Outbreak of immediate-type hydrolyzed wheat protein allergy due to a facial soap in Japan

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Outbreak of immediate-type hydrolyzed wheat protein allergy due to a facial soap in Japan

To the Editor:

Wheat proteins in hydrolyzed form have been widely used in cosmetic products. The number of patients allergic to hydrolyzed wheat protein (HWP) in cosmetic products seems to be small in Western countries (see Table E1 and this article's Online Repository at www.jacionline.org). However, in Japan, Fukutomi et al[1] first reported 5 Japanese patients with wheat-dependent exercise-induced anaphylaxis (WDEIA) after using the facial soap containing 0.3% of a specific type of HWP, Glupearl 19S, in 2009, and thousands of subjects showed allergic contact urticaria, anaphylaxis, and/or WDEIA after using the soap.

Here, we provide an overview of the outbreak of immediate-type wheat allergy caused by a specific HWP (HWP-IWA) by facial soaps in Japan.

A nationwide survey for HWP-IWA was conducted to collect the information on Glupearl 19S–containing soaps. A flowchart of the patient registration and the diagnostic criteria are shown in Fig E1 and Table E2, respectively, and details are also described in this article's Online Repository at www.jacionline.org.

On the basis of the diagnostic criteria listed in Table E2, the number of patients who satisfied the diagnostic criteria was 2111 (2025 females, 86 males; age, 1-93 years; average age, 45.8 $\pm$ 14.5 years). The age group with the largest share consisted of those in their 40s (see Fig E2 in this article's Online Repository at www.jacionline.org). Because sales of the soap containing Glupearl 19S were discontinued in May 2011, the number of reported patients has gradually decreased, and the nationwide survey for HWP-IWA ended in October 2014 (Fig 1).

The symptoms typically appeared 1 year after starting use of the soap. Most patients used the soap only for their faces, but some used it on other body parts as well. Symptoms observed in patients are listed in Table I. No patients had shown apparent wheat allergy before using this soap. Twenty-five percent of patients experienced anaphylactic shock, 43% experienced dyspnea, and 11% experienced vomiting. Most of the patients with food ingestion–related symptoms reacted to traditional wheat products such as bread and pasta. This was in contrast to non-Japanese patients allergic to HWP in cosmetic products, who tolerated traditional wheat products but showed the symptoms of allergic reaction after eating processed food such as ham and pâté (see Table E1 and Online Repository). Initial symptoms of anaphylaxis in the patients were facial symptoms, including swelling of the eyelids, urticaria/itchiness of the face, and runny nose, which were distinct from conventional WDEIA with initial symptoms of systemic reaction of itching and urticaria.

In contrast to conventional wheat allergies that react mainly with gliadin and high molecular weight glutenin in wheat protein,[2] immunoblot analysis and ELISA revealed that sera from patients allergic to the HWP-containing soap showed a pattern distinct from that of conventional wheat allergy.[1] Glupearl 19S that was produced by acid treatment of gluten (pH, 0.5-1.2) at 95°C for 40 minutes is the HWP responsible for the allergenicity of the soap. The SDS-PAGE analysis of Glupearl 19S showed a smear staining pattern from the low to high molecular weight range in contrast to the staining pattern of gluten. Fig E3 in this article's Online Repository at www.jacionline.org shows the SDS-PAGE of Glupearl 19S and IgE reactivity against Glupearl 19S by ELISA[3] (see this article's Methods section in the Online Repository at www.jacionline.org) using sera obtained from conventional patients with WDEIA, patients with HWP-IWA who satisfied the diagnostic criteria, subjects who had used soaps containing Glupearl 19S but did not meet the diagnostic criteria, and healthy controls. As shown in Fig E3, strong IgE reactions were observed only in those patients who satisfied the diagnostic criteria, and none of the sera obtained from patients with conventional WDEIA reacted with Glupearl 19S.

Because patients used the soap repeatedly on the face, it is likely that allergen exposure occurred through the eyelids and noses, leading to the strong allergic reactions with their eyelids that were not commonly observed in patients with conventional wheat allergy. Airaksinen et al[4] reported 2 patients of occupational rhinitis, asthma, and contact urticaria due to a sprayable hair conditioner containing HWP, and both of them showed exercise-induced eyelid edema and other symptoms after eating wheat-containing food.

