Letter to the Editor

Cord blood eosinophilia precedes neonatal onset of food-protein-induced enterocolitis syndrome (FPIES)

Dear Editor,

Food allergies can be categorized as IgE-mediated and non-IgE-mediated food allergies, depending on whether specific IgE antibodies are the primary mechanism for recognizing offending foods.[1] IgE-mediated food allergies are usually due to sensitization to a food through eczematous skin, and symptoms usually start several months after birth. However, in many patients with non-IgE-mediated gastrointestinal food allergy (non-IgE-GI-FA), symptoms start within one month after delivery.[2,3] Because non-IgE-GI-FAs involve immunity, we hypothesized that acquisition of immunity against offending foods precedes the onset of symptoms after birth, i.e., intrauterine antigen priming or acquisition of immunity. A recent study demonstrating the presence of major food allergens in amniotic fluid supports this hypothesis.[4] However, no studies have investigated the immunological cell types in cord blood of infants with non-IgE-GI-FAs. Because patients with a non-IgE-GI-FA often show significant blood eosinophilia,[5] we reviewed the data on cord blood eosinophils in those patients and compared them with matched controls to test our above hypothesis. Among the non-IgE-GI-FA subgroups, we focused on food-protein-induced enterocolitis syndrome (FPIES), for which there are well-developed diagnostic criteria.

This is a retrospective case−control study. The patient inclusion criteria were: (1) delivery in the National Center for Child Health and Development (NCCHD, Tokyo, Japan) and performance of cord blood examinations, (2) onset of symptoms before one month of age, (3) observation of vomiting more than twice at first presentation, without any other cause for the symptoms, and (4) elicitation of gastrointestinal symptoms (repetitive vomiting and/or frequent diarrhea) within 24 h after oral food challenge (OFC) performed at least 2 weeks after resolution of the initial symptoms. The exclusion criteria were: (1) cord blood clotting and thrombocytopenia of less than $100 \times 10^3/\mu L$, (2) cord blood nucleated red blood cells exceeding 5% and (3) absence of vomiting (to confirm that the symptoms are definitely induced by ingestion of the offending food). Namely, we excluded neonates who were diagnosed with food-protein-induced allergic proctocolitis (FPIAP) or food-protein-induced enteropathy (FPE), without vomiting. As matched controls, 30 babies were randomly extracted from 16,018 babies who did not have FPIES; they were comparable to

the 6 patients with FPIES in terms of the gestation period, sex, mode of delivery and birth weight. Supplementary Figure 1 shows the procedures that were followed for selection of the FPIES patients and matched controls. White blood cell (WBC) differentials of cord blood and peripheral blood samples were determined using an automated cell counter (Advia 120; Siemens Health Care Diagnostics; Tokyo, Japan). Blood cells were stained with peroxidase and differentiated by flow cytometry. The study was performed according to a protocol approved by the institutional review board of NCCHD (#2154).

Among 22,459 babies born in NCCHD between March 2002 and March 2015, 7 newborns who had valid cord blood data and experienced vomiting after being started on cow's milk were diagnosed with FPIES based on positive OFC results. One of those patients was excluded due to late onset of symptoms. The remaining 6 patients were enrolled, and their demographics were compiled (Table 1). Serum IgE specific to cow's milk protein was positive in one patient, but vomiting started 3 h after ingestion of milk. None of the 6 patients showed any immediate-type reactions such as acute urticaria or wheezing. The precise clinical data at onset and OFCs are shown in Table 1 and Supplementary Results. Patients 1 and 3 showed repetitive vomiting within 4 h after an OFC, and fulfilled the criteria for chronic FPIES set forth in the consensus guideline.[6] Patients 4 and 5 showed delayed onset (6−7 h) of vomiting after OFCs. Patients 2 and 6 showed frequent diarrhea within 24 h after OFCs. Among the three subgroups of non-IgE-GIFAs, Patients 2, 4, 5 and 6 showed strong similarity to FPIES, but not to FPE or FPIAP. Also, four patients fulfilled the earlier criteria for FPIES.[7] Therefore, all six patients were handled as FPIES in this report.

The laboratory data for the cord blood were compared between the FPIES patients and the matched controls (Fig. 1A). Among the cord blood hemoglobin, WBCs, platelet count and WBC differential, only the cord blood eosinophil percentage was significantly higher in FPIES ($P = .0002$). The absolute count of cord blood eosinophils was also significantly higher ($P = .0001$, Fig. 1B). There was no correlation between the eosinophil count in the maternal peripheral blood and the cord blood of babies with FPIES (Fig. 1C, D). Therefore, fetal eosinophilia is probably not due to simple transfer of eosinophilia-promoting factors from the mother. Eosinophilia may be a result of prolonged eosinophil survival or exaggerated eosinophilopoiesis due to IL-5 overproduction, presumably by

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Table 1
Patients' demographics.

