High-sensitivity C-reactive protein and allergic endpoints in German adolescents

Bo-Yi Yang, PhD[1,2,3,#]; Iana Markevych, PhD[2,3,4,#]; Carla Harris, PhD[3,6]; Marie Standl, PhD[3]; Tamara Schikowski, PhD[5]; Sibylle Koletzko, Prof[6]; Gunda Herberth, PhD[7]; Carl-Peter Bauer, Prof[8]; Andrea von Berg, PhD[9]; Dietrich Berdel, Prof[9]; Guang-Hui Dong, Prof[1]; Joachim Heinrich, Prof[2,3,10,*]

1. Guangdong Provincial Engineering Technology Research Center of Environmental Pollution and Health Risk Assessment, Guangzhou Key Laboratory of Environmental Pollution and Health Risk Assessment, Department of Preventive Medicine, School of Public Health, Sun Yat-sen University, Guangzhou, China.
2. Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, LMU Munich, Munich, Germany.
3. Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany.
4. Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, Munich, Ludwig Maximilian University of Munich, Munich, Germany.
5. IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany.
6. Division of Pediatric Gastroenterology and Hepatology, Dr. von Hauner

Children's Hospital Munich, Ludwig Maximilian University of Munich, Germany.

7. Department of Environmental Immunology/Core Facility Studies, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany.
8. Department of Pediatrics, Technical University of Munich, Munich, Germany.
9. Research Institute, Department of Pediatrics, Marien-Hospital Wesel, Wesel, Germany.
10. Allergy and Lung Health Unit, Melbourne School of Population and Global Health, University of Melbourne, Melbourne, Australia.

[#] These authors contributed equally to this article.

[*]**Corresponding author:**

Prof. Dr. Joachim Heinrich

Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, LMU Munich, Germany

Ziemssenstrasse 1, 80336 Muenchen

Tel: +49 89 440053251

e-mail: joachim.heinrich@med.uni-muenchen.de

Funding

The GINIplus study was mainly supported for the first 3 years by the Federal Ministry for Education, Science, Research and Technology (interventional arm) and Helmholtz Zentrum Munich (former GSF) (observational arm). The 4 year, 6 year and 10 year follow-up examinations of the GINIplus study were covered from the respective budgets of the 5 study centres (Helmholtz Zentrum Munich (former GSF), Marien-Hospital Wesel, LMU Munich, TU Munich and from 6 years onward also from IUF—Leibniz Research-Institute for Environmental Medicine) and a grant from the Federal Ministry for Environment (IUF, FKZ 20462296). The LISA study was mainly supported by grants from the Federal Ministry for Education, Science, Research and Technology and in addition from Helmholtz Zentrum Munich (former GSF), Helmholtz Centre for Environmental Research—UFZ, Leipzig, Marien-Hospital Wesel, Pediatric Practice, Bad Honnef for the first 2 years. The 4 year, 6 year and 10 year follow-up examinations of the LISA study were covered from the respective budgets of the involved partners (Helmholtz Zentrum Munich (former GSF), Helmholtz Centre for Environmental Research—UFZ, Leipzig, Marien-Hospital Wesel, Pediatric Practice, Bad Honnef, IUF—Leibniz-Research Institute for Environmental Medicine) and in addition by a grant from the Federal Ministry for Environment (IUF, FKZ 20462296). The recent 15 year follow-up examinations of the GINIplus and LISA studies were supported by the Commission of the European Communities, the 7th Framework Program (MeDALL project) and the Mead Johnson and Nestlé companies (GINIplus only). B-YY was supported by the

National Natural Science Foundation of China (No.81703179). The aforementioned funding sources had no involvement in the design of the study, collection, analysis and interpretation of data, writing of the report and decision to submit the article for publication.

Capsule Summary

High-sensitivity C-reactive protein levels are not associated with any of the allergic endpoints including allergic sensitization, asthma, eczema, and allergic rhinitis in German adolescents.

Keywords: high-sensitivity C-reactive protein, eczema, asthma, allergic rhinitis, allergic sensitization, adolescent

To the Editor:

According to the hygiene hypothesis, infections in childhood might be beneficial for modulating immune tolerance and the subsequent development of allergic disorders.[1] High-sensitivity C-reactive protein (hs-CRP) is a marker of low-grade systemic inflammation, which has been closely linked to many non-communicable diseases (NCD).[2] Childhood allergic diseases are considered as the earliest debuting NCD.[2] Thus, exploring the relationship between hs-CRP and allergies may be valuable not only for understanding the mechanisms of allergic disease development but also for early NCD prevention. However, only a handful of epidemiological studies so far have investigated the relationship between hs-CRP levels and concurrent or later allergic outcomes in children and adolescents, and the findings were mixed (these findings are summarized in Table S1).[3-7] Most of these studies adopted a cross-sectional design[3,4,7] and had a small sample size.[3-6] Therefore, we sought to re-examine the interrelation between hs-CRP levels and allergic outcomes using a larger population size and a longitudinal study design.

