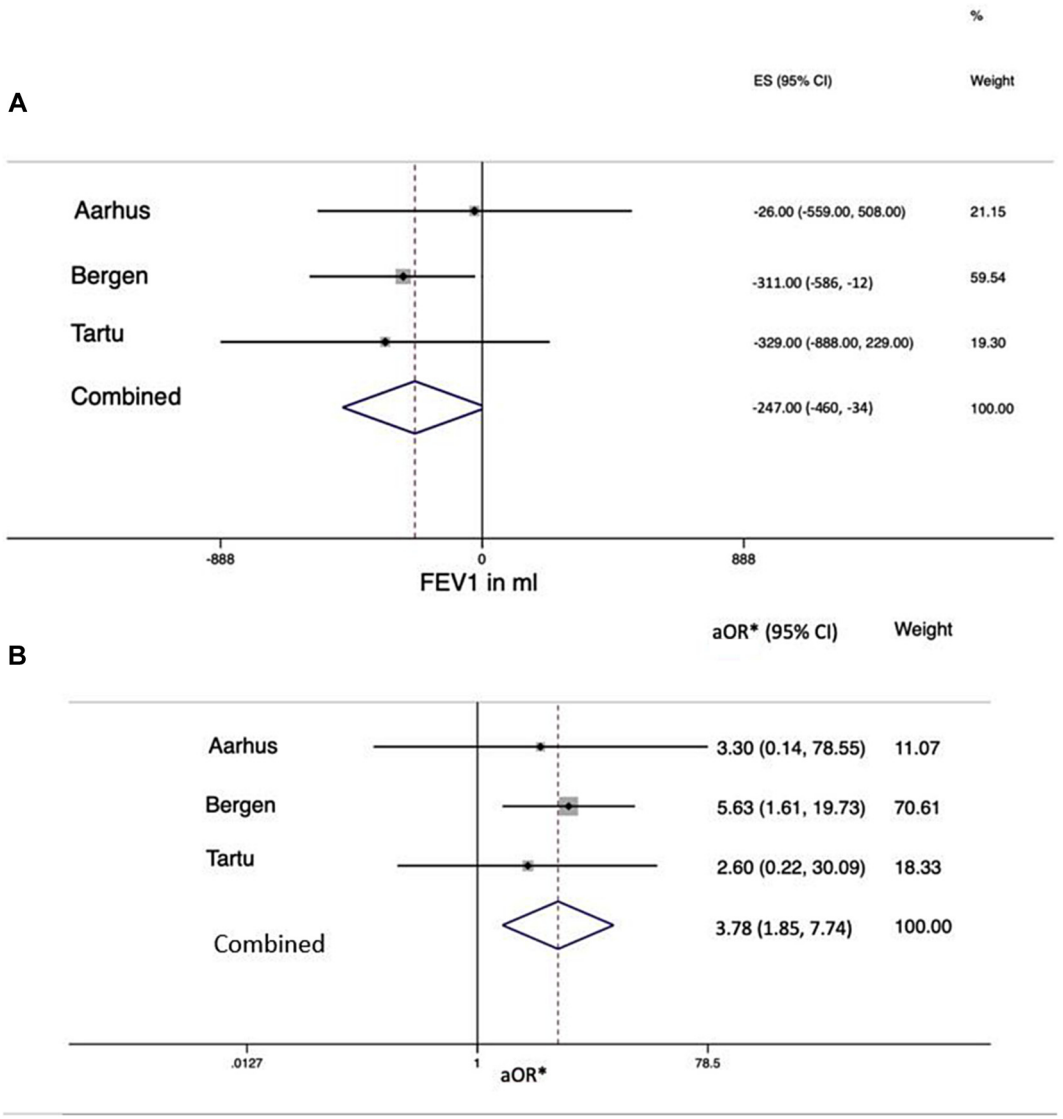

FIG 1. Meta-analysis of study centers specific associations among men of *Ascaris* seropositivity (≥90th percentile) with (**A**) FEV_1 (adjusted difference in mL) and (**B**) wheeze (aOR).