Patient number	1	2	3	4	5	6
Sex	F	M	F	F	F	F
Intrauterine						
Twins	−	+	−	−	−	−
Intrauterine infection	−	−	−	−	−	−
Amniotic fluid excess	+	−	−	+	−	−
Threatened abortion	−	+	−	−	+	−
Delivery						
Threatened premature delivery	−	+	−	+	−	−
Premature rupture	−	−	−	+	+	+
Placental abruption	−	−	−	−	+	+
Mode of delivery	vaginal	em-c/s	vaginal	em-c/s	c/s	em-c/s
Gestational age	41 w 5 d	33 w 6 d	37 w 4 d	36 w 6 d	36 w 2 d	32 w 4 d
Meconium-stained amniotic fluid	−	−	−	+	+	−
Birth weight (kg)	2.73	2.23	2.40	2.56	2.41	1.90
Onset (days after birth)	5	6	7	8	10	13
Blood eosinophils; %, (absolute counts/μL)						
Peripheral blood of the mother	1% (74)	2.3% (172)	1.7% (126)	0.7% (62)	0.9% (176)	2% (227)
Cord blood	4% (488)	11.3% (904)	5% (460)	13.5% (1242)	7% (1097)	5.3% (758)
Peak blood eosinophils of the baby after onset	ND	10% (997)	12% (1280)	23% (3680)	25% (4000)	38% (8007)
Cow's milk-specific IgE (class 0−6)	0	0	2	0	0	0
Offending food(s) confirmed by OFC	CM	CM, rice	CM	CM, rice	CM	CM, rice

Summary of first episode (onset) and positive oral-food challenge tests.

	Onset	OFC	Onset	OFC	Onset	OFC	Onset	OFC	Onset	OFC	Onset	OFC
Time after birth	5 d	5 mo 7 d	6 d	1 mo 8 d	7 d	6 mo	8 d	27 d	10 d	7 mo 4 d	13 d	2 y
Challenged food	CM	CM 60 mL	CM	CM	CM	CM 35 mL	CM	CM 30 mL	CM	CM 30 mL	CM	CM 0.8 mL
Latent time from the start of offending food to evocation of symptoms	5 d	3 h	5 d	8 h	7 d	3 h	7 d	6 h	9 d	7 h	11 d	3 h
Evoked symptom(s)	V, L	V, D	V, BS, apnea	D, apnea	V, BS	V, L	V, BS	V	V, BS	V	V, abd. Distension	D
Frequency of the symptom(s)	V several times	V, D: 5−6 times	V several	several	V (bile), several	V twice	V twice, BS 3 days	twice	V several	several	V several	several
Increase in blood neutrophils (>2500/μL)	ND	ND	−	−	−	+	−	−	ND	+	ND	ND
Stool eosinophilia[†]	ND	ND	+	+	+	−	+	+	ND	ND	+	ND
Stool Charcot-Leyden crystals[†]	ND	ND	+	−	+	−	+	+	ND	ND	+	ND

BS, bloody stool; CM, cow's milk; c/s, planned cesarean section; em-c/s, emergency cesarean section; D, diarrhea; L, lethargy; ND, not determined; OFC, oral food challenge test; V, vomiting.
Latent time; Patients ingested cow's milk formula every day before the onset of non-IgE-GI-FA. Blood neutrophils were examined on the same day that symptoms started. Stool mucus eosinophils and Charcot-Leyden crystals were examined on the day[†] after OFC.
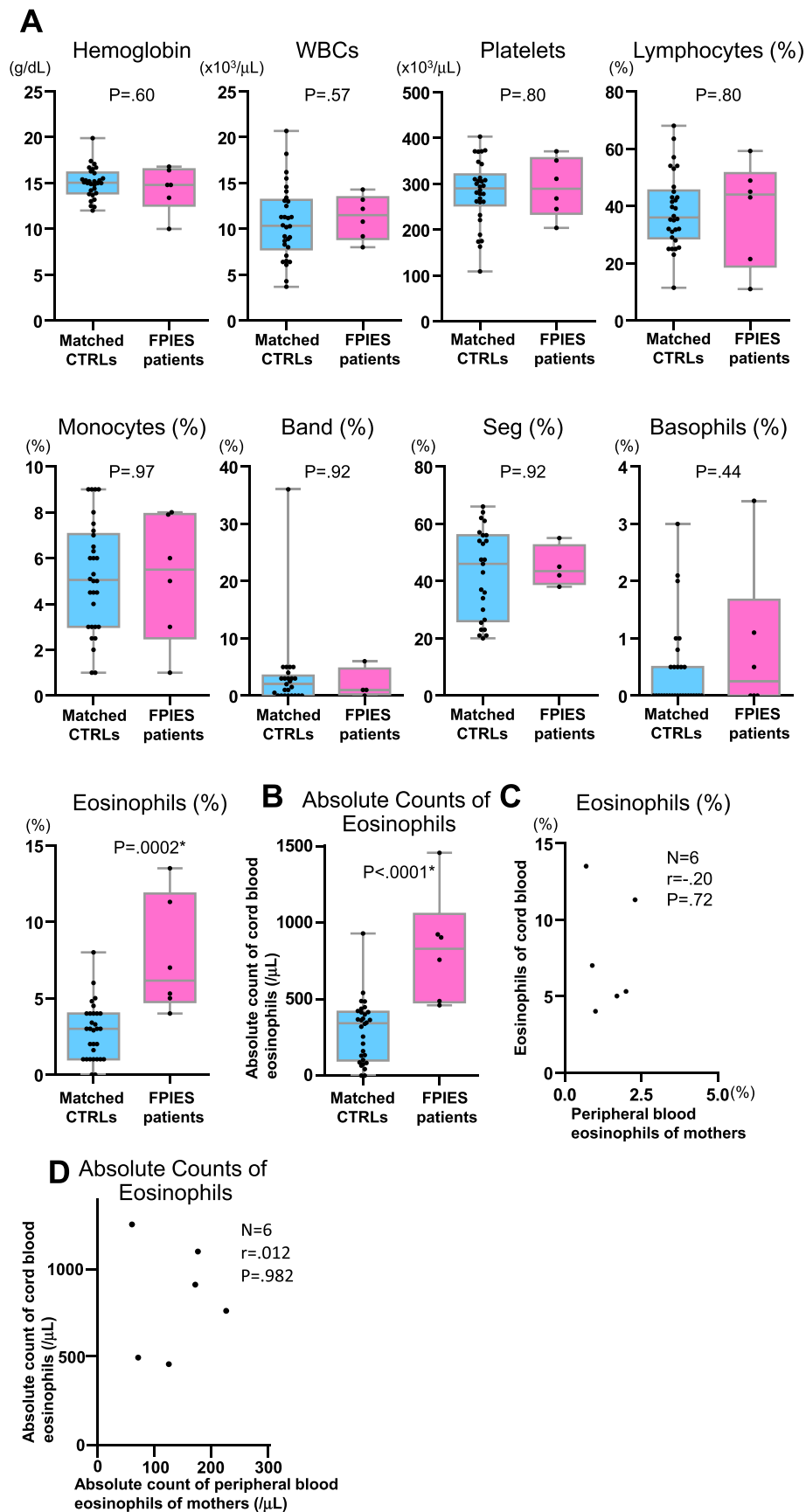
Fig. 1. Laboratory data for the cord blood were compared between the matched controls (CTRL, n = 30) and FPIES patients (n = 6). **A.** Hemoglobin, WBCs, platelets and the percentage of each cell type were compared. WBCs: white blood cells; Band: band neutrophils; Seg: segmented neutrophils. **B.** Absolute counts of the cord blood eosinophils were also compared. **C.** Relationship of eosinophil percentages between the cord blood of FPIES patients and the mothers' peripheral blood just before delivery. **D.** Relationship of absolute counts of eosinophils between the cord blood of FPIES patients and the mothers' peripheral blood just before delivery. *P < .05.

