

Letter to the Editor

Low-affinity allergen-specific IgE in cord blood and affinity maturation after birth

To the Editor:

Newborn blood and cord blood (CB) often contain allergen-specific IgE when the mother has a history of allergy and/or allergic inflammation during pregnancy.^{1,2} Such finding indicates intrauterine allergen-sensitization by maternofetal interactions.^{2,3} To our knowledge, there are no published data on fetal atopy and allergic disease despite the evidence of maternofetal passage of allergens across the placenta.³ The limited allergic responses in young infants and fetuses are considered to be due to immunological immaturity.⁴ Using an animal model, a recent study described 2 sequential immunological maturation class switching pathways that generate high- and low-affinity IgE.⁵ The high-affinity IgE is generated through sequential class switching ($\mu \rightarrow \gamma \rightarrow \epsilon$), in which an intermediary IgG phase is necessary for the affinity maturation by somatic hypermutation. In contrast, the low-affinity IgE is generated through direct class switching ($\mu \rightarrow \epsilon$) and is much less mutated. However, there are so far no reports of human low-affinity IgE.

We recently described a highly sensitive IgE microarray with high-density antigen immobilization on a diamond-like carbon (DLC)-coated chip⁶ and reported the allergen-specific IgE of fetal origin in CB.¹ The present study is an extension to our previous study and designed to further characterize allergen-specific IgE from birth to 6- and 14-month-old infants by using the same technology. The analysis identified the presence of low-affinity allergen-specific IgE in CB and detected affinity maturation after birth.

We used blood samples reported in a cohort study (the Gifu Allergy and Immunology Cohort Study).⁷ This study was approved by the Ethics Committees of the Graduate School of Medicine, Gifu University and Tokushima University Hospital. Paired samples ($n = 116$) of CB and peripheral blood at age 6 and 14 months from 37 subjects were positive for egg white-specific IgE (≥ 0.7 Ua/mL) on the UniCAP. Furthermore, the ovomucoid (OVM)-specific IgE levels in 9 paired CB and blood samples after birth from the 37 subjects (24.3%) were persistently 20 BUe/mL or more on the DLC-coated chip, whereas all OVM-specific IgE levels in CB were below the detection limit of the UniCAP system (see Fig E1 in this article's Online Repository available at www.jacionline.org). Atopic dermatitis (AD) was detected in 5 and 6 of the 9 infants at age 6 and 14 months, respectively (see Table E1 in this article's Online Repository available at www.jacionline.org). The affinity of OVM-specific IgE in CB and blood after birth was analyzed by using an OVM-immobilized DLC-coated chip by the OVM competition binding assay and binding inhibition using a mild chaotropic agent, diethylamine (DEA), as reported previously.⁸ Binding of OVM-specific IgE on the DLC-coated chip was detected specifically without cross-detection of IgM, IgG, IgA, and IgD (see Fig E2 in this article's Online Repository available at www.jacionline.org). We also performed functional analysis of OVM-specific IgE by cross-linking of human FcεRI on the activation of a luciferase-reporting mast cell line.⁹ Statistical analysis was conducted by using the Statistical Package for Social Sciences (version 18.0;

