Maternal plasma total neopterin and kynurenine/tryptophan levels during pregnancy in relation to asthma development in the offspring

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Background: Neopterin levels and kynurenine/tryptophan ratios (KTRs) increase with IFN-γ stimulation, indicating T_H1 immunity, and thus might be inversely associated with asthma.
Objective: We sought to examine the association of maternal neopterin levels and KTRs during pregnancy with asthma in the offspring.
Methods: We analyzed the associations of maternal plasma total neopterin levels and KTRs in midpregnancy with asthma at age 7 years among 2883 children in the Norwegian Mother and Child Cohort Study. Asthma was classified either based on registered dispensed asthma medications in the Norwegian Prescription Database or maternal report. We calculated adjusted relative risks using log-binomial regression.
Results: The median gestational week of blood sampling was 18 weeks (interquartile range, 17-19 weeks). The risk of dispensed asthma medications at age 7 years was highest among children of mothers in the highest quartile of neopterin levels, whereas the risk was similar in the 3 lowest quartiles. The adjusted relative risk of dispensed asthma medications was 1.66 (95% CI, 1.16-2.38) when comparing children of mothers in the highest quartile with those in the 3 lowest quartiles. A similar association was observed for maternal report of asthma at age 7 years. When we evaluated allergic versus nonallergic asthma, neopterin levels tended to be associated with nonallergic asthma. Maternal KTR was not associated with asthma development.
Conclusions: Our findings indicate that high maternal levels of neopterin, a marker of cellular immune activation, during pregnancy were positively associated with asthma in offspring. Experimental studies would be needed to further elucidate underlying mechanisms. (J Allergy Clin Immunol 2016;138:1319-25.)

Key words: *Asthma, kynurenine, neopterin, pregnancy, tryptophan*

Asthma is the most common chronic disease during childhood.[1] The disease shows a relatively modest genetic predisposition, and therefore uncovering contributing environmental factors is important to understand the cause of asthma.[2] Pregnancy and early childhood seem to be crucial windows of exposure for the development of asthma.[3] Therefore elucidating a plausible association with markers of immune activation during this time period might increase our understanding of the underlying causal pathways.

Neopterin is a pyrazino-pytamide compound derived from guanosine triphosphate and synthesized by human macrophages on stimulation with the cytokine IFN-γ, allowing monitoring of a T_H1 immune response.[4,5] Neopterin levels also increase during oxidative stress.[6,7] Circulating neopterin levels are reported to be higher among pregnant compared with nonpregnant women and to increase throughout pregnancy.[8,9] In case-control studies neopterin levels are reported to be increased during asthma exacerbations and among patients with a nonatopic asthma phenotype.[10-12] However, a study of cord blood neopterin levels reported no association with childhood asthma.[13] Therefore it remains uncertain whether neopterin is associated with asthma development.

Indoleamine 2,3-dioxygenase (IDO) is an enzyme that modulates immune cell activation status and disease through several molecular mechanisms, including enzyme-dependent deprivation of L-tryptophan, conversion of L-tryptophan into the aryl hydrocarbon receptor ligand kynurenine and other bioactive kynurenine pathway metabolites, or nonenzymatic cell-signaling actions involving tyrosine phosphorylation of IDO.[14] The observed variation in IDO activation is in line with the T_H1/T_H2 paradigm. IFN-γ induces expression of IDO in several tissues.[15,16] IDO is highly expressed in the placenta, and animal models have shown that inhibition of this enzyme among pregnant mice caused rejection of the fetus.[17,18] Therefore IDO expression during pregnancy seems to protect fetus from maternal immune attack. In case-control studies IDO activity has been observed to be lower among atopic subjects and subjects with an allergic asthma phenotype.[19,20] It has also been proposed

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Abbreviations used
IDO: Indoleamine 2,3-dioxygenase
IQR: Interquartile range
KTR: Kynurenine/tryptophan ratio
NorPD: Norwegian Prescription Database
RR: Relative risk

that lower IDO activity in response to early-life infections with respiratory syncytial virus might promote asthma development.[21]

There is currently limited knowledge regarding the potential influence of maternal immune system activation during pregnancy on asthma in the offspring. Therefore the objective of this study was to study the associations of maternal total neopterin levels and kynurenine/tryptophan ratios (KTRs; as a measure of IDO activation) during pregnancy with asthma in the offspring. We hypothesized that both of these markers of immune activation would be inversely associated with asthma development.

METHODS

Study subjects

The study included participants in the Norwegian Mother and Child Cohort Study (MoBa), which is conducted by the Norwegian Institute of Public Health.[22,23] MoBa recruited pregnant women between 1999 and 2008 at approximately 18 weeks of gestation. The participation rate was 41% of all eligible pregnant women. Mothers were allowed to participate in the cohort with more than 1 pregnancy, resulting in approximately 95,000 mothers and 114,500 children.

The current study used information from follow-up questionnaires at approximately 18 and 22 gestational weeks. Data from MoBa were linked to the Medical Birth Registry of Norway and the Norwegian Prescription Database (NorPD) by using national 11-digit personal identification numbers. The NorPD contains information on all dispensed prescriptions from all Norwegian pharmacies since January 2004.