antigen-specific Th2 cells[8] in the fetus. None of the mothers had allergies to cow's milk, so they had not eliminated cow's milk from their diet. The mechanisms underlying cord blood eosinophilia remain unclear. However, milk-antigen-specific Th2 cells were generated in the fetus and may have triggered GI inflammation after coming into contact with milk antigens from the mother. Furthermore, although it is unclear whether there is any causal relationship, very high frequencies of intrauterine and delivery abnormalities were observed (Table 1). Even though these abnormalities are not disease-specific, such high frequencies imply that priming of neonatal-onset FPIES may occur before birth, and one of the consequences may be cord blood eosinophilia. The development of clinical symptoms of FPIES after birth was followed by even further increases in the patients' peripheral blood eosinophils (997–8007/μL; Table 1).

When we looked at the relationship of the cord blood eosinophil percentage in the total 16,018 control babies to each of the 6 modes of delivery, emergency C/S showed a lower eosinophil percentage than spontaneous vaginal delivery and scheduled C/S (P < .001; Supplementary Results, Supplementary Fig. 2). However, although Patients 2, 4 and 6 were delivered by emergency C/S, their cord blood eosinophil percentages were high. They thus did not appear to be due to the mode of delivery.

Each of the 6 patients with FPIES developed atopic dermatitis during the period of 3–10 months of age, but only 11.1% of the matched control babies did (see Supplementary Results). This suggests a common pathogenesis between the two diseases.

Coincidentally, fetal MRI was performed for Patient 4 at 34 weeks of gestation and showed apparently abnormal gastrointestinal signals. This finding suggests the possibility that GI inflammation may have started before birth. The method of MRI and precise explanations are described in Supplementary Methods.

A limitation of this study is the small sample size of the FPIES cases (N = 6). However, accurate cord blood data for this rare disease cannot be easily obtained. Therefore, we thought that it is important to report our findings in spite of limited evidence.

In conclusion, our findings suggest that cord blood eosinophilia may precede neonatal onset of FPIES. In the future, our hypothesis needs to be more strongly proven by increasing the number of the patients, devising image diagnoses, and investigating the reactivity of cord blood immune cells to milk proteins.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2020.10.004.

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SUPPLEMENTARY METHODS

Preparation of cord blood

As soon as the babies were delivered—before delivery of the placenta—the midwife collected cord blood from the umbilical vein using a 20-mL syringe with an 18-gauge needle. The blood was promptly transferred to an EDTA tube for determination of the blood cell counts.

Selection of matched controls

From March 2002 to March 2015, 22,459 neonates were born in NCCHD, and their cord blood cells were examined. After exclusion of cases whose cord blood data showed a decreased platelet count (less than $100 \times 10^3/\mu L$) or a transient nucleated red cell increase, the data for 16,018 neonates were employed as the controls (unmatched controls). Thirty neonates were extracted as matched controls (Supplementary **Fig. 1,** Supplementary **Table 1**). To generate the matched controls, we performed 1:5 matching without replacement using a greedy matching algorithm.[E1] The confounding factors considered as matching variables were the birth weight, gestation period, sex and mode of delivery. The caliper widths of weight and the gestation period were set to 120 grams and five days, respectively. The other factors were set to exact matching. Matching was performed using SAS software, version 9.4 (SAS Institute; NC, USA).