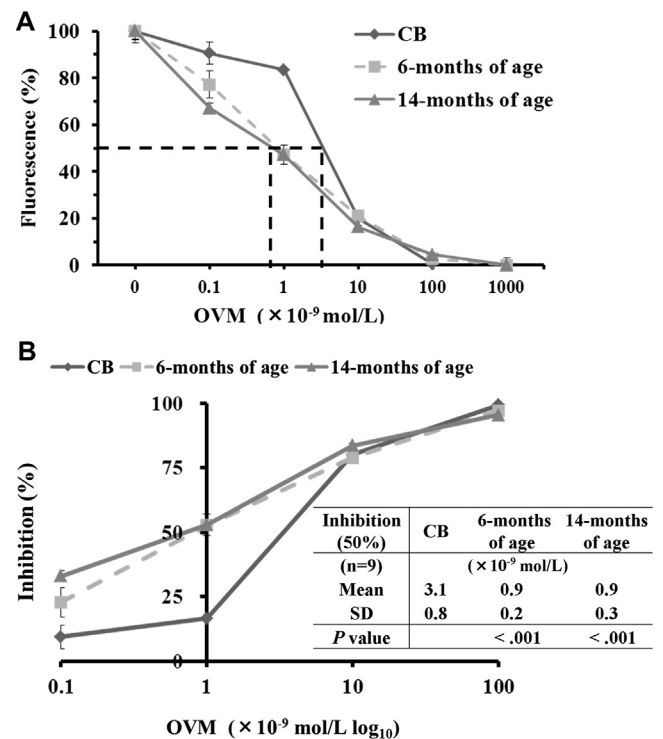


FIG 1. Competitive inhibition by OVM of the binding of OVM-specific IgE on the DLC-coated chip. CB and peripheral blood samples from 6- and 14-month-old infants ($n = 9$) were preincubated for 30 minutes with 0 to 1000×10^{-9} mol/L OVM and then the IgE bound on the chip was analyzed (A). Control binding without OVM was considered 100%. The % inhibition against OVM and OVM required for 50% inhibition (inset) as a measure of affinity were determined (B).

SPSS, Inc, Chicago, Ill). Differences between groups were assessed by using the Wilcoxon matched pairs signed rank test. A P value of less than .05 was considered significant.

Sera of 9 paired CB and peripheral blood samples after birth were treated by preincubation with 0 to 1×10^{-6} mol/L of OVM as a competitor for 30 minutes at 25°C and then the amount of IgE that could bind on the OVM-immobilized DLC-coated chip was determined by using a secondary antibody against IgE.¹ The binding of OVM-specific IgE at age 6 and 14 months was markedly and dose-dependently inhibited by low concentrations of OVM (0.1 – 1.0×10^{-9} mol/L), while that of OVM-specific IgE from CB was inhibited by higher concentrations (1 – 10×10^{-9} mol/L) (Fig 1, A). The % inhibition was plotted against the concentration of OVM, and OVM required for 50% inhibition was considered to reflect affinity (Fig 1, B). The concentration of OVM required for 50% inhibition was significantly higher (3.1×10^{-9} mol/L) in CB than in the blood of 6- and 14-month-old infants (0.9×10^{-9} mol/L, $P = .001$). The results indicated that OVM-specific IgE in CB was of lower affinity. The finding was further confirmed by measuring the amounts of IgE in serially diluted sera bound on the chip in the presence of 0 to 40×10^{-3} mol/L DEA⁸ (see Fig E3 in this article's Online Repository available at www.jacionline.org). Low-affinity antibodies are affected more than high-affinity antibodies by DEA in a dose-dependent manner.⁸ The leftward shift in the

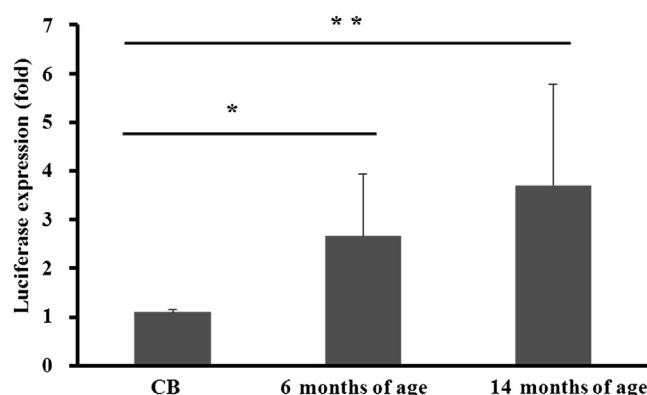


FIG 2. IgE-mediated human FcεRI cross-linking-induced luciferase expression in RS-ATL8 cells. Cells were preincubated with 100 BUe/mL OVM-specific IgE from CB and peripheral blood samples from 6- and 14-month-old infants. After washing, the cells were stimulated with 1×10^{-9} mol/L OVM and the induced luciferase was measured. Data are presented as fold increase with nonstimulated control considered as 1.0. * $P < .05$ and ** $P < .01$.