Glupearl 19S was produced by acid treatment of gluten (pH, 0.5-1.2) at 95°C for 40 minutes. It has been reported that gluten treated with 0.1 N hydrochloric acid for 30 minutes at 100°C markedly increased IgE-binding capacity of patients' sera, indicating that neoepitopes on the gluten might be generated after the treatment.[5] The acid treatments at high temperature for a short time produce random degradation of gluten, and mixed short and long peptides, leading to smear pattern by electrophoresis (Fig E3). Because most food products do not contain HWP, it was speculated that gastrointestinal enzyme reaction after ingestion of wheat protein might be responsible for acquiring allergenicity. Glupearl 19S itself is not deamidated by transglutaminase in the body, but deamidated peptides were produced during the process of acid and heat treatment of gluten,[6] and then specific IgE antibodies against Glupearl 19S were produced when patients used the soap repeatedly (Fig E3). Nakamura et al[7] showed that tissue transglutaminase treatment of gluten dramatically increased reactivity against IgE from the patients' sera by cell-based assay (EXiLE). Yokooji et al[8] reported that IgE-binding epitope QPQQPFPQ in γ-gliadin reacted more strongly with IgE of the patients in its deamidated form, PEEPFP.[8] Ingested wheat food product such as bread and/or pasta might be deamidated by transglutaminase in the body, and specific IgE antibodies against Glupearl 19S could cross-react with deamidated peptide derived from food gluten, which may lead to anaphylactic/allergic reaction in the patients.

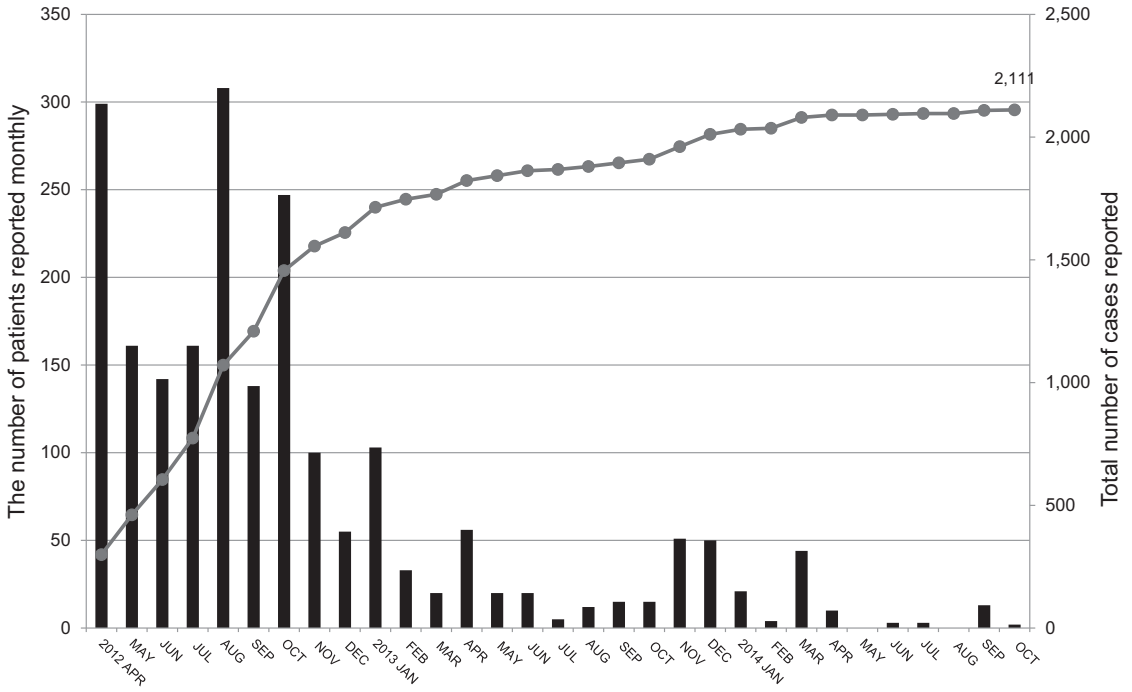

FIG 1. The number of patients registered per month and cumulative total number between 2012 and 2014.