DISCUSSION

Seropositivity to *Ascaris lumbricoides* consistently showed an association with substantially lower lung function in young adult men from a population-based study in Northern Europe. Men who were seropositive to *Ascaris* had lower FEV_1, unaltered FVC, and more asthma symptoms. A dose–response pattern was found: FEV_1 decreased with increased concentration of anti-*Ascaris* IgG. In women, no association was found with lung function, but asthma symptoms were significantly less common in seropositive women. Genome-wide analyses uncovered DNA methylation characteristics associated with *Ascaris* seropositivity, including differentially methylated sites related to lung pathology, such as regulation of airway muscle contraction and asthma pathogenesis, and to immune regulation and specifically to nematode infection. The associations of *Ascaris* seropositivity with differentially methylated DNA sites were different in men and women, supporting a sex-specific role of *Ascaris* exposure. The findings from epidemiologic analyses, supported by DNA methylation analyses, suggest that the lower lung function and higher asthma risk observed in *Ascaris*-exposed individuals may be mediated by infection-driven epigenetic changes.

Identification of a hypomethylated CpG close to DEAF1 binding site and the differential methylation in *Ascaris*-exposed individuals of genes associated with helminth infection, namely *NAV3* and *EGFR*,[45] suggests that *Ascaris*-associated changes have the potential to alter the function of genes involved in type 2 immunity and therefore lung function. Hypermethylation of deoxyhypusine hydroxylase linked to immune regulation,[46] chronic lung disease progression,[47] being a putative antiparasitic drug target,[48] and respiratory muscle contraction modulating *MYBPC1* (myosin-binding protein C, slow-type)[49] also support epigenetic change influencing lung function.

To our knowledge, our study is the first to investigate associations between serum *Ascaris* IgG antibody levels and lung function in humans. Our findings with respect to increased asthma (among men) associated with *Ascaris* is in accordance