We collected data on hs-CRP levels and six allergic outcomes (i.e., doctor-diagnosed asthma, eczema, and allergic rhinitis as well as any sensitization, food sensitization, and aeroallergen sensitization), in 10- and 15-year-old German adolescents from two German birth cohorts – the "German Infant Study on the influence of Nutrition Intervention plus environmental and genetic influences on allergy development" (GINIplus) study and the "Influence of Life-Style Factors on the Development of the Immune System and Allergies in East and West Germany" (LISA) study. Approval by

96 the local ethics committees and written consent from all families were obtained. For
97 detailed information on the flow chart of the study participants and study methods,
98 please see the supplemental materials (Figure S1 and Supplemental methods).

99 Compared to the original GINIplus and LISA participants (n = 9085), samples
100 included into the current analysis (n=1955) were more likely to be from the GINIplus
101 intervention or LISA studies, to have atopic parents and parents with high school
102 education (Table S2). Approximately 41.7% and 13.7% of the study participants had
103 hs-CRP levels below detection limit at the age of 10 and 15 years, respectively (Table
104 S3). The prevalence rates of eczema, asthma, and allergic rhinitis were similar at 10
105 and 15 years. The prevalence of food sensitization was higher at 10 years than in
106 15-year participants; aeroallergen sensitization showed an opposite trend.

107 We did not detect any significant association between hs-CRP levels and any of the
108 studied allergic outcomes in the main analysis (Table 1). This finding was consistent
109 across sensitivity analyses (using different definitions for asthma, eczema, and
110 allergic rhinitis (Table S4); restricting analyses to participants without infections
111 during the last 7 days (Table S5); restricting analyses to participants without asthma,
112 allergic rhinitis or eczema for sensitization outcomes only (Table S6); and including
113 additional adjustment (Table S7). Similarly, no associations were detected when
114 associations in 10- and 15-year old adolescents were tested cross-sectionally (data not
115 shown).

116 In agreement with our findings, Livnat and associates failed to detect any significant

association between hs-CRP levels and current asthma in 131 Israeli children aged 6-18 years.[3] In an analysis of 277 Danish children, higher hs-CRP levels at 7 years was associated with an elevated risk of concurrent allergic rhinitis, asthma, and sensitization to aeroallergens, food allergens, or any allergen; yet, no associations were observed between CRP levels at 6 months with later development of allergic outcomes until 7 years.[6] However, in two analyses by Mustonen and colleagues, children with elevated hs-CRP levels were at a decreased risk of allergic sensitization, though not with atopic dermatitis and asthma.[4,5] In addition, a study of 4111 USA children and adolescents (2-19 years) reported that increased hs-CRP levels were significantly associated with an elevated risk of atopy and food allergy.[7] The exact reasons for the mixed findings across the previous studies and our current analysis are unclear, but may be related to heterogeneity in study design, participants' age at assessment of hs-CRP and allergic endpoints, study area, or genetic background; furthermore, chance findings cannot be excluded.

Our study had several strengths in terms of the following three aspects: first, while most of the previous studies collected data on hsCRP or allergic outcomes once, we utilized repeated measurements on both hs-CRP levels and allergic outcomes; second, the population size of our study was large and a rich set of covariates was considered, which reduced a potential for residual confounding; and third, we performed several sensitivity analyses, in particular, to reduce reverse causality (Table S6) and to increase power (Table S4), which demonstrated consistency of the effect estimates.

However, our study is not without limitation. First, although our analysis was based on repeatedly collected data from the prospective cohorts and were analyzed using generalized estimation equation models, we had data on hs-CRP and allergic outcomes collected around the same time, which may have compromised the ability to judge the direction of the studied associations. Second, there can be a critical window (e.g., the first 1000 days of life) for early programing of the immune system,[8] thus measuring of the hs-CRP levels at 10 and 15 years may be too late to reflect the low-grade systemic inflammation status of early childhood. This can also help to explain the null findings observed in our study. Third, study participants were more likely to be initially recruited (and to further participate in the studies) from the families with higher socio-economic status compared to the general German population, which therefore reduces the generalizability of our findings. Fourth, we used only a single marker (hs-CRP) to reflect systemic inflammation, which is actually characterized by a range of indicators, such as higher levels of interleukin 6, interleukin 1B, Tumor Necrosis Factor, and adiponectin.[2] Hs-CRP is an acute-phase protein that raises quickly after a stimulus up to 48 hours, with the plasma half-life of 19 hours. There might hence be an association with other markers of systemic inflammation, but not with hs-CRP.

In summary, our study suggests that there is no association between hs-CRP levels and any of the allergic endpoints including allergic sensitization, asthma, eczema, and allergic rhinitis in German adolescents. More studies are needed to reach a definite conclusion on whether allergic diseases are inflammatory conditions and which

markers, and at which ages, might be most sensitive.

Acknowledgements

We thank all children and parents for their cooperation, and all technical and administrative support staff and medical and field work teams. We are also grateful to all members of the GINIplus and LISA Study Groups.

Conflict of interests

None.

Author information

Bo-Yi Yang, PhD, Guangdong Provincial Engineering Technology Research Center of Environmental Pollution and Health Risk Assessment, Guangzhou Key Laboratory of Environmental Pollution and Health Risk Assessment, Department of Preventive Medicine, School of Public Health, Sun Yat-sen University, Guangzhou, China; Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, LMU Munich, Munich, Germany; Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany.