TABLE I. Symptoms observed in immediate-type wheat allergy caused by Glupearl 19S (n = 899)

Skin symptoms during or after using soap, n (%)	
Skin symptoms	640 (71)
Swelling of eyelids	360 (40)
Urticaria, itching, and rubefaction	280 (31)
Skin symptoms negative	246 (27)
Unknown	13 (2)
Symptoms after eating wheat products, n (%)	
Swelling of eyelids	694 (77)
Urticaria	537 (60)
Dyspnea	385 (43)
Erythema	344 (38)
Itching	278 (31)
Anaphylactic shock	227 (25)
Diarrhea	148 (16)
Nausea	122 (14)
Nasal discharge	117 (13)
Vomiting	103 (11)
Nasal congestion	95 (11)

The detailed analysis of allergenicity of HWP and predisposition to type I allergy against HWP will lead to the safe use of cosmetic products containing wheat protein.

Hiragun et al[9] reported the status of remission of 110 patients with IWA-HWP who were part of the 2111 patients mentioned above, and the remission rate of 110 patients was still 56.1% at 60 months after stopping usage of HWP-containing soap. Therefore, it is necessary to find effective treatment for the long-lasting and refractory cases. Discovering the molecular mechanisms underlying the HWP-IWA, in comparison with conventional WDEIA and wheat intolerance such as celiac diseases, may lead to better understanding of the molecular basis of wheat protein–related diseases.

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Systems approach to uncover signaling networks in primary immunodeficiency diseases

To the Editor:

We describe here an approach to improve diagnoses and further our understanding of functional defects of primary immunodeficiency diseases (PIDs) using time-of-flight mass cytometry (CyTOF) to reveal the signaling of all circulating immune cells.

PIDs were historically diagnosed by a narrow, pathognomonic constellation of signs and symptoms. However, ever-broadening phenotypes have become apparent for diseases such as gain-of-function signal transducer and activator of transcription (STAT) 1. Moreover, distinct genetic mutations may share a single phenotype, especially if they share a signaling pathway (eg, LPS responsive beige-like anchor [LRBA] deficiency and cytotoxic T lymphocyte–associated antigen 4 haploinsufficiency). Thus, there has been an increasing reliance on genetic definitions of PIDs. However, sequencing cannot identify whether a novel mutation in a "known PID gene" will lead to a loss-of-function phenotype, a gain-of-function phenotype, or no phenotype at all. In this "postexome" era, identification of immune diseases would be greatly facilitated by a broad, unbiased *functional* analysis that parallels the broad, unbiased genetic analysis provided by next-generation sequencing.

This proof-of-concept study shows the potential of CyTOF to characterize a broad range of cells and signals. We began by testing the responses of circulating immune cells to canonical stimuli (cytokines and TLR agonists) in 5 healthy controls. Samples of whole blood were aliquoted and portions were stimulated with a cytokine or TLR agonists (IFN-α, IL-2, IL-5, IL-6, IL-7, IL-10, IL-17, IL-21, IL-25, LPS, and PMA) for 15 minutes; 1 aliquot was left unperturbed. We used CyTOF to measure more than 40 different markers simultaneously, including 9 intracellular phospho-proteins involved in signaling pathways (p38, ERK, PLCγ2, STAT1, STAT3, STAT5, S6 kinase, IκB, and AKT). We identified 18 types of circulating innate and adaptive immune cell types in the blood by gating (see Fig E1 in this article's Online Repository at www.jacionline.org) and examined phospho-signaling responses in these cell types at baseline and after stimulation (Fig 1; see Tables E1 and E2 in this article's Online Repository at www.jacionline.org). Examining responses after 15-minute stimulations minimized the impact of secondary signals that might arise at later time points.

This approach identified known patterns of stimuli and responses spanning both lymphoid and myeloid lineages including granulocytes, such as STAT5 in response to IL-2 and IL-7 and STAT3 in response to IL-6 and IL-10 (Fig 1). We noted that activated $CD4^+$ and $CD8^+$ T cells, respectively, had minimal or no increase in pSTAT5 in response to IL-7. In contrast, resting memory or naive T-cell lineages showed strong responses. These results can be explained by the reduced expression of IL-7 receptor in activated T cells.[1] Notably, IL-7R was not used in gating. Thus, our algorithm detected patterns of differential responses to IL-7 without an *a priori* understanding of IL-7R expression.

