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Intrauterine exposure to immunosuppressantsinfluences the development of postnatal allergic diseases

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Conflicts of interest

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To the Editor,

In pregnancies with connective tissue disease, controlling disease activity is important for mothers and children. Although tacrolimus, cyclosporine A, and azathioprineare important therapeutic agents that pregnant women can use[1, 2],evidence of long-term effects on offspring is stillinsufficient.Pediatric allergies are associated with the environment during the fetal period, including maternal therapeutic drugs[3]. Therefore, we conducted a retrospective cohort study using chart review and a questionnaire survey to investigate the relationship between intrauterine exposure to immunosuppressants and postnatal allergies. We obtained approval from the REC of the NCCHD for this study.Informed consent was obtained from the parents and school-age children using explanatory documents. The participants were shown in FigureS1. The prevalence of allergic symptoms was investigated mainly using the ISAAC questionnaire[4], which has a Japanese version with confirmed translational validation (TableS1). We compared the prevalence of allergic symptoms between children who were exposed to immunosuppressants in utero and children who were not exposed.

Among 85 children, 33 were exposed to immunosuppressants(FigureS1).Children in the exposed group were significantly younger (median age, 2 vs 8 years; P<0.0001). Other

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collected data, like socioeconomic status and clinical information, are shown in Table S2.As shown in Table 1,rash ever (33.3% vs 11.5%; *P*=0.01), current rash (30.3% vs 11.5%; *P*=0.03), and current wheeze (15.1% vs 1.9%, *P*=0.03) were significantly more prevalent in the exposed group in the univariate analysis. The difference in rash ever remainedsignificant in the multivariate analysis (OR, 3.64; 95% CI, 1.03–12.79, *P*=0.04).The analysis of 67 first children andthe analysis ofchildren exposed to only tacrolimusalso showed similar results to above.

To evaluate whether tacrolimus has a dose-dependent effect on rash, we divided the total tacrolimus dose during each trimester into three groups,as shown in Figure1.A-C, based on the daily doseand gestational age at delivery. Tacrolimus in the second and the third trimester had a dose-dependent effect on rash ever and current rash(Figure 1.B-C).

In this study, we revealed that intrauterine exposure to immunosuppressants might be associated with rash in offspring. Recently, it has been suggested that gene expressionrelated to the differentiation and maturation of T cells, is modified by various factors during the fetal period; this process is related to the development of allergies[3]. CNIsinhibit T cell activation, which leads to suppression of the Th1 reaction and shifting toward Th2 cytokine profiles. Azathioprine also inhibits T cell differentiation and proliferation. The fetal immune system is affected by various factors, including the maternal immune status, sointrauterine exposure to immunosuppressants with suppressive effects on Th1 and Treg function may affect T cell differentiation and maturation during the fetal period, resulting in postnatal allergies.In addition, the amount

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of tacrolimustransferred to the fetus may be greater after the second trimester due to decreasing levels of placental P-glycoprotein[5], and the fetal immune system mainly develops starting during the second trimester.Thus,the amount of exposure after the secondtrimestermight be more likely to affect the fetal immune system, resulting in dose-dependent increases in rash.Thus, although tacrolimus has a role as a therapeutic agent for atopic dermatitis, its exposure in utero may have the opposite effect by influence the immune development in the fetal period.

Major potential limitations arethe difference in child ageand small sample size. Also, we could not collect information on dietary and environmental factors that may affect allergic outcomes due to the retrospective study.Furthermore,this is a self-reported survey,and significant differences were observed in rash, not diagnosed eczema. However, we used the ISAAC questionnaire for the survey. It is possible that rash considered as eczema was observed more frequently in the exposed group at a younger age than other symptoms on the basis of atopic march[6].

Deterioration of SLE during pregnancy is related to adverse pregnancy outcomes. Immunosuppressants are useful agents for pregnant women with SLE. Therefore, it is recommended that pregnant women with SLE use immunosuppressants when necessary based on their disease activity, but in these cases, there is needto take prophylactic measures and observe allergic outcomes in offspring.Further research is needed for better long-term health outcomes in children with intrauterine exposure to immunosuppressants.