Iana Markevych, PhD, Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, LMU Munich, Munich, Germany; Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany; Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, Munich, Ludwig Maximilian University of Munich, Munich, Germany.

Carla Harris, PhD, Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany; Division of Paediatric Gastroenterology and Hepatology, Dr. von Hauner Children's Hospital Munich, Ludwig Maximilian University of Munich, Germany.

Marie Standl, PhD, Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany.

Tamara Schikowski, PhD, IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany.

Sibylle Koletzko, Prof, Division of Pediatric Gastroenterology and Hepatology, Dr. von Hauner Children's Hospital Munich, Ludwig Maximilian University of Munich, Germany.

Gunda Herberth, PhD, Department of Environmental Immunology/Core Facility Studies, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

Carl-Peter Bauer, Prof, Department of Pediatrics, Technical University of Munich,

Munich, Germany.

Andrea von Berg, PhD, Research Institute, Department of Pediatrics, Marien-Hospital Wesel, Wesel, Germany.

Dietrich Berdel, Prof, Research Institute, Department of Pediatrics, Marien-Hospital Wesel, Wesel, Germany.

Guang-Hui Dong, Prof, Guangdong Provincial Engineering Technology Research Center of Environmental Pollution and Health Risk Assessment, Guangzhou Key Laboratory of Environmental Pollution and Health Risk Assessment, Department of Preventive Medicine, School of Public Health, Sun Yat-sen University, Guangzhou, China.

Joachim Heinrich, Prof, Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, LMU Munich, Munich, Germany; Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany; Allergy and Lung Health Unit, Melbourne School of Population and Global Health, University of Melbourne, Melbourne, Australia.

Reference

1. Strachan DP. Hay fever, hygiene, and household size. BMJ 1989, 299: 1259-1260.
2. Prescott SL. Early-life environmental determinants of allergic diseases and the wider pandemic of inflammatory non-communicable diseases. J Allergy Clin Immunol 2013, 131: 23-30.
3. Livnat G, Yoseph RB, Nir V, Hakim F, Yigla M, Bentur L. Evaluation of high-sensitivity serum CRP levels compared to markers of airway inflammation and allergy as predictors of methacholine bronchial hyper-responsiveness in children. Lung 2015, 193: 39-45.
4. Mustonen K, Keski-Nisula L, Vaarala O, Pfefferle PI, Renz H, Riedler J, et al. Few associations between high-sensitivity C-reactive protein and environmental factors in 4.5-year-old children. Pediatr Allergy Immunol 2012, 23: 522-528.
5. Mustonen K, Orivuori L, Keski-Nisula L, Hyvarinen A, Pfefferle PI, Riedler J, et al. Inflammatory response and IgE sensitization at early age. Pediatr Allergy Immunol 2013, 24: 395-401.
6. Chawes BL, Stokholm J, Schoos AMM, Fink NR, Brix S, Bisgaard H. Allergic sensitization at school age is a systemic low-grade inflammatory disorder. Allergy 2017, 72: 1073-1080.
7. Visness CM, London SJ, Daniels JL, Kaufman JS, Yeatts KB, Siega-Riz Am, et al. Association of obesity with IgE levels and allergy symptoms in children and adolescents: results from the National Health and Nutritional Examination Survey

237 2005-2006. J Allergy Clin Immunol 2009, 123: 1163-1169.

238 8. Panders J, Stobberingh EE, van den Brandt PA, Thijs C. The role of the intestinal

239 microbiota in the development of atopic disorders. Allergy 2007, 62: 1223-1236.

Table 1. Adjusted ORs with 95% CIs for hs-CRP levels and allergic endpoints estimated using generalized estimating equations models*

		hs-CRP category†				
		I	II		III	
Endpoint	**No. of observations**	**Reference**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Any sensitization	1955	1	0.93 (0.79, 1.09)	0.353	0.99 (0.80, 1.23)	0.929
Food sensitization	1955	1	1.09 (0.87, 1.36)	0.453	1.04 (0.77, 1.41)	0.810
Aeroallergen sensitization	1955	1	0.95 (0.81, 1.11)	0.513	1.03 (0.83, 1.28)	0.762
Asthma	1951	1	0.98 (0.66, 1.46)	0.927	0.77 (0.42, 1.41)	0.400
Eczema	1929	1	1.03 (0.70, 1.51)	0.894	0.85 (0.48, 1.52)	0.592
Allergic rhinitis	1953	1	1.07 (0.82, 1.39)	0.609	1.11 (0.79, 1.58)	0.549

Abbreviations: CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio.

*All models adjusted for time of follow-up, study area, sex, parental income, body mass index, and child smoking at 15 years.

†CRP categories at 10 years: CRP-I, below detection limit (<0.02 mg/dl); CRP-II, ≥0.02 mg/dl and <75th sex-specific percentile of those with CRP ≥0.02 mg/dl; CRP-III, ≥0.02 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.02 mg/dl. CRP categories at 15 years: CRP-I, below detection limit (<0.016 mg/dl); CRP-II, ≥0.016 mg/dl and <75th sex-specific percentile of those with CRP ≥0.016 mg/dl; CRP-III, ≥0.016 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.016 mg/dl.

