ARTICLE

Genome-wide association study reveals an association between the HLA-DPB1*02:01:02 allele and wheat-dependent exercise-induced anaphylaxis

Koya Fukunaga,[1] Yuko Chinuki,[2] Yuto Hamada,[3] Yuma Fukutomi,[3] Akiko Sugiyama,[4] Reiko Kishikawa,[4] Atsushi Fukunaga,[5] Yoshiko Oda,[5] Tsukasa Ugajin,[6] Hiroo Yokozeki,[6] Naoe Harada,[7] Masataka Suehiro,[7] Michihiro Hide,[7] Yukinobu Nakagawa,[8] Emiko Noguchi,[9] Masashi Nakamura,[10,11] Kayoko Matsunaga,[10] Akiko Yagami,[12,13] Eishin Morita,[2,*] and Taisei Mushiroda[1,*]

Summary

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a life-threatening food allergy triggered by wheat in combination with the second factor such as exercise. The identification of potential genetic risk factors for this allergy might help high-risk individuals before consuming wheat-containing food. We aimed to identify genetic variants associated with WDEIA. A genome-wide association study was conducted in a discovery set of 77 individuals with WDEIA and 924 control subjects via three genetic models. The associations were confirmed in a replication set of 91 affected individuals and 435 control individuals. Summary statistics from the combined set were analyzed by meta-analysis with a random-effect model. In the discovery set, a locus on chromosome 6, rs9277630, was associated with WDEIA in the dominant model (OR = 3.95 [95% CI, 2.31–6.73], $p = 7.87 \times 10^{-8}$). The HLA-DPB1*02:01:02 allele displayed the most significant association with WDEIA (OR = 4.51 [95% CI, 2.66–7.63], $p = 2.28 \times 10^{-9}$), as determined via HLA imputation following targeted sequencing. The association of the allele with WDEIA was confirmed in replication samples (OR = 3.82 [95% CI, 2.33–6.26], $p = 3.03 \times 10^{-8}$). A meta-analysis performed in the combined set revealed that the HLA-DPB1*02:01:02 allele was significantly associated with an increased risk of WDEIA (OR = 4.13 [95% CI, 2.89–5.93], $p = 1.06 \times 10^{-14}$). Individuals carrying the HLA-DPB1*02:01:02 allele have a significantly increased risk of WDEIA. Further validation of these findings in independent multiethnic cohorts is needed.

Introduction

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is an immunoglobulin (Ig)E-mediated food allergy induced by exercise following the ingestion of wheat products.[1,2] The prevalence of wheat allergies, including WDEIA, has been reported to be 0.10%–0.79% in adults from Japan, Europe, Latin America, and North America.[3–8] Thus, there is little difference in the prevalence of wheat allergies among ethnicities. Moreover, it is well known that the phenotype of wheat allergies is age dependent, that is, immediate-type wheat allergy is dominantly observed in infants and both types of immediate-type wheat allergy and WDEIA are observed in children; however, in most adults, wheat allergy appears as WDEIA.[9] Therefore, several non-negligible potential individuals might suffer from WDEIA because wheat is the main ingredient of the most widely consumed foods, including bread, pasta, and beer.[10,11] Reported clinical WDEIA symptoms include severe reactions, such as urticaria, angioedema, generalized erythema, wheezing, and anaphylactic shock.[12] Kennard et al. reported that consuming a gluten-free diet and avoiding wheat in combination with exercise reduced allergic reactions by 67%–69% among individuals with WDEIA.[13] Therefore, the identification of WDEIA biomarkers is critical for preventing anaphylaxis in the individuals who might suffer from WDEIA in the future.