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Abbreviations

REC: Research Ethics Committee

NCCHD: National Center for Child Health and Development

ISAAC: International Study of Asthma and Allergies in Childhood

CNIs: Calcineurin inhibitors

Th1: T helper cells type 1

Th2: T helper cells type 2

Treg: Regulatory T cells

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	Total (n = 85)	In utero exposure (n = 33)	No exposure (n= 52)	Crude OR (95%CI)	Crude P value	Adjusted † OR (95%CI)	Adjusted † P value	Adjusted ‡ OR (95%CI)	Adjusted ‡ P value
Asthma									
Wheeze ever, *n* (%)	19 (22.3)	8 (24.2)	11 (21.1)	1.19 (0.42–3.37)	0.74	1.06 (0.35–3.27)	0.92	2.24 (0.53–11.72)	0.29
Current wheeze, *n* (%)	6 (7.1)	5 (15.1)	1 (1.9)	9.11 (1.01–81.86)	0.03*	6.53 (0.63–67.54)	0.12	18.52 (1.52–579.01)	0.04*
Diagnosed with asthma, *n* (%)	8 (9.4)	3 (9.1)	5 (9.6)	0.94 (0.21–4.22)	0.94	0.85 (0.17–4.24)	0.84	0.47 (0.07–2.85)	0.41
Current nocturnal cough, *n* (%)	7 (8.4)	4 (12.5)	3 (5.9)	2.29 (0.48–10.96)	0.29	2.29 (0.42–12.56)	0.34	1.70 (0.26–15.66)	0.60
Atopic dermatitis									
Rash ever, *n* (%)	17 (20.0)	11 (33.3)	6 (11.5)	3.83 (1.25–11.71)	0.01*	3.64 (1.03–12.79)	0.04*	2.09 (0.58–8.21)	0.27
Current rash, *n* (%)	16 (18.8)	10 (30.3)	6 (11.5)	3.33 (1.08–10.31)	0.03*	3.17 (0.88–11.39)	0.07	1.78 (0.48–7.06)	0.40
Rash in the specific region §, *n* (%)	14 (16.5)	8 (24.2)	6 (11.5)	2.45 (0.77–7.87)	0.12	2.83(0.79–11.06)	0.12	1.27 80.32–5.26)	0.73

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Resolution of rash, *n* (%)	8 (42.1)	6 (54.6)	2 (25.0)	3.60 (0.49–26.40)	0.35	5.55(0.35–237.37)	0.27	2.68 (0.22–42.14)	0.44
Diagnosed with atopic dermatitis, *n* (%)	6 (7.1)	2 (6.1)	4 (7.7)	0.77 (0.13–4.48)	0.78	0.70 (0.11–4.35)	0.70	0.58 (0.06–4.50)	0.60
Rhinitis									
Nasal symptoms ever, *n* (%)	39 (45.9)	15 (45.5)	24 (46.2)	0.97 (0.41–2.33)	0.95	1.19 (0.44–3.18)	0.74	3.71 (0.99–18.49)	0.07
Current nasal symptoms, *n* (%)	38 (44.7)	15 (45.5)	23 (44.2)	1.05 (0.44–2.53)	0.91	1.34 (0.50–3.59)	0.56	3.90 (1.04–19.48)	0.04*
Current nasal problems accompanied by eye symptoms, *n* (%)	14 (16.5)	5 (15.2)	9 (17.3)	0.85 (0.26–2.81)	0.79	1.00 (0.28–3.68)	0.99	2.00 (0.31–17.75)	0.49
Hay fever ever, *n* (%)	21 (29.6)	6(21.4)	15 (34.9)	0.51 (0.17–1.53)	0.22	0.62 (0.20–1.98)	0.42	6.92 (0.91–154.74)	0.11
Food Allergy									
Diagnosed with food allergy, *n* (%)	11 (12.9)	4 (12.1)	7 (13.5)	0.89 (0.24–3.30)	0.86	0.82 (0.21–3.24)	0.77	0.89 (0.18–4.48)	0.89
Other symptoms associated with meals ¶									
Rash after eating food, *n* (%)	13 (15.3)	7 (21.2)	6 (11.5)	2.06 (0.63–6.80)	0.23	1.72 (0.47–6.30)	0.41	4.28 (0.87–27.15)	0.09

