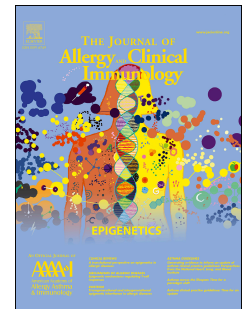


Accepted Manuscript

HLA-DQ and RBFOX1 as susceptibility genes for an outbreak of hydrolyzed wheat allergy



Emiko Noguchi, MD, PhD, Masato Akiyama, MD, PhD, Akiko Yagami, MD, PhD, Tomomitsu Hirota, DDS, PhD, Yukinori Okada, MD, PhD, Zenichiro Kato, MD, PhD, Reiko Kishikawa, MD, PhD, Yuma Fukutomi, MD, PhD, Michihiro Hide, MD, PhD, Eishin Morita, MD, PhD, Michiko Aihara, MD, PhD, Makiko Hiragun, MD, PhD, Yuko Chinuki, MD, PhD, Takahiro Okabe, MD, Akiko Ito, MD, PhD, Atsuko Adachi, MD, Atsushi Fukunaga, MD, PhD, Yumiko Kubota, MD, PhD, Toshiyuki Aoki, MD, PhD, Youko Aoki, MD, Kazue Nishioka, MD, PhD, Tetsuya Adachi, MD, Nobuo Kanazawa, MD, PhD, Hitoshi Miyazawa, MD, Hiroyuki Sakai, MD, PhD, Takehito Kozuka, MD, Hideo Kitamura, MD, PhD, Hideo Hashizume, MD, PhD, Chiharu Kanegane, MD, PhD, Koji Masuda, MD, PhD, Kumiya Sugiyama, MD, Reiko Tokuda, MD, PhD, Junichi Furuta, MD, PhD, Ikkou Higashimoto, MD, PhD, Atsuko Kato, MD, Mariko Seishima, MD, PhD, Akihiko Tajiri, MD, Atsuko Tomura, MD, PhD, Hiroko Taniguchi, MD, PhD, Hiroto Kojima, MD, Hidenori Tanaka, B.Pharm, Aiko Sakai, MD, PhD, Wataru Morii, M.Med.Sc, Masashi Nakamura, PhD, Yoichiro Kamatani, MD, PhD, Atsushi Takahashi, PhD, Michiaki Kubo, MD, PhD, Mayumi Tamari, MD, PhD, Hirohisa Saito, MD, PhD, Kayoko Matsunaga, MD, PhD

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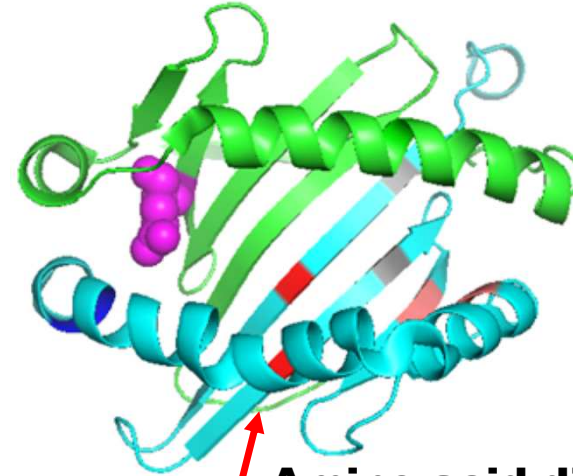
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HLA-DQ and *RBFOX1* as susceptibility genes for an outbreak of hydrolyzed wheat allergy



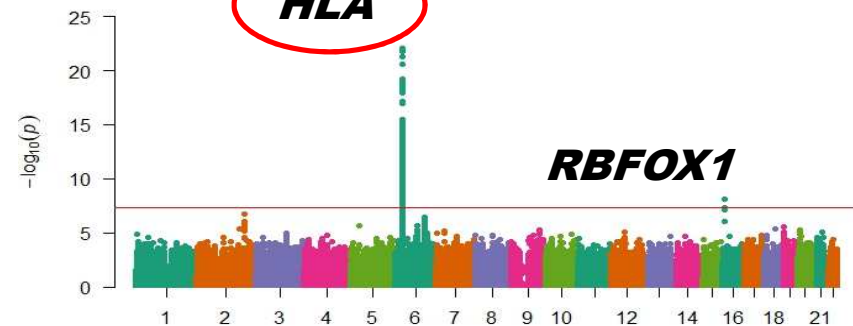
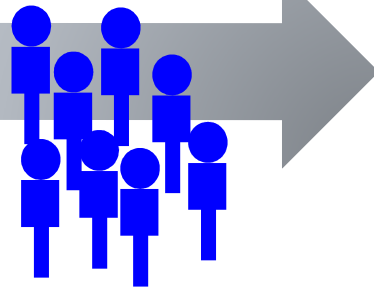
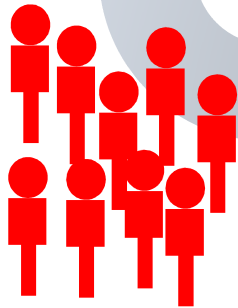
GLUTEN
(hydrolyzed wheat protein)



**Amino acid differences
in pocket 4 of HLA-DQ**

Wheat allergy

Controls



Genome-wide association study (GWAS, 452 cases, 2700 controls)

HLA-DQ and RBFOX1 as susceptibility genes for an outbreak of hydrolyzed wheat allergy

Emiko Noguchi^{a,*}, MD, PhD, Masato Akiyama^{b,c,*}, MD, PhD, Akiko Yagami^{d,*}, MD, PhD, Tomomitsu Hirota^{e,f}, DDS, PhD, Yukinori Okada^{g,h}, MD, PhD, Zenichiro Kato^{i,j}, MD, PhD, Reiko Kishikawa^k, MD, PhD, Yuma Fukutomi^l, MD, PhD, Michihiro Hide^m, MD, PhD, Eishin Moritaⁿ, MD, PhD, Michiko Aihara^o, MD, PhD, Makiko Hiragun^m, MD, PhD, Yuko Chinukiⁿ, MD, PhD, Takahiro Okabe^p, MD, Akiko Ito^q, MD, PhD, Atsuko Adachi^r, MD, Atsushi Fukunaga^s, MD, PhD, Yumiko Kubota^t, MD, PhD, Toshiyuki Aoki^u, MD, PhD, Youko Aoki^v, MD, Kazue Nishioka^w, MD, PhD, Tetsuya Adachi^x, MD, Nobuo Kanazawa^y, MD, PhD, Hitoshi Miyazawa^z, MD, Hiroyuki Sakai^{aa}, MD, PhD, Takehito Kozuka^{ab}, MD, Hideo Kitamura^{ac}, MD, PhD, Hideo Hashizume^{ad}, MD, PhD, Chiharu Kanegane^{ae}, MD, PhD, Koji Masuda^{af}, MD, PhD, Kumiya Sugiyama^{ag}, MD, Reiko Tokuda^{ah}, MD, PhD, Junichi Furuta^{ai}, MD, PhD, Ikkou Higashimoto^{aj}, MD, PhD, Atsuko Kato^{ak}, MD, Mariko Seishima^{al}, MD, PhD, Akihiko Tajiri^{am}, MD, Atsuko Tomura^{an}, MD, PhD, Hiroko Taniguchi^{ao}, MD, PhD, Hiroto Kojima^{ap}, MD, Hidenori Tanaka^{ap}, B.Pharm, Aiko Sakai^{aq}, MD, PhD, Wataru Morii, M.Med.Sc^a, Masashi Nakamura, PhD^{ar}, Yoichiro Kamatani^{b, as}, MD, PhD, Atsushi Takahashi^{b,at}, PhD, Michiaki Kubo^{au}, MD, PhD, Mayumi Tamari^{e,f}, MD, PhD, Hirohisa Saito^{av}, MD, PhD, Kayoko Matsunaga^{ar} MD, PhD

*These authors contributed equally to this work.

^aDepartment of Medical Genetics, Faculty of Medicine, University of Tsukuba, Ibaraki 305-8575, Japan

^bLaboratory for Statistical Analysis, RIKEN Center for Integrative Medical Sciences,
1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045 Japan

^cDepartment of Ophthalmology, Graduate school of Medical Sciences, Kyushu
University, Fukuoka 812-8582, Japan

^dDepartment of Allergology, Fujita Health University School of Medicine, 3-6-10,
Otobashi, Nakagawa-ku, Nagoya, Aichi 54-8509, Japan

^eLaboratory for Respiratory and Allergic Diseases, RIKEN Center for Integrative
Medical Sciences, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045
Japan

^fCore Research Facilities for Basic Science (Molecular Genetics), Research Center for
Medical Science, The Jikei University School of Medicine, 3-25-8, Nishi-shinbashi,
Minato-ku, Tokyo 105-8461, Japan

^gDepartment of Statistical Genetics, Osaka University Graduate School of Medicine,
2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

^hLaboratory of Statistical Immunology, Immunology Frontier Research Center
(WPI-IFReC), Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan.

