DR. MARIO BAUER (Orcid ID : 0000-0001-5752-038X)
PROF. IRINA LEHMANN (Orcid ID : 0000-0001-8875-5587)

Article type : Letter to the Editor

To the Editor

Longitudinal trends of serum IgE and *IL5RA* expression throughout childhood are associated with asthma but not with persistent wheeze

Short title: Longitudinal IgE and *IL5RA* in childhood asthma and wheeze

Matthias Klös, MSc[1,2], Loreen Thürmann, PhD[1,2], Mario Bauer, MD[2], Joachim Heinrich PhD[3,4], Marie Standl, PhD[4], Andrea von Berg, MD[5], Beate Schaaf, MD[6], Dieter Weichenhan, PhD[7], Oliver Mücke[7], Christoph Plass, PhD[7], Stefan Röder, PhD[2], Gunda Herberth, PhD[2], Ulrich Sack, MD[8], Michael Borte, MD[9], Gabriele I. Stangl, PhD[10], Roland Eils, PhD[11,12,], Saskia Trump, PhD[1,2#] & Irina Lehmann, PhD[1,2#*]

1 Molecular Epidemiology Unit, Charité - Universitätsmedizin Berlin† and Berlin Institute of Health, Berlin, Germany‡
2 Department of Environmental Immunology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany
3 Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, LMU, Munich, Germany
4 Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany
5 Department of Pediatrics, Research Institute, Marien-Hospital Wesel, Wesel, Germany

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13837

[6] Pediatric Outpatient Department, Bad Honnef, Germany
[7] Division of Cancer Epigenomics, German Cancer Research Center (DKFZ), Heidelberg, Germany
[8] University of Leipzig, Medical Faculty, Institute for Clinical Immunology, Leipzig, Germany
[9] Children's Hospital, Municipal Hospital "St. Georg", Leipzig, Germany
[10] Institute of Agricultural and Nutritional Sciences, Martin Luther University Halle-Wittenberg, Halle, Germany
[11] Charité - Universitätsmedizin Berlin† and Berlin Institute of Health, Center for Digital Health, Berlin, Germany
[12] Heidelberg University Hospital, Health Data Science Unit, Heidelberg, Germany

#equal contribution

† corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin

‡ associated partner of the German Center for Lung Research (DZL)

***Corresponding author:**

Prof. Dr. Irina Lehmann, Molecular Epidemiology Unit, Charité - Universitätsmedizin Berlin and Berlin Institute of Health, Kapelle-Ufer 2, 10117 Berlin, Phone: +4930-450-543081, Fax: +4930-450-7-576902, mail: irina.lehmann@charite.de

Source of funding:

This work was supported by the Helmholtz Initiative on Personalized Medicine (iMed) and the Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE). S. Trump was supported by the Federal Ministry of Education and Research grant 01ZX1402D.

Statement of conflicts of interest

All authors have declared that they have no conflict of interest.

Key words: asthma, IgE, *IL5RA*, LINA study, LISA study, wheeze

To the Editor

Wheezing episodes are common in young infants affecting every third child before the age of three (1, 2). While wheezing episodes in early life are mostly triggered by viral lower respiratory tract infection, the development of asthma and airway hyperresponsiveness seem to be promoted by Immunoglobulin E (IgE)-mediated lung inflammation (3-5). Although the development of an asthma phenotype not necessarily requires an atopic status, early childhood asthma is mostly associated with atopy while non-atopic, intrinsic asthma is a rather rare condition (3, 4). Considering the critical role of IgE in the development of impaired lung function in early childhood, several studies tried to differentiate children with transient wheezing episodes from those with persistent wheeze based on early childhood serum IgE levels providing evidence that persistently wheezing children seem to have elevated IgE levels already very early in life (6). Notably, although not every child with persistent or late-onset wheeze will be diagnosed with asthma, none of the earlier studies focusing on IgE, wheeze, and asthma distinguished between persistent wheeze and asthma (e.g. (4, 6)). Thus, one major aim of the present study was to evaluate the longitudinal association between serum IgE levels, wheeze, and asthma considering persistent wheeze and asthma as independent endotypes. In addition to serum IgE, we included interleukin 5 receptor α (*IL5RA*)

measured by qPCR from whole blood RNA in our analyses as *IL5RA*, has been closely linked to both IgE and asthma development (7).