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Vomiting after eating food, *n* (%)	7 (8.2)	3 (9.1)	4 (7.7)	1.20 (0.25–5.74)	0.82	1.33 (0.24–7.55)	0.74	1.32 (0.19–9.97)	0.78

Table 1.Prevalence of allergic symptoms in the group with immunosuppressant exposure and the unexposed group

† Model 1 adjusted for sex, light for date, and parental allergy history.

‡ Model 2adjusted for SLEPDAI ≥ 4 during the first trimester, total glucocorticoid dose during pregnancy, and age ≤ 6 years.

§ Rash in the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes.

¶ Symptoms associated with meals are separated from the field of food allergy because these questionnaires are based on the medical questionnaire rather than ISAAC.

Some symptoms were not shown because no one had those symptoms.

* Indicates statistical significance ($P< 0.05$).

CI: confidence interval; OR: odds ratio.

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Figure legends

Figure 1. TAC dose-dependency on rash ever and current rash in (A) first trimester, (B) second trImester, and (C) thirdtrimester

Results of the TAC dose dependency analysis for rash ever and current rash. Figure 1. A shows the result of Cochran-Armitage trend test about total TAC dose in the first trimester and the prevalence of rash ever or current rash. Figure 1.B shows in the second trimester, and figure 1.C in the third trimester. TAC dose was divided into 3 groups. For the first and second trimesters, the following categories were used: 0 mg, 0 mg < TAC ≤ 250 mg, and TAC > 250 mg. For the third trimester, 0 mg, 0 mg < TAC ≤ 150 mg, and TAC > 150 mg were used, based on gestational age at delivery. *Indicates statistical significance (P< 0.05), Cochran-Armitage trend test.

TAC: tacrolimus.

Supplementary Figure S1. Selection of patients for this study

Children born to participating mothers at another hospital in the past were not included in this cohort because of the lack of perinatal data. We regarded the first child born under initial perinatal management at our center as the first child. Intrauterine exposure to immunosuppressants was defined as the case when the mother used at least one of the following drugs during pregnancy, regardless of duration: tacrolimus, cyclosporine A, and azathioprine.

To evaluate whether tacrolimus has a dose-dependent effect on allergic symptoms,

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children were divided into two groups based on whether they had exposure to TAC in utero.

TAC: tacrolimus.