ⁱStructural Medicine, The United Graduate School of Drug Discovery and Medical
Information Sciences, Gifu University, Yanagido 1-1, Gifu, Gifu 501-1194, JAPAN

^jDepartment of Pediatrics, Graduate School of Medicine, Gifu University, Yanagido 1-1,
Gifu, Gifu 501-1194, JAPAN

^kDepartment of Allergology, National Hospital Organization Fukuoka National Hospital,
4-39-1, Yakatabaru, Minami-ku, Fukuoka 811-1394, Japan

^lClinical Research Center for Allergy and Rheumatology, National Hospital
Organization Sagamihara Hospital, 18-1, Sakuradai, Minami-ku, Sagamihara,

Kanagawa 252-0392, Japan

^mDepartment of Dermatology, Graduate School of Biomedical and Health Sciences,
Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima 734-8551, Japan

ⁿDepartment of Dermatology, Shimane University Faculty of Medicine, 89-1, Enya-cho,
Izumo, Shimane 693-8501, Japan

^oDepartment of Dermatology, Yokohama City University School of Medicine, 3-9,
Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-0004, Japan

^pOkabe Allergy Clinic, 1-5-3, Bashaku Kokurakitaku, Kitakyushu, Fukuoka 802-0077,
Japan

^qDepartment of Dermatology, Niigata University Medical & Dental Hospital, 1-754,
Asahimachidori, Chuo-ku, Niigata-city, Niigata 951-8520, Japan

^rDepartment of dermatology, Hyogo Prefectural Kakogawa Hospital, 231, Kanno,
Kanno-Cho, Kakogawa City, Hyogo 675-8555, Japan

^sDivision of Dermatology, Department of Internal Related, Kobe University Graduate
School of Medicine, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

^tDepartment of Dermatology, Fukuoka Sanno Hospital, 3-6-45, Momochihama,
Sawara-ku Fukuoka, 814-0001, Japan

^uAoki Skin and Allergy Clinic for Itchy Skin Diseases, 1-8-15, Daido, Tennoji-ku,
Osaka 543-0052, Japan

^vAoki dermatology, 103, Horikawa-cho, Miyazaki, Miyazaki 880-0873, Japan

^wJoy Dermatological Clinic, 6-4-1, Nakagawa, Sanyo-Onoda, Yamaguchi 756-0806,
Japan

^xMusashino Allergy Clinic, 2-8-30, Akebono-cho, Tachikawa-shi, Tokyo 190-0012,
Japan

^yDepartment of Dermatology, Wakayama Medical University, 811-1, Kimiidera,
Wakayama 641-0012, Japan

^zNishi-Sapporo Skin and Allergy Clinic, 2-11, 7-Kita, Nishi-mati, Nishi-ku, Sapporo
063-0061, Japan

^{aa}Department of Dermatology, Asahikawa City Hospital, 1-1-65, Kinsei-cho,
Asahikawa, Hokkaido 070-8610, Japan

^{ab}Section of Dermatology, Hino Clinic, 1248, Fukai-Nakamachi, Naka-Ku, Sakai,
Osaka 599-8272, Japan

^{ac}Department of Dermatology, Aomori Prefectural Central Hospital, 2-1-1,
Higashi-Tsukurimichi, Aomori 030-8553, Japan

^{ad}Department of Dermatology, Shimada Municipal Hospital, 1200-5, Noda, Shimada,
Shizuoka 427-8502, Japan

^{ae}Department of Allergy, National Hospital Organization Toyama Hospital, 2135,
Shin-machi, fucyu-machi, Toyama, Toyama 939-2692, Japan

^{af}Department of Dermatology, Kyoto Prefectural University of Medicine, 465, Kajicho,
Kawaramachi Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan

^{ag}Department of Respiratory Medicine and Clinical Immunology, Dokkyo Medical
University Saitama Medical Center, 2-1-50, Minami-koshigaya, Koshigaya, Saitama
343-8555, Japan

^{ah}National Mie Hospital, 357, Oosatokubota-cho, Thu, Mie 514-0125, Japan

^{ai}Department of dermatology, Faculty of Medicine, University of Tsukuba, 1-1-1,
Tennodai, Tsukuba, Ibaraki 305-8575, Japan

^{aj}Department of Pulmonary Medicine, Graduate School of Medical and Dental Sciences,
Kagoshima University, 8-35-1, Sakuragaoka, Kagoshima, Kagoshima 890-8520, Japan

^{ak}Department of Dermatology, Osaka Kaisei Hospital, 1-6-10, Miyahara Yodogawa-ku,
Osaka-shi, Osaka 532-0003, Japan

^{al}Department of Dermatology, Gifu University Graduate School of Medicine, 1-1,
Yanagido, Gifu 501-1194, Japan

^{am}Tajiri Dermatology Clinic, 41-1, 2-Kano, Kiyotake-cho, Miyazaki 889-1607, Japan

^{an}Tomura Clinic Dermatology, 11-22, Tanaka Tegata Akita, Akita 010-0862, Japan

^{ao}Department of Dermatology, Kudanzaka Hospital, 1-6-12, Kudanminami, Chiyoda-ku,
Tokyo 102-0074, Japan

^{ap}HLA Foundation Laboratory, 2F#1 Kyoto Research Park, Shimogyo-ku, Kyoto
600-8813, Japan

^{aq}Department of Child Health, Faculty of Medicine, University of Tsukuba, Ibaraki
305-8575, Japan

^{ar}Department of Integrative Medical Science for Allergic Disease, Fujita Health
University School of Medicine, 3-6-10, Otobashi, Nakagawa-ku, Nagoya, Aichi
454-8509, Japan

^{as}Kyoto-McGill International Collaborative School in Genomic Medicine, Kyoto
University Graduate School of Medicine, 53 Kawara-cho, Shogo-in, Sakyo-ku, Kyoto
606-8507, Japan

^{at}Department of Genomic Medicine, Research Institute, National Cerebral and
Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 562-0011, Japan

^{au}RIKEN Center for Integrative Medical Sciences, 1-7-22 Suehiro-cho, Tsurumi-ku,
Yokohama, Kanagawa 230-0045 Japan

^{av}Department of Allergy and Clinical Immunology, National Research Institute for
Child Health & Development, 2-10-1 Taishido, Setagaya-ku, Tokyo 157-8535, Japan

Correspondence should be sent to:

Emiko Noguchi, MD, PhD

Department of Medical Genetics, Faculty of Medicine, University of Tsukuba, 1-1-1,
Tennoudai, Tsukuba-city, Ibaraki 305-8575, Japan
Tel.: (+81)-29-853-3177, Fax: (+81)-29-853-3333; E-mail: enoguchi@md.tsukuba.ac.jp

Kayoko Matsunaga, MD, PhD

Department of Integrative Medical Science for Allergic Disease, Fujita Health
University School of Medicine, 3-6-10, Otobashi, Nakagawa-ku, Nagoya, Aichi
454-8509, Japan
Tel.: (+81)-562-93-9441, Fax: (+81)-562-95-2915; E-mail: kamatsu@fujita-hu.ac.jp

Hirohisa Saito, MD, PhD

Department of Allergy and Clinical Immunology, National Research Institute for Child
Health & Development, 2-10-1 Taishido, Setagaya-ku, Tokyo 157-8535, Japan
Tel.: (+81)-3-5494-7120, Fax: (+81)-3-5494-7028; E-mail: saito-hr@ncchd.go.jp

CONFLICTS OF INTERESTS

The authors declare no competing interests.

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ABSTRACT

Background:

Food allergy is a growing health problem worldwide because of its increasing prevalence, life-threatening potential, and shortage of effective preventive treatments. In an outbreak of wheat allergy in Japan, thousands of patients showed allergic reactions to wheat after using soap containing hydrolyzed wheat protein (HWP).

Objectives:

The aim of the present study was to investigate genetic variation that may contribute to susceptibility to HWP allergy.

Methods:

We conducted a genome-wide association study (GWAS) of HWP allergy in 452 cases and 2,700 controls using 6.6 million genotyped or imputed single-nucleotide polymorphisms (SNPs). Replication was assessed by genotyping SNPs in independent samples comprising 45 HWP allergic patients and 326 controls.

Results:

Through the GWAS, we identified significant associations with the class II human leukocyte antigen (HLA) region on 6p21 ($P = 2.16 \times 10^{-24}$ for rs9271588, $P = 2.96 \times 10^{-24}$ for HLA-DQ α 1 amino acid position 34) and with the *RBFox1* locus at 16p13 (rs74575857, $P = 8.4 \times 10^{-9}$). The associations were also confirmed in the replication dataset. Both amino acid polymorphisms (HLA-DQ β 1 amino acid positions 13 and 26) located in the P4 binding pockets on the HLA DQ molecule achieved the genome-wide significance level ($P < 5.0 \times 10^{-8}$).

Conclusions:

Our data provide the first demonstration of genetic risk for HWP allergy and show that this genetic risk is mainly represented by multiple combinations of HLA variants.

Key Messages

- IgE reactivity to HWP sensitized through skin was not correlated with that to omega-5-gliadin.
- In this first GWAS of patients allergic to HWP, we found strong associations with HLA-DQ variants and that the properties of the P4 binding pocket on the DQ molecule seem to influence the allergenicity of HWP.
- Variants in the HLA-DQ region associated in the literature with increased risk of peanut allergy were associated with a protective effect of HWP allergy.
- The *RBFOX1* region on chromosome 16q13, which has been reported to be associated with food allergy in a European ancestry cohort, is also associated with HWP allergy.