SUPPLEMENTAL METHODS

Study population

The current analysis is based on the data from two ongoing multicenter population-based prospective birth cohort studies in Germany: the "German Infant Study on the influence of Nutrition Intervention plus environmental and genetic influences on allergy development" (GINIplus) study and the "Influence of Life-Style Factors on the Development of the Immune System and Allergies in East and West Germany" (LISA) study. Detailed information on the cohorts has been published elsewhere[E1, E2]. Briefly, the GINIplus is a two-armed study consisting of 5,991 healthy full-term and normal birth weight newborns recruited at selected maternity wards in Munich and Wesel between 1995 and 1998. The interventional arm included newborns with family history of allergy. The newborns participated in the randomized, double-blind controlled intervention trial with hydrolyzed formulas, including partially hydrolyzed whey, extensively hydrolyzed whey, extensively hydrolyzed casein, or a conventional cow's milk. The observational arm included newborns without family history of allergy, and those whose parents declined participation in the intervention trial. The LISA cohort is a population-based cohort consisting of 3,094 full-term and normal birth weight newborns recruited at selected maternity wards in Munich, Leipzig, Wesel, and Bad Honnef from 1997 to 1999.

In both cohorts, parent-completed questionnaires were administered at birth and when children were 1, 2, 3, 4, 6, 10 and 15 years of age in GINIplus and at 6, 12, 18, and 24 months and 4, 6, 10 and 15 years of age in LISA. Additionally, blood samples were drawn at 6, 10, and 15 years from subgroups of the cohorts.

Approvals for the two cohorts have been obtained from the local ethics committees (Bavarian General Medical Council, University of Leipzig, Medical Council of North-Rhine-Westphalia). All families have signed informed consent.

hs-CRP assessment

Serum hs-CRP concentrations at 10- and 15- years were measured using the Roche (Mannheim, Germany) Tina-quant CRP (latex) high-sensitivity assay. The measured hs-CRP concentrations had highly right-skewed distribution, as many hs-CRP observations were below the detection limits. To facilitate data analysis, we categorized hs-CRP levels into three age- and sex-specific levels[E3]. The hs-CRP categories at 10 years were: CRP-I, below detection limit (<0.02 mg/dl); CRP-II, ≥0.02 mg/dl and <75th sex-specific percentile of those with CRP ≥0.02 mg/dl; and CRP-III, ≥0.02 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.02 mg/dl. hs-CRP categories at 15 years old were: CRP-I, below detection limit (<0.016 mg/dl); CRP-II, ≥0.016 mg/dl and <75th sex-specific percentile of those with CRP ≥0.016 mg/dl; and CRP-III, ≥0.016 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.016 mg/dl.

Allergic endpoints

For the main analysis, all allergic endpoints were defined based on the information collected at the 10- and 15-year follow-ups. Doctor-diagnosed eczema and asthma were defined based on a positive response to the questions "In the past 12 months, was your child diagnosed with eczema/asthma?" Doctor-diagnosed allergic rhinitis was defined based on a positive response to one of the following two questions: "In the past 12 months, has your child been diagnosed with hay fever/allergic rhinitis?"

Specific IgE against common allergens was assessed in serum collected at the 10- and 15-year follow-ups using the standardized CAP-RAST FEIA method (ThermoFischer, Freiburg, Germany). Allergic sensitization to aeroallergens (SX1: house dust mites, cats, dogs, mold, birch, rye, mugwort and timothy grass), as well as allergic sensitization to food allergens (FX5: milk, peanut, eggs, soya, cod and wheat flour), was defined as a specific IgE value

above 0.35 kU/L against SX1 and FX5 allergens, respectively. Any sensitization was defined as an allergic sensitization to either aero- or food allergens.

For a sensitivity analysis, eczema, asthma and allergic rhinitis were defined based on the information collected from birth (eczema) or from 3 years onwards (asthma and allergic rhinitis)[E4]. This was done due to the difficulty of accurate diagnosis of asthma and allergic rhinitis at very young ages[E5]. Each of these three outcomes was defined as satisfying two out three following criteria: (1) doctor diagnosis ever, (2) having symptoms in the last 12 months, and (3) taking medication in the last 12 months.

Covariates

The following potentially important covariates were considered for this analysis: sex, study (GINIplus intervention vs. GINIplus observation vs. LISA), study area (Munich vs. Leipzig vs. Wesel vs. Bad Honnef), time-specific net equivalent household income (defined as time- and city-specific income tertiles due to large income difference among cities, time-specific body mass index (BMI, kg/m^2), time-specific exposure to tobacco smoke at home in the last 12 months, child's smoking status (as ever smoking) at 15 years, parental education level (based on highest parental level of education: both parents with less than 10 years of school (low), at least one parent with 10 years of school (medium), at least one parent with more than 10 years of school (high), classified according to the German education system), and parental history of allergic diseases (self-report of doctor diagnosis of asthma, allergic rhinitis or eczema, collected at birth). Missing values in income variables, which were many, were coded as a separate category.

Statistical analysis

We used generalized estimation equation (GEE) models[E6] with log link and exchangeable correlation structure to assess the associations between hs-CRP levels and allergic endpoints

at 10 and 15 years of age because of the longitudinal design of the current study (i.e., exposure and outcomes). Thus, the results are presented as odds ratios (OR) with corresponding 95% confidence intervals (CIs).