The major causative WDEIA allergen is ω-5 gliadin, and not glutenin, in the salt-insoluble wheat protein fraction.[14,15] In addition to the provocation test with wheat plus exercise and skin prick test,[16] measuring the serum ω-5 gliadin level with enzyme-linked immunosorbent assay with specific IgE antibodies to ω-5 gliadin is useful for diagnosing WDEIA.[17] The positive rate of specific IgE antibodies to ω-5 gliadin is reportedly 94.7% in individuals with WDEIA aged ≤20 years.[18] The serum levels of not only ω-5 gliadin but also histamine and IL-10 mRNA are high in these individuals.[19] Therefore, IgE-dependent mast cell degranulation and histamine release may be

[1]Laboratory for Pharmacogenomics, RIKEN Center for Integrative Medical Sciences, Yokohama, Kanagawa 230-0045, Japan; [2]Department of Dermatology, Shimane University Faculty of Medicine, Shimane 693-0021, Japan; [3]Clinical Research Center for Allergy and Rheumatology, National Hospital Organization Sagamihara National Hospital, Kanagawa 252-0392, Japan; [4]Department of Allergology, National Hospital Organization Fukuoka National Hospital, Fukuoka 810-0062, Japan; [5]Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan; [6]Department of Dermatology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo 113-8510, Japan; [7]Department of Dermatology, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima 734-8551, Japan; [8]Department of Dermatology, Course of Integrated Medicine, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan; [9]Department of Medical Genetics, Faculty of Medicine, University of Tsukuba, Tsukuba 305-8575, Japan; [10]Department of Integrative Medical Science for Allergic Disease, Fujita Health University School of Medicine, Nagoya 454-8509, Japan; [11]General Research and Development Institute, Hoyu, Nagakute 454-8509, Japan; [12]Department of Allergology, Fujita Health University School of Medicine, Nagoya 454-8509, Japan; [13]Fujita Health University General Allergy Center in Bantane Hospital, Nagoya 454-8509, Japan

*Correspondence: emorita@med.shimane-u.ac.jp (E.M.), mushiroda@riken.jp (T.M.)

https://doi.org/10.1016/j.ajhg.2021.06.017.

critical in WDEIA, but the underlying mechanisms and the genetic background remain unclear. The aim of this study was to delineate the genetic factors contributing to WDEIA by conducting a genome-wide association study (GWAS) of WDEIA in a Japanese population. To the best of our knowledge, this is the first report of GWAS for WDEIA.

Subjects and methods

Study populations and the definition of WDEIA

One hundred and seven subjects (77 and 30 for the discovery and replication sets, respectively) with WDEIA were recruited from seven sites (National Hospital Organization Sagamihara National Hospital, Shimane University Hospital, National Hospital Organization Fukuoka National Hospital, Kobe University Hospital, Tokyo Medical and Dental University Medical Hospital, Hiroshima University Hospital, and Osaka University Hospital) in Japan between July 2019 and January 2021. Sixty-one individuals with WDEIA were recruited for a replication study from Fujita Health University Bantane Hospital between October 2018 and November 2020. We first performed a GWAS by using the genomes of 77 individuals with WDEIA collected by June 2020 and then conducted a replication study by using the genomes of 61 individuals with WDEIA independently collected by Fujita Health University Bantane Hospital and the genomes of our additional 30 individuals with WDEIA collected from June 2020 to January 2021. The diagnostic criteria of the Health Labour Sciences Research Grant study group for WDEIA are as follows (Table S1): (1) occurrence of immediate-type allergic reactions, such as urticaria, after taking wheat products owing to secondary factors, including exercise, non-steroidal anti-inflammatory drugs and/or alcohol consumption; (2) induction of immediate-type allergic reactions by oral wheat provocation test[15] (wheat intake + exercise, aspirin + wheat intake, or aspirin + wheat intake + exercise); (3) detection of wheat protein (including $\geq$0.70 kUa/L to ω-5 gliadin)-specific IgE in serum; and (4) positive results in wheat protein prick test. Food-dependent exercise-induced anaphylaxis (FDEIA) was diagnosed when criteria 1 and 2 were satisfied or 1 was repeated more than once and (3) or (4), or both, were satisfied. Serum-specific IgE levels were determined with the Immuno-CAP system (Thermo Fisher Scientific, Waltham, MA, USA). The specific IgE antibody levels for the three allergens, wheat, gluten, and ω-5 gliadin, were measured. Subjects with $\geq$0.70 kUa/L specific IgE to ω-5 gliadin were defined as individuals with WDEIA.