Comparison of unmatched controls, matched controls and FPIES patients

Differences between the study groups were analyzed using the Mann-Whitney U test, performed with IBM SPSS Statistics for Windows, version 25 (IBM Corp.; Armonk, NY, USA).

The cord blood eosinophils differed significantly between the unmatched controls and FPIES. Emergency cesarean section (C/S) was performed to deliver 50% of the FPIES neonates and matched control neonates, but only 12.6% of the unmatched control neonates (Supplementary **Table 1**).

SUPPLEMENTARY RESULTS

The clinical courses of six patients with FPIES, especially the initial events and results of OFCs.

Patient 1: A girl, born at gestational age 41 weeks and five days by vaginal delivery. Her birth weight was 2.73 kg. After birth, she was mainly breastfed. When cow's milk formula was added to her nutrition, she vomited several times and experienced lethargy. A similar reaction occurred several times, so cow's milk was eliminated from her diet. When she was five months old, her parents gave her 60 mL of cow's milk. Three hours later she started repeated projectile vomiting, six times. She was taken to a regional pediatric clinic. Specific IgE antibody to cow's milk was found to be negative. When she was six months old, she was fed 10 mL of partially hydrolyzed casein formula (E-AKACHAN; Morinaga Milk Industry Co., Ltd.; Tokyo, Japan). Three hours later she experienced lethargy and vomited six times. Based on these events, she was diagnosed with chronic FPIES. She subsequently gained weight and developed well. When she was one year and 11 months of age, she was admitted to NCCHD for OFC. The result was negative, and remission of the FPIES was confirmed.

Patient 2: A boy. He was one of two chorions, i.e., amniotic twins. At the gestational age of 33 weeks and six days, he was delivered by emergency C/S because of the start of intense labor pains. After delivery, he experienced transient tachypnea. Because breast milk was not sufficient, he was fed cow's formula from day two after birth. After the start of cow's milk, blood acidosis and apnea gradually worsened. On day six, he exhibited intermittent vomiting, frequent apnea and bloody stool. Stool mucus eosinophilia and Charcot-Leyden crystals were observed. The cow's formula was

discontinued, and the apnea and vomiting ceased. He was placed on an amino-acid formula (Elemental-formula; Meiji; Tokyo, Japan), and his weight increased. On day 38 after birth, he was again introduced to cow's formula. Apnea restarted. Diarrhea started and contained many stool-mucus-eosinophils and was positive for hemoglobins. At that time, he was diagnosed with FPIES. The cow's formula was stopped, and he was again fed an amino-acid formula. His growth and development were normal. When he was three years old, he went to another region of Japan due to his father's job transfer. As a result, OFCs aimed at confirmation of remission were unable to be performed.

Patient 3: A baby girl, born by natural vaginal delivery at the gestational age of 37 weeks and four days. Because the mother did not produce enough breast milk, she was fed cow's milk from day 0. On day seven, she developed intermittent vomiting and bloody stool. An upper gastrointestinal series showed no stenosis, dilation or malrotation of her GI tract. Stool mucus cytology revealed eosinophilia. The cow's milk was discontinued, and the vomiting and bloody stool ceased. Non-IgE-GI-FA was suspected, and amino acid milk was started. She gained weight. When she was six months old, she was admitted to NCCHD for OFC. Three hours after ingestion of 35 mL of cow's milk, profuse vomiting started, together with lethargy. She was diagnosed with FPIES. Her subsequent growth and development were normal. When she was two years old, she was able to ingest cow's milk without reaction, and remission of FPIES was confirmed.

Patient 4: Fetal MRI was performed on the 17th day before birth because of excess amniotic fluid. The baby, a girl, was delivered by emergency C/S, at which time meconium aspiration occurred. Cow's milk was introduced soon after birth. On the same day, she defecated bloody stool once; cytological examination showed the stool to contain many eosinophils. Vomiting and bloody stool occurred on the 8th day after birth. The

cow's milk was discontinued and changed to an amino-acid formula (Elental-P; Ajinomoto Pharmaceuticals Co., Ltd.; Tokyo, Japan). With the amino-acid formula, she was in good health, and her body weight increased. On the 26th day, cow's milk was re-started, and vomiting occurred 6 hours later. Peripheral blood eosinophils were increased to 23% of total WBCs. Cow's milk was discontinued, but it was re-started again on the 62nd day; vomiting and diarrhea developed seven days later. Peripheral blood eosinophils increased by up to 30%. Therefore, she was diagnosed as having FPIES. She was given soy milk at seven months old, and 24 hours later she developed bloody stool that continued for five days. She developed normally after cow's milk and soy were eliminated from her diet. She was able to safely ingest cow's milk formula and soy at one year and nine months of age, indicating that her FPIES was in remission.