We adjusted main models for time of follow-up and the covariates, which were associated with hs-CRP, as well as at least one of the outcome endpoints. Thus, the main models were adjusted for sex, study area, net equivalent household income, BMI, and child's smoking at 15 years. We also performed several sensitivity analyses. First, we re-ran the models for eczema, asthma, and allergic rhinitis using alternative definitions (Table S4). This was done to achieve larger power to detect possible associations, as prevalence of asthma and eczema based exclusively on doctor diagnosis in the past 12 months were low (Table S3). Second, participants who had infections during the last 7 days prior to blood collection at 10 or 15 years, or participants with such information missing were excluded from the analytic sample, as their CRP levels could have been affected (Table S5). Third, participants who had asthma, eczema, and allergic rhinitis (alternative definitions), or participants with such information missing, were excluded from the analysis with sensitization outcomes (Table S6). Fourth, models were additionally adjusted for the covariates, which were associated with either hs-CRP, or at least one of the allergic endpoints – study, parental education level and parental history of allergic diseases (Table S7). Finally, we explored cross-sectional associations in 10- and 15- year old participants separately by running logistic (instead of GEE) models.

We performed all the statistical analyses using the program R, version 3.5.0 (Vienna, Austria).[E7] GEE models were fitted by the *geeglm* function from the *geepack* package.[E8]

References

E1. von Berg A, Krämer U, Link E, et al. Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course - the GINIplus study up to the age of 6 years. Clin Exp Allergy 2010;40(4);627-636.

E2. Zutavern A, Brockow I, Schaaf B, Bolte G, von Berg A, Diez U, Borte M, Herbarth O, Wichmann HE, Heinrich J; LISA Study Group. Timing of solid food introduction in relation to atopic dermatitis and atopic sensitization: results from a prospective birth cohort study. Pediatrics 2006;117:401–411.

E3. Harris C, Demmelmair H, von Berg A, et al. Associations between fatty acids and low-grade inflammation in children from the LISAplus birth cohort study. Eur J Clin Nutr 2017, 71: 1303-1311.

E4. Lodrup Carlsen KC, Haland G, et al. Asthma in every fifth child in Oslo, Norway: a 10-year follow up of a birth cohort study. Allergy. 2006;61:454–60.

E5. Markevych I, Baumbach C, Standl M, et al. Early life travelling does not increase risk of atopic outcomes until 15 years: results from GINIplus and LISAplus. Clin Exp Allergy 2017, 47: 395-400.

E6. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 1986, 73: 13-22.

E7. R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing: 2012. Available at: http://www.R-project.org/.

E8. Højsgaard S, Halekoh U, Yan J. The R Package geepack for generalized estimation equations. J Stat Software 2006, 15: 1-11.