We included a dataset of 924 and 435 healthy subjects from the Pharma SNP Consortium (PSC) as the discovery set and the Japan Biological Informatics Consortium (JBIC) as the replication set, resulting in a total of 1,359 control subjects. The study protocol was approved by the research ethics committees of RIKEN and all participating hospitals. We performed the study according to the provisions of the Declaration of Helsinki. All participants provided written informed consent.

Genetic analysis

The GWAS analysis (flowchart, Figure S1) included 77 individuals with WDEIA as a discovery set, for which 659,184 variants were genotyped via the Illumina Infinium Asian Screening Array-24 kit (version 1, Illumina, San Diego, CA, USA). For the 927 controls from PSC, 951,738 variants were genotyped via the Illumina Infinium OmniExpressExome-8 kit (Illumina) before quality control (QC). The QC of subjects was performed with the following exclusion criteria: (1) sample call rate $<$ 0.98, (2) closely related individuals identified via PLINK v.1.9.0 software,[20] and (3) non-Asian outliers estimated by principal-component analysis (PCA) via EIGENSOFT software[21] (version 7.2.1). Variants satisfying the following criteria were also excluded: (1) single-nucleotide polymorphism (SNP) call rate $<$ 0.99, (2) minor allele frequency (MAF) $<$ 0.01, and (3) Hardy–Weinberg equilibrium p value $\leq 1.0 \times 10^{-6}$, as described elsewhere.[22]

Because the number of overlapping variants between different SNP arrays was very small (115,250 SNPs), we initially performed whole-genome imputation for the datasets of affected individuals and control individuals separately. We then combined the imputed variants of affected individuals and control individuals after whole-genome imputation. The same QC filters for the imputed variants were further applied, and 3,879,004 variants were used for the association study (see details in the supplemental methods).

We performed human leukocyte antigen (HLA) imputation separately for each dataset by using the genotyped SNPs located in a region that included the entire major histocompatibility complex (MHC) (29–34 Mb on chromosome 6, hg19). We adopted the Japanese imputation reference panel constructed previously.[23] We applied HLA imputation by using SNP2HLA[24] software (version 1.0.3) to impute all HLA variants, including the selected SNPs, two- and four-digit classical HLA alleles, and amino acid polymorphisms of the *HLA* genes. Combining the imputed variants of affected individuals and control individuals yielded a total of 12,092 imputed variants for the association study.

Target sequencing

We preformed target sequencing of 168 affected individuals and 1,359 control individuals to validate the imputed alleles of *HLA-DPA1* (MIM: 142880) and *HLA-DPB1* (MIM: 142858) in the discovery set and determine the alleles of *HLA-DPA1*, *-DPB1*, and *-DPB2* in the replication set. To genotype two variants (rs480413 and rs2775248) that were suggestive of an association with WDEIA in the discovery set, we also performed targeted sequencing of 168 affected individuals and 1,359 control individuals, as described above, by using specific primers (Table S2).

Transcriptomic data analysis

Tissue-specific expression profiles of *HLA-DPA1*, *-DPB1*, and *-DPB2* were obtained from the GTEx Portal database. The expression quantitative trait locus (eQTL) of variants was obtained from the eQTL catalog, NESDA NTR Conditional eQTL Catalog, and JENGER databases.

Statistical analysis

Age is summarized as median and interquartile range (IQR), and it was compared via Mann–Whitney test. Individuals of each sex are summarized as number and percentage, and the data were compared via Fisher's exact test.

