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Maternal iron status during early pregnancy and school-age, lung function, asthma, and allergy: The Generation R Study

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Abstract

Background: Iron deficiency during early life could affect the developing lung and immune system, and influence child's respiratory or allergy outcomes in later life.

Objective: To examine the associations of maternal iron status during early pregnancy with child's lung function, asthma, inhalant allergic sensitization, and physician-diagnosed inhalant allergy at school-age.

Methods: In a population-based cohort study, among 3825 mother–child pairs, ferritin, transferrin concentrations, and transferrin saturation were measured from maternal venous blood samples during early pregnancy. In children at the age of 10 years, spirometry was used to determine child's lung function, current asthma and physician-diagnosed inhalant allergy were assessed by questionnaires, and inhalant allergic sensitization was measured by skin prick tests. We used multivariable regression models to examine the associations.

Results: After adjustment for gestational age at maternal iron status measurement and sociodemographic or lifestyle-related confounders, a higher maternal transferrin concentration was associated with a higher risk of physician-diagnosed inhalant allergy (odds ratio [95% confidence interval]: 1.13 [1.01 to1.26]), but not with lung function, asthma, or inhalant allergic sensitization. This association did not attenuate after further adjustment for maternal hemoglobin levels or early growth factors. We observed no consistent association of maternal ferritin concentrations or transferrin saturation with child's respiratory or allergy outcomes.

Conclusion: Higher maternal transferrin concentrations during pregnancy, reflecting lower serum iron levels, were associated with an increased risk of child's physician-diagnosed inhalant allergy but not lung outcomes. Underlying mechanisms and clinical implications need to be explored.

KEYWORDS

asthma, child, cohort study, iron, lung function

1 | INTRODUCTION

Approximately 22% of all pregnant women in developed countries have anemia.[1] Iron deficiency is the most common cause.[1] Maternal iron deficiency during pregnancy is associated with increased risk of intrauterine growth retardation,[2] which subsequently might lead to a higher risk of respiratory morbidity.[3] A potential underlying mechanism might be that maternal iron deficiency during pregnancy leads to a lower oxygen binding and therefore inadequate oxygen supply through the placenta to the developing fetus.[4] Also, animal studies showed that hypoxic conditions could result in redistribution of the fetal cardiac output leading to a reduction of blood flow to the lungs, and consequently a reduction in lung growth.[5] Additionally, maternal iron deficiency during fetal development may result in a T helper cell 2 (Th2) dominance, which might lead to later life development of asthma and related diseases.[6] Thus, maternal iron deficiency during pregnancy might have an adverse effect on the developing lung and immune system. A population-based exploratory study among 157 subjects found that a lower maternal iron status during pregnancy was associated with a lower forced expiratory volume in 1 s (FEV_1), and a higher risk of wheezing and atopic sensitization in children at the age of 10 years.[7] Also, a population-based prospective cohort study showed that maternal genetic proxies of lower iron status were weakly associated with a lower lung function in children, but only among mothers without iron supplementation.[8] In addition, we previously found a relation of higher maternal hematocrit concentrations during pregnancy with a lower lung function.[9] A randomized control trial showed that, as compared to selective iron supplementation, routine maternal iron supplementation during pregnancy reduced the risk of asthma diagnosis in the children.[10] However, the number of studies on maternal iron status during pregnancy with childhood respiratory and allergy outcomes are scarce, small sample-sized, used genetic variants or hematocrit as a proxy for maternal iron status, or were limited in asthma diagnosis ascertainment. Therefore, the present study aimed to examine among 3825 children and their mothers participating in a population-based prospective cohort study, the associations of maternal iron status during early pregnancy with lung function, asthma, inhalant allergic sensitization, and physician-diagnosed inhalant allergy in school-aged children.

2 | METHODS

2.1 | Design

The current study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in Rotterdam, the Netherlands.[11] The study has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center in Rotterdam (MEC-2012-165-NL40020.078.12). Written informed consent was obtained from the parents or legal representatives of the children. A total of 3825 mother–child pairs were included for current analyses (Figure S1).