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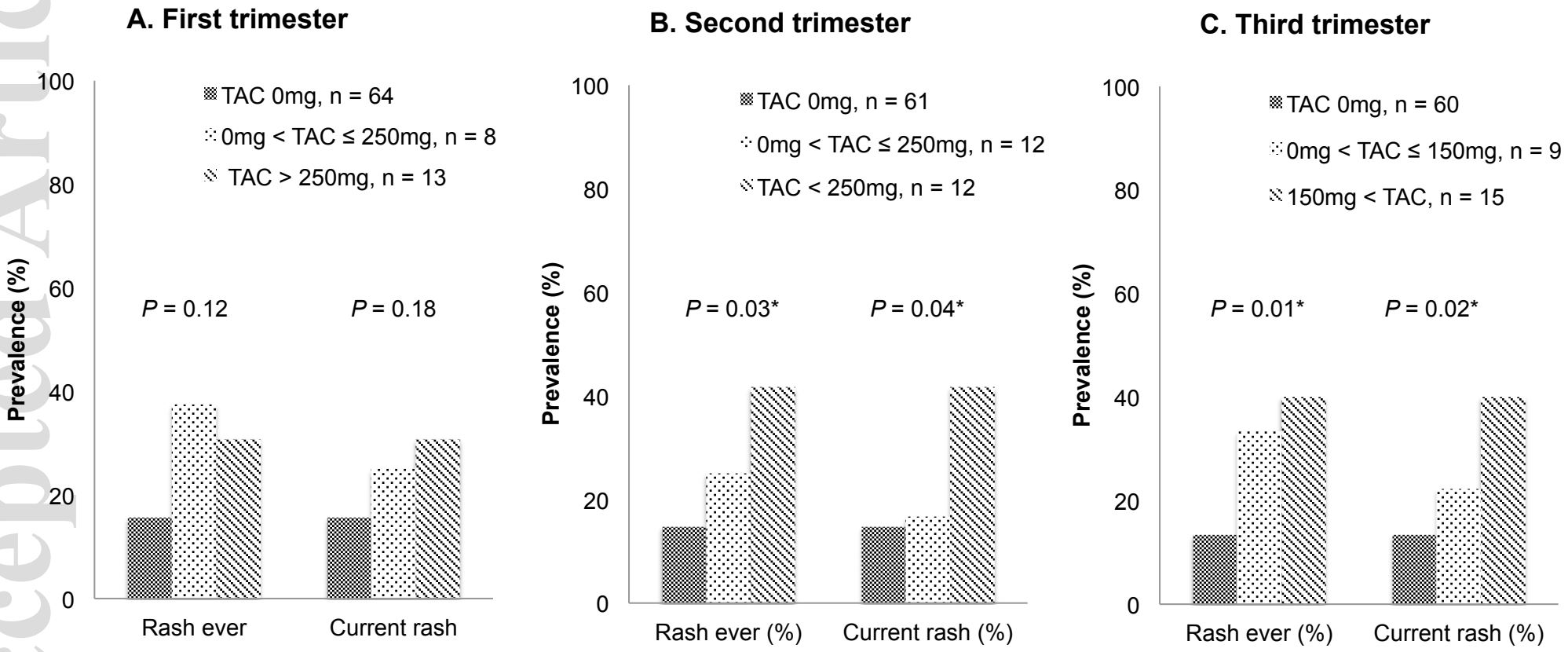
Figure 1. TAC dose-dependency on rash ever and current rash in (A) first trimester, (B) second trImester, and (C) third trimester



Results of the TAC dose dependency analysis for rash ever and current rash. Figure 1. A shows the result of Cochran-Armitage trend test about total TAC dose in the first trimester and the prevalence of rash ever or current rash. Figure 1.B shows in the second trimester, and figure 1.C in the third trimester. Among exposed children, three were exposed starting in the second trimester and one starting in the third trimester. One exposed child was born at 25 weeks of gestation. we divided the total tacrolimus dose during each trimester into three groups based on the daily dose (approximately 2.5 mg) and gestational age at delivery. For the first and second trimesters, the following categories were used: 0 mg, over 0 mg to 250 mg, and over 250 mg. For the third trimester, 0 mg, over 0 mg to 150 mg, and over 150 mg were used. *Indicates statistical significance ($P < 0.05$), Cochran-Armitage trend test.
TAC: tacrolimus.