Patient 5: A girl. At a gestational age of 36 weeks and two days, a decreased fetal heart sound and early placental ablation were detected. Aspiration delivery was performed. The amniotic fluid was meconium-stained, and tarry stools were discharged. The Apt test was positive and revealed the blood to be of fetal origin. Breast milk was started, and then supplemented with cow's milk. Initially she looked well. However, on day 10, intermittent vomiting and bloody diarrhea started. She lost appetite the next day, and the cow's milk was discontinued. FPIES was suspected, and an amino-acid formula (Elemental-formula) was started. She regained her appetite and increased in weight. When she was seven months old, she was admitted to NCCHD and underwent OFC. Seven hours after ingestion of 30 mL of cow's milk, she vomited twice. Peripheral blood neutrophils were increased by 5,800/μL from the baseline. She was diagnosed with FPIES. She developed normally after eliminating cow's milk from her diet. When she was one year and nine months old, she was able to ingest cow's milk without reaction, indicating

that remission had been achieved.

Patient 6: A girl. She was delivered by emergency C/S due to premature abruption of the placenta. After birth, she was fed breast milk and cow's milk. On day 13, she started intermittent vomiting and diarrhea. Metabolic acidosis, increased blood eosinophils, and stool mucus eosinophilia were detected. Her nutrition was changed to breast milk (with maternal elimination of dairy products) and amino-acid formula (Elemental-formula). Her symptoms then disappeared, and her laboratory data became normal. FPIES was suspected. She developed normally after eliminating cow's milk from her diet. Her parents did not want her to undergo OFC in the hospital setting. When she was two years old, she was administered 0.8 mL of cow's milk in her home. Three hours after, she started severe diarrhea that continued for 2-3 days. This was repeated more than three times during the next several months. She was diagnosed with FPIES. When she was three years old, she was able to freely ingest cow's milk, and remission of the FPIES was thus confirmed.

Detailed clinical information regarding emergency C/S cases.

In this study, four of the six infants with FPIES had been delivered by C/S, three of which were emergency C/S (**Table 1**).

Patient 2 was delivered by emergency C/S because his mother had a twin pregnancy and experienced strong labor contractions. No fever developed after birth. Cord blood C-reactive protein (CRP) was under 0.2 mg/dL. Bacterial cultures of the blood, nasal mucus and gastric juice were all negative. No antibiotics were administered to the baby.

Patient 4 was delivered by emergency C/S because of premature rupture of the membranes. No fever developed after birth. Cord blood CRP was 0.3-0.4 mg/dL.

Bacterial cultures of the blood, nasal mucus and gastric juice were all negative or found only normal flora. Intravenous ampicillin sodium and gentamycin were given prophylactically. No apparent infections were observed.

Patient 6 was delivered by emergency C/S because of premature abruption of the placenta. No fever developed after delivery. Cord blood CRP was under 0.2 mg/dL. Bacterial cultures of the blood, nasal mucus and gastric juice were all negative. No antibiotics were administered to the baby.

Development of atopic dermatitis in the FPIES infants and matched controls.

All six patients with FPIES developed atopic dermatitis afterward. The diagnosis was made by an experienced allergy specialist using the diagnostic criteria of the UK Working Party. Five patients showed bilateral flexural involvement at more than two areas of the elbows, knees and foot joints.

Patient 1: Skin dryness started at seven months of age. Whole-body eczema was seen at eight months. When she was 10 months old, she developed eczema on the bilateral lower legs and was scratching it. She was diagnosed with atopic dermatitis, and application of hydrocortisone butyrate was started.

Patient 2: When he was three months old, eczema was observed on the bilateral cheeks. At four months, whole-body skin dryness started. Moreover, bilateral flexural involvement was seen at the neck, elbows and knees. He was scratching those sites. He was diagnosed with atopic dermatitis. He was treated by application of dexamethasone valerate on his body. Remission was maintained with proactive treatment (twice a week) using the same topical glucocorticoid.

Patient 3: At 2 months of age, she developed diffuse-mild eczema over her whole body. Typical bilateral flexural involvement was seen on the wrists, knees and foot joint at three months. She was scratching those sites. Thus, she was diagnosed with atopic dermatitis and treated with topical hydrocortisone butyrate. Remission was maintained with proactive treatment using the same topical steroid.

Patient 4: Bilateral eczema on the cheeks started when she was three months old. At four months, eczema appeared on the chest, ears and upper eyelids. She was scratching bilateral flexural eczema on her knees. A diagnosis of atopic dermatitis was made. Hydrocortisone butyrate achieved remission induction, and remission was maintained with proactive use of this steroid.

Patient 5: When she was two months old, she developed eczema on the bilateral cheeks. At five months, bilateral flexural involvement of the wrists and knees was seen, and she was scratching those sites. When she was one year old, eczema on the nape of the neck started, and betamethasone valerate was applied. There was no reactivation after two months of treatment.

Patient 6: When she was 2 months old, she had eczema on the left cheek. At 5 months, she developed eczema bilaterally on her cheeks, neck, elbows and chest. She was scratching those sites. She was diagnosed with atopic dermatitis and treated with hydrocortisone butyrate. Remission was maintained with proactive treatment using the same topical steroid. When she was one year old, she showed reactivation of papular erythema on the truncal skin.