Hierarchical clustering indeed showed that functional signaling responses largely mirrored developmental lineages (see Fig E2 in this article's Online Repository at www.jacionline.org). Interestingly, we found that myeloid dendritic cells, plasmacytoid dendritic cells, and $CD16^+$ monocytes clustered with lymphoid cells, while $CD16^-$ monocytes clustered with myeloid cells. This grouping may reflect the functional propensity of $CD16^+$ monocytes to differentiate into dendritic cells.[2] These results show that even cells within the same developmental lineages may have varying degrees of responses to stimuli.

To demonstrate the utility of CyTOF in elucidating PIDs with broad phenotypes, we studied 2 patients with PID as a proof-of-principle. We started with an adolescent patient with chronic mucocutaneous candidiasis (CMC) identified with a monoallelic mutation in STAT1 (p.R274W), producing a GOF phenotype. CMC in these patients has been attributed to defective T_H17 immunity.[3] We first examined whether any *baseline* phosphorylation in our GOF STAT1 subject fell outside the 95% CI established in controls. At baseline, we unexpectedly found increased STAT3 phosphorylation in T cells (Fig 2, *A*). We did not find increased STAT1 phosphorylation at baseline, consistent with many previous studies. Next, we examined responses of the GOF STAT1 subject to stimuli as compared with controls (see Fig E3 in this

METHODS

MEDLINE searches for patients allergic to HWP in cosmetic products outside of Japan

We used PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) to search the National Library of Medicine and MEDLINE to collect information about patients allergic to HWP. For the electronic searches, we used a combination of keywords related to "hydrolyzed wheat protein" and "allergy/anaphylaxis," then manually excluded patients allergic to HWP in food. We included articles published in English or those with English translations available. We also used the information published in Scientific Committee on Consumer Safety in 2014.[E1]

Nationwide survey for HWP-IWA

The soap, Yuuka-no-sekken, commonly known as Cha-no-shizuku soap (Yuuka, Fukuoka, Japan) containing Glupearl 19S (Katayama Chemical Industries, Osaka, Japan), was sold only by mail order between March 2004 and December 2010. Glupearl 19S was derived from wheat gluten, and had not been exported to other domestic/foreign companies. The soap was very popular, especially among women, due to the advertised effects of beautifying and whitening the skin. The number of registered customers at Yuuka, the company selling the soap, was 4,667,000, and the total number of soaps sold to the customers was 46,508,000.[E2] On the basis of the number of adult Japanese women, 54,444,000,[E3] it was estimated that approximately 1 in 12 Japanese adult women used the soap. The Special Committee for the Safety of Protein Hydrolysates in Cosmetics was formed by the Japanese Society of Allergology, and the diagnostic criteria were established in October 2011, with patient registration beginning in April 2012. Among hospitals/clinics eligible for proper diagnosis, 270 clinics/hospitals reported confirmed cases of HWP-IWA by the facial soaps to the Special Committee. Fig E1 shows the flowchart of patient registration. Subjects who experienced any symptoms after using the soap and/or ingestion of wheat products contacted the company that sold the soaps (Yuuka), the Ministry of Health, Labour, and Welfare of Japan, the Consumer Affairs Agency of Japan, or the Japanese Society of Allergology. They provided information regarding which clinics/hospitals provided proper diagnosis for HWP-IWA and which could perform skin prick tests using Glupearl 19S and/or specific IgE antibody detection tests for Glupearl 19S. The Glupearl 19S specific IgE antibody detection test was provided in the laboratory organized by the Special Committee. Some consumers visited clinics/hospitals directly, and some of them were referred to allergy/dermatology specialists for proper diagnosis. Because the information regarding allergic symptoms and usage of the soaps was provided from many resources, including the company (Yuuka), the Consumer Affairs Agency of Japan, the Japanese Society of Allergology, and the Ministry of Health, Labour, and Welfare of Japan, and the incident was also covered in the mass media, it is considered that we covered most of the cases of the incident.