2.2 | Maternal iron status

Maternal blood serum samples were collected during early pregnancy (median gestational age 13.5 weeks (95% range: 9.8–17.4 weeks)), and transported and stored as previously described in detail.[12] We focussed on the iron biomarkers ferritin as a measure of iron stores, transferrin as iron transport capacity, and transferrin saturation reflecting the iron-bound part of the total iron-binding capacity.[13] Ferritin was determined by electrochemiluminescence immunoassay on the Cobas e411 analyzer (Roche). Iron was determined by colorimetric assay and transferrin by immunoturbidimetric assay both by the C502 module on the Cobas 8000 (Roche). Transferrin saturation was calculated as (serum iron × 100)/(transferrin × 25.1).[13] To meet the homoscedasticity assumption for the regression models, we natural log-transformed ferritin. Standard deviation scores (SDS; [observed value − mean]/SD) for all iron biomarkers were calculated to enable comparison of the effect estimates.

2.3 | School-age lung function, asthma, and allergy outcomes

Children visited the research center at a mean (*SD*) age of 9.8 (0.4) years. Lung function measures were obtained by spirometry according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations.[14] If spirometry measurements did not meet the criteria for reproducibility ($n = 277$), a single curve that was technically acceptable was included for the analyses, as this did not influence our results. Lung function measures included FEV_1, forced vital capacity (FVC), FEV_1/FVC ratio, and forced expiratory flow at 75% of FVC (FEF_{75}), and values were converted into sex-, height-, age-, and ethnicity-adjusted *z*-scores according to the Global Lung Initiative reference data.[15] Questions translated and validated from the International Study on Asthma and Allergy in Childhood (ISAAC) questionnaires were used to obtain information on physician-diagnosed asthma and inhalant allergy to cats, dogs, house dust mite, or pollen. Current asthma was defined as ever physician-diagnosed asthma with either wheezing or asthma medication use in the past 12 months.[16] Sensitization for birch, cat or dog, or five grass mixture was measured with skin prick tests using the scanned area method at age 10 years (ALK-Abelló B.V.).[17] For a better clinical interpretation, we additionally categorized allergy outcomes into the groups "no inhalant allergic sensitization, no physician-diagnosed inhalant allergy," "inhalant allergic sensitization but no physician-diagnosed inhalant allergy," "no inhalant allergic sensitization but physician-diagnosed inhalant allergy," and "inhalant allergic sensitization and physician-diagnosed inhalant allergy."

2.4 | Covariates

We obtained information on sociodemographic, lifestyle, and growth-related covariates. Information on maternal age, daily energy intake, education (low: no education or primary education; middle: secondary Phase 1 or 2 finished; high: vocational or university degree), European ethnic background based on the country of birth of the participant and her parents (yes; no), parity (nulliparous or multiparous), body mass index, smoking during pregnancy (yes/no), psychological distress during pregnancy (yes; no), and history of asthma or atopy (yes; no) was obtained from questionnaires during pregnancy. Maternal hemoglobin and high sensitivity C-reactive protein (CRP) concentrations were measured from venous blood samples as previously described in detail.[18,19] Information on child's sex, gestational age at birth, and birth weight were obtained from midwife or hospital records. Postnatal questionnaires provided information on every breastfeeding of the child (yes; no).