Among MoBa children born between July 1, 2002, and December 31, 2003, with follow-up information from questionnaires at gestational weeks 18 and 22 (n = 17,073 eligible children), we measured maternal midpregnancy total neopterin, kynurenine, and tryptophan levels in a random sample of 3,021 children (Fig 1). The maternal characteristics were similar among the eligible and randomly sampled subjects (see Table E1 in this article's Online Repository at www.jacionline.org). The primary analysis in the current study included the 2,883 children from this random sample who were singletons, still alive, and residing in Norway as of January 2014.

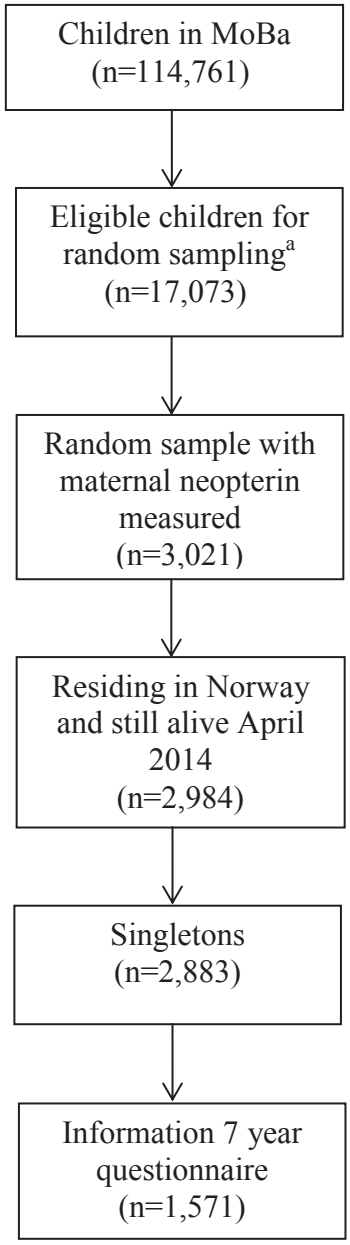

FIG 1. Study sample. [a]Subjects born between July 1, 2002, and December 31, 2003, with information from the Medical Birth Registry of Norway and the first 2 follow-up questionnaires at 18 and 22 gestational weeks.

Ethics

All participants in MoBa provided written informed consent at the time of enrollment. The Norwegian Data Inspectorate approved the ongoing data collection in MoBa (reference no. 01/4325-69/HTL) and the linkage between MoBa and NorPD (reference no. 08/00854-2/IUR). Ethical approval was obtained by the Regional Committee for Medical and Health Research Ethics of South/East Norway (reference no. 2011/2313b).

Exposure

Nonfasting venous blood samples were collected from mothers during the routine ultrasound screening at approximately gestational week 18.[24] Plasma was transported by regular mail to the biobank at the Norwegian Institute of Public Health and stored at −80°C until analysis.[24] Maternal plasma total neopterin, kynurenine, and tryptophan levels were measured by using liquid chromatography–tandem mass spectrometry at Bevital AS (Bergen, Norway; www.bevital.no).[25] This assay measures total neopterin levels as the sum of neopterin and 7,8-dihydroneopterin levels. Neopterin and total neopterin are of equal value to assess immune responses.[25,26] The KTR, as a measure of IDO activation, was calculated by dividing the plasma concentration of kynurenine (in nanomoles per liter) by the concentration of tryptophan (in micromoles per liter). The within- and between-day coefficients of variation for total neopterin, kynurenine, and tryptophan are 2.5% to 10%.[25]

Outcome

The primary outcome of interest was whether the child had dispensed asthma medications registered in the NorPD. Asthma at age 7 years was classified based on at least 1 dispensed prescription for asthma medications in the past 12 months at age 7 years in addition to at least a second dispensed

prescription within 12 months after the first. Medications and their ATC codes defined as asthma medications included inhaled short- and long-acting β_2-agonists (R03AC), inhaled corticosteroids (R03BA), fixed-dose combinations of inhaled β_2-agonists and corticosteroids (R03AK), and leukotriene antagonists (R03DC).

Among subjects whose mothers responded to a questionnaire administered when the child was 7 years old (n = 1571, Fig 1), we also examined maternal report that the child had asthma at age 7 years as a secondary outcome. Current asthma at age 7 years was defined according to 3 positive criteria: maternal report of ever doctor-verified asthma in addition to experiencing either asthma symptoms during the past year and/or using medications for asthma in the past year. Medications defined as asthma medications included R03AC, R03BA, R03AK, and R03DC.

Finally, to distinguish between allergic versus nonallergic asthma, we classified mutually exclusive outcomes based on maternal report at age 7 years: asthma only, allergies only, and asthma in combination with allergies. Allergies were classified based on maternal report of doctor-verified eczema, hay fever, and/or animal hair allergies with symptoms in the past 12 months.