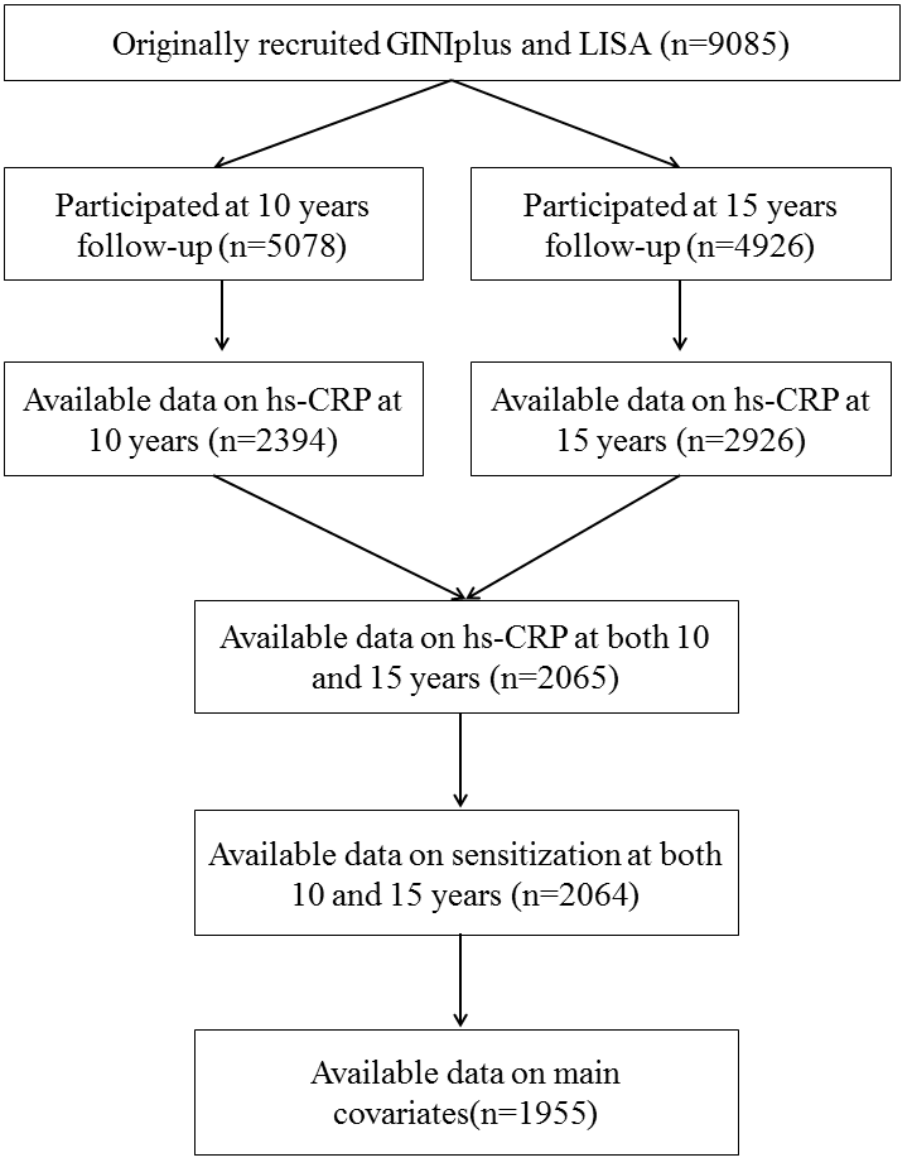

**Figure S1. Flow chart of study participants. ***Main covariates included study area, sex, BMI and child's smoking at 15 years. Missing values in income variables were coded as a separate category.

Table S1 Prior studies on hs-CRP levels and childhood allergic endpoints

Authors (year)	**Country**	**Study design**	**Age of hs-CRP and outcomes assessment**	**Sample size**	**Main findings**
Visness et al. (2009)	United States	Cross-sectional	2-19 years	4111	Elevated CRP levels were associated with a significant increased risk of food allergy (OR = 1.25, 95% CI = 1.01-1.55), and a borderline significant increased risk of atopy (OR = 1.22, 95% CI = 1.00-1.49).
Mustonen et al. 2012	Finland, Germany, Austria, France, Switzerland	Cross-sectional analysis in birth cohort study	4.5 years	653	Children with CRP levels lower than the 75th percentile had a lower risk of sensitization to inhaled allergens and seasonal allergens compared to those with CRP levels below the detection limit. However, no significant further decrease in risk of different sensitizations was observed in those with CRP levels higher than 75th percentile. In addition, no association was detected when CRP was used as a continuous variable.
Mustonen et al. (2013)	Finland, Germany, Austria, France, Switzerland	Longitudinal	1 year and 4.5 years	636	Increased CRP levels at the age of 1 year were associated with a decreased risk of allergic sensitization at the age of 4.5 years only in non-sensitized children at 1 year old. However, no association was observed for the overall population.
Livnat et al. (2015)	Israel	Cross-sectional	6-18 years	131	No significant association was observed between CRP levels and current asthma in children aged 6-18 years.

Chawes et al. (2017)	Denmark	Longitudinal (7 follow-up years)	6 months and 7 years	277	Elevated CRP levels at 7 years were associated with a concurrent (6 years) higher risk of any sensitization, aeroallergen sensitization, food sensitization, asymptomatic sensitization, allergic rhinitis, and asthma. However, CRP levels at 6 months were not associated with later development of allergy endpoints in longitudinal analyses.

Abbreviations: CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio.

Table S2 Baseline characteristics of the originally recruited participants and the participants from the analytic sample, n (%)

Variable	Recruited participants	Analytic sample	p-value*
Study			<0.0001
GINIplus observation	3739 (41.2)	582 (30)	
GINIplus intervention†	2252 (24.8)	652 (33)	
LISA	3094 (34.1)	721 (37)	
Area			<0.0001
Munich	4413 (48.6)	1060 (54)	
Leipzig	976 (10.7)	205 (11)	
Bad Honnef	306 (3.4)	91 (4.7)	
Wesel	3390 (37.3)	599 (31)	
Sex			0.848
Female	4349 (47.9)	957 (49)	
Male	4575 (50.4)	998 (51)	
Missing	161 (1.8)	0 (0.0)	
Parental education§			<0.0001
Low (<10 years)	969 (10.7)	93 (4.8)	
Medium (10 years)	2656 (29.2)	500 (26)	
High (>10 years)	5379 (59.2)	1356 (69)	
Missing	81 (0.9)	6 (0.3)	
Parental history of allergic diseases¶			<0.0001
No	4081 (44.9)	738 (38)	
Yes	4841 (53.3)	1199 (61)	
Missing	163 (1.8)	18 (0.9)	

Abbreviations: GINIplus, the German Infant Study on the influence of Nutrition Intervention plus environmental and genetic influences on allergy; LISAplus, the Immune System and the development of Allergies in childhood study.

*p-value from Chi-Square test.

†Group that participated in the intervention trial with hypoallergenic formulae.

§Definition based on highest parental level of education: both parents with less than 10 years of school (low), at least one parent with 10 years of school (medium), at least one parent with more than 10 years of school (high), classified according to the German education system.