Capsule summary

This study provides the first demonstration of genetic susceptibility to adverse reactions related to cosmetic products and wheat allergy and that susceptibility to food allergies may be influenced by the different allergen-binding properties of HLA alleles.

Key Words

Hydrolyzed wheat protein, allergy, percutaneous, GWAS, HLA, *RBFOX1*

Abbreviations used

HWP: hydrolyzed wheat protein

194	HWP-IWA:	immediate-type wheat allergy due to hydrolyzed wheat protein
195	GWAS:	genome-wide association study
196	HLA:	human leukocyte antigen
197	FLG:	filaggrin
198	Q-Q plot:	quantile–quantile plot
199	SNP:	single nucleotide polymorphism
200	WDEIA:	wheat-dependent exercise-induced anaphylaxis
201	TTG:	anti-tissue transglutaminase
202	EDC:	epidermal differentiation complex
203		
204		

Introduction

Food allergy is a growing health problem worldwide because of its increasing prevalence, life-threatening potential, and shortage of effective preventive treatments. The prevalence of food allergy in infants and preschool children is 5-10% in Japan,¹ 4.4-5.3% in the United Kingdom,² and 7.6% in the United States.³ Food allergy can be a lifelong burden to many subjects, and currently, no effective treatments are available. The major allergens underlying most food reactions include a relatively small number of foods: milk, eggs, peanuts, tree nuts, seafood, shellfish, soy, and wheat.⁴ Food allergies are thought to be caused by both genetic and environmental factors. Many genetic studies of food allergy have focused on peanut allergy, and loss-of-function mutations in filaggrin (*FLG*) have been reported to be associated with peanut allergy and peanut sensitization, possibly due to skin barrier dysfunction.⁵ So far, 5 genome-wide association studies have been conducted for food allergy. Variation within the human leukocyte antigen (HLA) region have been reported to be associated with peanut allergy⁶⁻⁹ and with self-reported allergic reactions to shrimp and peaches.¹⁰ *SERPINB7/B2* has also been reported to be associated with hen's egg allergy⁷ and *C11orf30* with peanut allergy.¹¹ However, few genetic studies of wheat allergy have been conducted.

Wheat is one of the most widely consumed foods in the world, and the gluten in wheat has been found to be linked with various diseases, such as celiac disease, wheat allergy and gluten sensitivity.¹² These diseases are termed "gluten-related disorders" and have an estimated global prevalence of approximately 5%.¹³ However, gluten-related disorders are rare in Japan, unlike in European countries, possibly due to the low consumption of wheat in the diet.

Wheat proteins have also been widely used in a hydrolyzed form in food as well as cosmetics. Christensen *et al.*, reported hydrolyzed wheat protein (HWP) allergic patients with a case history of anaphylaxis related to ingestion of food containing HWP called Meripro 711 and showed that these patients were monosensitized to HWP and tolerated ingestion of nonhydrolyzed wheat products.¹⁴ Patients allergic to HWP in cosmetic products have been reported in Western countries, albeit only 20 or so patients reported so far.¹⁵ It has been suggested that percutaneous food sensitization can occur by the application of skin oil containing a food product (peanuts), resulting in increased prevalence of peanut allergy.¹⁶ In Japan, a total of 2,111 subjects showed allergic contact urticaria, anaphylaxis, and/or wheat-dependent exercise-induced allergy after using a soap containing a specific type of HWP, Glupearl 19S®, causing public and social concern.^{15, 17} Glupearl 19S was produced by acid treatment (pH, 0.5-1.2) of gluten at 95°C for 40 minutes. The soap containing Glupearl 19S® was very popular, especially among women, and the total number of soaps sold to the customers was 46,508,000 (http://www.jsaweb.jp/modules/en/index.php?content_id=11). Based on the number of adult Japanese women (54,444,000), approximately 1 in 12 Japanese adult women were estimated to have used the soaps.¹⁵ However, the number of patients that satisfy the diagnostic criteria of this HWP allergy was 2,111; therefore, only a small fraction (< 0.1%) of HWP soap consumers developed allergy/anaphylaxis. In the case of the patients using HWP-containing soap, no patients had shown an apparent allergy to wheat before using the soap. The symptoms typically appeared 1 year after starting use of the soap, and most of the patients with ingestion-related symptoms reacted to traditional wheat products, such as pasta, noodles, and bread, unlike non-Japanese patients allergic to HWP, who tolerated traditional wheat products.^{14, 15} The factors and

mechanisms underlying HWP allergy are largely unknown. However, considering the fact that only a small fraction of consumers developed allergy/anaphylaxis, it is speculated that genetic components are involved in the development of diseases, similar to other allergic diseases such as asthma, atopic dermatitis and food allergy. In the present study, we conducted the first genome-wide association study (GWAS) of immediate-type wheat allergy due to hydrolyzed wheat protein (HWP-IWA) related to facial soaps, a unique cosmetic-induced hydrolyzed wheat allergy.

Methods

Study design and patient characteristics

A flowchart of this study is presented in Fig. 1, and the characteristics of the participants are shown in Table E1 (see Table E1 in this article's Online Repository at www.jacionline.org). Because the majority (>95%) of HWP-IWA patients were female, due to the great popularity of the soap among women,¹⁵ cases and controls were restricted to females. Diagnostic criteria for HWP-IWA is as follows: (1) usage of soap containing HWP, (2) allergic symptoms within several to 30 min after using the soap and/or allergic symptoms after eating wheat products, (3) positive laboratory test result, skin prick test using $\leq 0.1\%$ Glupearl 19S solution, enzyme-linked immunosorbent assay (ELISA), and/or basophil activation test (Table E2). The age group with the largest share to use the soaps were those in their 40's,¹⁵ and it reflected age distribution of cases of the present study. Serum specific immunoglobulin E (IgE) levels for Glupearl 19S was quantified by the ELISA method,¹⁸ and specific IgE levels for wheat, gluten and omega-5-gliadin was determined with ImmunoCAP system (Thermo Fisher Diagnostics K.K, Tokyo, Japan).

GWAS and quality controls

Four hundred and eighty patients with HWP-IWA who satisfied the diagnostic criteria described in Table E2 were recruited as GWAS cases between June 2013 and April 2014. As GWAS controls, we included a dataset of 2,364 female subjects without asthma, atopic dermatitis, Graves' disease, rheumatoid arthritis, viral hepatitis, or drug eruption in the BioBank Japan project.¹⁹⁻²¹ We also included a dataset of 336 healthy female subjects from the Pharma SNP Consortium, resulting in a total of 2,700 subjects as controls. Subjects for GWAS were genotyped using the Illumina HumanOmniExpress BeadChip (Illumina, Inc., San Diego, CA, USA) following the manufacturer's protocols. Of the 480 GWAS patients, 452 HWP-IWA patients passed the quality controls (QC) as described in the Online Repository Method. We used East Asian samples (EAS) from Phase 1, version 3, of the 1,000 Genomes Project as a reference and evaluated 6,575,663 imputed SNPs that passed QC in GWAS. A quantile–quantile plot (Q–Q plot) revealed no evidence of genomic inflation (genomic inflation factor [λ_{GC}] 1.013, Fig E1), suggesting minimal confounding of the analysis by cryptic relatedness and population stratification. The threshold for the genome-wide statistically significant association level was set at $P = 5 \times 10^{-8}$. Detailed methods on genotyping, quality control, and statistical analysis are shown in the Methods section in this article's Online Repository at www.jacionline.org.

Conditional analysis of HLA regions for GWAS

A stepwise conditional analysis was applied to the top associated variants, and the analysis was repeated until no variants reached the genome-wide significant association

threshold ($P = 5.0 \times 10^{-8}$). Detailed methods are shown in the Methods section in this article's Online Repository at www.jacionline.org.

Replication analysis

Patients who satisfied the diagnostic criteria described in Table E2 were also recruited between April 2015 and January 2016, and 45 patients gave informed consent to participate in the study. A total of 326 female Japanese general population dataset was used as replication controls.²² We considered it to be replicated if the lead SNP or another correlated SNP in the region showed P values $< 0.05/2$ independent tests ($P < 0.025$). Detailed methods are shown in the Methods section in this article's Online Repository at www.jacionline.org.

HLA allele determination, HLA allele replication analysis, HLA DQ haplotype analysis, and HLA-DQ protein structure

To obtain HLA allelic types in GWAS, we used SNP2HLA²³ to impute classical 2- and 4-digit HLA alleles and amino acid polymorphisms in HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DPA1, and HLA-DPB1 using Japanese-population-specific HLA reference panels ($n = 908$).²⁴ We also performed HLA-DQA1 and HLA-DQB1 genotyping for 452 discovery cohort cases for GWAS, replication study cases ($n = 45$) and replication study controls ($n = 326$) using the polymerase chain reaction-reverse sequence-specific oligonucleotide probe (PCR-rSSO) method. We considered alleles to be replicated when the HLA allelic association in the replication dataset (45 cases vs 326 controls) showed P values $< 0.05/22$ tests ($P < 0.0023$).