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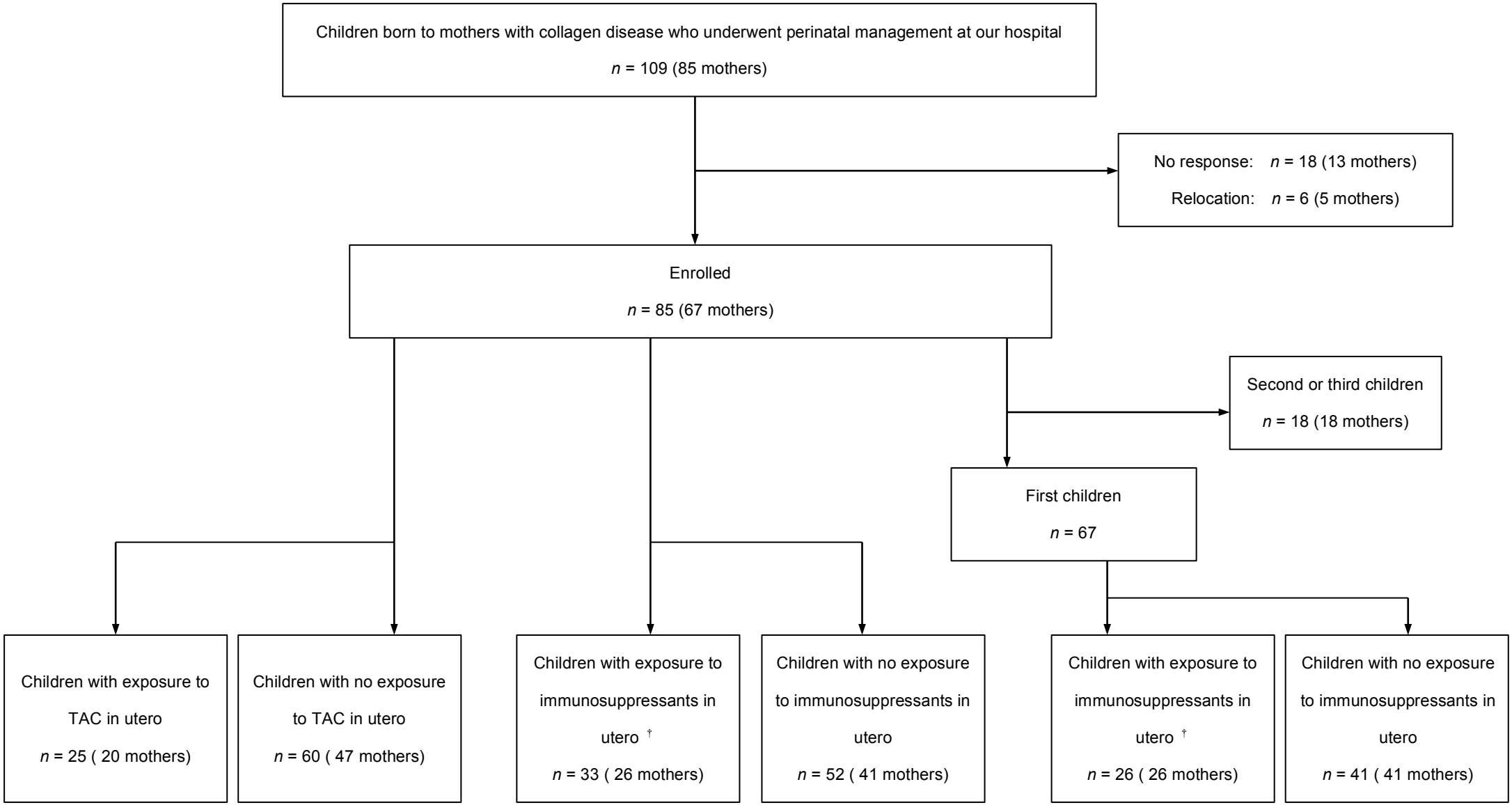
Supplementary Figure S1. Patient selection for this study



The participants were 67 mothers and 85 children who underwent perinatal management in our hospital from January 2002 to December 2018. Children born to participating mothers at another hospital in the past were not included in this cohort because of the lack of perinatal data. We regarded the first child born under initial perinatal management at our center as the first child.

† Intrauterine exposure to immunosuppressants was defined as the case when the mother used at least one of the following drugs during pregnancy, regardless of duration: tacrolimus, cyclosporine A, and azathioprine. TAC: tacrolimus.

Supplementary Table S1.