The genomic inflation factor (λ) was calculated with PLINK software. We conducted a GWAS in the discovery set to identify WDEIA-associated variants and validated the significant variant with the lowest p value and two variants suggestive of an association with WDEIA in the replication set. Association analyses were conducted with Fisher's exact test in three different genetic models (allelic, dominant, and recessive) with PLINK software. The lowest p value among these models was utilized. Summary statistics from the discovery and replication sets were analyzed by a fixed-effect

Table 1. Demographic and clinical characteristics of the discovery and replication sets

Variables	Discovery set		Replication set	
	Affected individuals (n = 77)	Control individuals (n = 924)	Affected individuals (n = 91)	Control individuals (n = 435)
Age, median (IQR), years	51 (42–63)	36 (29–46)	44 (36–54)	37 (29–47)
Female sex, no. (%)	30 (39)	354 (38)	33 (36)	199 (46)
Wheat-specific IgE, median (IQR), Ua/mL	0.27 (0.10–1.14)	–	0.48 (0.17–1.23)	–
Gluten-specific IgE, median (IQR), Ua/mL	0.75 (0.17–1.98)	–	1.33 (0.44–3.12)	–
ω-5 gliadin-specific IgE, median (IQR), Ua/mL	6.36 (3.28–11.90)	–	6.73 (2.79–10.90)	–

Abbreviation: IQR, interquartile range. Age available for 77 affected individuals and 924 control individuals in the discovery set and 91 affected individuals and 432 control individuals in the replication set. Sex available for 77 affected individuals and 924 control individuals in the discovery set and 91 affected individuals and 435 control individuals in the replication set. Wheat-specific IgE available for 76 affected individuals in the discovery set and 87 affected individuals in the replication set. Gluten-specific IgE available for 75 affected individuals in the discovery set and 89 affected individuals in the replication set. ω-5 gliadin-specific IgE available for 77 affected individuals in the discovery set and 91 affected individuals in the replication set.

and random-effect meta-analysis with codes based on the meta package in R software (version 3.5.0). We generated a Manhattan plot by using the qqman package in R software to visualize overall associations with SNPs. Regional plots of genome-wide significant loci were created via LocusZoom[25] software (version 2.7.2). The genome-wide significance and suggestive significance thresholds were 5.0×10^{-8} and 1.0×10^{-5}, respectively.

The associations of HLA variants with WDEIA were evaluated with additive logistic regression models implemented in R software. We defined the HLA variants as bi-allelic single-nucleotide variants in the MHC region, two- and four-digit bi-allelic HLA alleles, and bi-allelic HLA amino acid variants corresponding to their respective residues. We conducted conditional association analysis of the HLA variants by additionally including the HLA-DPB1*02:01 or -DPB1*02:01:02 allele as a covariate.

Genotyping validation of discovery set variants and alleles was conducted by targeted sequencing. Replication set genotype data were assessed via the targeted sequencing results as validated in the discovery set. Results with p values ≤ 0.05 obtained with Fisher's exact test in the replication set were considered significant.

Results

Individuals, genotyping, and quality control

After applying stringent QC filters for excluding samples with low call rates, closely related subjects, and outliers in the PCA (Figure S2), the discovery set comprised 77 individuals with WEDIA and 924 control subjects from a general Japanese population, whereas the replication set had 91 affected individuals and 435 control individuals. The demographic characteristics of the subjects after QC are shown in Table 1. Our study included 168 affected individuals and 1,359 control individuals with no significant sex-related differences.

Genome-wide association analysis for WDEIA

After performing whole-genome imputation and applying QC for the variants, we obtained 3,879,004 variants. Common variants with an MAF > 0.01 were investigated for associations with WDEIA in three different genetic models, namely, allelic, dominant, and recessive. The genomic inflation factor (λ) in the discovery set was 1.0444. We plotted the minimal p (pmin) values from these genetic models as $-\log_{10}$ (pmin) against the chromosome position across the genome to construct a Manhattan plot (Figure 1 and Table S3). A substantial signal with genome-wide significance was detected on chromosome 6. The regional plot of this position demonstrated that all genome-wide associated variants were located near *HLA-DPA1*, *-DPB1*, and *-DPB2* (Figure 2). We validated the lead SNP (rs9277630) on chromosome 6 with a genome-wide significant p value by targeted sequencing. After validation, the dominant model had the lowest p value among the three models (Table S4, odds ratio [OR] = 3.95; 95% confidence interval [CI], 2.31–6.73; $p = 7.87 \times 10^{-8}$).