The haplotype association analyses for DQA1 and DQB1 were performed using the Arlequin version 3.5.²⁵ We performed the haplotype analysis using 452 GWAS cases, 45 replication cases, and 326 replication controls whose DQA1 and DQB1 genotypes were available.

To visualize the molecular structure of HLA-DQ, its crystal structure was obtained from the Protein Data Bank (PDB ID: 2NNA²⁶). The amino acid residues were visualized using the PyMOL Molecular Graphics System (Version 2.0, Schrödinger, LLC, URL: <https://www.pymol.org/>).²⁷

Results

Study design and patient characteristics

Fig 1 shows the flowchart of the present study. Serum IgE levels of Glupearl 19S quantified by ELISA were available for 297 patients, and the median (range) was 18.6 (<3 – >100; ranging from the lower and upper bounds) (Fig 2, A). Skin prick test results were positive for all of the HWP-IWA patients with serum IgE levels of Glupearl 19S negative. Serum levels of wheat, gluten, and omega-5-gliadin specific IgE were available for 400, 390, and 377 patients, respectively, and the median (range) was 0.63 (<0.35-63.4), 0.77 (<0.35-63.5), and <0.35 (<0.35-9.39), respectively (Fig 2, B). Significant correlation was observed between serum-specific IgE levels of Glupearl 19S and those of wheat ($Rho = 0.59$, $P < 0.0001$ Fig 2, C) and gluten ($Rho = 0.66$, $P < 0.0001$, Fig 2, D), but not those of omega-5-gliadin ($Rho = 0.072$, $P = 0.26$, Fig 2, E).

Loci associated with HWP-IWA by GWAS and HLA imputation analysis

Fig 3, A shows a Manhattan plot of the GWAS, identifying 1 locus on chromosome 6 and 1 locus on chromosome 16 that exceeded the genome-wide significance level ($P < 5.0 \times 10^{-8}$). Details of the SNPs that are strongly associated based on GWAS analysis are described in Table E3 (see Table E3 in this article's Online Repository at www.jacionline.org).

Because the most significant association was observed in the HLA regions on chromosome 6 (Fig 3, B, rs9271763, $P = 8.8 \times 10^{-23}$, odds ratio (OR) 2.65 [95% confidence interval (CI) 2.19-3.22] for the G allele), we performed HLA imputation with SNP2HLA²⁴ using an HLA Japanese population reference panel.²⁵ The association results of variants, including SNPs, HLA amino acid variants, and HLA allelic variants are shown in Table E4 (see Table E4 in this article's Online Repository at www.jacionline.org). The most significant association in the GWAS and HLA imputation analysis was observed at rs9271588 ($P = 2.16 \times 10^{-24}$, OR 2.2 [95% confidence interval (CI) 1.88-2.59] for the G allele), that was not included in GWAS imputation reference panel and that is in strong linkage disequilibrium with rs9271763 ($r^2 = 0.94$, $D' = 0.98$, Fig 3, B). One locus located in the intron of *RBFOX1* on chromosome 16 (rs74575857, $P = 8.42 \times 10^{-9}$, OR 3.28 [95% CI 2.19-4.91] for the C allele, Fig 3, C) that reached the genome-wide significance level was also detected.

Conditional analysis of HLA regions for GWAS

To identify independently associated loci in the HLA region, we performed conditional analysis on the top associated variants, and the analysis was repeated until no variants reached the genome-wide significant association threshold. Fig E2, A and E2, B show the results of the conditional analysis. The association analysis of HLA amino acid

polymorphisms with HWP-IWA revealed that the most significant association in amino acid polymorphisms was observed at amino acid position 34 of HLA-DQ α 1 ($P_{\text{binary}} = 2.96 \times 10^{-24}$, OR 2.20 [95% CI 1.88-2.58] for the Glu allele, Fig E2, B, Table E4), and this amino acid polymorphism was in complete linkage disequilibrium with the top SNP, rs9271588 ($r^2 = 1$). After conditioning on rs9271588 (position 34 of HLA-DQ α 1), a significant association was observed with HLA-DQA1*03:03 ($P = 8.14 \times 10^{-9}$, Table E4), but most of the associations in the HLA-DR/DQ regions disappeared (Fig E2, B). Then, conditioning on both rs9271588 and HLA-DQA1*03:03 identified rs9263827 as an independent association signal ($P = 3.49 \times 10^{-8}$, OR 6.93 [95% CI 3.66-13.12] for the A allele, Table E4). SNP rs9263827 is near HLA-C. After conditioning on rs9271588, HLA-DQA1*03:03 and rs9263827, no variants reached the genome-wide significant association threshold (Fig E2).

Replication analysis using independent case-control samples and meta-analysis

We genotyped rs9271588 on chromosome 6 and rs59325236 on chromosome 16 in independent replication samples comprising 45 HWP-IWA patients and 326 female replication controls (Table 1). rs9271588 on chromosome 6 was confirmed to be associated with HWP-IWA in the replication samples ($P = 1.96 \times 10^{-6}$, OR 3.90 [95% CI 2.23-6.84], Table 1).

We also performed HLA-DQA1 and HLA-DQB1 4-digit allelic typing in the replication dataset. A similar effect size to that of the GWAS discovery samples was observed in the replication samples (Fig. 4 and Table E5). HLA-DQA1*01:03 was confirmed to be associated with HWP-IWA in the replication samples ($P = 7.11 \times 10^{-4}$, OR 0.3 [95% CI 0.12–0.62]; Table E5), and DQB1*03:02 reached the GWAS significance level in the

meta-analysis.

Table E6 shows the results of the conditional analysis of the HLA-DQA1 and HLA-DQB1 4-digit allele on rs9271588 in both the GWAS and the replication dataset. In the replication dataset, DQA1*03:03 was not associated with HWP-IWA after conditioning on rs9271588 ($P = .65$, OR 1.15 [95% CI 0.62–2.15]). The meta-analysis results showed that no HLA-DQ alleles achieved GWAS significance levels after conditioning on rs9271588.

The top associated SNP in the chromosome 16 region was rs74575857 ($P = 8.4 \times 10^{-9}$, Table E3), but we could not perform genotyping of rs74575857 for the replication study because a suitable TaqMan assay design was not obtained. Therefore, we performed genotyping using the second most associated SNP in the chromosome 16 region (rs59325236), which was in strong linkage disequilibrium with rs74575857 ($D' = 1$, and $r^2 = 0.8$). Although rs59325236 did not achieve $P < 0.025$ in the replication samples ($P = 0.097$, OR 2.27 [95% CI 0.87-5.98], Table 1) because of the reduced statistical power caused by its low allele frequency, both SNPs had similar effect sizes, which were observed in the GWAS discovery samples, and rs59325236 reached the genome-wide significance level in the meta-analysis ($P = 1.31 \times 10^{-8}$, OR 2.57 [95% CI 1.85-3.55], test for heterogeneity, $P = 0.79$).

Haplotype analysis of DQA1 and DQB1

Because we have observed a strong association between HLA class II alleles and HWP-IWA, we performed haplotype association analysis using actual genotypes of HLA-DQA1 and HLA-DQB1 (all HWP-IWA cases; $n = 497$, and replication control; $n = 326$). DQA1*01:03-DQB1*06:01 haplotype achieved the genome-wide significance

level ($P = 3.34 \times 10^{-10}$, Table E7). No HLA-DQ haplotypes achieved GWAS significance levels after conditioning on rs9271588 (Table E7).

***FLG* loss-of-function mutation analysis**

Because *FLG* loss-of-function mutations have been reported to be associated with food allergy,²⁸ we performed genotyping of the 5 most common *FLG* loss-of-function mutations in the Japanese population. As shown in Table E8, the frequencies of these *FLG* loss-of-function mutations did not differ between HWP-IWA patients and the Japanese general population ($P > 0.05$), suggesting that skin barrier dysfunction did not play a major role in the development of HWP-IWA.

Association analysis of HWP-IWA with previously reported HLA variants/SNPs of food allergy/gluten-related diseases by GWAS

Table 2 shows the previously reported association results of HLA alleles and amino acid polymorphisms in food allergy⁶ and provides a comparison with the results of HWP-IWA in the present study. The opposite direction of association, except at position 26 of HLA-DQ β 1, was observed between peanut allergy and HWP-IWA in the HLA 2- and 4-digit and amino acid variant analyses. We also examined possible associations of HWP-IWA with published food allergy loci⁷⁻¹¹ and celiac disease loci²⁹ by GWAS (Table E9). Three of the examined variants were nominally significant at $P < 0.05$, and all were within the HLA region.

Discussion

This is the first GWAS in patients allergic to HWP, and we found strong associations of

HWP allergy with HLA-DQ variants.

Many HWP-IWA patients experienced serious symptoms, and we previously reported that 25% of patients experienced anaphylactic shock and 43% experienced dyspnea.¹⁵ Most patients with conventional wheat-dependent exercise-induced anaphylaxis (WDEIA) are specific IgE positive for omega-5-gliadin. In the present study, specific IgE levels for Glupearl 19S were statistically significantly correlated with those of wheat and gluten-specific IgE, but not with omega-5-gliadin specific IgE (Fig. 2), suggesting distinct allergenicity of Glupearl 19S from omega-5-gliadin.