Covariates

We adjusted for a number of maternal characteristics that might influence both the mother's immune system activation during pregnancy and the child's risk of asthma. These characteristics were maternal age (continuous), parity (primiparous and 1, 2, and ≥3 previous births), education (less than high school, high school, up to 4 years of college, and >4 years of college), prepregnancy body mass index (continuous), smoking during pregnancy (yes/no), history of asthma/allergies (yes/no), respiratory tract infections and/or influenza by 18 gestational weeks (yes/no), autoimmune-related disorders (yes/no), and quartiles of maternal 25-hydroxyvitamin D level during pregnancy (<53.9, 54.0-72.5, 72.6-88.1, and >88.1 nmol/L). Maternal respiratory tract infections included upper (ear, throat, and/or sinus infections) and lower (pneumonia and/or bronchitis) respiratory tract infections. Maternal autoimmune-related disorders included insulin-dependent diabetes, systemic lupus erythematosus, multiple sclerosis, psoriasis, rheumatoid arthritis, celiac disease, and/or thyroid disease. Additionally, we adjusted for gestational week of blood sample collection (≤16, 17, 18, 19, and ≥20 weeks), calendar month of blood sample collection (January-March, April-June, July-September, and October-December) and creatinine level (continuous) as a measure of renal function. Creatinine and maternal 25-hydroxyvitamin D levels were measured in plasma at the same time that the total neopterin levels and KTRs were measured by using liquid chromatography–tandem mass spectrometry.

Statistical analysis

Distribution of the mother's total neopterin level and KTR by the covariates was tested by using robust linear regression. We examined potential nonlinear associations of the mother's total neopterin level and KTR with the child's risk of asthma at age 7 years by using restricted cubic splines with 4 knots. The number of knots was chosen based on log-likelihood tests comparing models with an increasing number of knots. We then estimated the association of maternal neopterin levels and KTRs with the child's risk of asthma using 2 different approaches by entering maternal neopterin level and KTR continuously and divided into quartiles. The associations were estimated by using relative risks (RRs) and 95% CIs with log-binomial regression models with the log-link function. Analyses were performed by unadjusted models, as well as after adjustment for all potential confounders described above. Categorical covariates were entered by using dummy variables.

We conducted several sensitivity analyses. The amount of missing information on individual covariates was generally low (<2%), but across all covariates, this percentage increased to 8%. Therefore we conducted multiple imputation of missing covariate information by using chained equations, imputing a total of 20 data sets, to evaluate the potential influence of missing information on the observed associations with the mi impute chained command in Stata software (StataCorp, College Station, Tex). Furthermore, we conducted sensitivity analyses excluding preterm children, children of mothers who smoked during pregnancy, and children of mothers with asthma/allergies.

The statistical significance level was 5% or a 95% CI not including 1. We conducted all analyses using Stata software (version 14).

RESULTS

Distribution of total neopterin levels and KTRs

The median gestational week of blood sampling was 18 weeks (interquartile range [IQR], 17-19). The median was 7.16 nmol/L (IQR, 6.22-8.40 nmol/L) for total neopterin level and 18.56 nmol/μmol (IQR, 16.47-21.01 nmol/μmol) for KTR. The Spearman rank correlation coefficient between these 2 biomarkers of immune activation was 0.48. The mother's total neopterin level was positively associated with age, parity, prepregnancy body mass index, gestational week of sample collection, calendar month of sample collection, and creatinine level (Table I). Furthermore, the total neopterin level was less among smokers compared with nonsmokers. The mother's KTR was positively associated with parity, prepregnancy body mass index, and creatinine levels (Table I). There was a nonlinear association of maternal vitamin D status with both total neopterin level and KTR.

Maternal total neopterin level and dispensed asthma medications in offspring at age 7 years

A total of 5% of children had dispensed asthma medications at age 7 years according to the NorPD. The probability of dispensed asthma medication at age 7 years according to the mother's total neopterin level is indicated in Fig 2, *A*. The risk of dispensed asthma medications was similar among children of mothers in the 3 lowest quartiles of total neopterin levels ($P = .6$). When we compared children of mothers in the highest quartile of total neopterin levels to children of mothers in the 3 lower quartiles, the adjusted RR of dispensed asthma medications at age 7 years was 1.66 (95% CI. 1.16-2.38; Table II). Overall, the results were similar for maternal report of asthma at age 7 years (see Table E2 in this article's Online Repository at www.jacionline.org).

Maternal KTR and dispensed asthma medications in offspring at age 7 years

The probability of dispensed asthma medication at age 7 years according to the mother's KTR during pregnancy is indicated in Fig 2, *B*. The mother's KTR showed a tendency for a nonlinear/U-shaped association with dispensed asthma medications at age 7 years (Table II). However, this tendency was not statistically significant (the *P* value for the second-order term was .291). The results for maternal report of asthma at age 7 years also showed no significant associations (see Table E2).

Maternal total neopterin levels and KTRs in relation to maternal report of allergic and nonallergic asthma in offspring at age 7 years

A total of 57 (4%) of the children only had asthma, 147 (10%) only had allergies, and 28 (2%) had both asthma and allergies based on maternal report at age 7 years. The maternal total

TABLE I. Distribution of maternal total neopterin levels and KTRs during pregnancy by background characteristics (n = 2883)