When we extended the threshold p value to $<1.0 \times 10^{-5}$, we identified two SNPs with suggestive associations: rs480413 near GLYATL2 (glycine-N-acyltransferase like 2 [MIM: 614762]) on chromosome 11 and rs2775248 near OR4L1 (olfactory receptor 4L1) on chromosome 14 (Table S4 and Figure S5).

Analysis of the association of the HLA-imputed variants with WDEIA

By performing HLA imputation with the large-scale population-specific HLA reference panel of Japanese individuals, we obtained imputed genotypes of 12,092 imputed variants in the MHC region. We conducted a dominant logistic regression analysis of HLA alleles, amino acid substitutions, and variants within the MHC region (for regional associations, see Table S5 and Figure 3). The HLA allele located in the MHC class 2 region had the most significant association with WDEIA (HLA-DPB1*02:01, OR = 5.2 [95% CI, 3.0–8.9], $p = 6.19 \times 10^{-11}$), which was in a strong linkage disequilibrium with rs9277630 ($r^2 = 0.930$). The HLA-DPB1*02:01:02 allele had the second highest frequency

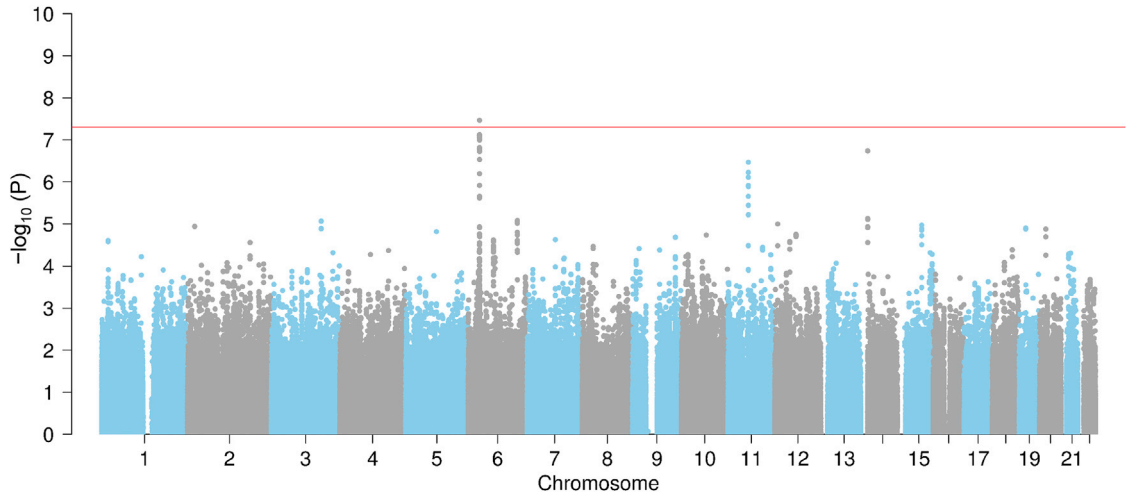

Figure 1. Manhattan plot of a genome-wide association of SNPs with WDEIA in the discovery set of 77 affected individuals and 924 control individuals

The x axis indicates chromosomal positions and the y axis shows the −log10 minimum p values calculated with three genetic models. Each dot represents a SNP. The dotted line indicates the genome-wide significance level.

among the *HLA-DPB1* alleles in the Japanese population (Table S6). When we conditioned on *HLA-DPB1*02:01* to identify an independently associated locus in the HLA region, no other variant satisfied the significance p value (5.70×10^{-6}) after Bonferroni correction (Figure 3). The HLA-DPB2*01:01:02 allele's p value was close to the genome-wide significance threshold (OR = 3.95 [95% CI, 2.31−6.37], $p = 7.87 \times 10^{-8}$). Genotype validation by targeted sequencing yielded six-digit HLA alleles from all coding regions. In the WDEIA association study on all alleles in *HLA-DPA1*, *-DPB1*, and *-DPB2*, the p value of the HLA-DPB1*02:01:02 allele was below the genome-wide significance threshold (Table S6). The frequency of HLA-DPB1* 02:01:02 allele carriers in the discovery set was 0.740