2.5 | Statistical analysis

Characteristics of children included and non-included in our analyses were compared using independent sampled *t*-test for continuous normally distributed variables, Mann–Whitney *U* test for not normally distributed variables, and X^2 test for categorical variables. We used linear and logistic regression models to study the associations of maternal iron status with child's respiratory and allergy outcomes. In the basic model, we adjusted for gestational age at maternal iron status measurement. In the main model, we additionally adjusted for sociodemographic and lifestyle-related factors including maternal education, ethnic background, parity, body mass index, smoking during pregnancy, psychological distress during pregnancy, history of asthma or atopy, and child's sex and breastfeeding. Selection of confounders was based on literature, if they were related to both maternal iron status during pregnancy and at least one of the respiratory or allergy outcomes, or if the effect estimate changed ≥10% when we included the variable in our model. Consequently, maternal age and daily energy intake during pregnancy showed a changed in ≥10% of the effect estimate and were considered as confounders but not included in our models based on a lack of association with the outcome in the directed acyclic graph (DAG), and a lack of association with both exposure and outcome in the univariate analysis. Additionally, for better visualization of causal assumptions and potential confounders, we created a DAG using DAGitty version 2.3 (Figure S2). For clinical interpretation, ferritin was categorized based on clinical cutoffs and we defined iron deficiency as serum ferritin <15 μg/L and iron overload as serum ferritin >150 μg/L.[20] In addition, we examined the associations of maternal iron status with the risk of inhalant allergic sensitization and physician-diagnosed inhalant allergy combined in groups, by using multinomial logistic regression models. Sensitivity analyses included an additional adjustment of our main model for maternal hemoglobin concentrations, CRP concentrations, and early growth factors including gestational age at birth and birth weight. We tested for nonlinearity of the associations by applying natural cubic splines (three degrees of freedom). Missing data for covariates were <15%, except for breastfeeding (16.6%). To reduce bias and imprecision, we imputed missing data of the covariates with multiple imputations ($m = 10$) by using the fully conditional specification method. All measures of association are presented as effect estimates with their 95% confidence intervals (95% CIs). Multiple testing adjustment was not used given the

TABLE 1 Maternal and child characteristics

	n = 3825
Maternal characteristics	
Age (years)	30.7 (4.7)
Daily energy intake (kcal)	2055.1 (545.6)
Educational level, higher (%)	50.6 (1934)
Parity, nullipara (%)	59.7 (2282)
Ethnic background, European (%)	68.1 (2604)
Body mass index at enrollment (kg/m^2)[a]	23.5 (18.7–35.4)
Smoking during pregnancy, yes (%)	25.5 (976)
Psychological distress, yes (%)[a]	8.9 (341)
History of asthma or atopy, yes (%)	38.6 (1477)
Ferritin concentration during early pregnancy (μg/L)[a]	56.0 (10.2–209.1)
Iron deficient (%)	6.0 (230)
Normal (%)	86.5 (3307)
Iron overload (%)	7.5 (288)
Transferrin concentration (g/L)	2.8 (0.4)
Transferrin saturation (%)	24.9 (10.6)
Hemoglobin (g/dl)	12.4 (0.9)
C-reactive protein (mg/L)[a]	4.3 (0.6–24.7)
Child characteristics	
Sex, female (%)	50.5 (1933)
Gestational age at birth (weeks)[a]	40.3 (36.0–42.3)
Birth weight (grams)	3438.4 (549.0)
Ever breastfeeding, yes (%)	92.1 (3521)
FEV_1 (*z*-score)	0.16 (0.98)
FVC (*z*-score)	0.19 (0.94)
FEV_1/FVC (*z*-score)	−0.10 (0.96)
FEF_{75} (*z*-score)	0.03 (0.92)
Current asthma, yes (%)	5.8 (185)
Inhalant allergic sensitization, yes (%)	32.8 (891)
Physician-diagnosed inhalant allergy, yes (%)	12.4 (387)

Note: Values are means (*SD*).

[a]Medians (2.5–97.5th percentile) or valid percentages (absolute numbers) based on imputed data. Forced expiratory volume in 1 s (FEV_1; $n = 471$), forced vital capacity (FVC; $n = 471$), FEV_1/FVC ratio ($n = 471$), forced expiratory flow after exhaling 75% of FVC (FEF_{75}; $n = 471$), current asthma ($n = 626$), inhalant allergic sensitization ($n = 1107$) and physician-diagnosed inhalant allergy ($n = 707$) was not imputed.

hypothesis-driven analysis.[21] Statistical analyses were performed using SPSS version 25 for Windows (IBM Corp.) and R version 3.5.0 (R Foundation).