IgE measurements in patients with allergy to Glupearl 19S and other wheat-related allergies

SDS-PAGE. SDS-PAGE was performed using Novex NuPAGE 4% to 12% gels and MOPS buffer (Thermo Fisher Scientific, Waltham, Mass) according to the manufacturer's instructions. Separated proteins of Glupearl 19S and gluten were stained with SimplyBlue SafeStain (Thermo Fisher Scientific).

ELISA for Glupearl 19S. *Subjects.* Sera were obtained from patients with conventional WDEIA who reacted to ω-5 gliadin (n = 7), patients with HWP-IWA who satisfied the diagnostic criteria described in Table E2 (n = 20), subjects who had used soaps containing Glupearl 19S but who did not satisfy the diagnostic criteria (ie, skin prick test negative using 0.1% Glupearl 19S solution, n = 20), and healthy controls without wheat allergy (n = 7). The study was approved by the Ethics Committee of Fujita Health University (11-210), and informed consent was obtained from each patient. This study was carried out in accordance with the Declaration of Helsinki.

ELISA. Details of the ELISA method were described previously.[E4] Briefly, 1 mg/mL Glupearl 19S was plated on a Nunc MaxiSorp flat-bottom 96-well plate (Thermo Fisher Scientific). The plate was blocked with 1% skim milk/PBS containing 0.1% Tween 20. After washing, diluted patients' sera was added to each well, and the plate was incubated. The plate was washed, 0.1 μg/mL antihuman IgE antibody horseradish peroxidase conjugate (KPL, Gaithersburg, Md) was added to each well, and the plate was incubated. The plate was washed, and the colorimetric reaction was developed by adding 1-Step Ultra TMB-ELISA (Thermo Fisher Scientific). Absorbance values at 450 nm were measured by VersaMax (Molecular Devices, Sunnyvale, Calif), and were converted into units, as described previously, and cutoff values were set at 5.0 units.

RESULTS AND DISCUSSION

MEDLINE searches for patients allergic to HWP in cosmetic products outside of Japan

A list of non-Japanese patients allergic to HWP in cosmetic products is given in Table E1.[E4-E15] Many of the patients used facial creams, and some used sprayable hair conditioner containing HWP.[E15] As shown in Table E1, some had no symptoms related to ingestion of food, whereas others showed allergic symptoms after eating foods containing wheat. Interestingly, most of the patients tolerated traditional wheat products, such as bread and pasta, but allergic reactions occurred after eating processed food containing HWP, such as ham and liver pâté.[E9,E12,E13]

Among hospitals/clinics eligible for proper diagnosis, 270 clinics/hospitals reported confirmed cases of HWP-IWA by the facial soaps to the Special Committee. After a careful examination at the clinics/hospitals, 2111 subjects were confirmed to be satisfied with the diagnostic criteria. Doctors who treated the confirmed cases were asked to participate in an online survey, and clinical details of 899 patients were reported to the Special Committee (Table I).

Causality between Glupearl 19S and HWP-IWA was considered as follows. (1) Patients showed symptoms related to type I allergy after using soap containing Glupearl 19S, and showed symptoms repeatedly. (2) Skin prick test, ELISA, or basophil activation test against Glupearl 19S was positive. This reactivity was not observed in patients with traditional WDEIA and in healthy controls (Fig E3). (3) These symptoms, including allergic reactions after ingestion of wheat products, had not been observed before starting use of soap containing Glupearl 19S.

Among 2111 confirmed cases, the past/present history of allergic diseases was available in 899 patients. Allergic rhinitis/pollinosis, atopic dermatitis, urticaria, and asthma were observed in 297 (33%), 107 (12%), 42 (5%), and 17 (2%), respectively. There was no increase in the prevalence of allergic rhinitis/pollinosis and asthma in HWP-allergic patients compared with the prevalence in a general population survey of allergic rhinitis/pollinosis (47.2%) and adult asthma (5.4%), but the incidence of atopic dermatitis, the prevalence of which in the general population was reported to be 9.4% for those in their 20s, 8.3% in the 30s, and 4.8% in the 40s, slightly increased.[E16]