Japanese version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire

Core questionnaire for asthma

1. Have you (has your child) ever had wheezing or whistling in the chest at any time in the past?

   Yes [] No [] □

   IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 6 □

2. Have you (has your child) had wheezing or whistling in the chest in the past 12 months?

   Yes [] No [] □

   IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 6 □

3. How many attacks of wheezing have you (has your child) had in the past 12 months?

   None [] 1 to 3 [] 4 to 12 [] More than 12 [] □

4. In the past 12 months, how often, on average, has your (your child's) sleep been disturbed due to wheezing?

   Never woken with wheezing []□

   Less than one night per week []

   One or more nights per week [] □

5. In the past 12 months, has wheezing ever been severe enough to limit your (your child's) speech to only one or two words at a time between breaths?

   Yes [] No [] □

6. Have you (has your child) ever had asthma?

   Yes [] No [] □

7. In the last 12 months, has your (your child's) chest sounded wheezy during or after exercise?

   Yes [] No [] □

8. In the past 12 months, have you (has your child) had a dry cough at night, apart from a cough associated with a cold or a chest infection?

   Yes [] No [] □

Core questionnaire for eczema

1. Have you (has your child) ever had an itchy rash that was coming and going for at least 6 months*? (*If your child is <2 years old, at least 2 months)

   Yes [] No [] □

   IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 7 □

2. Have you (has your child) had this itchy rash at any time in the past 12 months?

   Yes [] No [] □

   IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 7 □

3. Has this itchy rash at any time affected any of the following sites: in the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes?

   Yes [] No [] □

4. At what age did this itchy rash first occur?

   < 2 years [] 2–4 years [] ≥5 years [] □

5. Has this itchy rash cleared completely at any time during the past 12 months?

   Yes [] No [] □

6. In the past 12 months, how often, on average, have you (has your child) been kept awake at night by this itchy rash?

   Never in the last 12 months []□

   Less than one night per week []

   One or more nights per week [] □

7. Have you (has your child) ever had eczema?

   Yes [] No [] □

8. In the last 12 months, has your child had dry skin? (only if your child is <2 years old)

   Yes [] No []

Core questionnaire for rhinitis

All questions are about problems that occur when you DO NOT (your child DOES NOT) have a cold or the flu.

1. Have you (has your child) ever had a problem with sneezing, a runny nose, or a blocked nose when you (your child) DID NOT have a cold or the flu?

   Yes [] No [] □

   IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 6 □
2. In the past 12 months, have you (has your child) had a problem with sneezing, a runny nose, or a blocked nose when you (your child) DID NOT have a cold or the flu?

   Yes [] No [] □

   IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 6 □
3. In the past 12 months, has this nasal problem been accompanied by itchy, watery eyes?

   Yes [] No [] □
4. In which of the past 12 months did this nasal problem occur? (Please tick all that apply)

   January [] February [] March [] April [] May [] June []□July []

   August [] September [] October [] November [] December [] □
5. In the past 12 months, how much did this nasal problem interfere with your (your child's) daily activities?

   Not at all [] A little [] □A moderate amount [] A lot [] □
6. Have you (has your child) ever had hay fever?

   Yes [] No [] □

Core questionnaire for food allergy †

1. Have you (has your child) ever had a rash after eating food?

   Yes [] No []
2. Have you (has your child) ever had a wheezing or cough after eating food?

   Yes [] No []
3. Have you (has your child) ever had nausea or vomiting after eating food?

   Yes [] No []
4. Have you (has your child) ever had syncope or loss of consciousness after eating food?

   Yes [] No []
5. Have you (has your child) ever been diagnosed with food allergy?

   Yes [] No []

† Questionnaires for food allergy is based on a medical questionnaire rather than the ISAAC.

Supplementary Table S2. Characteristics of children in this study.