dose-response curves in the presence of DEA was measured at 50% of the maximum fluorescence, and quantitative data were expressed as $\log_{10}$ of this shift (Fig E3, C). Suppression by DEA was significantly higher in CB than in peripheral blood after birth. Next, we conducted functional analysis of OVM-specific IgE elicitation by using a human FcεRI-expressing luciferase-reporting mast cell line (RS-ATL8)⁹ (Fig 2). The levels of OVM-specific IgE in CB and peripheral blood of 6- and 14-month-old infants were adjusted at 100 BUe/mL, and then the sera were incubated overnight with RS-ATL8 cells. After washing, the cells were induced for 3 hours with 0 to 10×10^{-9} mol/L OVM at 37°C (see Fig E4 in this article's Online Repository available at www.jacionline.org). IgE-mediated FcεRI cross-linking-induced luciferase expression was presented as fold increase relative to the background expression.⁹ CB with low-affinity OVM-specific IgE did not elicit luciferase expression by OVM compared with the background expression, whereas peripheral blood of 6- and 14-month-old infants showed a significant increase in luciferase expression by OVM with a plateau at concentrations exceeding 100×10^{-12} mol/L (Fig E4), although the differences in the values of 6- and 14-month-old infants at 1×10^{-9} mol/L OVM were not significant (Fig 2). A high correlation ($r_s = 0.903$) of OVM-specific IgE value was found between the DLC-coated chip and UniCAP in peripheral blood after birth, but the difference between infants with AD (+) and infants without AD (−) was not significant, probably due to the limited number of subjects (Table E1). Low-affinity IgE can compete with high-affinity IgE for binding to Fcε receptors,⁵ and thus prevents anaphylaxis in the fetus. However, it can change to high affinity by antigen stimulation with somatic hypermutation of immunoglobulin genes after birth. Further studies are required to assess the prognostic value

of low-affinity IgE present in CB and the mechanisms of affinity maturation after birth.

In conclusion, we identified low-affinity OVM-specific IgE in CB and affinity maturation in peripheral blood of 6- and 14-month-old infants. We also described the methods used for the detection of low- and high-affinity allergen-specific IgE.

Norio Kamemura, PhD^{a,*}

Norio Kawamoto, MD, PhD^{b,*}

Ryosuke Nakamura, PhD^c

Reiko Teshima, PhD^c

Toshiyuki Fukao, MD, PhD^b

Hiroshi Kido, MD, PhD^a

From ^athe Division of Enzyme Chemistry, Institute for Enzyme Research, University of Tokushima, Tokushima, Japan; ^bthe Department of Pediatrics, Graduate School of Medicine, Gifu University, Gifu, Japan; and ^cthe Division of Novel Foods and Immunochimistry, National Institute of Health Science, Tokyo, Japan. E-mail: kido@ier.tokushima-u.ac.jp.

*These authors contributed equally to this work.

This study was supported in part by Grants-in-Aid 18659293, 21659245, and 19659253 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; by a supporting program for creating university ventures from the Science and Technology Agency of Japan; and by Health and Labor Sciences Research Grants for Research on Allergic Diseases and Immunology from the Ministry of Health, Labor and Welfare of Japan.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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<http://dx.doi.org/10.1016/j.jaci.2013.09.034>

METHODS

OVM-specific IgE competition assay

OVM in PBS was added to sera of 9 paired CB and peripheral blood samples from 6- and 14-month-old infants at final concentrations of 0, 0.1, 1, 10, 100, and 1000×10^{-9} mol/L. After incubation of the mixture at 25°C for 30 minutes, each sample was applied to an OVM-immobilized DLC-coated chip⁶ and competitive binding assay was conducted at 37°C for 60 minutes. After washing 3 times, the bound antibody was detected with a HiLyte Fluor 555 (Dojindo Molecular Technologies, Kumamoto, Japan)-labeled secondary antibody against human IgE and the resulting images were analyzed as described previously.¹ Before analysis, the amount of anti-OVM-specific IgE in each serum bound on the DLC-coated chip was adjusted at a fluorescence intensity of 2000 by dilution with PBS containing 1% BSA, 0.3 mol/L KCl, and 0.05% Tween 20.

Effect of DEA on DLC-coated chip assay

Measurement of OVM-specific IgE on the DLC-coated chip was conducted in the presence of a mild chaotropic agent, DEA, as described previously.⁸ DEA was added to serially buffer-diluted serum described above at a final concentration of 0, 10, 20, and 40×10^{-3} mol/L, and each sample was applied to an OVM-immobilized DLC-coated chip. After incubation at 37°C for 60 minutes and washing, the bound antibody was detected by secondary antibody against human IgE. Fluorescence was plotted against serum dilution, and the

leftward shift in the dose-response curve in the presence of DEA was measured at 50% of the maximum fluorescence.