3 | RESULTS

3.1 | Subject characteristics

Table 1 shows the maternal and child characteristics. The median maternal ferritin concentration was 56.0 µg/L (95% range: 10.2–209.1), the mean (*SD*) transferrin concentration was 2.8 g/L (0.4), and the mean (*SD*) transferrin saturation was 24.9% (10.6). The prevalence of current asthma was 5.8% ($n = 185$), of inhalant allergic sensitization 32.8% ($n = 891$), and of physician-diagnosed inhalant allergy 12.4% ($n = 387$). Nonresponse analyses showed that non-included children more often had mothers who were lower educated, had a non-European ethnic background, were less often nulliparous, smokers, and had more often psychological distress during pregnancy, and the children had a lower mean gestational age at birth, and were less often breastfed (Table S1).

3.2 | Maternal iron status during pregnancy and child's respiratory and allergy outcomes

In our basic models, higher maternal ferritin concentrations during early pregnancy were associated with a lower FEV_1/FVC and FEF_{75} in children aged 10 years (*Z*-score difference [95% CI]: −0.05 [−0.08 to −0.01], −0.05 [−0.08 to −0.02] per SDS increase in log ferritin, respectively; Table 2). A higher maternal transferrin was associated with a higher FEF_{75} (*Z*-score difference [95% CI] 0.04 [0.01 to 0.07] per SDS increase in transferrin), and a higher risk of physician-diagnosed inhalant allergy (odds ratio [OR] [95% CI]: 1.15 [1.03 to 1.28] per SDS increase in transferrin). A higher maternal transferrin saturation was associated with a lower FEV_1/FVC and a lower FEF_{75} (*Z*-score difference [95% CI]: −0.04 [−0.07 to −0.01], −0.04 [−0.07 to −0.01] per SDS increase in transferrin saturation, respectively). After adjustment for sociodemographic and lifestyle confounders, only the association of higher maternal transferrin concentrations during early pregnancy with a higher risk of physician-diagnosed inhalant allergy in children aged 10 years remained (OR [95% CI]: 1.13 [1.01 to1.26] per SDS increase in transferrin). We observed no associations of any other measure of maternal iron status with FEV_1, FVC, asthma, or inhalant allergic sensitization.

When we studied maternal ferritin concentrations in categories based on clinical cutoffs, we observed no associations of maternal iron deficiency or overload in pregnancy with child's respiratory or allergy outcomes (Table 3). In our study, most of the children (85%) with physician-diagnosed inhalant allergy had inhalant allergic sensitization. In the multinomial regression, maternal ferritin, transferrin, and transferrin saturation during pregnancy were not consistently associated with the risk of inhalant allergic sensitization and physician-diagnosed inhalant allergy combined (Table S2). Additional adjustment for maternal hemoglobin concentrations during early pregnancy or early growth characteristics of the child did not materially change the effect size or direction of the association of maternal transferrin concentrations with child's physician-diagnosed inhalant allergy (results not shown). Also, additional adjustments for CRP concentrations showed similar strengths and directions of the effect estimates as our main model (Table S3). There was no indication of nonlinearity for the associations of maternal iron biomarkers with child's respiratory or allergy outcomes (results not shown).

4 | DISCUSSION

In this population-based prospective cohort study, we observed that, after adjustment for socioeconomic and lifestyle-related confounders, higher maternal transferrin concentrations during early pregnancy were associated with a higher risk of physician-diagnosed inhalant allergy at school age, but not with lung function, asthma, or inhalant allergic sensitization. Using clinical cutoffs of ferritin, no associations of maternal iron deficiency or iron overload during pregnancy with child's respiratory or allergy outcomes were observed.