¶Definition based on either of the parents having ever doctor-diagnosed asthma, allergic

rhinitis or eczema

Table S3 Characteristics of the study participants (n=1955)

	Baseline		10 years		15 years	
Variable	**n/N or mean**	**% or SD**	**n/N or mean**	**% or SD**	**n/N or mean**	**% or SD**
Covariates						
Area						
Munich	1060/1955	54.2	-	-	-	-
Leipzig	205/1955	10.5	-	-	-	-
Bad Honnef	91/1955	4.7	-	-	-	-
Wesel	599/1955	30.6	-	-	-	-
Study						
GINIplus observation	582/1955	29.8	-	-	-	-
GINIplus intervention*	652/1955	33.4	-	-	-	-
LISA	721/1955	36.9	-	-	-	-
Sex - female	957/1955	49.0	-	-	-	-
Parental history of allergic diseases - yes	1199/1937	61.9	-	-	-	-
Parental education†						
Low (<10 years)	93/1949	4.8	-	-	-	-
Medium (10 years)	500/1949	25.7	-	-	-	-
High (>10 years)	1356/1949	69.6	-	-	-	-
Child smoking - yes	-	-			142/1955	7.3
Household income						

Low	-	-	563/1955	28.8	545/1955	27.9
Medium	-	-	659/1955	33.7	587/1955	30.0
High	-	-	583/1955	29.8	585/1955	29.9
Missing	-	-	150/1955	7.7	238/1955	12.2
BMI (kg/m^2)[§]	-	-	17.33	2.43	20.79	3.18
Infections last 7 days - yes	-	-	437/1898	23.0	425/1955	21.7
hs-CRP [¶]						
I	-	-	815/1955	41.7	267/1955	13.7
II	-	-	884/1955	45.2	1260/1955	64.5
III	-	-	256/1955	13.1	428/1955	21.9
Outcomes						
Any sensitization - yes	-	-	854/1955	43.7	943/1955	48.2
Food sensitization - yes	-	-	359/1955	18.4	220/1955	11.3
Aeroallergen sensitization - yes	-	-	760/1955	38.9	916/1955	46.9
Asthma - yes [£]	-	-	72/1904	3.8	75/1911	3.9
Asthma using alternative definitions - yes[ø]	-	-	118/1923	6.1	131/1909	6.9
Eczema - yes[£]	-	-	89/1900	4.7	61/1896	3.2
Eczema using alternative definitions - yes[ø]	-	-	215/1924	11.2	176/1907	9.2
Allergic rhinitis - yes[£]	-	-	199/1882	10.6	211/1884	11.2
Allergic rhinitis using alternative definitions - yes[ø]	-	-	224/1881	11.9	351/1888	18.6

Abbreviations: BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; GINIplus, the German Infant Study on the influence of Nutrition Intervention plus environmental and genetic influences on allergy; LISA, the Immune System and the development of Allergies in childhood study.

[*]Group that participated in an intervention trial with hypoallergenic formulae.

[†]Definition based on highest parental level of education: both parents with less than 10 years of school (low), at least one parent with 10 years of school (medium), at least one parent with more than 10 years of school (high), classified according to the German education system.

[§]Mean ± Standard Deviation

[¶]CRP categories at 10 years: CRP-I, below detection limit (<0.02 mg/dl); CRP-II, ≥0.02 mg/dl and <75th sex-specific percentile of those with CRP ≥0.02 mg/dl; CRP-III, ≥0.02 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.02 mg/dl. CRP categories at 15 years: CRP-I, below detection limit (<0.016 mg/dl); CRP-II, ≥0.016 mg/dl and <75th sex-specific percentile of those with CRP ≥0.016 mg/dl; CRP-III, ≥0.016 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.016 mg/dl.

[£]Defined as a parental report of doctor diagnosis during the last 12 months.

[ø]The definitions are based on satisfying two out of three criteria: (1) ever doctor diagnosis from 1 (eczema) or 3 years onwards (asthma and allergic rhinitis), (2) medication use during last 12 months, and (3) allergic diseases symptoms during last 12 months.

Table S4 Adjusted ORs with 95% CIs[*] for hs-CRP levels and alternative definitions of eczema, asthma, and allergic rhinitis [†] estimated using generalized estimation equation models

		hs-CRP category[§]				
		I	**II**		**III**	
Outcome	**No. of observations**	**Reference**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Asthma	1953	1	1.05 (0.76, 1.45)	0.778	0.84 (0.54, 1.33)	0.465
Eczema	1951	1	1.00 (0.77, 1.30)	0.982	0.86 (0.60, 1.24)	0.428
Allergic rhinitis	1948	1	0.92 (0.73, 1.17)	0.506	0.84 (0.61, 1.15)	0.275

Abbreviations: CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio.

[*]All models adjusted for time of follow-up, study area, sex, parental income, body mass index, and child's smoking at 15 years.

[†]The definitions are based on satisfying two out of three criteria: (1) ever doctor diagnosis from 1 (eczema) or 3 years onwards (asthma and allergic rhinitis), (2) medication use during last 12 months, and (3) allergic diseases symptoms during last 12 months.

[§]CRP categories at 10 years: CRP-I, below detection limit (<0.02 mg/dl); CRP-II, ≥0.02 mg/dl and <75th sex-specific percentile of those with CRP ≥0.02 mg/dl; CRP-III, ≥0.02 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.02 mg/dl. CRP categories at 15 years: CRP-I, below detection limit (<0.016 mg/dl); CRP-II, ≥0.016 mg/dl and <75th sex-specific percentile of those with CRP ≥0.016 mg/dl; CRP-III, ≥0.016 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.016 mg/dl.