Data and samples from two population-based German birth cohorts, LINA (8) and LISA (9), were used for this study. While the majority of birth cohorts dispose of only few blood sampling time points, the LINA study with annual clinical visits and blood sampling until the age of eight offers the opportunity to study the timing of sensitization, wheezing symptoms, and asthma onset in a tightly time-resolved manner. The LISA study with available blood samples at age two, six, ten and 15 was used as a replication cohort. For the current analyses, only children with longitudinal blood samples (LINA age one to eight; n=98; LISA age two, six, ten, and 15, n=453) and available RNA (LINA age one, four and eight; LISA age 15) were included (for the description of the study population see Figure S1, Table S1, supplementary methods). Wheezing children without an asthma diagnosis were grouped according to their wheezing endotypes: transient (reported wheezing up to the age of three but not thereafter), late-onset (wheezing reported after age three), and persistent wheeze (wheezing during the first three years of life and at least one time thereafter). These children were compared to apparently healthy controls and children diagnosed with asthma respectively. The asthma outcome was defined according to the question asked at each follow-up (see Figure S1 in the Supporting Information): "Has a physician diagnosed your child with asthma during the past 12 months?". Within the studied LINA and LISA subpopulations 10.2% (10/98) and 10.8% (49/453) of the children were ever diagnosed with asthma respectively.

As expected, in both cohorts the specific IgE concentrations (sIgE) (Figure 1A) and sensitization percentages (Figure S2 in the Supporting Information) against aeroallergens (sx1/rx1) were significantly different between groups over time with

highest levels in asthmatic children (One-way Repeated Measurement ANOVA, Figure 1A). Compared to healthy controls, LINA children diagnosed with asthma later in life showed significantly higher sIgE levels already starting at age four (post hoc comparison by Dunnett's test, Table 1A). From age six onward in both cohorts, asthmatic children also showed significantly higher sIgE concentrations compared to all wheezing endotypes including persistent wheeze (Table 1 A/B). Similarly, total IgE (tIgE) was significantly different between groups over time (Figure 1A). Although, differences in tIgE between wheezing endotypes and asthmatic children were less clear and consistent compared to sIgE (Table 1C/D).

Asthmatic children twice as often had high IgE concentrations (sIgE> 0.35 kU/l; tIgE>100 kU/l) compared to healthy controls or any of the wheezing endotypes (Figure S2 in the Supporting Information). Only in LINA, late-onset wheezers also showed high sIgE levels, which most likely was related to those children not yet diagnosed with asthma during our observation period until the age of eight. Noteworthy, the persistent wheezing group was rather more similar to the healthy controls than to the asthma group.

Similarly to IgE, *IL5RA* mRNA expression was significantly increased only in children with asthma compared to controls and all wheezing endotypes starting at age four (Figure 1B). As demonstrated by adjusted general estimation equations (adjGEE), tIgE, sIgE, and *IL5RA* showed a significant longitudinal association with the development of asthma (Table 1E) but not with persistent wheezing or any other of the wheezing endotypes in both cohorts (IgE: Figure S3 in the Supporting Information, *IL5RA*: data not shown). Within the LINA study, an increased expression of *IL5RA* was observed already at the age of four in children diagnosed with asthma (Figure 1B). Subsequent mediation analyses revealed that *IL5RA* expression transmits the effect of tIgE on

asthma, as shown by a significant direct (unstandardized b=0.41, CI=0.19-0.64) and indirect (unstandardized b=0.16, CI=0.06-0.30) effect in a meta-analysis based on LINA and LISA data (Figure 1C).

IL5RA is expressed on eosinophils, basophils, mast cells, and B cells. Unfortunately, we neither determined these cell types in the blood nor do we have protein data that might account for functional properties. Therefore, we were not able to identify the cellular source of this receptor in the context of asthma development within this study. A further limitation of this study is the parent reported physician-diagnosed asthma, which might be less accurate as a diagnosis made in a clinical setting. In addition, there might be misdiagnosed asthma children within the persistent and late-onset wheezing subgroups. Together with the low numbers of patients in the different subgroups, these limitations might have reduced the power of this study.