4.1 | Comparison with previous studies

We previously observed in our cohort that higher maternal hematocrit concentrations in pregnancy were related to a lower FEF_{75} in the children at the age of 10 years.[9] Our current results suggest that this association is not driven by maternal iron status. Previous studies that examined the associations of maternal iron status during pregnancy with respiratory and allergy outcomes in children are scarce.[8] In detail, a previous cohort study among 157 mother–child pairs found that one unit increase in maternal ferritin concentrations (ng/ml) at 11 weeks of gestation was consistently related to 0.14–0.20 higher FEV_1, FVC, and peak expiratory flow *z*-scores, and that a higher soluble transferrin receptor concentration, a marker of a lower ferritin status, was associated with an increased risk of wheezing in children up to the age of 10 years.[7] However, they observed no association of maternal iron status with doctor-diagnosed asthma or hay fever. In a Mendelian randomization study among 6002 subjects, maternal genetic proxies for a lower iron status were associated with a lower school age FEV_1 and FVC, but only in children whose mothers did not receive iron supplementation in late pregnancy, and not in case of asthma or inhalant allergic sensitization.[8] A follow-up of a randomized controlled trial, limiting unmeasured and residual confounding, found that children of mothers who received routine supplementation of 100 mg iron per day had a lower risk of an asthma diagnosis between 10 and 32 years of age, as compared to children of mothers who received only two times a day 50 mg of iron supplementation if the mother was diagnosed with

TABLE 2 Associations of maternal iron status during pregnancy with respiratory and allergy outcomes in children aged 10 years

Iron measure (z-score)	FEV_1 Z-score change (95% CI) *n* = 3354	FVC Z-score change (95% CI) *n* = 3354	FEV_1/FVC Z-score change (95% CI) *n* = 3354	FEF_{75} Z-score change (95% CI) *n* = 3354	Current asthma OR (95% CI) *n* = 3199	Inhalant allergic sensitization OR (95% CI) *n* = 2718	Physician-diagnosed inhalant allergy OR (95% CI) *n* = 3118
Ferritin							
Basic model	−0.01	0.01	**−0.05****	**−0.05****	0.95	0.98	1.00
	(−0.05 to 0.02)	(−0.02 to 0.04)	**(−0.08 to −0.01)**	**(−0.08 to −0.02)**	(0.81 to 1.11)	(0.90 to 1.07)	(0.89 to1.12)
Main model	0.01	0.02	−0.02	−0.01	0.98	1.01	1.02
	(−0.02 to 0.05)	(−0.01 to 0.06)	(−0.05 to 0.02)	(−0.04 to 0.02)	(0.83 to 1.15)	(0.92 to 1.10)	(0.91 to 1.15)
Transferrin							
Basic model	0.01	−0.00	0.03	**0.04***	1.12	1.09	**1.15***
	(−0.02 to 0.05)	(−0.04 to 0.03)	(0.00 to 0.07)	**(0.01 to 0.07)**	(0.96 to 1.30)	(1.00 to 1.18)	**(1.03 to1.28)**
Main model	−0.01	−0.02	0.02	0.01	1.05	1.06	**1.13***
	(−0.04 to 0.03)	(−0.05 to 0.02)	(−0.02 to 0.05)	(−0.02 to 0.04)	(0.90 to 1.24)	(0.97 to 1.15)	**(1.01 to 1.26)**
Transferrin saturation							
Basic model	−0.01	0.00	**−0.04***	**−0.04****	0.98	0.98	1.03
	(−0.05 to 0.02)	(−0.03 to 0.04)	**(−0.07 to −0.01)**	**(−0.07 to −0.01)**	(0.84 to1.13)	(0.90 to 1.06)	(0.93 to 1.15)
Main model	0.01	0.02	−0.03	−0.02	1.05	1.01	1.06
	(−0.02 to 0.05)	(−0.01 to 0.06)	(−0.06 to 0.01)	(−0.05, 0.01)	(0.89 to 1.22)	(0.93 to 1.10)	(0.95 to 1.18)