Characteristics	No.	Neopterin (nmol/L), median (IQR)	*P* value	KTR (nmol/μmol), median (IQR)	*P* value
Maternal age (y)					
<25	361	6.90 (5.96-8.08)	<.001	18.48 (16.31-20.92)	.202
25-29	990	7.06 (6.15-8.35)		18.37 (16.31-20.76)	
30-34	1095	7.18 (6.25-8.43)		18.64 (16.61-21.08)	
≥35	437	7.56 (6.56-8.71)		18.93 (16.64-21.16)	
Maternal parity					
Primiparous	1197	6.84 (5.98-7.85)	<.001	18.26 (16.12-20.52)	<.001
1	1078	7.37 (6.47-8.73)		18.80 (16.68-21.47)	
2	451	7.50 (6.42-8.65)		18.64 (16.80-21.13)	
≥3	157	7.63 (6.44-8.99)		19.18 (16.65-21.62)	
Maternal education					
Less than high school	262	7.16 (6.26-8.41)	.996	18.67 (16.58-21.39)	.051
High school	1022	7.10 (6.20-8.44)		18.81 (16.65-21.32)	
Up to 4 y of college	1113	7.16 (6.23-8.35)		18.46 (16.34-20.98)	
More than 4 y of college	475	7.24 (6.22-8.40)		18.30 (16.31-20.69)	
Missing	11	6.69 (6.13-7.17)		16.39 (14.77-19.41)	
Maternal prepregnancy BMI (kg/m^2)					
Underweight (<18.5)	78	6.74 (6.09-8.13)	<.001	18.11 (16.08-21.47)	<.001
Normal weight (18.5-24.9)	1805	7.01 (6.11-8.12)		18.30 (16.17-20.62)	
Overweight (25-29.9)	589	7.27 (6.27-8.61)		18.83 (16.86-21.27)	
Obese (≥30)	299	8.10 (7.05-9.41)		20.09 (17.63-23.16)	
Missing	112	6.97 (6.29-8.10)		18.64 (16.33-20.86)	
Maternal smoking during pregnancy					
No	2441	7.19 (6.25-8.44)	<.001	18.62 (16.50-21.08)	.156
Yes	424	6.93 (5.99-8.04)		18.32 (16.35-20.67)	
Missing	18	7.20 (5.85-7.53)		17.36 (16.38-20.16)	
Maternal asthma/allergies*					
No	2359	7.15 (6.20-8.39)	.504	18.48 (16.42-20.94)	.074
Yes	524	7.22 (6.27-8.42)		18.87 (16.59-21.42)	
Maternal autoimmune disorders†					
No	2744	7.16 (6.22-8.40)	.909	18.55 (16.47-20.96)	.658
Yes	139	7.30 (6.02-8.39)		19.14 (16.39-21.81)	
Maternal respiratory tract infections/influenza by 18 gestational wk‡					
No	2498	7.15 (6.23-8.40)	.740	18.57 (16.47-20.98)	.586
Yes	385	7.23 (6.15-8.33)		18.51 (16.50-21.27)	
Gestational week of blood sampling					
≤16	232	7.10 (6.05-8.37)	<.001	18.44 (16.36-21.08)	.822
17	673	7.10 (6.12-8.28)		18.51 (16.34-20.86)	
18	1105	7.15 (6.22-8.31)		18.68 (16.53-21.19)	
19	637	7.19 (6.26-8.44)		18.51 (16.47-21.00)	
≥20	222	7.57 (6.47-9.20)		18.23 (16.50-20.98)	
Missing	14	6.64 (5.85-7.04)		18.49 (16.28-20.26)	
Season of sample collection					
January-March	431	6.87 (6.00-8.09)	.004	18.48 (16.28-20.72)	.187
April-June	1148	7.19 (6.22-8.44)		18.54 (16.40-21.00)	
July-September	986	7.19 (6.28-8.39)		18.56 (16.54-20.96)	
October-December	318	7.27 (6.36-8.58)		18.89 (16.65-21.50)	
Maternal creatinine level (nmol/L)					
First quartile (<44.9)	714	6.84 (5.88-7.95)	<.001	17.53 (15.59-19.70)	<.001
Second quartile (44.9-48.7)	722	6.95 (6.07-8.21)		18.37 (16.17-20.76)	
Third quartile (48.8-53.0)	722	7.25 (6.37-8.46)		18.81 (16.92-21.08)	
Fourth quartile (>53.0)	725	7.59 (6.62-9.02)		19.72 (17.50-22.65)	
Maternal vitamin D level (nmol/L)					
First quartile (<53.9)	713	7.10 (6.06-8.40)	.009	18.68 (16.62-21.07)	.039
Second quartile (54.0-72.5)	713	7.08 (6.19-8.35)		18.35 (16.34-20.72)	
Third quartile (72.6-88.1)	713	7.11 (6.19-8.26)		18.39 (16.31-20.79)	
Fourth quartile (>88.1)	713	7.38 (6.39-8.53)		18.92 (16.50-21.53)	

*Asthma/allergies were defined as current asthma, hay fever, animal hair allergy, atopic eczema, and/or other allergies at 18 gestational weeks.

†Autoimmune disorders were defined as diabetes, lupus erythematosus, multiple sclerosis, psoriasis, rheumatoid arthritis, celiac disease, and thyroid disease.

‡Respiratory tract infections include upper (ear, sinus, and/or throat infections) and lower (pneumonia and/or bronchitis) respiratory tract infections.