Nevertheless, this study provides interesting new data of potential clinical relevance. For the first time, we show high resolved longitudinal IgE and *IL5RA* data in children with wheezing symptoms and asthma compared to healthy controls. Based on these data a clear difference between persistent wheezing and asthma evolved, underlining the necessity to consider both endotypes as different entities. Results of the mediation analyses suggest that the development of asthma requires both a high IgE level and an increase in *IL5RA* mRNA expression. Furthermore, there is some evidence from the LINA study that *IL5RA* activation, studied here as *IL5RA* mRNA expression, may occur already before the asthma phenotype is established (see Figure S4 in the Supporting Information showing *IL5RA* mRNA expression at the age of four in those 7 children diagnosed with asthma later in life). Although this result is based on a small case number and therefore has to be interpreted with caution, our data may encourage further studies in this direction. In early life, high IgE levels and concomitant increase in

IL5RA mRNA expression might help to distinguish children developing asthma from those children with wheezing symptoms but who never will suffer from asthma.

Acknowledgements

We cordially thank the participants of the LINA and LISA studies and Beate Fink, Anne Hain, and Melanie Bänsch for their excellent technical assistance and fieldwork.

Statement of contribution

Each named author has substantially contributed to this paper. Irina Lehmann, Gunda Herberth, Stefan Röder, and Michael Borte were involved in the development of the study design and the field work. Mario Bauer, Gunda Herberth, Ulrich Sack, Dieter Weichenhan, Oliver Mücke, and Christoph Plass were involved in sample analyses. Matthias Klös, Loreen Thürmann, Stefan Röder, Saskia Trump, and Irina Lehmann contributed to the statistical analysis. Saskia Trump, Gabriele I. Stangl, and Roland Eils contributed to the discussion of the data. Matthias Klös, Saskia Trump, and Irina Lehmann wrote the paper.

References

1. Bloomberg GR. Recurrent wheezing illness in preschool-aged children: assessment and management in primary care practice. Postgrad Med. 2009;121(5):48-55.
2. Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. Tucson children's respiratory study: 1980 to present. Journal of Allergy and Clinical Immunology. 2003;111(4):661-75.
3. Just J, Bourgoin-Heck M, Amat F. Clinical phenotypes in asthma during childhood. Clin Exp Allergy. 2017;47(7):848-55.
4. Peat JK, Toelle BG, Dermand J, van den Berg R, Britton WJ, Woolcock AJ. Serum IgE levels, atopy, and asthma in young adults: results from a longitudinal cohort study. Allergy. 1996;51(11):804-10.
5. Kusel MM, de Klerk NH, Kebadze T, Vohma V, Holt PG, Johnston SL, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. J Allergy Clin Immunol. 2007;119(5):1105-10.
6. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med. 1995;332(3):133-8.
7. Virkud YV, Kelly RS, Croteau-Chonka DC, Celedon JC, Dahlin A, Avila L, et al. Novel eosinophilic gene expression networks associated with IgE in two distinct asthma populations. Clin Exp Allergy. 2018;48(12):1654-64.
8. Trump S, Bieg M, Gu Z, Thurmann L, Bauer T, Bauer M, et al. Prenatal maternal stress and wheeze in children: novel insights into epigenetic regulation. Sci Rep. 2016;6:28616.
9. Tischer C, Standl M, Lehmann I, Schaaf B, von Berg A, Heinrich J. Sleeping on animal fur is related to asthma outcomes in later childhood. Eur Respir J. 2015;46(1):107-14.

Table 1A: Dunnett's test (p-value) comparing specific IgE against aeroallergens (sx1/rx1) between asthmatic and non-asthmatic children of controls and wheezing endotypes in the LINA cohort. P-value < 0.05 are indicated in red.

compared phenotypes			age 1	age 2	age 3	age 4	age 5	age 6	age 8
asthma	vs	controls	1.0	0.952	0.291	**0.028**	**0.0001**	**0.0001**	**0.0001**
		persistent	1.0	0.978	0.525	0.095	**0.0006**	**0.0001**	**0.0001**
		late	1.0	1.000	0.977	0.946	0.138	**0.044**	**0.011**
		transient	1.0	0.958	0.460	**0.040**	**0.0002**	**0.0001**	**0.0001**

Table 1B: Dunnett's test (p-value) comparing specific IgE against aeroallergens (sx1/rx1) between asthmatic and non-asthmatic children of controls, and wheezing endotypes in the LISA cohort.