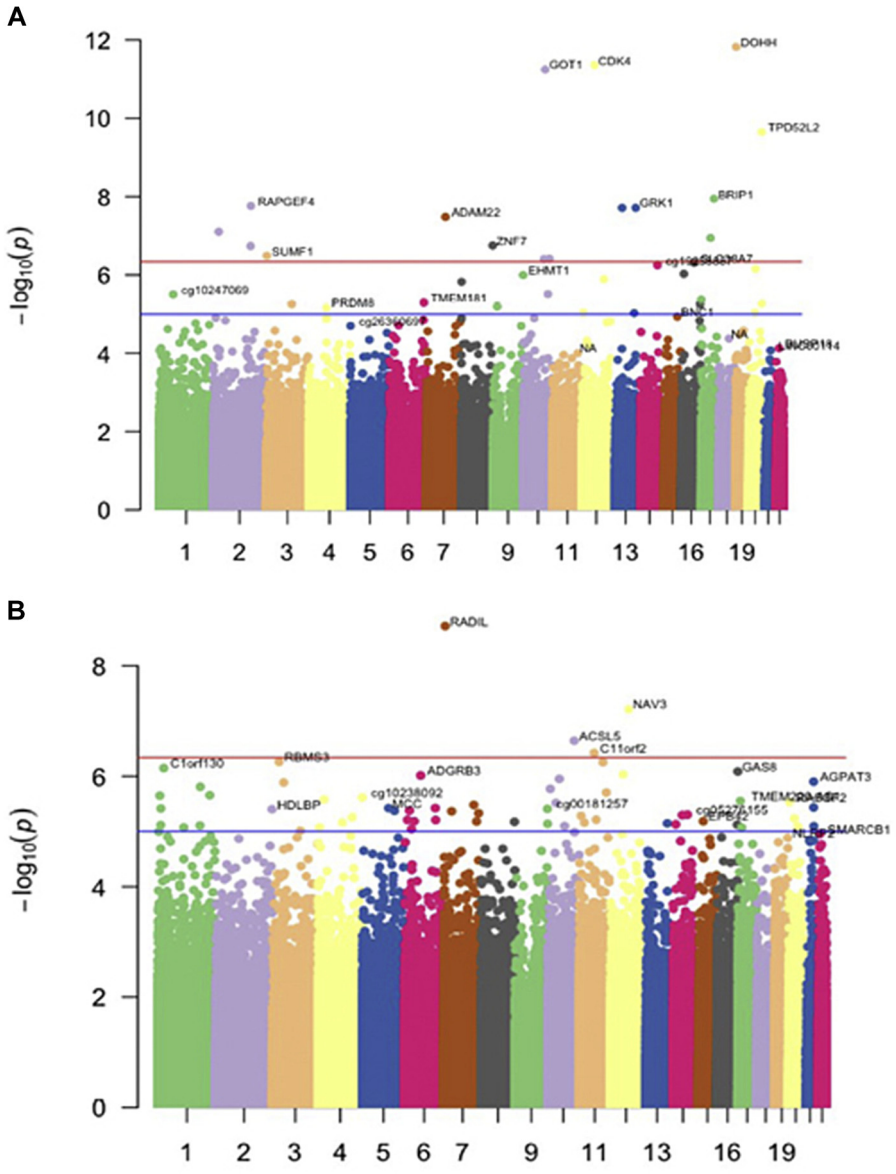

FIG 2. Manhattan plot for *Ascaris* seropositivity EWAS showing autosomal chromosomes in male (**A**) and female (**B**) subjects. The *vertical axis* ($\log_{10}$ transformed) indicates observed *P* values; *horizontal axis,* chromosomal positions with the points indicating individual CpGs. The *red line* indicates the multiple testing correction threshold (false discovery rate <0.05); *blue,* the suggestive line.

with other publications; however, most previous research has been based on pediatric cohorts.[50-54] The scale of lung damage evidenced by computed tomographic scan in *Ascaris*-infected persons strongly supports the likelihood that reduced long-term lung function would not be unexpected after infection. Our finding of impaired lung function in seropositive young men from Northern Europe therefore breaks new ground in presenting *Ascaris* infection as potentially important cause of long-term reduction in lung function.

The sex differences in the associations of *Ascaris* seropositivity with lung function and asthma are striking and have not been described before. The intensity of *A lumbricoides* can be influenced by sex-related behavioral and environmental factors that contribute to risk of exposure to infectious inoculum.[23] Higher infection rates have previously been reported in women,[25,26] which is in accordance with our results. Higher seroprevalence rates among women were seen for all measured helminth antibodies. Differences in sex hormone levels could hypothetically influence the pathogenic outcomes from *Ascaris* exposure. Estradiol, the main female sex hormone, is known to be important for many tissue repair processes, notably inflammation and regranulation,[55,56] and tissue damage is an essential element in the pathology caused by *Ascaris.* Moreover, murine models have shown that female mice can have a delayed T_H2 response to the murine nematode *Trichuris muris* compared to male mice.[57] On the one hand, such mechanisms could possibly lead to a higher worm burden in female subjects and therefore higher *Ascaris* IgG antibody levels. Sustained T_H2 responses in male subjects, on the other hand, could enhance pathology such as pulmonary inflammation. Interestingly, we discovered hypomethylation in dmCpGs in *NAV3* (a