Antigenic peptides are bound in the binding groove of HLA class II molecules in an extended conformation, and the binding grooves of HLA class II isotypes are mainly characterized by the properties of the P1, P4, P6, and P9 pockets.³⁰ The molecular structure of the extracellular region of the HLA-DQ molecule, position 34 of HLA-DQ α 1, the top associated variant; and the positions of the P1, P4, P6 and P9 pockets of HLA-DQ β 1 in the crystal structure of the HLA-DQ molecules are shown in Fig. 5, along with the associated variants in each binding pocket. The risk-associated amino acid residue 34 of HLA-DQ α 1 (Fig. 5, pink sphere) is located in the second hypervariable region of the first domain in the floor of the antigen binding cleft. Both amino acid polymorphisms located in the P4 binding pockets achieved the genome-wide significance level. Therefore, the effect of HWP exposure on skin and nasal/eye mucosa may be influenced by the properties of the P4 binding pocket on the DQ molecule.

Celiac disease is a gluten-intolerance disorder, and its major genetic factors are the HLA-DQA1 and DQB1 loci. Approximately 95% of patients possess DQ2.5 (DQA1*05:01/DQB1*02:01), while the remaining 5% carry DQ8 (DQA1*03:01/DQB1*03:02), and the estimated heritability for classical HLA-DQ

alleles and independent MHC variants outside of HLA-DQ has been reported as 0.232 and 0.18, respectively.³¹ Celiac disease is considered a common disease in Europe and USA³² but is very rare in Japan, possibly due to low allele frequencies for the predisposing DQ alleles and low consumption of gluten-related food products. Fukunaga et al. reported that high levels (>10 U/mL) of anti-tissue transglutaminase (TTG) immunoglobulin A antibody were observed in 4 of 2,008 nonclinical Japanese subjects tested (0.2%), and only 1 of them (0.05% among nonclinical subjects) was confirmed to have celiac disease by duodenal histopathological findings.³³ The allele frequencies of the predisposing alleles, DQB1*02:01 and DQB1*03:02, in the Japanese general population are 0.134% and 9.6%, respectively,³⁴ and this allele frequency of DQB1*02:01 is extremely low compared with those in other East Asian and European countries (<http://pypop.org/popdata/2008/byfreq-DQ.php.html>).³⁵ In the present study, DQB1*03:02 (DQ8) was also a risk allele for HWP-IWA. Another risk allele for HWP-IWA, DQB1*03:03 (DQ9), is not a predisposing allele for celiac disease, although only 1 amino acid change (position 57 of DQβ1) in the antigen binding pocket (P9) differentiates DQ8 and DQ9. Glupearl 19S contains a variety of gluten-derived peptides with or without deamination, and our data suggest that common as well as different antigenic peptides for celiac disease and HWP-IWA may exist.

Genome-wide significant associations were also observed in the SNPs rs74575857 and rs59325236, located in the intron of *RBFOX1*. *RBFOX1* is an RNA-binding protein that plays important roles in alternative splicing³⁶ and has been reported to be associated with neurodevelopmental diseases.³⁷ A recent report showed that copy number variants of *RBFOX1* are associated with food allergy in a European-ancestry cohort.³⁸ Although copy number variation analysis was not applied to our dataset, it is possible that copy

number variation in *RBFOX1* plays a role in the development of HWP-IWA. Compared with other allergic diseases such as asthma and atopic dermatitis, the number of GWAS/genetic studies related to food allergy is small. Hong et al. conducted GWAS for patients with food allergy, including egg, milk, and peanut, and they found that rs7192 and rs9275596, located in the HLA-DQ region, were strongly associated with peanut allergy.⁶ The opposite direction of association, except at position 26 of HLA-DQ β 1, was observed between peanut allergy and HWP-IWA in both SNP and HLA 2- and 4-digit associations (Tables 2 and Table E4), suggesting that the same variant in this HLA-DQ region may exert an effect either enhancing or reducing allergen-binding property depending on the structure of food allergens. Recently, 5 GWASs related to food allergy have been published,⁷⁻¹¹ and a comparison between the previously published GWAS results and those in the present study revealed no associations of HWP-IWA with previously reported variants, except those in the HLA regions.

Skin barrier disruption in early life has been shown to be associated with both food allergen sensitization and clinical food allergy, even in those without atopic dermatitis, supporting the notion that a defective skin barrier might be a route of sensitization to food allergens.³⁹ Exposure to environmental food allergens can occur through the skin, and loss-of-function mutations in the *FLG* gene are strong predisposing factors for the development of food allergy.⁴⁰ In addition, a recent GWAS reported that rs12123821, located in the epidermal differentiation complex (EDC) near the *FLG* gene on chromosome 1q21, was associated with food allergy.⁷ In the present study, we examined whether skin barrier dysfunction was associated with the development of HWP-IWA by genotyping the 5 most common *FLG* loss-of-function mutations in the

Japanese population. As shown in Table E6, *FLG* loss-of-function mutations are not associated with HWP-IWA. HWP-IWA patients were exposed to wheat protein extensively through the cutaneous and mucosal route when they used facial soaps containing HWP and detergent; therefore, it is possible that allergen sensitization occurred in patients with HWP regardless of their skin barrier status, due to repeated use of these soaps.

In conclusion, we conducted the first GWAS for HWP allergy, and we identified associations with HLA-DQ variants and the *RBFOX1* locus. To the best of our knowledge, this is the first GWAS combining adverse reactions related to cosmetic products and wheat allergy. HWP is widely used in food as well as cosmetic products. In a recent report, the Cosmetic Ingredient Review (CIR) Expert Panel reported that the use of HWP was safe in cosmetics when they were restricted to average molecular weight of 3.5 kDa or less,⁴¹ and the results obtained in the present study can be used to support safer manufacturing and use of HWP. Although further studies are needed to understand the precise mechanisms underlying gluten-related disorders, our data suggest that subjects genetically susceptible and tolerant to HWP exist when exposed through the skin and/or mucosa, leading to an increased understanding of immunologic disorders, such as wheat allergy and celiac disease, and of wheat protein risk prediction.

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References

1. Ebisawa M, Ito K, Fujisawa T, Committee for Japanese Pediatric Guideline for Food Allergy TJSOPA, Clinical Immunology TJSOA. **Japanese guidelines for food allergy 2017.** *Allergol Int* 2017; 66:248-64.
2. Venkataraman D, Erlewyn-Lajeunesse M, Kurukulaaratchy RJ, Potter S, Roberts G, Matthews S, et al. **Prevalence and longitudinal trends of food allergy during childhood and adolescence: Results of the Isle of Wight Birth Cohort study.** *Clin Exp Allergy* 2018; 48:394-402.
3. Gupta RS, Warren CM, Smith BM, Blumenstock JA, Jiang J, Davis MM, et al. **The Public Health Impact of Parent-Reported Childhood Food Allergies in the United States.** *Pediatrics* 2018.
4. Ho MH, Wong WH, Chang C. **Clinical spectrum of food allergies: a comprehensive review.** *Clin Rev Allergy Immunol* 2014; 46:225-40.
5. Brough HA, Simpson A, Makinson K, Hankinson J, Brown S, Douiri A, et al. **Peanut allergy: effect of environmental peanut exposure in children with filaggrin loss-of-function mutations.** *J Allergy Clin Immunol* 2014; 134:867-75 e1.
6. Hong X, Hao K, Ladd-Acosta C, Hansen KD, Tsai HJ, Liu X, et al. **Genome-wide association study identifies peanut allergy-specific loci and evidence of epigenetic mediation in US children.** *Nat Commun* 2015; 6:6304.
7. Marenholz I, Grosche S, Kalb B, Ruschendorf F, Blumchen K, Schlags R, et al. **Genome-wide association study identifies the SERPINB gene cluster as a susceptibility locus for food allergy.** *Nat Commun* 2017; 8:1056.
8. Martino DJ, Ashley S, Koplin J, Ellis J, Saffery R, Dharmage SC, et al.

Genomewide association study of peanut allergy reproduces association with amino acid polymorphisms in HLA-DRB1. *Clin Exp Allergy* 2017; 47:217-23.

9. Asai Y, Eslami A, van Ginkel CD, Akhabir L, Wan M, Yin D, et al. **A Canadian genome-wide association study and meta-analysis confirm HLA as a risk factor for peanut allergy independent of asthma.** *J Allergy Clin Immunol* 2018; 141:1513-6.

10. Khor SS, Morino R, Nakazono K, Kamitsuji S, Akita M, Kawajiri M, et al. **Genome-wide association study of self-reported food reactions in Japanese identifies shrimp and peach specific loci in the HLA-DR/DQ gene region.** *Sci Rep* 2018; 8:1069.

11. Asai Y, Eslami A, van Ginkel CD, Akhabir L, Wan M, Ellis G, et al. **Genome-wide association study and meta-analysis in multiple populations identifies new loci for peanut allergy and establishes C11orf30/EMSY as a genetic risk factor for food allergy.** *J Allergy Clin Immunol* 2018; 141:991-1001.

12. Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M, et al. **Spectrum of gluten-related disorders: consensus on new nomenclature and classification.** *BMC Med* 2012; 10:13.

13. Valenti S, Corica D, Ricciardi L, Romano C. **Gluten-related disorders: certainties, questions and doubts.** *Ann Med* 2017; 49:569-81.

14. Christensen MJ, Stahl Skov P, Poulsen LK, Bindslev-Jensen C, Mortz CG. **Clinical relevance of sensitization to hydrolyzed wheat protein in wheat-sensitized subjects.** *J Allergy Clin Immunol* 2018; 141:802-5 e1.

15. Yagami A, Aihara M, Ikezawa Z, Hide M, Kishikawa R, Morita E, et al.
Outbreak of immediate-type hydrolyzed wheat protein allergy due to a facial soap in Japan. *J Allergy Clin Immunol* 2017; 140:879-81 e7.
16. Lack G, Fox D, Northstone K, Golding J, Avon Longitudinal Study of P, Children Study T. **Factors associated with the development of peanut allergy in childhood.** *N Engl J Med* 2003; 348:977-85.
17. Fukutomi Y, Itagaki Y, Taniguchi M, Saito A, Yasueda H, Nakazawa T, et al.
Rhinoconjunctival sensitization to hydrolyzed wheat protein in facial soap can induce wheat-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 2011; 127:531-3 e1-3.
18. Nakamura M, Yagami A, Hara K, Sano A, Kobayashi T, Aihara M, et al. **A new reliable method for detecting specific IgE antibodies in the patients with immediate type wheat allergy due to hydrolyzed wheat protein: correlation of its titer and clinical severity.** *Allergol Int* 2014; 63:243-9.
19. Hirata M, Kamatani Y, Nagai A, Kiyohara Y, Ninomiya T, Tamakoshi A, et al.
Cross-sectional analysis of BioBank Japan clinical data: A large cohort of 200,000 patients with 47 common diseases. *J Epidemiol* 2017; 27:S9-S21.
20. Nagai A, Hirata M, Kamatani Y, Muto K, Matsuda K, Kiyohara Y, et al.
Overview of the BioBank Japan Project: Study design and profile. *J Epidemiol* 2017; 27:S2-S8.
21. Okada Y, Momozawa Y, Ashikawa K, Kanai M, Matsuda K, Kamatani Y, et al.
Construction of a population-specific HLA imputation reference panel and its application to Graves' disease risk in Japanese. *Nat Genet* 2015; 47:798-802.

- 612 22. Morii W, Sakai A, Ninomiya T, Kidoguchi M, Sumazaki R, Fujieda S, et al.
 613 **Association of Japanese cedar pollinosis and sensitization with HLA-DPB1**
 614 **in the Japanese adolescent.** *Allergol Int* 2018; 67:61-6.
- 615 23. Jia X, Han B, Onengut-Gumuscu S, Chen WM, Concannon PJ, Rich SS, et al.
 616 **Imputing amino acid polymorphisms in human leukocyte antigens.** *PLoS*
 617 *One* 2013; 8:e64683.
- 618 24. Hirata J, Hirota T, Ozeki T, Kanai M, Sudo T, Tanaka T, et al. **Variants at**
 619 **HLA-A, HLA-C, and HLA-DQB1 Confer Risk of Psoriasis Vulgaris in**
 620 **Japanese.** *J Invest Dermatol* 2018; 138:542-8.
- 621 25. Excoffier L, Laval G, Schneider S. **Arlequin ver. 3.0: An integrated software**
 622 **package for population genetics data analysis.** *Evol Bioinform Online* 2007;
 623 1:47-50.
- 624 26. Henderson KN, Tye-Din JA, Reid HH, Chen Z, Borg NA, Beissbarth T, et al. **A**
 625 **structural and immunological basis for the role of human leukocyte antigen**
 626 **DQ8 in celiac disease.** *Immunity* 2007; 27:23-34.
- 627 27. DeLano W. **The PyMOL Molecular Graphics System.** San Carlos, CA:
 628 **DeLano Scientific.** 2002.
- 629 28. Hirota T, Nakayama T, Sato S, Yanagida N, Matsui T, Sugiura S, et al.
 630 **Association study of childhood food allergy with genome-wide association**
 631 **studies-discovered loci of atopic dermatitis and eosinophilic esophagitis.** *J*
 632 *Allergy Clin Immunol* 2017; 140:1713-6.
- 633 29. Dubois PC, Trynka G, Franke L, Hunt KA, Romanos J, Curtotti A, et al.
 634 **Multiple common variants for celiac disease influencing immune gene**
 635 **expression.** *Nat Genet* 2010; 42:295-302.

- 636 30. Jones EY, Fugger L, Strominger JL, Siebold C. **MHC class II proteins and**
637 **disease: a structural perspective.** *Nat Rev Immunol* 2006; 6:271-82.
- 638 31. Gutierrez-Achury J, Zhernakova A, Pulit SL, Trynka G, Hunt KA, Romanos J, et
639 al. **Fine mapping in the MHC region accounts for 18% additional genetic**
640 **risk for celiac disease.** *Nat Genet* 2015; 47:577-8.
- 641 32. Catassi C, Gatti S, Lionetti E. **World perspective and celiac disease**
642 **epidemiology.** *Dig Dis* 2015; 33:141-6.
- 643 33. Fukunaga M, Ishimura N, Fukuyama C, Izumi D, Ishikawa N, Araki A, et al.
644 **Celiac disease in non-clinical populations of Japan.** *J Gastroenterol* 2018;
645 53:208-14.
- 646 34. Ikeda N, Kojima H, Nishikawa M, Hayashi K, Futagami T, Tsujino T, et al.
647 **Determination of HLA-A, -C, -B, -DRB1 allele and haplotype frequency in**
648 **Japanese population based on family study.** *Tissue Antigens* 2015; 85:252-9.
- 649 35. Solberg OD, Mack SJ, Lancaster AK, Single RM, Tsai Y, Sanchez-Mazas A, et
650 al. **Balancing selection and heterogeneity across the classical human**
651 **leukocyte antigen loci: a meta-analytic review of 497 population studies.**
652 *Hum Immunol* 2008; 69:443-64.
- 653 36. Jin Y, Suzuki H, Maegawa S, Endo H, Sugano S, Hashimoto K, et al. **A**
654 **vertebrate RNA-binding protein Fox-1 regulates tissue-specific splicing via**
655 **the pentanucleotide GCAUG.** *EMBO J* 2003; 22:905-12.
- 656 37. Conboy JG. **Developmental regulation of RNA processing by Rbfox proteins.**
657 *Wiley Interdiscip Rev RNA* 2017; 8.
- 658 38. Li J, Fung I, Glessner JT, Pandey R, Wei Z, Bakay M, et al. **Copy Number**
659 **Variations in CTNNA3 and RBFOX1 Associate with Pediatric Food Allergy.**

- 660 *J Immunol* 2015; 195:1599-607.
- 661 39. Kelleher MM, Dunn-Galvin A, Gray C, Murray DM, Kiely M, Kenny L, et al.
- 662 **Skin barrier impairment at birth predicts food allergy at 2 years of age.** *J*
- 663 *Allergy Clin Immunol* 2016; 137:1111-6 e8.
- 664 40. Irvine AD, McLean WH, Leung DY. **Filaggrin mutations associated with skin**
- 665 **and allergic diseases.** *N Engl J Med* 2011; 365:1315-27.
- 666 41. Burnett C, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, et al.
- 667 **Safety Assessment of Hydrolyzed Wheat Protein and Hydrolyzed Wheat**
- 668 **Gluten as Used in Cosmetics.** *Int J Toxicol* 2018; 37:55S-66S.

Table 1. Results of 2 SNPs for the development of HWP-IWA in GWAS and replication samples

		Discovery				Replication				Meta-analysis		
SNP	Allele	AF _{CASE}	AF _{Control}	OR (95% CI)	<i>P</i>	AF _{CASE}	AF _{Control}	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	<i>P^{HET}</i>
rs9271588	A/ G	0.701	0.523	2.21 (1.88-2.59)	2.16×10^{-24}	0.779	0.494	3.90 (2.23-6.84)	1.96×10^{-6}	2.30 (1.98-2.70)	1.11×10^{-26}	0.056
rs59325236	A /G	0.057	0.024	2.60 (1.84-3.68)	5.19×10^{-8}	0.068	0.033	2.27 (0.87-5.98)	0.097	2.57 (1.85-3.55)	1.31×10^{-8}	0.79

In the Allele column, the first of the 2 alleles is the minor allele. The risk allele is indicated in bold, and the effect of the risk allele is examined. The association analysis was performed using the additive genetic model. AF, allele frequency of the risk allele; OR, odds ratio; 95% CI, 95% confidence interval; P^{HET} , *P* value for test of heterogeneity.

Table 2. Association of HLA alleles and amino acid polymorphisms with HWP-IWA and comparison with Hong et al's findings regarding peanut-allergy-associated alleles/polymorphisms.