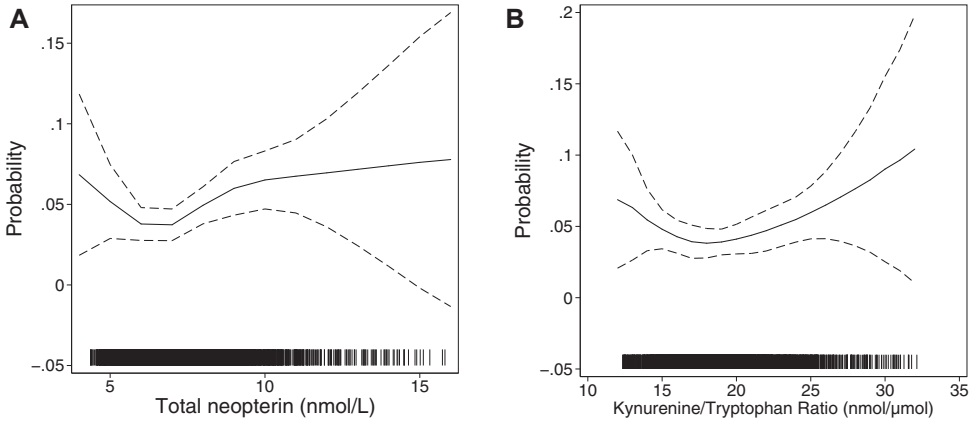

FIG 2. Plot of the probability of dispensed asthma medications at age 7 years by maternal total neopterin levels **(A)** and KTRs **(B)** during pregnancy. Probabilities are based on a log-binomial regression model using restricted cubic splines with 4 knot points. The knot points were 5.16, 6.64, 7.78, and 10.70 nmol/L for the total neopterin level, and the knot points were 14.18, 17.37, 19.92, and 25.28 nmol/μmol for the KTR. For the purpose of plot visibility/interpretation, we restricted the graph to exclude the first and 99th percentiles because of the uncertainty of the estimated probabilities at the outer points of distribution.

TABLE II. Association of maternal total neopterin levels and KTRs during pregnancy with child's use of asthma medications at age 7 years (n = 2883)

	No.	Cases (%)	Unadjusted RR (95% CI)	Adjusted RR (95% CI)*
Total neopterin level (nmol/L)				
Continuous†	2883	NA	1.13 (1.01-1.28)	1.12 (0.99-1.27)
First quartile (<6.22)	720	4.4	1	1
Second quartile (6.22-7.16)	721	4.3	0.97 (0.60-1.57)	1.08 (0.66-1.78)
Third quartile (7.17-8.40)	721	3.5	0.78 (0.47-1.30)	0.83 (0.48-1.42)
Fourth quartile (>8.40)	721	6.8	1.53 (0.99-2.36)	1.60 (0.99-2.57)
First-third quartile	2162	4.1	1	1
Fourth quartile	721	6.8	1.67 (1.19-2.34)	1.66 (1.16-2.38)
KTR (nmol/μmol)				
Continuous†	2878	NA	1.10 (0.96-1.27)	1.09 (0.94-1.26)
First quartile (<16.47)	720	5.6	1	1
Second quartile (16.47-18.56)	718	3.6	0.65 (0.40-1.06)	0.63 (0.39-1.04)
Third quartile (18.57-21.01)	720	3.8	0.67 (0.42-1.09)	0.67 (0.41-1.09)
Fourth quartile (>21.01)	720	6.0	1.08 (0.71-1.63)	1.00 (0.63-1.56)

The multivariable analysis is based on a complete case analysis, in which approximately 8% of observations had missing data on 1 or more covariates.
NA, Not applicable.
*Adjusted for maternal age, parity, education, smoking during pregnancy, prepregnancy BMI, asthma/allergies, autoimmune disorders, respiratory tract infections and/or influenza, gestational week of blood sampling, calendar month of sample collection, creatinine level, and vitamin D status.
†Associations reflect associations per SD increase.

neopterin level showed a nonsignificant positive association with nonallergic asthma (RR, 1.18 [95% CI, 0.95-1.46] per SD increase in total neopterin level), whereas the association with allergies alone or allergic asthma was overall null (Table III). Maternal KTR showed a nonsignificant inverse association with allergic asthma (Table III).

Sensitivity analyses

We conducted sensitivity analyses to evaluate the robustness of our findings. The multiple imputation analysis yielded similar results as the complete case analysis (see Tables E3 and E4 in this article's Online Repository at www.jacionline.org). The sensitivity analyses excluding preterm children, children of mothers who smoked during pregnancy, and children of mothers with a history of asthma/allergies also yielded similar results (data not shown).

DISCUSSION

This is the first study to show that there is an association between maternal neopterin levels during pregnancy and asthma development in offspring. Our findings indicated that children of mothers in the highest quartile of total neopterin levels during pregnancy (>8.4 nmol/L) had an increased risk of asthma by 7 years of age. Maternal KTRs during pregnancy showed no significant association with asthma in the offspring.