Fig E3 shows an SDS-PAGE image of Glupearl 19S and gluten. Glupearl 19S was produced by acid treatment of gluten (pH, 0.5-1.2) at 95°C for 40 minutes. The acid treatments at high temperature for a short time produce random degradation of gluten, and mixed short and long peptides, leading to smear pattern by electrophoresis. Other researchers also showed a similar smear pattern of electrophoresis using Glupearl 19S.[E17] Acid treatment of gluten at high temperature for a long period

(24 hours) produced only small peptides that are not allergenic,[E18] but Glupearl 19S contains short and long peptides that could cause allergic reaction in the patients. Glupearl 19S itself is not deamidated by transglutaminase in the body, but deamidated peptides were produced during the process of acid and heat treatment of gluten,[E18] and then specific IgE antibodies against Glupearl 19S were produced while patients used the soap repeatedly (Fig E3). Ingested wheat food product such as bread and/or pasta might be deamidated by transglutaminase in the body, and specific IgE antibodies against Glupearl 19S could cross-react with deaminated peptide derived from food gluten, which may lead to anaphylactic/allergic reaction in the patients.

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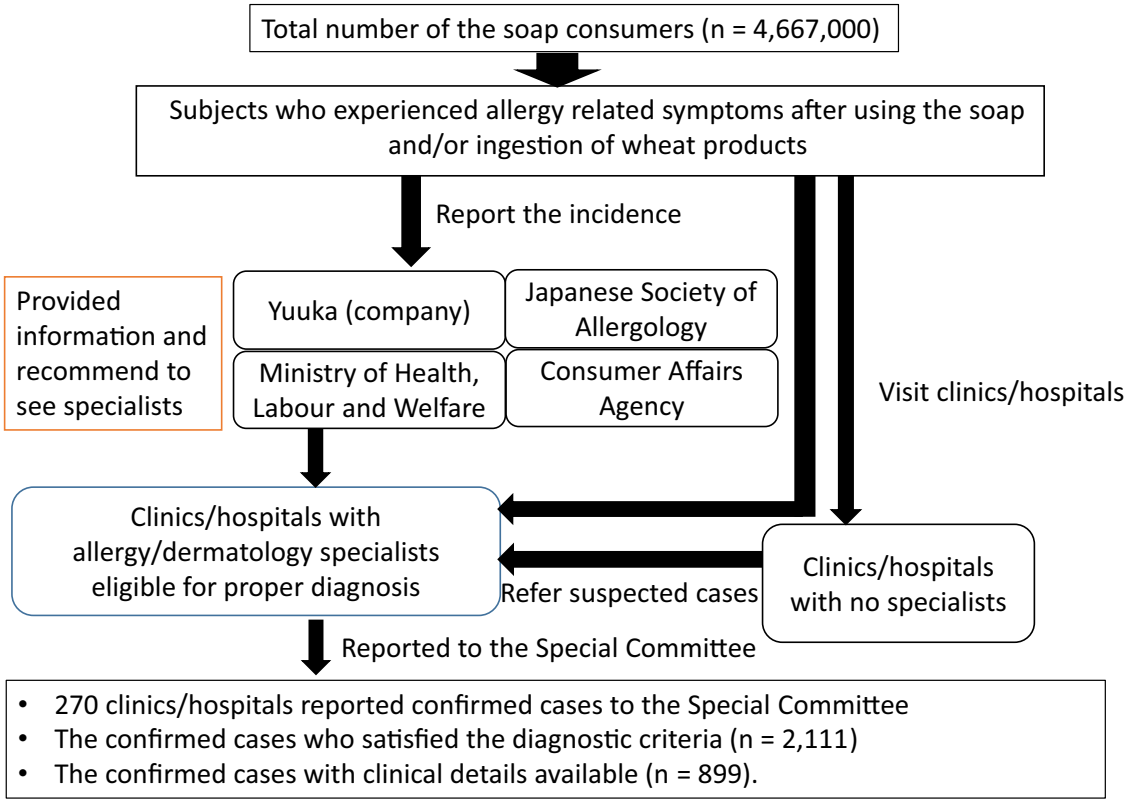

FIG E1. Flowchart of the patients' registration for immediate-type wheat allergy caused by a specific HWP (HWP-IWA).