Note: Values are change in *Z*-scores or odds ratios (OR) with 95% confidence interval (95% CI), derived from linear or logistic regression models, respectively. Bold indicates $p < .05$. Forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), and forced expiratory flow after exhaling 75% of FVC (FEF_{75}). Basic model was adjusted for gestational age at maternal iron status measurement. Main model was the basic model additionally adjusted for maternal education, ethnic background, parity, body mass index, smoking during pregnancy, psychological distress during pregnancy and history of asthma/atopy, and child's sex and breastfeeding.

*$p < .05$.

**$p < .01$.

TABLE 3 Associations of maternal iron deficiency and iron overload during pregnancy with respiratory and allergy outcomes in children aged 10 years

Iron measure (z-score)	FEV_1 Z-score change (95% CI) $n = 3354$	FVC Z-score change (95% CI) $n = 3354$	FEV_1/FVC Z-score change (95% CI) $n = 3354$	FEF_{75} Z-score change (95% CI) $n = 3354$	Current asthma OR (95% CI) $n = 3199$	Inhalant allergic sensitization OR (95% CI) $n = 2718$	Physician-diagnosed inhalant allergy OR (95% CI) $n = 3118$
Iron deficiency ($n = 230$)	−0.10 (−0.24 to 0.04)	−0.13 (−0.27 to 0.00)	0.06 (−0.08 to 0.20)	−0.03 (−0.16 to 0.10)	0.87 (0.44 to 1.71)	0.80 (0.56 to 1.14)	0.90 (0.56 to 1.47)
Normal ($n = 3307$)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Iron overload ($n = 288$)	−0.02 (−0.15 to 0.10)	−0.03 (−0.15 to 0.09)	0.01 (−0.11 to 0.14)	0.01 (−0.11 to 0.13)	0.90 (0.50 to 1.63)	0.96 (0.70 to 1.32)	1.07 (0.72 to 1.58)

Note: Values are change in Z-scores or odds ratios (OR) with 95% confidence interval (95% CI), derived from linear or logistic regression models, respectively. Forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), and forced expiratory flow after exhaling 75% of FVC (FEF_{75}). Iron deficiency was defined as serum ferritin <15 μg/L and overload as >150 μg/L. Models were adjusted for gestational age at maternal iron status measurement, maternal education, ethnic background, parity, body mass index, smoking during pregnancy, psychological distress during pregnancy and history of asthma/atopy, and child's sex and breastfeeding.

anemia.[10] Differences with our results might be due differences in the populations, a selected or mono-ethnic population versus our multi-ethnic population, timing of iron status assessment in pregnancy, higher ferritin levels at baseline, and potential unmeasured confounders in our population. Additionally, it could be that only maternal iron deficiency below a certain threshold affects child's respiratory and allergy outcomes. If this would be the case, potential beneficial effects of maternal iron supplementation on detailed measurements of child's respiratory and allergy outcomes at different ages should be evaluated.