Strengths and limitations

The strengths of the study include the prospective pregnancy cohort design, the large sample size, adjustment for a large number of potential confounding factors, and the use of 2 independent data sources to classify asthma at age 7 years. A limitation of the current study is that we only measured maternal total neopterin levels and KTRs at 1 time point during

TABLE III. Association of maternal total neopterin levels and KTRs during pregnancy with maternal report of asthma and allergies at age 7 years in offspring (n = 1571)

	Asthma only (n = 57)		Allergies only (n = 147)		Asthma and allergies (n = 28)	
	Unadjusted RR (95% CI)	Adjusted RR (95% CI)*	Unadjusted RR (95% CI)	Adjusted RR (95% CI)*	Unadjusted RR (95% CI)	Adjusted RR (95% CI)*
Total neopterin level (nmol/L)						
Continuous†	1.23 (1.02-1.47)	1.18 (0.95-1.46)	1.00 (0.83-1.19)	0.97 (0.79-1.19)	0.87 (0.54-1.40)	0.99 (0.62-1.58)
First quartile (<6.22)	1	1	1	1	1	1
Second quartile (6.22-7.16)	1.60 (0.72-3.58)	1.73 (0.73-4.11)	0.82 (0.50-1.34)	0.81 (0.48-1.37)	0.64 (0.24-1.66)	0.75 (0.27-2.04)
Third quartile (7.17-8.40)	0.94 (0.38-2.34)	0.86 (0.32-2.32)	1.18 (0.74-1.87)	1.17 (0.71-1.91)	0.38 (0.12-1.20)	0.33 (0.09-1.24)
Fourth quartile (>8.40)	2.28 (1.06-4.89)	1.97 (0.82-4.70)	0.96 (0.59-1.55)	0.91 (0.53-1.55)	0.57 (0.21-1.55)	0.79 (0.26-2.39)
KTR (nmol/μmol)						
Continuous†	1.00 (0.77-1.31)	0.83 (0.60-1.15)	0.85 (0.70-1.04)	0.80 (0.65-1.00)	0.58 (0.35-0.97)	0.61 (0.35-1.06)
First quartile (<16.47)	1	1	1	1	1	1
Second quartile (16.47-18.56)	0.93 (0.45-1.92)	0.83 (0.38-1.81)	1.14 (0.73-1.79)	1.28 (0.79-2.07)	0.89 (0.36-2.23)	0.98 (0.36-2.63)
Third quartile (18.57-21.01)	0.75 (0.36-1.59)	0.63 (0.28-1.42)	0.58 (0.34-0.98)	0.62 (0.36-1.08)	0.37 (0.12-1.20)	0.32 (0.08-1.19)
Fourth quartile (>21.01)	0.73 (0.34-1.56)	0.44 (0.19-1.06)	0.85 (0.53-1.37)	0.79 (0.47-1.35)	0.48 (0.16-1.43)	0.59 (0.19-1.87)

*Adjusted for maternal age, parity, education, smoking during pregnancy, prepregnancy BMI, asthma/allergies, autoimmune disorders, respiratory tract infections and/or influenza, gestational week of blood sampling, calendar month of sample collection, creatinine level, and vitamin D status.
†Associations reflect associations per SD increase.

pregnancy, raising the possibility of regression dilution bias. Total neopterin levels and KTRs show relatively good reproducibility among healthy nonpregnant subjects, with intraclass correlation coefficients of 0.52 to 0.67 and 0.67 to 0.74, respectively.[27] However, we cannot be sure that the same reproducibility is observed among pregnant women. Using maternal report of asthma and allergies might have resulted in misclassification, but we have previously shown the validity of maternal report that the child used asthma medications on the 7-year questionnaires by comparison with dispensed asthma medications in the NorPD.[28] We also cannot exclude the possibility of selection bias because of the initial participation rate of 41% of eligible subjects in the MoBa cohort or because of loss to follow-up.[23] Information from NorPD was available for all MoBa participants, including nonresponders to follow-up questionnaires, and therefore we were able to evaluate a potential selection bias caused by loss to follow-up. Notably, the results were overall consistent for the 2 different asthma definitions at 7 years of age.

Comparison with previous studies

Limited knowledge is currently available regarding the association of maternal neopterin levels and IDO activation during pregnancy in relation to asthma in the offspring.

One previous study found a median neopterin concentration of 13.0 nmol/L (IQR, 10.6-15.5 nmol/L) in cord blood from 408 newborns but did not demonstrate any association with asthma by 6 years of age.[13] However, this study addresses a different research question from our study. Cord blood levels are likely to be a marker of the mother's neopterin level during late pregnancy and/or the child's level very early in life, whereas we measured maternal neopterin levels during midpregnancy.

Three previous case-control studies have compared the neopterin level between adult asthmatic patients and nonasthmatic subjects or stable versus exacerbated asthma cases, overall indicating that neopterin levels can be increased during exacerbations and among patients with nonallergic asthma.[10-12]

Results from case-control studies also indicate that IDO activity was lower among atopic subjects and lower among subjects with an allergic asthma phenotype and that lower IDO activity in response to early-life infections with respiratory syncytial virus might promote asthma development.[19-21]

However, these case-control studies cannot answer the research question of whether neopterin or IDO activation is associated with subsequent asthma development because the exposure and outcome are defined at the same time point.

Interpretation of findings

The maternal immune system is subject to strict temporal control during the course of a normal pregnancy.[29] In line with previous studies, neopterin levels among MoBa participants increased during pregnancy.[8,9] Therefore pregnancy itself seems to be a condition associated with activated cell-mediated immunity. In contrast, we observed no change in IDO activation by gestational week of sample collection, where the literature indicates that IDO activation is often increased during pregnancy, to protect the fetus from maternal immune attack.[17,18] However, IDO activation in the human placenta seems to be variably expressed during different periods of pregnancy.[30,31] Furthermore, the range of gestational week of sample collection was relatively narrow in the current study.