OVM-specific IgE elicitation in FcεRI-expressing luciferase-reporting mast cell line

This assay was conducted by using a human FcεRI-expressing mast cell line, RS-ATL8, reported previously.⁹ RS-ATL8 cells (5×10^4 cells/50 μL/well) were plated onto a clear-bottom white 96-well plate (ViewPlate; Perkin Elmer, Waltham, Mass) and incubated for 3 hours at 37°C in a 5% CO₂ incubator. Then, the cells were sensitized with CB and peripheral blood samples from 6- and 14-month-old infants that were adjusted at 100 BUe/mL of OVM-specific IgE by dilution with minimal essential medium supplemented with 10% heat-inactivated FCS. After overnight incubation, the cells were washed once with sterile PBS and then stimulated with OVM at various concentrations ranging from 1×10^{-12} mol/L to 10×10^{-9} mol/L in minimal essential medium containing 10% FCS (50 μL/well) for 3 hours at 37°C. After stimulation, 50 μL of luciferase substrate solution containing cell lysis reagent (ONE-Glo, Promega, Tokyo) was added to the cells, and chemiluminescence was measured by using an EnVision multilabel plate reader (Perkin Elmer). Luciferase expression levels were represented as the fold increase in light units compared with the background expression after subtraction of a blank control (without cells). Measurements were conducted in duplicates, and the average value was used for analysis.

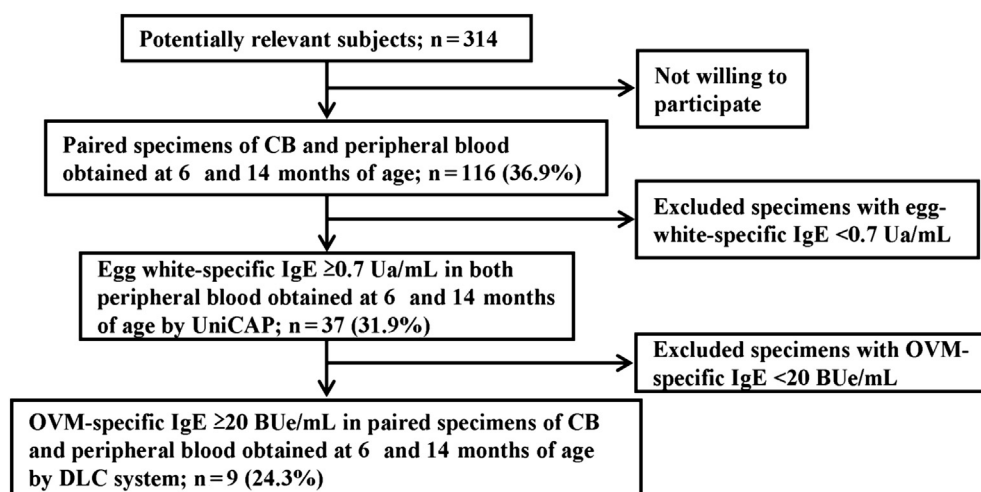
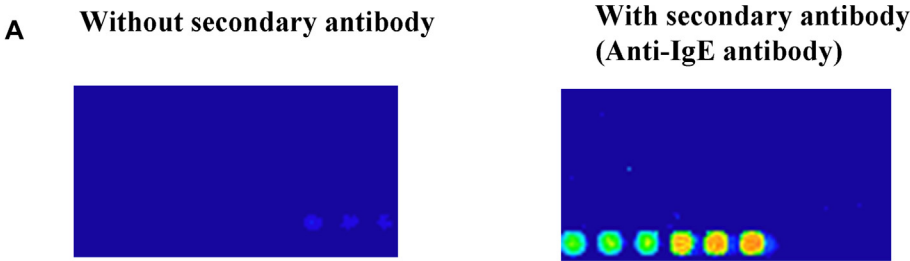


FIG E1. Flow diagram of the specimen selection process.



Layout on DLC chip

Blank	Human IgG (1×10^{-7} mol/L)	Human IgG (2×10^{-7} mol/L)
Human IgA (1×10^{-7} mol/L)	Human IgA (2×10^{-7} mol/L)	Human IgD (1×10^{-7} mol/L)
Human IgD (2×10^{-7} mol/L)	Human IgM (1×10^{-7} mol/L)	Human IgM (2×10^{-7} mol/L)
Human IgE (1×10^{-7} mol/L)	Human IgE (2×10^{-7} mol/L)	