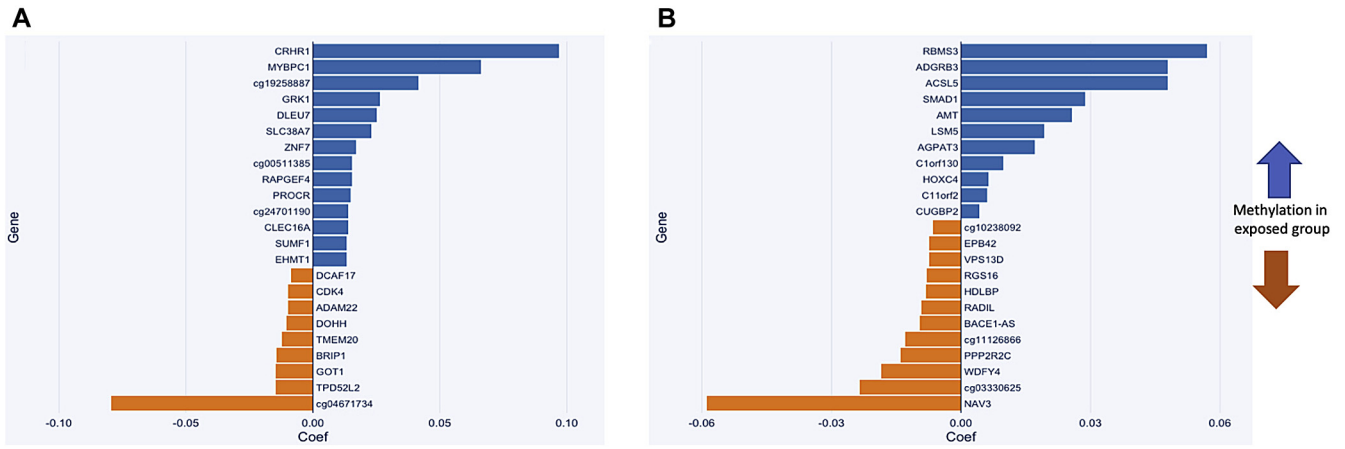

FIG 3. The top dmCpGs showing a different methylation pattern between exposed and unexposed groups in male (**A**) and female (**B**) subjects. *Positive regression coefficient shows that the mean methylation is higher in the exposed group, whereas negative regression coefficient shows lower mean methylation.

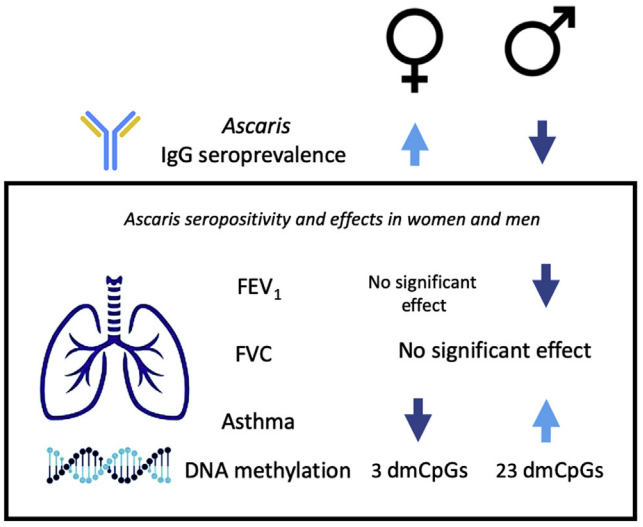

FIG 4. Sex differences in relation to *Ascaris* exposure.

gene involved in immunoregulatory processes through IL-2) in women, suggestive of sex differences in immunoregulation of helminth responses. The apparent *lower* likelihood of asthma risk in seropositive women is still surprising, and we speculate that altered immunoregulation may play a role.

Our findings suggest that men and women might respond differently to *Ascaris* infections and that these responses could have long-term outcomes. The EWAS analyses also showed that lower lung function in men may be explained by changes in the function of signaling pathways related to asthma pathogenesis and muscle contraction. The likelihood of a long-term effect is supported by findings from others that demonstrate long-term changes in DNA methylation after helminth infection.[31]

A strength of the present study is the extensive data on respiratory symptoms and spirometry data, as well as *Ascaris* serology, for population-based cohorts. Further, the inclusion of 3 study centers from different countries contributes to higher credibility and generalizability of the results. Interestingly, the Estonian study center had the lowest seroprevalence compared to Norway and Denmark.