Matched control babies: NCCHD pediatricians monitored the skin of 23 of the 30 matched control babies until at least one year of age. While 20 patients were not diagnosed with atopic dermatitis, three patients were diagnosed with atopic dermatitis by

the referred dermatologists. Namely, 11.1% of the matched control babies developed atopic dermatitis. The cord blood eosinophil percentages of these three babies were 1.0%, 1.0% and 6.0%.

Atopic dermatitis morbidity is known to increase in FPIES patients. Th2-prone immunity and eosinophilia are common features of both diseases. Given the early immunological changes in the uterus, it is no wonder that skin in extensive contact with the amniotic fluid is exposed to some stimuli simultaneously with the gastrointestinal tract. After birth, a robust immune response may occur first in the gastrointestinal tract after exposure to large amounts and high concentrations of antigen, followed by inflammation of skin that has been exposed to relatively low concentrations of some antigens or stimuli.

Cord blood eosinophils and mode of delivery.

The cord blood eosinophil percentages of the total 16,018 control babies were compared among six modes of delivery (Supplementary **Fig. 2**). Cord blood from babies delivered by emergency C/S showed lower eosinophils than spontaneous vaginal deliveries and scheduled C/S (P<.001). Stress hormones like cortisol are increased in complicated labor compared with uncomplicated labor.[E2] Cortisol decreases circulating eosinophils. This might be why cord blood eosinophils were lower in the emergency C/S deliveries compared with the scheduled C/S deliveries.

Fetal Magnetic Resonance Imaging (MRI) of Patient 4 and age-matched controls

Fetal MRI was performed at 34 weeks of gestation on Patient 4 and on 6 gestational-age-matched control subjects. A blinded radiologist compared the signal intensities of the meconium in the colon in T1-weighted images and the signal intensities of the contents of the small intestine in T2-weighted images (single-shot fast spin-echo).

The fetal MRI of Patient 4 did not show a high T1 signal for meconium in her colon (**Supplementary Fig. 3 D**), whereas such signals were observed in all the control subjects (**Supplementary Fig. 3 C, G**). On the other hand, the contents of the small intestine of Patient 4 showed a very high T2 signal (**Supplementary Fig. 3 F**) compared with each of the controls (**Supplementary Fig.3 E, G**). These findings are interpreted as indicating that intestinal inflammation increased the intraluminal fluid, which flushed the meconium out of the terminal portion of the GI tract or diluted the meconium in the colon. Very similar MRI findings were reported for congenital chloride diarrhea.[E3] However, after delivery, this baby girl did not develop chloride diarrhea, but instead manifested FPIES. This finding may suggest that GI inflammation started before birth. Moreover, a very recent report from another hospital demonstrated similar fetal-MRI findings in a case of very early neonatal onset FPIES,[E4] suggesting that such findings are common in FPIES patients.

Considerations about OFCs

Powell,[E5] Sicherer, et al.,[E6] and Nowak-Wegrzyn, et al.[E7] each described criteria for a positive reaction in oral food challenge tests (OFCs) for diagnosing FPIES. Sicherer et al. wrote that "standardized food challenge elicited diarrhea and/or vomiting within 24 hours after administration of the food." However, the international guideline[E7] released in 2017

focused on patients who show repetitive vomiting within 4 hours after food challenge and clearly defined a restricted phenotype of FPIES. The latter can be expected to reduce confusion regarding FPIES.

Of the six patients in this study, only two met Nowak-Wegrzyn's criteria[E7] of repetitive vomiting within 4 hours (**Table 1**) and can be diagnosed as having chronic FPIES. Patients 4 and 5 had repetitive vomiting in response to the challenge tests, but they occurred 6-7 hours (not 4) after challenge. Patients 2 and 6 did not vomit in response to the OFCs, but they experienced frequent diarrhea. Thus, those four patients cannot be classified as FPIES according to the latest FPIES criteria. However, they still fulfilled Sicherer et al.'s earlier FPIES criteria.[E6] Among the three subgroups of non-IgE-GIFAs, Patients 2, 4, 5 and 6 showed strong similarity to FPIES, but not to FPE or FPIAP. Furthermore, there is still insufficient evidence regarding neonatal onset FPIES. To set strict diagnostic criteria, we must wait for future research results. Therefore, all six patients were handled as FPIES in this report.