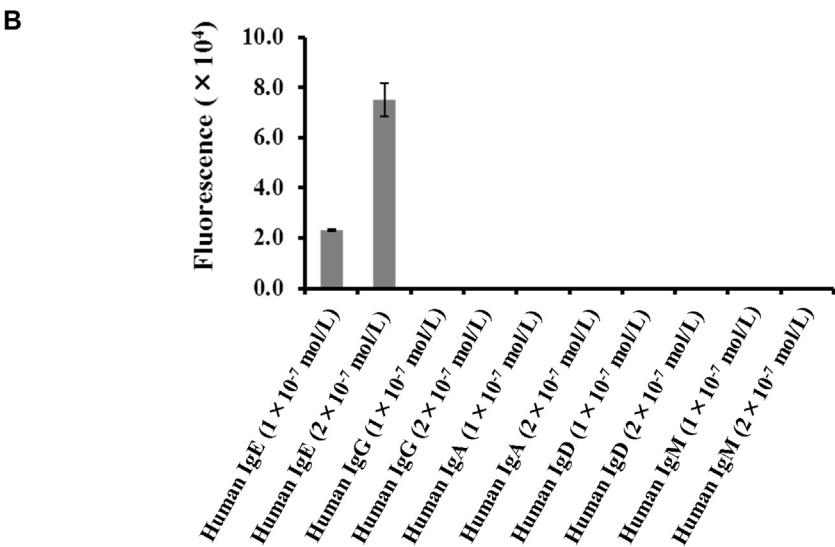


FIG E2. OVM-specific IgE binding on the DLC-coated chip without cross-reaction with IgM, IgG, IgA, and IgD. Layout of various human immunoglobulins immobilized on a DLC-coated chip (100 and 200×10^{-9} mol/L) and rainbow display after reaction with fluorescence-labeled anti-IgE secondary antibody (**A**). Fluorescence value of each spot (**B**).

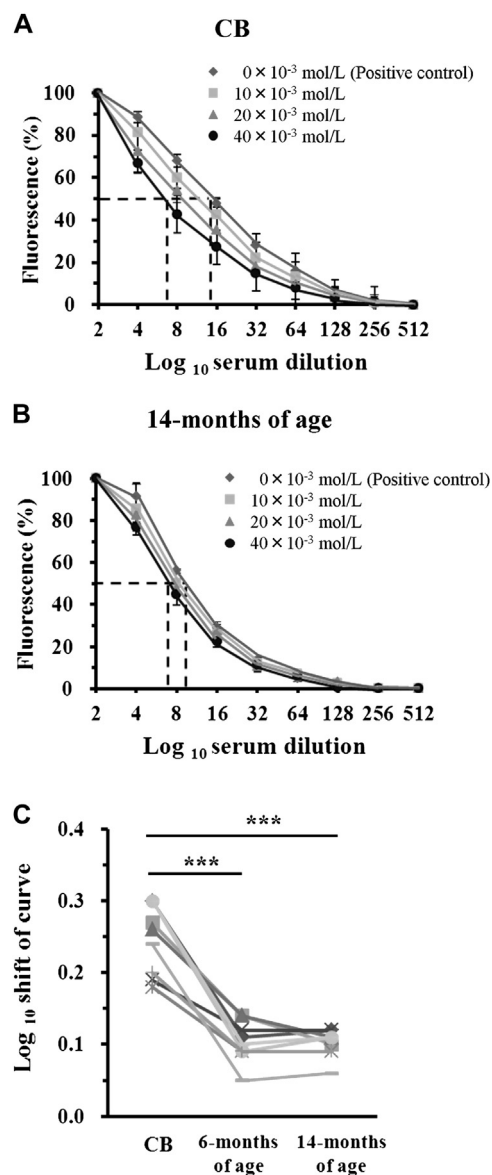


FIG E3. Effect of 0 to 40×10^{-3} mol/L DEA on the binding of serially diluted sera of CB and peripheral blood samples from 6- and 14-month-old infants to the OVM-immobilized DLC-coated chip. The curves of the binding of OVM-specific IgE to the chip in the presence of DEA (10 – 40×10^{-3} mol/L) were obtained by serial dilutions of CB (**A**) and blood samples from 14-month-old infants ($n = 9$, each) (**B**). Fluorescence without DEA for each dilution was considered 100%. The leftward shift by 40×10^{-3} mol/L DEA was measured at 50% of maximum fluorescence, and the results were expressed as $\log_{10}$ of this shift (**C**). *** $P < .001$.