Defining *Ascaris* exposure is a challenge because of potential cross-reactivity with other helminths and allergens. For Bergen samples, we used an additional method in which serum was preincubated on plates covered with *Toxocara* somatic antigen before transferring to *Ascaris* plates; this should reduce cross-reactivity. Moreover, using the same methodology, anti-*Ascaris* IgG_4 seropositive serum has been shown to react with live larvae.[58] Both strategies strengthen our findings and suggest that we are truly measuring antibodies toward *Ascaris*. Exposure to *Anisakis simplex* due to fish consumption could be a potential cause of cross-reactivity; however, a study of Norwegian blood donors showed a prevalence of almost 0 for IgE sensitization toward *Anisakis*.[59] HDM sensitization is commonly assumed to be a confounder as a result of possible cross-reactivity and the close structural homology between HDM and *Ascaris* proteins, such as tropomyosin, enolase, and enoyl-CoA hydratase.[60] However, adjusting our analyses for HDM sensitivity did not alter the results for either lung function or asthma. Thus, we believe that anti-*Ascaris* IgG as measured in our study reflects exposure to *Ascaris* rather than any other helminth or cross-reactivity with other allergens. For defining HDM sensitivity, we used a single SPT procedure, harmonized with the European Community Respiratory Health Survey protocol. We do note that a study by Thomsen et al[61] found that performing SPT twice with the same allergen batch did not enhance the validity of the test. Still, we do not know whether *Ascaris* IgG antibody seropositivity reflects past infection, current infection, or parasite exposure without disease manifestation. The reference standard for diagnosing ongoing infection is real-time quantitative PCR or microscopy of eggs in stool. Past infection, however, cannot be assessed that way.

EWAS analyses found that *Ascaris* seropositivity was associated with DMR *PRSS22*. This region has been associated with nematode infection, and this finding strengthens the interpretation that the participants who are *Ascaris* seropositive have actually been exposed to the helminth. A limitation of the epigenetic analysis is that it was carried out on DNA extracted from whole blood while other relevant tissues were not available. Further, although the epigenetic analysis was adjusted for blood cell type composition, it is possible that differences in cell subtype composition

between *Ascaris*-exposed and -unexposed individuals may partly account for the observed associations. Finally, our study cannot address whether the identified DNA methylation changes lie on the causal pathway between *Ascaris* exposure and lung function.

In conclusion, our findings show that detection of higher serum IgG antibody levels against *Ascaris* is associated with substantially lower lung function and more asthma among Northern European men. The effect magnitude for lung function was larger than that of current smoking. In women, no association was found with lung function, but asthma symptoms were significantly less common in seropositive women. A wide range of sex-specific DNA methylation markers associated with *Ascaris* exposure were identified in genes linked to asthma pathogenesis, lung function, and immunoregulation. These findings support the notion that DNA methylation changes due to helminth exposure may contribute to the pathogenesis underlying reduced lung health. Further, our findings identify a need to investigate the role of helminths on long-term lung health globally, including in high- and middle-income countries, as well as in low-income countries where helminth exposure is highly prevalent.

We thank all the study participants, clinical field workers, and laboratory personnel involved.

Clinical implications: *Ascaris* exposure may be an important public health challenge in terms of respiratory function. Our results highlight a need for further research on long-term effects of helminth exposure on host lung health.