4.2 | Interpretation of the results

Our results suggest no consistent association of maternal iron status during early pregnancy with child's lung function or asthma. Higher transferrin concentrations during early pregnancy, which reflect iron deficiency, were associated with a higher risk of physician-diagnosed inhalant allergy. This association might be explained by the effect of low iron levels on the development of the fetal immune system including the T helper cell balance.[6,22] A Th1/Th2 imbalance may lead to allergy.[23] Cellular studies showed that iron deficiency could lead to a Th2 predominance through the high sensibility of Th1 cells to iron-deficient status, while Th2 cells show a high level of resistance due to larger iron storage pools.[6] This might in part be responsible for a shift in the immune system into a Th2 phenotype, resulting in a clinical expression of allergy.[24] Additionally, a human study showed an association of low fetal iron status with an increase in eosinophilia during infancy, a clinical marker for allergic diseases.[25] Although ferritin is often used to define iron deficiency in pregnancy, this biomarker has limitations as it might be influenced by inflammation.[26] Thus, transferrin concentrations might indicate an iron deficiency in presence of normal ferritin concentration.[27] This might partly explain the different observations for the associations of transferrin but not of ferritin with child's physician-diagnosed inhalant allergy. We did not find a consistent association of higher transferrin concentrations with inhalant allergic sensitization, or of other iron measures in pregnancy with allergy outcomes in the children. When we studied the association of transferrin with the risk of inhalant allergic sensitization and physician-diagnosed inhalant allergy combined, we observed a similar direction and magnitude of the association as with physician-diagnosed inhalant allergy as the outcome only, although the association was less strong which might be due to a lack of power. Thus, we need to interpret our observations with care since we used maternal iron status as a proxy for the fetal iron status, although iron actively crosses the placenta the metabolism and transferred amount might differ between periods of pregnancy.[28] More studies that examine the underlying mechanisms and randomized controlled trials that focus on the effect of maternal iron status at different time points in pregnancy on child's allergy outcomes are needed.

4.3 | Strengths and limitations

The strengths of our study were the population-based prospective design with detailed measurements of maternal iron status during pregnancy, and respiratory and allergy outcomes in childhood. Some limitations need to be addressed. Nonresponse analyses suggest the selection of a more healthy and affluent population. This is partly reflected by the low prevalence of iron deficiency in our cohort of 6%, as compared to other European studies reporting iron deficiency in 10%–32% of pregnant women.[29] To date, no pregnancy-specific definitions for iron deficiency or overload are available. However, for clinical interpretation and comparison to previous studies, we defined groups based on widely used guidelines for the general population. We measured maternal iron status in early pregnancy, but we did not have information on maternal iron status or maternal iron intake from supplements in later stages of pregnancy. After detection of iron deficiency or anemia in pregnancy, mothers may have received iron supplementation as treatment later in pregnancy which might have influenced our results. However, lung development starts already in the fourth week of pregnancy and it has been hypothesized that especially early stages of pregnancy are crucial for later life lung disease development.[30] Although data on current asthma and physician-diagnosed inhalant allergy was obtained from questions adapted from validated questionnaires, misclassification bias cannot be excluded. However, this misclassification is most likely non-differential and might therefore have led to an underestimation of the effect estimates. Finally, residual confounding due to medication use and other dietary factors or nutrients might have influenced our results.

4.4 | Conclusion

Our study suggests that higher transferrin concentrations during pregnancy are associated with a higher risk of physician-diagnosed inhalant allergy, but not with lung function, asthma, or inhalant allergic sensitization. Although the observed association was small and may reflect a chance finding, it could be of interest from an etiological perspective. Future studies which focus on the effect of maternal iron status from pre-conception onwards on child's respiratory or allergy outcome in the long term are needed to get more insight into the clinical relevance of our findings.

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AUTHOR CONTRIBUTIONS

Hugo G. Quezada-Pinedo: Conceptualization (equal); formal analysis (lead); funding acquisition (lead); methodology (lead); project administration (lead); visualization (lead); writing-original draft (lead); writing review & editing (lead). **Sara M. Mensink-Bout**: Conceptualization (lead); formal analysis (lead); methodology (lead); project administration (equal); visualization (equal); writing-original draft (lead); writing-review & editing (lead). **Irwin K. Reiss**: Conceptualization (lead); funding acquisition (lead); methodology (equal); writing original draft (equal); writing review & editing (equal). **Vincent W. V. Jaddoe**: Conceptualization (equal); methodology (lead); writing original draft (equal); writing review & editing (equal). **Marijn J. Vermeulen**: Conceptualization (lead); writing original draft (equal); writing review & editing (equal). **Liesbeth Duijts**: Conceptualization (lead); formal analysis (lead); funding acquisition (lead); methodology (lead); project administration (lead); visualization (lead); writing original draft (lead); writing review & editing (lead).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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