compared phenotypes			age 2	age 6	age 10	age 15
asthma	vs	controls	1.0	**0.0001**	**0.0001**	**0.0001**
		persistent	1.0	**0.0009**	**0.0001**	**0.0001**
		late	1.0	**0.0019**	**0.0001**	**0.0001**
		transient	1.0	**0.0001**	**0.0001**	**0.0001**

Table 1C: Dunnett's test (p-value) comparing total IgE between asthmatic and non-asthmatic children of controls and wheezing endotypes in the LINA cohort.

compared phenotypes			age 1	age 2	age 3	age 4	age 5	age 6	age 8
asthma	vs	controls	0.969	0.204	0.062	**0.005**	**0.041**	**0.001**	**0.017**
		persistent	0.983	0.253	0.150	**0.017**	0.123	0.058	0.074
		late	0.936	0.292	0.196	0.090	0.182	**0.026**	0.218
		transient	1.000	0.515	0.318	0.051	0.169	**0.011**	**0.004**

Table 1D: Dunnett's test (p-value) comparing total IgE between asthmatic and non-asthmatic children of controls, and wheezing endotypes in the LISA cohort.

compared phenotypes			age 2	age 6	age 10	age 15
asthma	vs	controls	0.917	**0.0001**	**0.0001**	**0.0001**
		persistent	0.998	**0.015**	**0.0001**	**0.0001**
		late	0.997	**0.024**	**0.0001**	**0.0001**
		transient	0.993	**0.015**	**0.0001**	**0.0001**

Table 1E: Longitudinal association of specific IgE (aeroallergens Sx1/Rx1), total IgE, and *IL5RA* expression of asthmatic children compared to healthy controls in the LINA and LISA cohort (wheezing children were excluded from these analyses; results for wheezing endotypes see Figure S3). Given are ORs with upper and lower 95% confidence intervals and p-values from adjusted* general estimation equations.

	LINA			LISA		
	OR	**CI**	**p-value**	**OR**	**CI**	**p-value**
sIgE	1.13	1.03-1.22	0.006	1.46	1.33-1.59	<0.0001
tIgE	1.20	1.06-1.35	0.003	1.71	1.38-2.11	<0.0001
IL5RA	1.04	1.03-1.06	0.0001		---#	

*adjusted for: gender, maternal history of atopy, parental educational level, mode of delivery, and prenatal tobacco smoke exposure (cotinine level).
RNA only available in the LISA cohort for age 15.

Figure captions

Figure 1 A: Longitudinal pattern of specific and total serum IgE levels in the LINA and LISA cohorts. Given are mean +/- SEM. A repeated measurement one-way ANOVA was applied to determine the difference between groups over time. **B: Comparison of relative *IL5RA* gene expression** in asthma, different wheezing endotypes, and controls in the LINA and the LISA cohort, respectively. One-way ANOVA followed by Dunnett´s post hoc test was applied to determine the difference between asthma and the other groups. **C: Mediation meta-analysis for the relationship of tIgE, *IL5RA* expression and asthma development** for 8-year-old children in LINA and 15-year-old children in LISA together. Models were adjusted for gender, maternal history of atopy, mode of delivery and prenatal tobacco smoke exposure. The effect sizes for each path of the mediation analysis are given as unstandardized b-values with lower and upper confidence intervals ($p< 0.05$ *; $p<0.01$**; $p<0.001$***; $p<0.0001$****).

Figure S1: Overview of the subcohorts with complete longitudinal questionnaire and IgE data at all timepoints. An additional prerequisite was the availability of mRNA samples at selected time points.

Figure S2: Percentages of children with a positive sensitization to aeroallergens (>0.35 kU/l) or elevated total serum IgE (>100 kU/l) given for each time point. Fisher exact test was applied to determine differences between asthmatic children compared to controls and wheezing endotypes (* p-value< 0.05).

Figure S3: Longitudinal association of specific serum IgE against aeroallergens (A) and total IgE (B) to respiratory outcomes in the LINA and LISA cohorts. GEEs considered IgE concentrations determined at age one to six, and age eight for LINA, for LISA IgE concentrations from age two, six, ten and 15 were considered. Given are ORs with upper and lower 95% CIs from GEEs adjusted for gender, maternal history of atopy, parental educational level, mode of delivery and prenatal tobacco smoke exposure.

Figure S4: Differential expression of *IL5RA* in 4-year-old children considering in the asthma group only those children who were first diagnosed with asthma later in life (n=7).

Figure 1