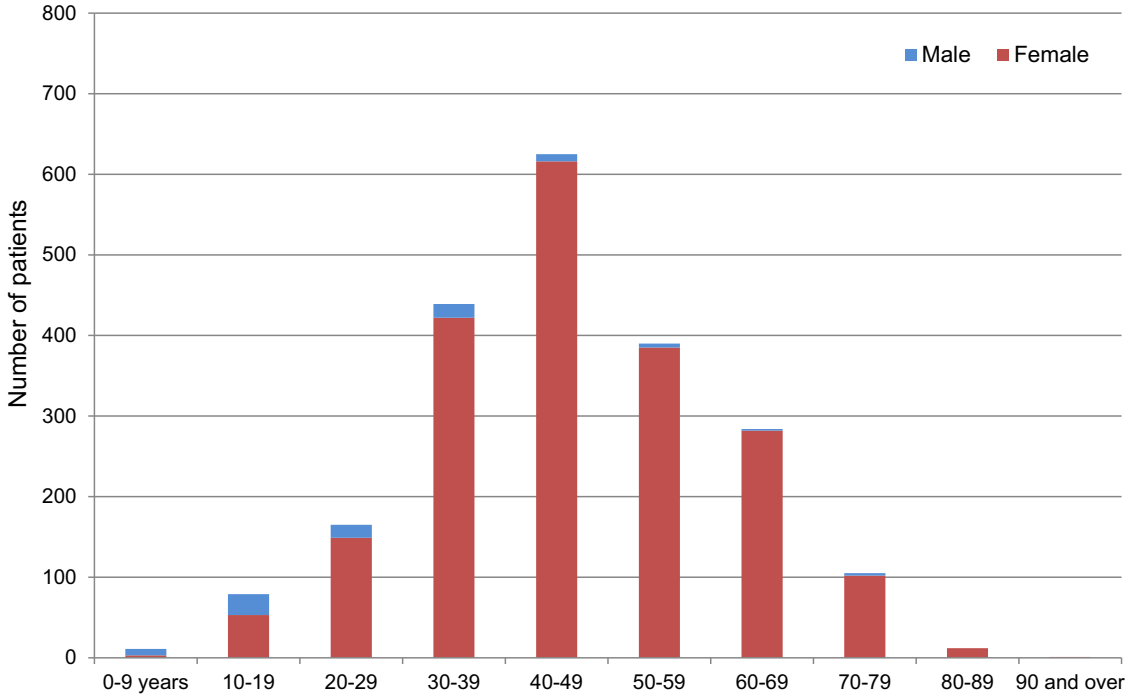

FIG E2. Age and sex distribution of the Japanese patients allergic to HWP in facial soaps.