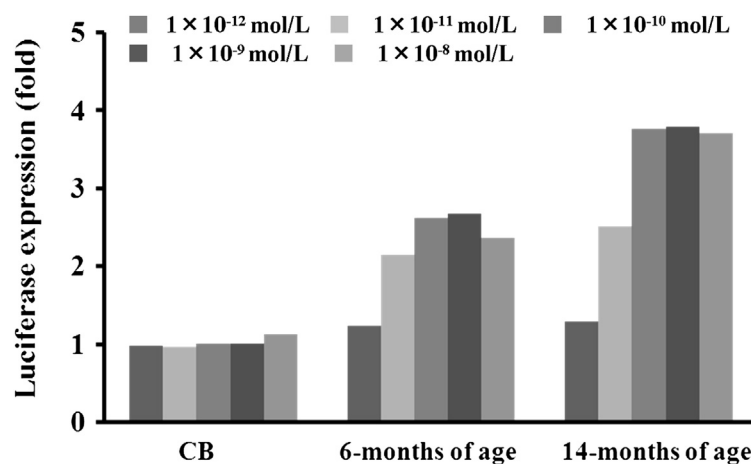


FIG E4. Evaluation of anti-OVM-specific IgE-mediated Fc ϵ RI cross-linking in CB and peripheral blood samples obtained after birth in a luciferase-reporting mast cell line. OVM-specific IgE in CB and blood samples from 6- and 14-month-old infants were adjusted at 100 BUe/mL and then preincubated overnight with RS-ATL8 cells. After washing, the cells were induced with 0 to 10×10^{-9} mol/L OVM for 3 hours at 37°C. IgE-mediated Fc ϵ RI cross-linking-induced luciferase expression is presented as fold increase, with nonstimulated control as 1.0.

TABLE E1. Characteristics of the 9 participants whose sera were analyzed in detail

Case ID	Sex	Gestational age (wk)	Birth weight (g)	CB		Age 6 mo						Age 14 mo							
				Total IgE (IU/mL)	OVM IgE (BUe/mL)	AD	RW	Total IgE (IU/mL)	Egg white IgE (Ua/mL)	OVM IgE (Ua/mL)	OVM IgE (BUe/mL)	AD	RW	FA*	Total IgE (IU/mL)	Egg white IgE (Ua/mL)	OVM IgE (Ua/mL)	OVM IgE (BUe/mL)	
86	F	38	2524	2.13	595	+	—	14.87	5.74	4.27	247	+	—	—	14.60	2.49	0.75	61	
129	F	38	2820	0.38	223	—	—	32.73	8.55	4.10	164	+	—	+	57.00	4.31	3.78	333	
131	M	40	3472	0.32	252	+	—	74.05	9.53	3.26	315	+	—	+	45.10	7.83	7.49	623	
181	M	40	3050	0.32	318	+	—	156.29	20.20	16.60	948	+	—	—	238.24	38.50	34.90	404	
191	F	40	2860	1.87	324	—	—	148.72	47.10	0.12	46	+	—	—	527.20	43.90	1.71	121	
194	M	40	3628	0.18	363	+	—	86.48	12.20	3.38	163	—	—	—	86.64	4.75	2.02	74	
248	F	41	3244	0.21	91	—	—	102.28	19.90	5.57	456	—	—	—	151.07	28.20	11.40	375	
283	F	40	2994	0.51	278	—	—	8.24	3.71	1.06	91	—	—	—	5.00	1.28	0.71	90	
294	F	41	2932	0.36	200	+	—	135.68	23.20	2.12	176	+	—	+	201.95	30.00	7.50	422	

AD was diagnosed by a pediatric allergist in Gifu Allergy and Immunology Cohort Study.⁷

AD, Atopic dermatitis; CB, Cord blood; F, female; FA, food allergy; M, male; OVM, ovomucoid; RW, recurrent wheezing.

*Three participants (case numbers 129, 131, and 294) were diagnosed with FA by their physicians though oral food challenge was not performed. Two of them (case numbers 131 and 294) avoided egg consumption, and 1 (case no. 129) had half-cooked egg consumption. Two participants (case numbers 86 and 191) avoided consumption of eggs on the basis of parents' decision until age 14 months. The remaining 4 participants consumed eggs before age 14 months, and 2 participants (case numbers 194 and 248) had no adverse reaction after egg consumption. Case numbers 181 and 283 developed certain adverse reactions at least twice before age 14 months. All OVM-specific IgE levels in CB were below the detection limit of the UniCAP system.