			HWP-IWA (present study)				Peanut allergy (Hong et al, 2015)		
Gene	Allele		AF _{CASE}	AF _{Control}	OR (95%CI)	P value	AF _{CASE}	AF _{Control}	P value
HLA-DQA1	DQA1*01:02	Present	0.108	0.135	0.79 (0.63-0.99)	0.043	0.28	0.2	2.7×10^{-8}
HLA-DQB1	DQB1*06	Present	0.216	0.337	0.53 (0.44-0.63)	5.5×10^{-14}	0.35	0.25	5.4×10^{-9}
	amino acid	position	AF _{CASE}	AF _{Control}	OR (95%CI)	P value	AF _{CASE}	AF _{Control}	P value
HLA-DRβ1	Arg	71	0.821	0.735	1.69 (1.40-2.03)	6.84×10^{-9}	0.38	0.45	2.3×10^{-10}
HLA-DQα1	Met	207	0.109	0.136	0.79 (0.63-0.99)	0.045	0.28	0.2	2.7×10^{-8}
HLA-DQβ1	Leu	26	0.522	0.398	1.73 (1.49-2.00)	2.45×10^{-13}	0.71	0.63	1.1×10^{-8}
HLA-DQβ1	Gly	86	0.065	0.068	0.97 (0.72-1.29)	0.82	0.1	0.06	1.6×10^{-8}

HWP-IWA (present study): AF_{CASE}, allele frequency of GWAS cases; AF_{Control}, allele frequency of GWAS controls; OR, odds ratio; 95% CI, 95% confidence interval; The association analysis was performed using the additive genetic model.

Peanut allergy (Hong et al, reference 6): AF_{CASE} and AF_{Controls}, allele frequencies of HLA classical alleles and amino acid polymorphisms imputed by SNP2HLA for peanut allergy patients and controls of discovery samples (reference 6 in the main text).

Figure Legends

Figure 1. Flowchart of the present study.

EAS samples of 1KGP, east Asian samples from Phase 1, version 3, of the 1000 Genomes Project; SNP, single-nucleotide polymorphism.

Figure 2. Specific IgE values of HWP-IWA patients.

(A) Specific IgE values for Glupearl 19S in HWP-IWA patients ($n = 297$). Values < lower limit detection values (3 unit) were recorded as 1 unit, and those $\geq$ upper limit detection values as 100 units for analysis. **(B)** Wheat ($n = 400$), gluten ($n = 390$), and omega-5-gliadin ($n = 377$) specific IgE levels in HWP-IWA patients. Bars indicate median values. Specific IgE values for wheat, gluten, and omega-5-gliadin < lower limit detection values ($0.35 \text{ U}_A/\text{ml}$) were recorded as $0.1 \text{ U}_A/\text{ml}$, and those $\geq$ upper limit detection values ($100 \text{ U}_A/\text{ml}$) were recorded as $100 \text{ U}_A/\text{ml}$. Spearman rank correlation between Glupearl 19S specific IgE and wheat **(C)**, Glupearl 19S specific IgE and gluten **(D)**, and Glupearl 19S specific IgE and omega-5-gliadin **(E)** are shown.

Figure 3. Manhattan plots of the GWAS and regional association plots on HWP-IWA.

(A) The Manhattan plots for genotyped and imputed SNPs are shown for the GWAS on HWP-IWA (452 cases vs. 2700 controls). The X-axis indicates the chromosomal position, and the Y-axis shows $-\log_{10}(P \text{ value})$. The red line indicates the thresholds for genome-wide significance ($P < 5.0 \times 10^{-8}$).

(B) Regional association plot on chromosome 6 that reached genome-wide significance.

Each dot represents the $-\log_{10}(P \text{ value})$ for genotyped and imputed SNPs with

Minimac3 software using the EAS samples of 1KGP (phase1v3). Genomic locations of genes within the regions of interest are shown at the bottom. SNP color represents pairwise linkage disequilibrium (r^2) between the index SNP (rs9271763) and each genotyped and imputed SNP based on 1000 Genomes Mar 2012 ASN (hg19) as the reference.

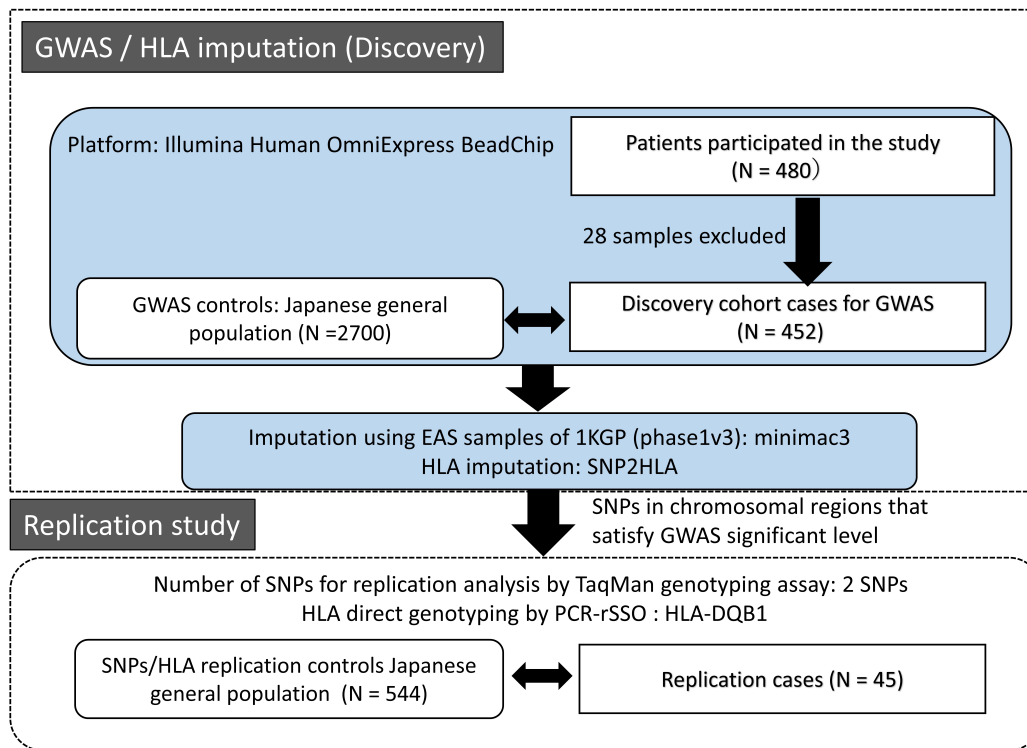
(C) Regional association plot on chromosome 16 that reached genome-wide significance. Each dot represents the $-\log_{10}$ (P value) for genotyped and imputed SNPs with Minimac3 software using the EAS samples of 1KGP (phase1v3). SNP color represents pairwise linkage disequilibrium (r^2) between the index SNP (rs74575857) and each genotyped and imputed SNP based on 1000 Genomes Mar 2012 ASN (hg19) as the reference.

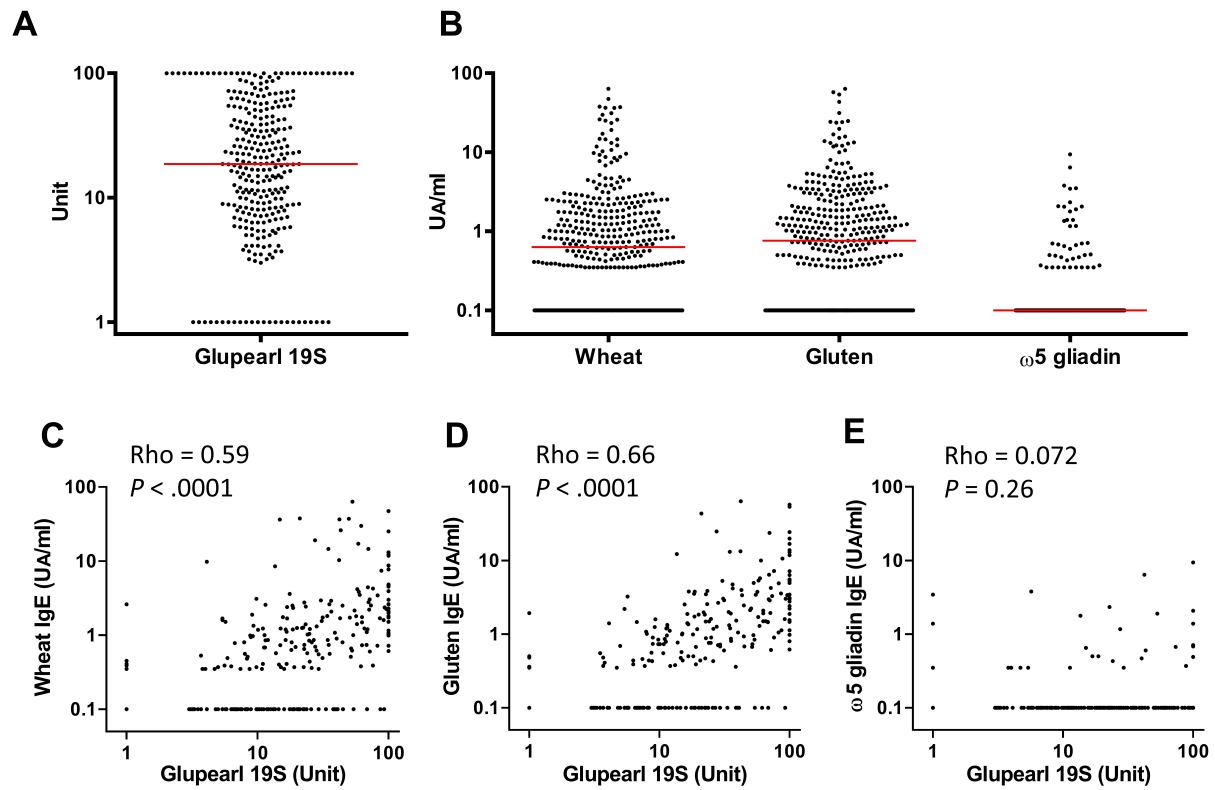
Figure 4. Association results for HLA DQA1 **(A)** /DQB1 **(B)** 4-digit alleles in GWAS (discovery), replication, and meta-analysis. Odds ratio for GWAS (■), replication (○), and meta-analysis (▲) and 95% confidence interval (bars) are shown for each HLA-DQ 4-digit allele. The P values that achieve GWAS significance ($P < 5 \times 10^{-8}$) are marked with asterisks.