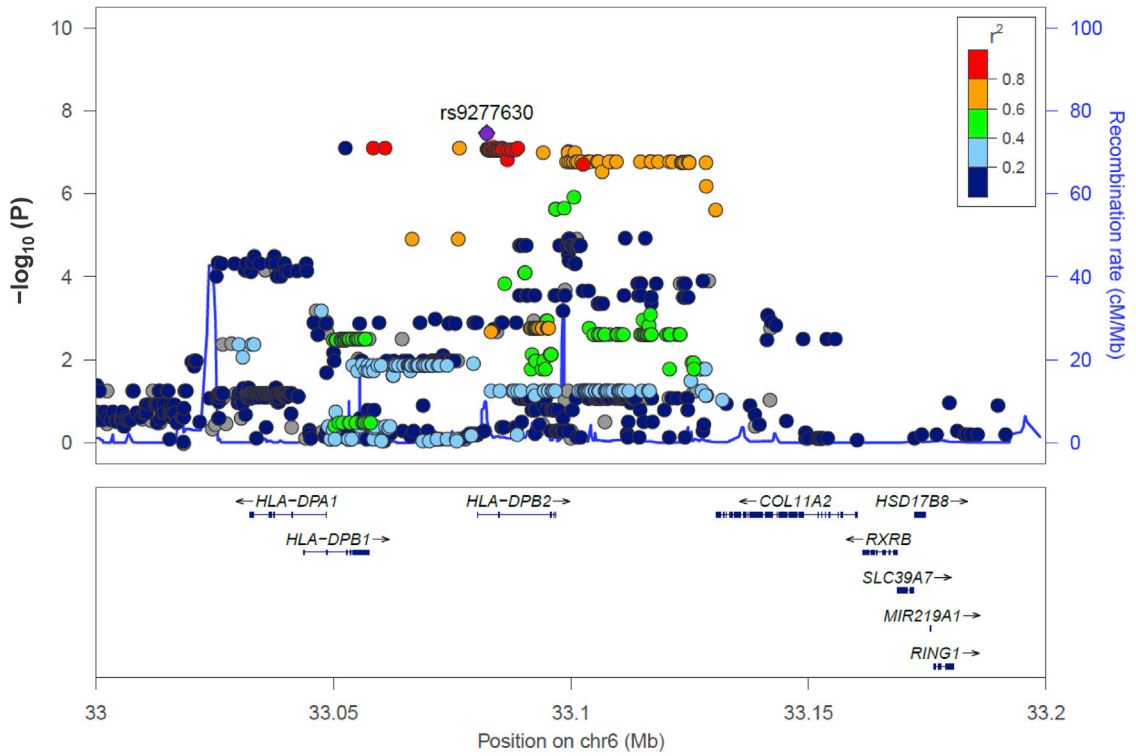

Figure 2. Regional association plot of susceptibility locus associated with WDEIA in the discovery set of 77 affected individuals and 924 control individuals

SNPs are colored according to their linkage disequilibrium (LD; based on the 1000 Genomes Project, phase 3, ASN reference panel), and the lead SNP, rs9277630, is marked with a purple diamond.