Interestingly, we did not observe an association between maternal autoimmune-related conditions and neopterin levels or KTRs, with many of these diseases traditionally considered to be associated with a T_H1-skewed immune profile. This might due to the fact that these conditions were well regulated during pregnancy.

Asthma is traditionally considered to be associated with a T_H2-skewed immune profile. However, emerging evidence suggests that there exists asthma phenotypes characterized by a T_H1 and T_H17 immune profile, and several parts of the innate immune system are believed to play a role in asthma pathogenesis.[32,33] Both neopterin and IDO activation are stimulated by IFN-γ and are therefore considered markers of T_H1 immune activation.[4,5,15,16] In contrast to our hypothesis, neither neopterin nor IDO activation was inversely associated with asthma development. However, when we evaluated allergic versus nonallergic asthma, a tendency for a positive association with neopterin levels was only observed with nonallergic asthma, whereas KTRs showed a tendency for an inverse association with an allergic asthma phenotype. Therefore these findings

were more in line with results reported from previous case-control studies and our predefined hypothesis. Notably, we had reduced power to detect associations with allergic versus nonallergic asthma because this analysis relied on follow-up information from the 7-year questionnaire, which was only available for about half of the study sample.

Potential explanatory mechanisms for a positive association between maternal neopterin levels during pregnancy and asthma development include their increase during inflammation, infectious disease, or oxidative stress.[4,6,7,14] Maternal prepregnancy body mass index, a marker of systemic inflammation, is reported to be positively associated with asthma development.[34] In our study maternal neopterin levels increased with prepregnancy body mass index. Furthermore, studies have reported a positive association between both maternal infectious disease and oxidative stress during pregnancy with asthma development in the offspring.[35]

In conclusion, our findings indicate that a high level of maternal neopterin, a marker of cellular immune activation, during pregnancy was positively associated with asthma in the offspring. Because this is the first study, confirmatory studies in other populations are warranted. Experimental studies would be needed to further elucidate the underlying mechanisms.

We thank all the families participating in the MoBa.

Key messages

- **Our findings indicate that high levels of maternal neopterin, a marker of cellular immune activation, during pregnancy were positively associated with asthma in the offspring.**
- **Experimental studies would be needed to further elucidate the underlying mechanisms.**

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TABLE E1. Distribution of characteristics among eligible and randomly selected participants

Characteristics	Eligible (n = 17,073[%])	Random sample (n = 3,021 [%])	*P* value
Maternal age (y)			.828
<25	12.4	12.6	
25-29	34.5	34.0	
30-34	37.6	38.1	
≥35	15.6	15.3	
Maternal parity			.014
Primiparous	40.7	42.2	
1	36.8	37.2	
2	17.3	15.3	
≥3	5.2	5.3	
Maternal education			.028
Less than high school	10.3	9.2	
High school	34.6	35.4	
Up to 4 y of college	39.0	38.2	
More than 4 y of college	15.6	16.9	
Missing	0.5	0.4	
Maternal prepregnancy BMI (kg/m^2)			.165
Underweight (<18.5)	2.9	2.8	
Normal weight (18.5-24.9)	61.7	62.5	
Overweight (25-29.9)	21.8	20.4	
Obese (≥30)	10.0	10.5	
Missing	3.7	3.8	
Maternal smoking during pregnancy			.090
No	85.9	85.0	
Yes	13.4	14.4	
Missing	0.6	0.6	
Maternal asthma/allergies*			.011
No	83.4	81.8	
Yes	16.6	18.2	
Singletons			.189
No	96.1	96.5	
Yes	3.9	3.5	

*Maternal asthma/allergies include maternal history of asthma, eczema, hay fever, animal hair allergies and/or other allergies.

TABLE E2. Association of maternal total neopterin levels and KTRs during pregnancy with maternal report of asthma at 7 years in offspring (n = 1571)

	No.	Cases (%)	Unadjusted RR (95% CI)	Adjusted RR (95% CI)*
Total neopterin level (nmol/L)				
Continuous†	1571	NA	1.17 (1.02-1.34)	1.18 (1.02-1.36)
First quartile (<6.22)	400	5.3	1	1
Second quartile (6.22-7.16)	397	5.9	1.11 (0.62-1.97)	1.18 (0.64-2.15)
Third quartile (7.17-8.40)	381	3.7	0.69 (0.36-1.35)	0.65 (0.32-1.32)
Fourth quartile (>8.40)	393	7.5	1.41 (0.82-2.42)	1.43 (0.79-2.60)
First-third quartile	1178	5.0	1	1
Fourth quartile	393	7.5	1.50 (0.98-2.31)	1.54 (0.97-2.44)
KTR (nmol/μmol)				
Continuous†	1568	NA	0.94 (0.76-1.18)	0.86 (0.67-1.10)
First quartile (<16.47)	394	7.0	1	1
Second quartile (16.47-18.56)	394	6.1	0.88 (0.52-1.49)	0.82 (0.47-1.43)
Third quartile (18.57-21.01)	392	4.4	0.63 (0.35-1.14)	0.57 (0.30-1.06)
Fourth quartile (>21.01)	388	4.7	0.67 (0.38-1.20)	0.53 (0.29-1.00)

The multivariable analysis is based on a complete-case analysis, in which approximately 8% of observations had data missing on 1 or more covariates.
NA, Not applicable.
*Adjusted for maternal age, parity, education, smoking during pregnancy, prepregnancy BMI, asthma/allergies, autoimmune disorders, respiratory tract infections and/or influenza, gestational week of blood sampling, calendar month of sample collection, creatinine level, and vitamin D status.
†Associations reflect associations per SD increase.