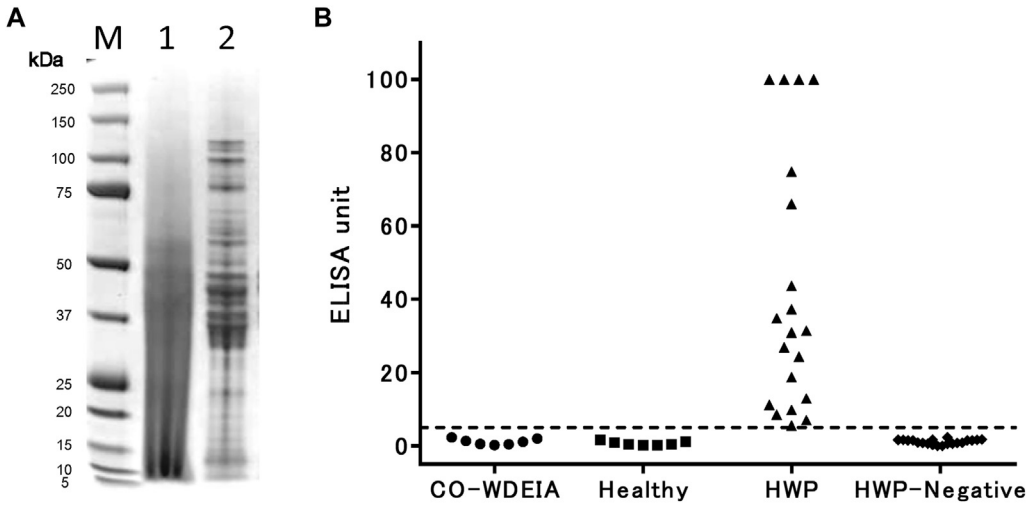

FIG E3. IgE antibody levels against Glupearl 19S. **A,** SDS-PAGE image of Glupearl 19S and gluten. M: molecular weight marker; lane 1: Glupearl 19S, lane 2: gluten. **B,** Dot plots of serum Glupearl 19S specific IgE levels were quantified by ELISA using sera from patients with conventional WDEIA (CO-WDEIA, n = 7), HWP-IWA who satisfied the diagnostic criteria described in Table E2 (HWP, n = 20), subjects who had used the soaps but who did not satisfy diagnostic criteria (HWP-Negative, n = 20), and healthy controls without wheat allergy (Healthy, n = 7). Units greater than 100 are described as 100. *Dashed line* indicates a cutoff value (5 units).

TABLE E1. Non-Japanese patients allergic to HWP in cosmetic products

Year	Authors	No. of patients	Country	Cosmetic products	Symptoms after applying cosmetics	Symptoms after eating wheat-containing food	Reference
2000	Sanchez-Perez et al	1	Spain	Moisturizing cosmetic cream	Contact urticaria	NA	E5
2000	Varjonen et al	1	Finland	Body cream	Contact dermatitis/urticaria	NA	E4
2002	Pecquet et al	1	France	Eyelid cream, body moisturizer	Generalized urticaria	Generalized urticaria	E6
2004	Pecquet et al	7	France	Facial creams	Contact dermatitis	Anaphylaxis/urticaria (6 patients)	E7
2006	Codreanu et al	3	France	Shower gel, shampoo, mascara	Generalized erythema, contact eczema, facial angioedema with generalized urticaria	WDEIA (1 patient)	E8
2006	Lauriere et al	9	France	Facial creams, body moisturizer, shower gel, hair conditioner	Contact urticaria	Generalized urticaria (3 patients), anaphylaxis (2 patients), WDEIA (1 patient)	E9
2007	Hann et al	1	United Kingdom	Moisturizing cosmetic cream	Contact dermatitis	NA	E10
2007	Livideanu et al	1	France	Emollient	Contact dermatitis	NA	E11
2010	Bouchez-Mahiout et al	4	France	Skin tensing cosmetic, facial cream	Contact urticaria	Urticaria (1 patient), exercise-induced food allergy (1 patient)	E12
2010	Olaiwan et al	2	France	Cosmetics	Contact urticaria	Generalized urticaria (1 patient)	E13
2012	Barrientos et al	1	Spain	Skin cream	Contact dermatitis	No symptoms related to ingestion of the food	E14
2013	Airaksinen et al	2	Finland	Sprayable hair conditioner	Occupational rhinitis, asthma, contact urticaria	WDEIA (1 patient), exercise-induced food allergy (1 patient)	E15

NA, Not available.

TABLE E2. Diagnostic criteria for immediate wheat allergy to HWP (Glupearl 19S)

Individual must meet all the following criteria[E4-E6]
Criteria 1 (usage of soaps):
1. History of usage of Cha-no-Shizuku soap or other products containing hydrolyzed wheat (Glupearl 19S)
Criteria 2 (symptoms), either of the following:
1. Itching, eyelid edema, nasal discharge, and/or wheals within several to 30 min after using Cha-no-Shizuku soap or other products containing hydrolyzed wheat (Glupearl 19S)
2. General symptoms, such as itching, wheals, eyelid edema, nasal discharge, dyspnea, nausea, vomiting, abdominal pain, diarrhea, and decreased blood pressure, within 4 h after eating wheat products
Criteria 3 (laboratory test positive), either of the following:
1. Skin prick test using ≤0.1% Glupearl 19S solution
2. Immunoassay, such as dot blot, ELISA, and/or western blot to identify specific IgE antibody to Glupearl 19S in the serum/plasma
3. Basophil activation test using Glupearl 19S
Exclusion criterion
Skin prick test negative using 0.1% Glupearl 19S solution

Defined by the Special Committee for the Safety of Protein Hydrolysates in Cosmetics on October 11, 2011.