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Supplementary Table 1. Demographics of unmatched controls, matched controls and FPIES patients.
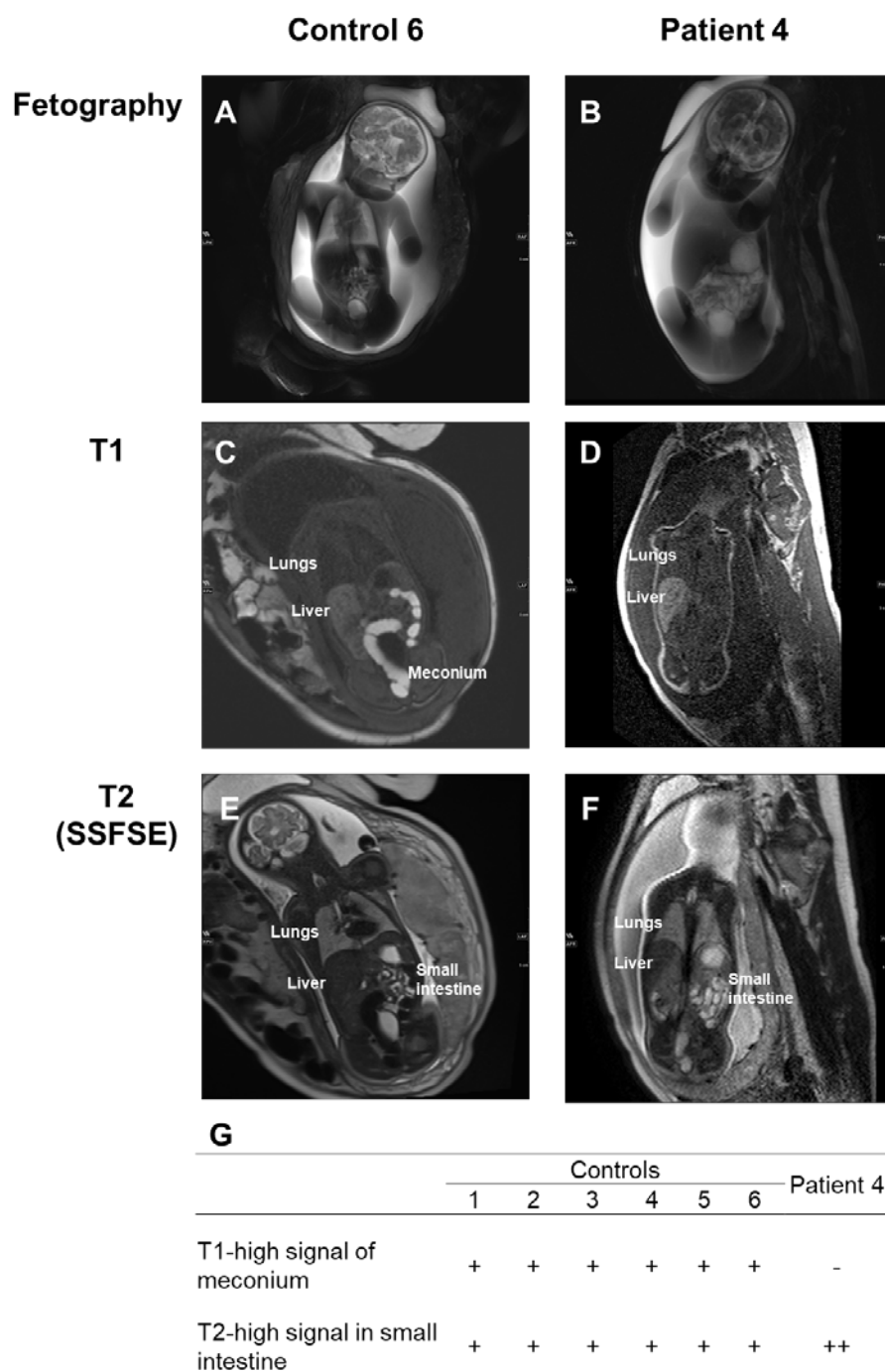
	Unmatched controls N=16,018	Matched controls N=30	FPIES patients N=6
Gestation period (days), median (range)	273 (145-298)	257 (227-290)	256 (228-291)
Birth weight (g), median (range)	2,930 (285-5,045)	2,405 (1,796-2,740)	2,405 (1,902-2,725)
Emergency cesarean section, N (%)	2,023 (12.6)	15 (50.0)	3 (50.0)
Sex: female (%)	7,631 (47.6)	25 (83.3)	5 (83.3)
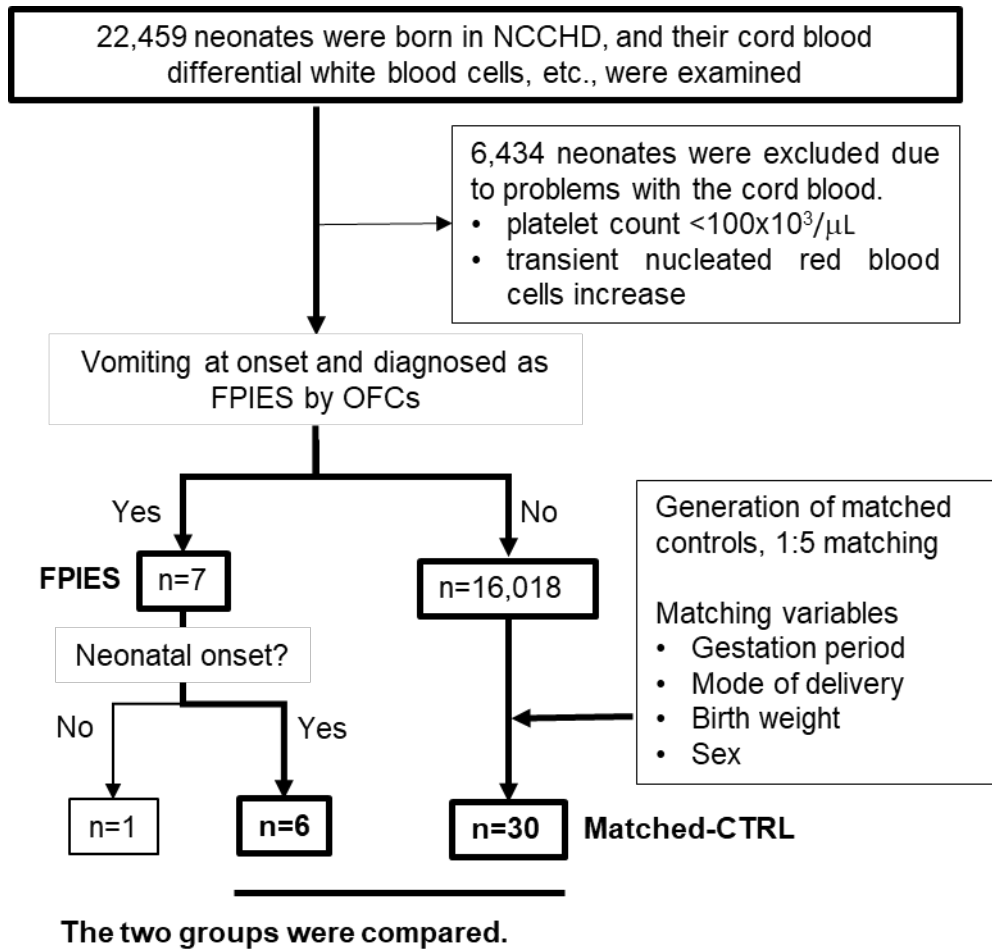
Supplementary Fig. 1: Selection of the patients and matched controls. Out of 22,459 neonates delivered in NCCHD, 9 patients were identified as having FPIES. Two were excluded because of problems with the cord blood examination. Onset in one patient was late (79th day after birth). Finally, 6 FPIES patients with neonatal-onset were included. For the other 16,018 neonates with valid cord blood data, we performed 1:5 matching. The matching variables were the gestation period, mode of delivery, birth weight and sex. Thirty neonates were extracted.