TABLE E3. Association of maternal total neopterin levels and KTRs during pregnancy with the child's use of asthma medications at age 7 years (n = 2883)

	No.	Cases (%)	Unadjusted RR (95% CI)	Adjusted RR (95% CI)*
Total neopterin level (nmol/L)				
Continuous†	2883	NA	1.13 (1.01-1.28)	1.12 (0.99-1.27)
First quartile (<6.22)	720	4.4	1	1
Second quartile (6.22-7.16)	721	4.3	0.97 (0.60-1.57)	0.95 (0.58-1.54)
Third quartile (7.17-8.40)	721	3.5	0.78 (0.47-1.30)	0.77 (0.46-1.30)
Fourth quartile (>8.40)	721	6.8	1.53 (0.99-2.36)	1.47 (0.93-2.32)
First-third quartile	2162	4.1	1	1
Fourth quartile	721	6.8	1.67 (1.19-2.34)	1.64 (1.15-2.33)
KTR (nmol/μmol)				
Continuous†	2878	NA	1.10 (0.96-1.27)	1.09 (0.94-1.26)
First quartile (<16.47)	720	5.6	1	1
Second quartile (16.47-18.56)	718	3.6	0.65 (0.40-1.06)	0.62 (0.38-1.01)
Third quartile (18.57-21.01)	720	3.8	0.67 (0.42-1.09)	0.63 (0.39-1.02)
Fourth quartile (>21.01)	720	6.0	1.08 (0.71-1.63)	0.97 (0.62-1.50)

Multiple imputation of missing covariate information was conducted by using chained equations, in which a total of 20 data sets were imputed.
NA, Not applicable.
*Adjusted for maternal age, parity, education, smoking during pregnancy, prepregnancy BMI, asthma/allergies, autoimmune disorders, respiratory tract infections and/or influenza, gestational week of blood sampling, calendar month of sample collection, creatinine level, and vitamin D status.
†Associations reflect associations per SD increase.

TABLE E4. Association of maternal total neopterin levels and KTRs during pregnancy with maternal report of asthma and allergies at 7 years in offspring (n = 1571)

	Asthma only (n = 57)		Allergies only (n = 147)		Asthma and allergies (n = 28)	
	Unadjusted RR (95% CI)	Adjusted RR (95% CI)*	Unadjusted RR (95% CI)	Adjusted RR (95% CI)*	Unadjusted RR (95% CI)	Adjusted RR (95% CI)*
Total neopterin level (nmol/L)						
Continuous†	1.23 (1.02-1.47)	1.14 (0.92-1.40)	1.00 (0.83-1.19)	1.00 (0.82-1.22)	0.87 (0.54-1.40)	0.94 (0.58-1.52)
First quartile (<6.22)	1	1	1	1	1	1
Second quartile (6.22-7.16)	1.60 (0.72-3.58)	1.65 (0.73-3.77)	0.82 (0.50-1.34)	0.78 (0.47-1.29)	0.64 (0.24-1.66)	0.66 (0.25-1.78)
Third quartile (7.17-8.40)	0.94 (0.38-2.34)	0.92 (0.36-2.34)	1.18 (0.74-1.87)	1.10 (0.68-1.78)	0.38 (0.12-1.20)	0.39 (0.12-1.28)
Fourth quartile (>8.40)	2.28 (1.06-4.89)	1.89 (0.83-4.29)	0.96 (0.59-1.55)	0.95 (0.57-1.59)	0.57 (0.21-1.55)	0.70 (0.24-2.05)
KTR (nmol/μmol)						
Continuous†	1.00 (0.77-1.31)	0.86 (0.63-1.17)	0.85 (0.70-1.04)	0.83 (0.67-1.03)	0.58 (0.35-0.97)	0.61 (0.35-1.06)
First quartile (<16.47)	1	1	1	1	1	
Second quartile (16.47-18.56)	0.93 (0.45-1.92)	0.80 (0.37-1.69)	1.14 (0.73-1.79)	1.24 (0.78-1.98)	0.89 (0.36-2.23)	1.07 (0.41-2.80)
Third quartile (18.57-21.01)	0.75 (0.36-1.59)	0.60 (0.27-1.33)	0.58 (0.34-0.98)	0.60 (0.35-1.02)	0.37 (0.12-1.20)	0.38 (0.11-1.25)
Fourth quartile (>21.01)	0.73 (0.34-1.56)	0.47 (0.21-1.08)	0.85 (0.53-1.37)	0.80 (0.48-1.34)	0.48 (0.16-1.43)	0.56 (0.18-1.76)

Multiple imputation of missing covariate information was conducted by using chained equations, in which a total of 20 data sets were imputed.

*Adjusted for maternal age, parity, education, smoking during pregnancy, prepregnancy BMI, asthma/allergies, autoimmune disorders, respiratory tract infections and/or influenza, gestational week of blood sampling, calendar month of sample collection, creatinine level, and vitamin D status.

†Associations reflect associations per SD increase.