Table S5 Adjusted ORs with 95% CIs[*] for hs-CRP and allergic outcomes in participants without infections during last 7 days estimated using generalized estimation equation models

		hs-CRP category[†]				
		I	**II**		**III**	
Outcome	**No. of observations**	**Reference**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Any sensitization	1857	1	0.90 (0.75, 1.08)	0.250	0.99 (0.77, 1.28)	0.958
Food sensitization	1857	1	0.96 (0.75, 1.24)	0.777	0.95 (0.66, 1.35)	0.770
Aeroallergen sensitization	1857	1	0.94 (0.79, 1.13)	0.534	1.07 (0.83, 1.38)	0.594
Asthma	1836	1	1.01 (0.65, 1.56)	0.982	0.82 (0.41, 1.64)	0.583
Eczema	1821	1	1.18 (0.76, 1.84)	0.465	0.86 (0.44, 1.68)	0.653
Allergic rhinitis	1824	1	1.00 (0.74, 1.34)	0.994	1.26 (0.85, 1.87)	0.251

Abbreviations: CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio.

[*]All models adjusted for time of follow-up, study area, sex, parental income, body mass index, and child's smoking at 15 years.

[†]CRP categories at 10 years: CRP-I, below detection limit (<0.02 mg/dl); CRP-II, ≥0.02 mg/dl and <75th sex-specific percentile of those with CRP ≥0.02 mg/dl; CRP-III, ≥0.02 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.02 mg/dl. CRP categories at 15 years: CRP-I, below detection limit (<0.016 mg/dl); CRP-II, ≥0.016 mg/dl and <75th sex-specific percentile of those with CRP ≥0.016 mg/dl; CRP-III, ≥0.016 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.016 mg/dl.

Table S6 Adjusted ORs with 95% CIs[*] for hs-CRP and sensitization outcomes in participants without allergic manifestation[†] estimated using generalized estimation equation models

		hs-CRP category[§]				
		I	**II**		**III**	
Outcome	**No. of observations**	**Reference**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Any sensitization	1571	1	0.98 (0.80, 1.20)	0.858	1.13 (0.87, 1.48)	0.354
Food sensitization	1571	1	1.10 (0.81, 1.49)	0.544	0.97 (0.64, 1.48)	0.884
Aeroallergen sensitization	1571	1	1.01 (0.82, 1.24)	0.922	1.20 (0.92, 1.58)	0.181

Abbreviations: CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio.

[*]Adjusted for time of follow-up, study area, sex, parental income, body mass index, and child smoking at 15 years.

[†]Allergic manifestation is defined as no asthma, allergic rhinitis, or eczema using two out of the following three criteria: (1) ever doctor diagnosis from 1 (eczema) or 3 years onwards (asthma and allergic rhinitis), (2) medication use during last 12 months, and (3) allergic diseases symptoms during last 12 months.

[§]CRP categories at 10 years: CRP-I, below detection limit (<0.02 mg/dl); CRP-II, ≥0.02 mg/dl and <75th sex-specific percentile of those with CRP ≥0.02 mg/dl; CRP-III, ≥0.02 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.02 mg/dl. CRP categories at 15 years: CRP-I, below detection limit (<0.016 mg/dl); CRP-II, ≥0.016 mg/dl and <75th sex-specific percentile of those with CRP ≥0.016 mg/dl; CRP-III, ≥0.016 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.016 mg/dl.

Table S7 Additionally adjusted ORs with 95% CIs for hs-CRP levels and allergic outcomes estimated using generalized estimation equation models*

		hs-CRP category†				
		I	II		III	
Outcome	**No. of observations**	**Reference**	**OR (95% CI)**	**p value**	**OR (95% CI)**	**p value**
Any sensitization	1932	1	0.93 (0.79, 1.09)	0.355	0.99 (0.79, 1.23)	0.900
Food sensitization	1932	1	1.08 (0.86, 1.35)	0.512	1.02 (0.75, 1.39)	0.878
Aeroallergen sensitization	1932	1	0.95 (0.80, 1.12)	0.515	1.03 (0.83, 1.28)	0.794
Asthma	1928	1	0.97 (0.65, 1.45)	0.894	0.78 (0.42, 1.43)	0.420
Eczema	1907	1	1.02 (0.69, 1.49)	0.934	0.80 (0.45, 1.44)	0.465
Allergic rhinitis	1930	1	1.07 (0.82, 1.40)	0.607	1.11 (0.77, 1.58)	0.577

Abbreviations: CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio.

*All models adjusted for time of follow-up, study area, sex, parental income, body mass index, and child's smoking at 15 years, study, atopic parents, and parental education level.

† CRP categories at 10 years old: CRP-I, below detection limit (<0.02 mg/dl); CRP-II, ≥0.02 mg/dl and <75th sex-specific percentile of those with CRP ≥0.02 mg/dl; CRP-III, ≥0.02 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.02 mg/dl. CRP categories at 15 years old: CRP-I, below detection limit (<0.016 mg/dl); CRP-II, ≥0.016 mg/dl and <75th sex-specific percentile of those with CRP ≥0.016 mg/dl; CRP-III, ≥0.016 mg/dl and ≥75th sex-specific percentile of those with CRP ≥0.016 mg/dl.