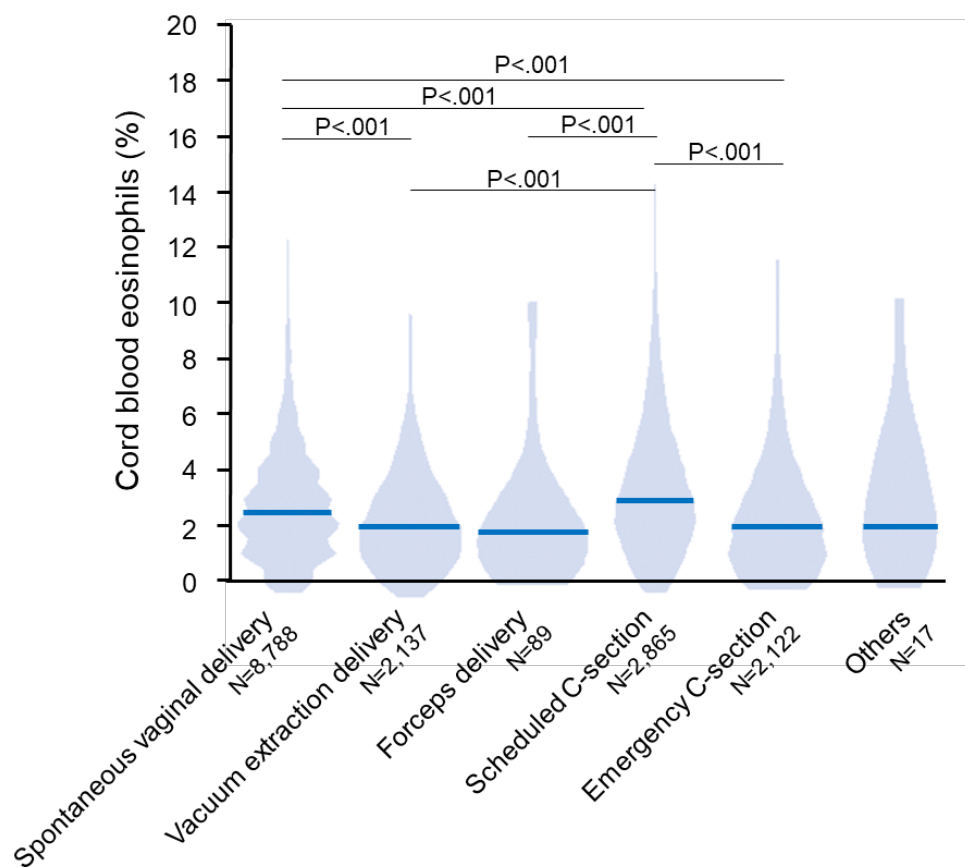
Supplementary Fig. 2. Cord blood eosinophils and modes of delivery. Cord blood eosinophil percentages were compared among 6 modes of delivery. Violin plots were generated using JMP11. The median of each group is shown as a dark-blue line. The statistical methods employed were ANOVA, and Dunnett's T3 test for multiple comparisons.

Supplementary Fig. 3. Fetal MRI of age-matched controls and FPIES Patient 4 at 34 weeks of gestation. **A** and **B** show MR fetography images. Meconium-related T1-high signals in the colon and rectum were present in Control 6 (**C**) and all the other controls (**G**), but absent in Patient 4 (**D, G**). The T2 signal (SSFSE; single-shot fast spin-echo) in the small intestine was very high in Patient 4 (**F, G**) compared with Control 6 (**E**). **G.** Similar results were obtained for each of the other five age-matched controls.