REFERENCES

1. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit Vectors 2014;7:37.
2. Strunz EC, Addiss DG, Stocks ME, Ogden S, Utzinger J, Freeman MC. Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis. PLoS Med 2014;11:e1001620.
3. Mughini-Gras L, Harms M, van Pelt W, Pinelli E, Kortbeek T. Seroepidemiology of human *Toxocara* and *Ascaris* infections in the Netherlands. Parasitol Res 2016; 115:3779-94.
4. Jogi NO, Svanes C, Siiak SP, Logan E, Holloway JW, Igland J, et al. Zoonotic helminth exposure and risk of allergic diseases: a study of two generations in Norway. Clin Exp Allergy 2018;48:66-77.
5. Lassen B, Janson M, Viltrop A, Neare K, Hütt P, Golovljova I, et al. Serological evidence of exposure to globally relevant zoonotic parasites in the Estonian population. PLoS One 2016;11:e0164142.
6. Zdybel J, Karamon J, Dabrowska J, Rozycki M, Bilska-Zajac E, Klapec T, et al. Parasitological contamination with eggs *Ascaris* spp, *Trichuris* spp and *Toxocara* spp of dehydrated municipal sewage sludge in Poland. Environ Pollut 2019;248: 621-6.
7. O'Lorcain P, Holland CV. The public health importance of *Ascaris lumbricoides*. Parasitology 2000;121(suppl):S51-71.
8. Sakai S, Shida Y, Takahashi N, Yabuuchi H, Soeda H, Okafuji T, et al. Pulmonary lesions associated with visceral larva migrans due to *Ascaris suum* or *Toxocara canis:* imaging of six cases. Am J Roentgenol 2006;186:1697-702.
9. Izumikawa K, Kohno Y, Izumikawa K, Hara K, Hayashi H, Maruyama H, et al. Eosinophilic pneumonia due to visceral larva migrans possibly caused by *Ascaris suum:* a case report and review of recent literatures. Jpn J Infect Dis 2011;64:428-32.
10. Xu Z, Fan Y, Wang GS, Wu GM. Muscle pain, fever, cough, and progressive dyspnea in a woman with eosinophilic pneumonia. Genet Mol Res 2015;14:4189-94.
11. Okada F, Ono A, Ando Y, Yotsumoto S, Yotsumoto S, Tanoue S, et al. Pulmonary computed tomography findings of visceral larva migrans caused by *Ascaris suum*. J Comput Assist Tomogr 2007;31:402-8.
12. Moncayo AL, Vaca M, Oviedo G, Workman LJ, Chico ME, Platts-Mills TA, et al. Effects of geohelminth infection and age on the associations between allergen-specific IgE, skin test reactivity and wheeze: a case–control study. Clin Exp Allergy 2013;43:60-72.
13. Buendía E, Zakzuk J, Mercado D, Alvarez A, Caraballo L. The IgE response to *Ascaris* molecular components is associated with clinical indicators of asthma severity. World Allergy Organ J 2015;8:8.
14. Alcantara-Neves NM, Badaro SJ, dos Santos MC, Pontes-de-Carvalho L, Barreto ML. The presence of serum anti–*Ascaris lumbricoides* IgE antibodies and of *Trichuris trichiura* infection are risk factors for wheezing and/or atopy in preschool-aged Brazilian children. Respir Res 2010;11:114.
15. Takeuchi H, Khan AF, Yunus M, Hasan MI, Hawlader MDH, Takanashi S, et al. Anti-*Ascaris* immunoglobulin E associated with bronchial hyper-reactivity in 9-year-old rural Bangladeshi children. Allergol Int 2016;65:141-6.
16. Figueiredo CA, Barreto ML, Rodrigues LC, Cooper PJ, Silva NB, Amorim LD, et al. Chronic intestinal helminth infections are associated with immune hyporesponsiveness and induction of a regulatory network. Infect Immun 2010;78: 3160-7.
17. McSharry C, Xia Y, Holland CV, Kennedy MW. Natural immunity to *Ascaris lumbricoides* associated with immunoglobulin E antibody to ABA-1 allergen and inflammation indicators in children. Infect Immun 1999;67:484-9.