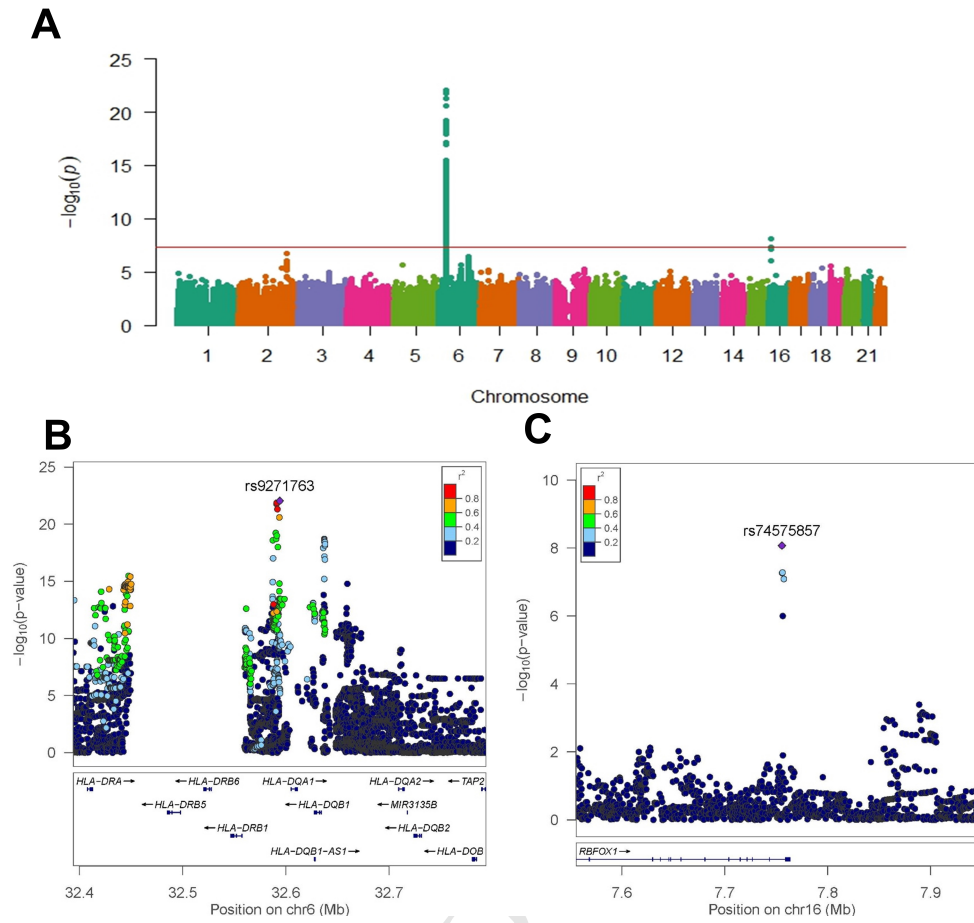
Figure 5. HLA-DQ protein structure. The molecular structure of the extracellular region of the HLA-DQ molecule (PDB ID: 2NNA), along with amino acid position 34 of HLA-DQ α 1 (pink sphere), and the locations of pocket 1, pocket 4, pocket 6, and pocket 9 of HLA-DQ α 1/HLA-DQ β 1 and their polymorphic amino acid (AA_) positions are shown. An arrow indicates each pocket location on the HLA-DQ molecule. Amino acid

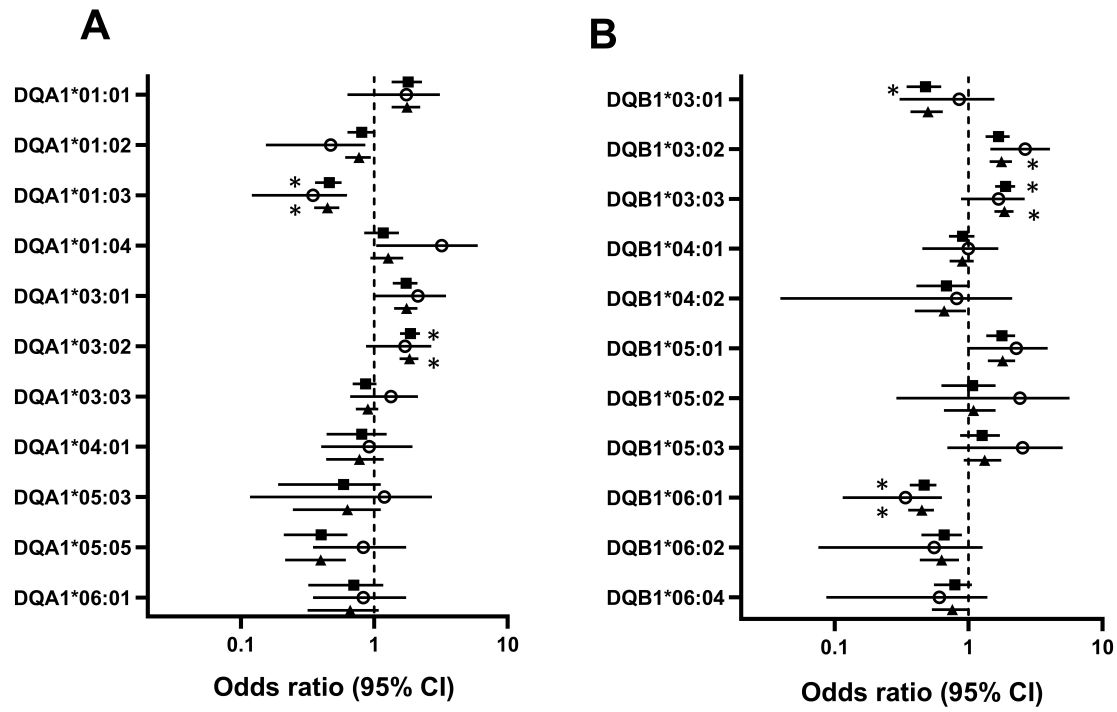
48 polymorphisms with asterisks (*) indicate those that reached the genome-wide
49 significance level ($P < 5.0 \times 10^{-8}$) in GWAS and/or the meta-analysis, and amino acid
50 polymorphisms with asterisks (**) indicate those that kept the genome-wide
51 significance level after conditioning on rs9271588. DQA1*01:03, DQB1*06:01, and
52 DQB1*03:01 are protective alleles (marked with blue color), while DQA1*03:02,
53 DQB1*03:02, and DQB1 *03:03 are susceptibility alleles (marked with red color) for
54 HWP-IWA that reached the genome-wide significance level for HLA classical allelic
55 association analysis.

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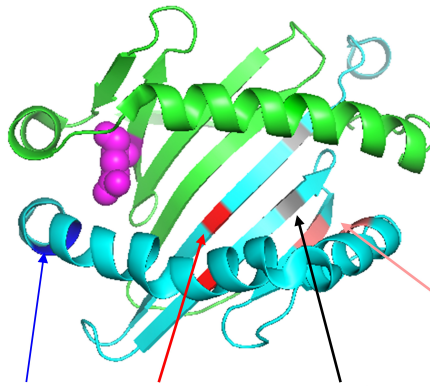








Allele	Pocket 1	Pocket 4	Pocket 6	Pocket 9	
	AA_52	Not polymorphic	AA_66	AA_69	AA_76
DQA1*01:03	S	N.A	M	A	M
DQA1*03:02	R	N.A	I	L	V*



Allele	Pocket 1		Pocket 4		Pocket 6		Pocket 9		
	AA_86	AA_87	AA_13	AA_26	AA_9	AA_30	AA_37	AA_38	AA_57
DQB1*06:01	A	F*	A*	Y*	L*	Y	D*	V	D*
DQB1*03:01	E	L	A*	Y*	Y**	Y	Y*	A	D*
DQB1*03:02	E	L	G*	L**	Y**	Y	Y*	A	A
DQB1*03:03	E	L	G*	L**	Y**	Y	Y*	A	D*

ACCEPTED