	Total (*n* = 85)	In utero exposure (*n* = 33)	No exposure (*n* = 52)	*P* value
Age of child at the time of the questionnaire (y)				
Median (IQR)	4 (2-9)	2 (0.5–3)	8 (3.25–10)	<.0001*
≤6, *n* (%)	51 (60.0)	30 (90.9)	21 (40.4)	<.0001*
0, n (%)	9 (10.6)	8 (24.2)	1 (1.9)	
1, n (%)	7 (8.2)	5 (15.2)	2 (3.8)	
2, n (%)	11 (12.9)	7 (21.2)	4 (7.7)	
3, n (%)	12 (14.1)	6 (18.2)	6 (11.5)	
4, n (%)	7 (8.3)	3 (9.1)	4 (7.7)	
5, n (%)	3 (3.5)	1 (3.0)	2 (3.9)	
6, n (%)	2 (2.4)	0 (0)	2 (3.9)	
7, n (%)	3 (3.5)	1 (3.0)	2 (3.9)	
8, n (%)	8 (9.4)	1 (3.0)	7 (13.4)	
9, n (%)	6 (7.1)	0 (0)	6 (11.5)	
10, n (%)	8 (9.4)	1 (3.0)	7 (13.4)	
11, n (%)	3 (3.5)	0 (0)	3 (5.8)	
12, n (%)	3 (3.5)	0 (0)	3 (5.8)	
13, n (%)	2 (2.4)	0 (0)	2 (3.9)	
14, n (%)	0 (0)	0 (0)	0 (0)	
15, n (%)	0 (0)	0 (0)	0 (0)	
16, n (%)	1 (1.2)	0 (0)	1 (1.9)	
Gestational age (weeks), median (IQR)	37 (34–38)	37 (34.5–38)	37 (34–39)	0.73
Preterm delivery (<37 weeks), *n* (%)	33 (38.8)	13 (39.4)	20 (38.5)	0.93
Birth weight (g), median (IQR)	2425.0(1847.0–2795.0)	2588.0 (1887.0–2902.0)	2401.5(1833.5–2740.6)	0.37
Sex, *n* (%)				
Male	38 (44.7)	19 (57.6)	19 (36.5)	0.06
Female	47 (55.3)	14 (42.4)	33 (63.5)	
Neonatal complication				
LFD, *n* (%)	26(30.9)	7(21.2)	19 (37.3)	0.12
Neonatal asphyxia, *n* (%)	14 (17.1)	7 (21.2)	7 (14.3)	0.41
Breastfeeding duration [†], *n* (%)				
<3 months	12 (14.6)	3 (9.4)	9 (18.0)	0.84
3–6 months	11 (13.4)	7 (21.9)	4 (8.0)	
≥6 months	59 (72.0)	22 (68.7)	37 (74.0)	
Missing, *n* = 3				
Maternal history of allergy, *n* (%)	33 (39.2)	9 (28.1)	24 (46.2)	0.10
Paternal history of allergy, *n* (%)	30 (35.7)	10 (31.3)	20 (38.5)	0.50

Pet exposure, *n* (%)	12 (14.1)	2 (6.1)	10 (19.2)	0.11
Paternal employment status, *n* (%)				0.64
Regular employee	81 (95.3)	31 (93.9)	50 (96.2)	
Non-regular employee	0 (0)	0 (0)	0 (0)	
Entrepreneur/leader	4 (4.7)	2 (6.1)	2 (3.8)	
Unemployed	0 (0)	0 (0)	0 (0)	
Maternal employment status, *n* (%)				0.55
Regular employee	19 (22.4)	7 (21.2)	12 (23.1)	
Non-regular employee	1 (1.2)	0 (0)	1 (1.9)	
Entrepreneur/leader	2 (2.3)	0 (0)	2 (3.8)	
Unemployed	63 (74.1)	26 (78.8)	37 (71.2)	
Household income (JPY), *n* (%)				0.67
< 6,000,000	28 (33.3)	11 (33.3)	17 (33.3)	
6,000,000 ≤, <10,000,000	37 (44.1)	13 (39.4)	24 (47.1)	
≥ 10,000,000	19 (22.6)	9 (27.3)	10 (19.6)	

† Including mixed nutrition

* Indicates statistical significance ($P < 0.05$). IQR: interquartile range; LFD: light for date