18. Bhattacharyya T, Santra A, Majumder DN, Chatterjee BP. Possible approach for serodiagnosis of ascariasis by evaluation of immunoglobulin G_4 response using *Ascaris lumbricoides* somatic antigen. J Clin Microbiol 2001;39:2991-4.
19. Nogueira DS, Gazzinelli-Guimaraes PH, Barbosa FS, Resende NM, Silva CC, de Oliveira LM, et al. Multiple exposures to *Ascaris suum* induce tissue injury and mixed Th2/Th17 immune response in mice. PLoS Negl Trop Dis 2016;10: e0004382.
20. Chen F, Wu W, Millman A, Craft JF, Chen E, Patel N, et al. Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. Nat Immunol 2014;15:938.
21. Marsland BJ, Kurrer M, Reissmann R, Harris NL, Kopf M. *Nippostrongylus brasiliensis* infection leads to the development of emphysema associated with the induction of alternatively activated macrophages. Eur J Immunol 2008;38:479-88.
22. Long SR, Lanter BB, Pazos MA, Mou H, Barrios J, Su CW, et al. Intestinal helminth infection enhances bacteria-induced recruitment of neutrophils to the airspace. Sci Rep 2019;9:15703.
23. Zuk M, McKean KA. Sex differences in parasite infections: patterns and processes. Int J Parasitol 1996;26:1009-23.
24. López-Griego L, Nava-Castro KE, López-Salazar V, Hernández-Cervantes R, Tiempos Guzmán N, Muñiz-Hernández S, et al. Gender-associated differential expression of cytokines in specific areas of the brain during helminth infection. J Interferon Cytokine Res 2015;35:116-25.
25. Feleke BE, Beyene MB, Feleke TE, Jember TH, Abera B. Intestinal parasitic infection among household contacts of primary cases, a comparative cross-sectional study. PLoS One 2019;14:e0221190.
26. Pham-Duc P, Nguyen-Viet H, Hattendorf J, Zinsstag J, Phung-Dac C, Zurbrügg C, et al. *Ascaris lumbricoides* and *Trichuris trichiura* infections associated with wastewater and human excreta use in agriculture in Vietnam. Parasitol Int 2013; 62:172-80.
27. Chen L, Zhang S, Pan X, Hu X, Zhang YH, Yuan F, et al. HIV infection alters the human epigenetic landscape. Gene Ther 2019;26:29-39.
28. Chiariotti L, Coretti L, Pero R, Lembo F. Epigenetic alterations induced by bacterial lipopolysaccharides. Adv Exp Med Biol 2016;879:91-105.
29. Marimani M, Ahmad A, Duse A. The role of epigenetics, bacterial and host factors in progression of *Mycobacterium tuberculosis* infection. Tuberculosis (Edinb) 2018;113:200-14.
30. Sagonas K, Meyer BS, Kaufmann J, Lenz TL, Häsler R, Eizaguirre C. Experimental parasite infection causes genome-wide changes in DNA methylation. Mol Biol Evol 2020;37:2287-99.
31. DiNardo AR, Nishiguchi T, Mace EM, Rajapakshe K, Mtetwa G, Kay A, et al. Schistosomiasis induces persistent DNA methylation and tuberculosis-specific immune changes. J Immunol 2018;201:124-33.
32. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, et al. Standardisation of the measurement of lung volumes. Eur Respir J 2005;26:511-22.
33. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012;40:1324-43.
34. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 2014;30:1363-9.
35. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics 2012;28:882-3.
36. Teschendorff AE, Marabita F, Lechner M, Bartlett T, Tegner J, Gomez-Cabrero D, et al. A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. Bioinformatics 2013;29: 189-96.

37. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47.
38. Houseman EA, Kile ML, Christiani DC, Ince TA, Kelsey KT, Marsit CJ. Reference-free deconvolution of DNA methylation data and mediation by cell composition effects. BMC Bioinformatics 2016;17:259.
39. Turner S. Qqman: an R package for visualizing GWAS results using Q-Q and Manhattan plots. J Open Source Softw 2018;3:751.
40. Peters TJ, Buckley MJ, Statham AL, Pidsley R, Samaras K, R VL, et al. *De novo* identification of differentially methylated regions in the human genome. Epigenetics Chromatin 2015;8:6.
41. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res 2016;44:W90-7.
42. Breeze CE, Reynolds AP, van Dongen J, Dunham I, Lazar J, Neph S, et al. eFORGE v2.0: updated analysis of cell type-specific signal in epigenomic data. Bioinformatics 2019;35:4767-9.
43. Hamilton RGMP, Adkinson FJ, Chan S, Hovanec-Burns D, Kleine-Tebbe J, et al. Immunological assays for human immunoglobulin E antibodies of defined allergen specificities. Wayne, Pa: Clinical and Laboratory Standards Institute; 2016.
44. Li M, Zou D, Li Z, Gao R, Sang J, Zhang Y, et al. EWAS Atlas: a curated knowledgebase of epigenome-wide association studies. Nucleic Acids Res 2018;47(Database issue):D983-8.
45. May K, Scheper C, Brügemann K, Yin T, Strube C, Korkuć P, et al. Genome-wide associations and functional gene analyses for endoparasite resistance in an endangered population of native German Black Pied cattle. BMC Genomics 2019;20:277.
46. Colvin SC, Maier B, Morris DL, Tersey SA, Mirmira RG. Deoxyhypusine synthase promotes differentiation and proliferation of T helper type 1 (Th1) cells in autoimmune diabetes. J Biol Chem 2013;288:36226-35.
47. Kersting D, Krüger M, Sattler JM, Mueller AK, Kaiser A. A suggested vital function for *eIF-5A* and *dhs* genes during murine malaria blood-stage infection. FEBS Open Bio 2016;6:860-72.
48. Chawla B, Kumar RR, Tyagi N, Subramanian G, Srinivasan N, Park MH, et al. A unique modification of the eukaryotic initiation factor 5A shows the presence of the complete hypusine pathway in *Leishmania donovani*. PLoS One 2012;7:e33138.
49. Ong BA, Li J, McDonough JM, Wei Z, Kim C, Chiavacci R, et al. Gene network analysis in a pediatric cohort identifies novel lung function genes. PLoS One 2013;8:e72899.
50. Cooper PJ. Interactions between helminth parasites and allergy. Curr Opin Allergy Clin Immunol 2009;9:29-37.
51. Mohammadzadeh I, Rostami A, Darvish S, Mehravar S, Pournasrollah M, Javanian M, et al. Exposure to *Ascaris lumbricoides* infection and risk of childhood asthma in north of Iran. Infection 2019;47:991-9.
52. Bragagnoli G, Silva MT. *Ascaris lumbricoides* infection and parasite load are associated with asthma in children. J Infect Dev Ctries 2014;8:891-7.
53. Hawlader MD, Ma E, Noguchi E, Itoh M, Arifeen SE, Persson L, et al. *Ascaris lumbricoids* infection as a risk factor for asthma and atopy in rural Bangladeshi children. Trop Med Health 2014;42:77-85.
54. Rîpă C, Bahnea RG, Cojocaru I, Luca MC, Leon M, Luca M. [Sensitization to *Ascaris lumbricoides* and asthma severity in children]. Rev Med Chir Soc Med Nat Iasi 2011;115:387-91.
55. Wilkinson HN, Hardman MJ. The role of estrogen in cutaneous ageing and repair. Maturitas 2017;103:60-4.
56. Wend K, Wend P, Krum SA. Tissue-specific effects of loss of estrogen during menopause and aging. Front Endocrinol (Lausanne) 2012;3:19.
57. Hepworth MR, Hardman MJ, Grencis RK. The role of sex hormones in the development of Th2 immunity in a gender-biased model of *Trichuris muris* infection. Eur J Immunol 2010;40:406-16.
58. Coakley G, Volpe B, Bouchery T, Shah K, Butler A, Geldhof P, et al. Immune serum-activated human macrophages coordinate with eosinophils to immobilize *Ascaris suum* larvae. Parasite Immunol 2020;e12728.
59. Lin AH, Nepstad I, Florvaag E, Egaas E, Van Do T. An extended study of seroprevalence of anti–*Anisakis simplex* IgE antibodies in Norwegian blood donors. Scand J Immunol 2014;79:61-7.
60. Gazzinelli-Guimaraes PH, Bennuru S, de Queiroz Prado R, Ricciardi A, Sciurba J, Kupritz J, et al. House dust mite sensitization drives cross-reactive immune responses to homologous helminth proteins. PLoS Pathog 2021;17:e1009337.
61. Thomsen GF, Schlünssen V, Skadhauge LR, Malling TH, Sherson DL, Omland Ø, et al. Are allergen batch differences and the use of double skin prick test important? BMC Pulm Med 2015;15:33.