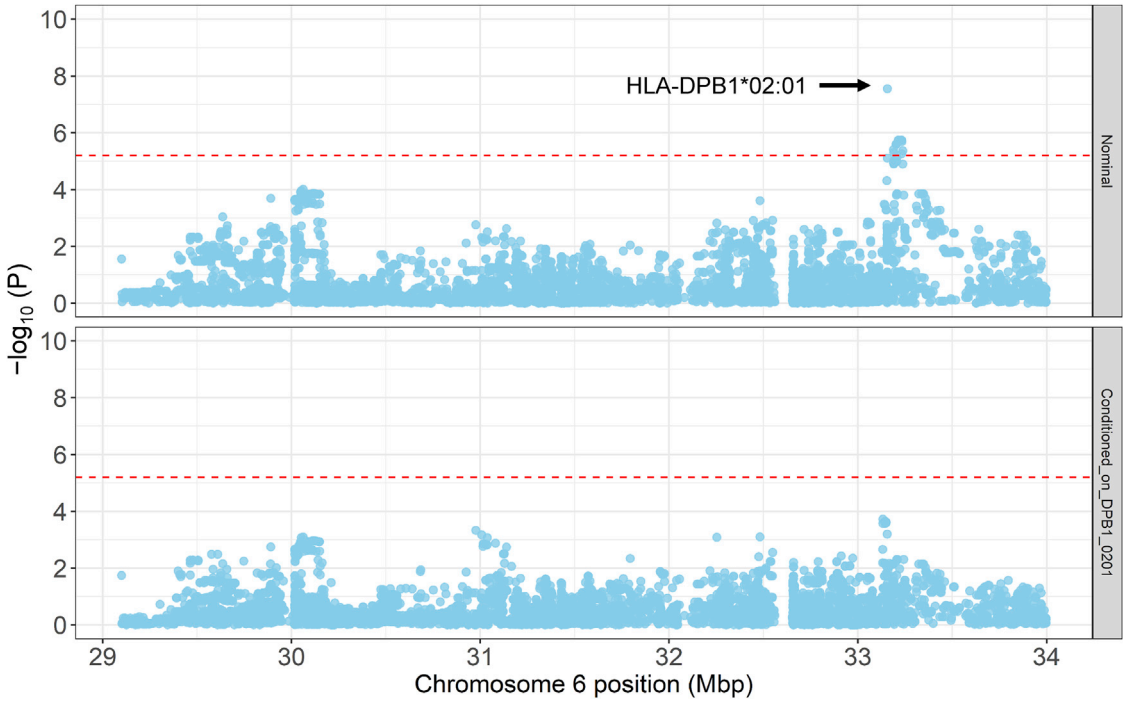

Figure 3. Conditional regression results of the HLA locus independently associated with WDEIA susceptibility
The lowest p value was observed for a classical allele (HLA-DPB1*02:01) in the HLA-DPB1 locus (top). After adjustment for HLA-DPB1*02:01, none of the remaining variants in the HLA region reached the Bonferroni-adjusted significance level (bottom).

among affected individuals and 0.387 among control individuals (OR = 4.51 [95% CI, 2.66–7.63], $p = 2.28 \times 10^{-9}$).

Replication study and meta-analysis

We genotyped rs9277630, rs480413, and rs2775248, along with all alleles in *HLA-DPA1*, *-DPB1*, and *-DPB2*, by targeted sequencing in an independent replication set consisting of 91 individuals with WDEIA and 435 general Japanese control subjects (Table 1). We confirmed rs9277630 and the HLA-DPB1*02:01:02 allele to be significantly associated with WDEIA by using the dominant genetic model in replication samples (Tables S4 and S6, rs9277630: OR = 3.19 [95% CI, 1.93–5.28], $p = 2.97 \times 10^{-6}$; HLA-DPB1*02:01:02: OR = 3.82 [95% CI, 2.33–6.26], $p = 3.03 \times 10^{-8}$). The results of the fixed-effect and random-effect meta-analyses in the combined set for rs9277630 and HLA-DPB1*02:01:02 are shown in Figure 4. The HLA-DPB1*02:01:02 allele had the most significant association with WDEIA (OR = 4.13 [95% CI, 2.89–5.93], $p = 1.06 \times 10^{-14}$). The conditional analysis of the HLA-DPB1*02:01:02 allele in the combined set indicated that the remaining alleles in *HLA-DPA1*, *-DPB1*, and *-DPB2* did not reach the Bonferroni-adjusted significance level (Table S7). The associations of rs480413 and rs2775248 were not confirmed for the replication samples (Table S4).

Functional *in-vitro* assay of the lead SNP from a public database

We examined the biological function of the HLA-DP locus by assessing tissue-specific expression profiles of *HLA-DPA1*, *-DPB1*, and *-DPB2* obtained from the GTEx database. The mRNA expression levels of *HLA-DPA1* and *-DPB1* in Epstein-Barr virus-transformed lymphocytes were, respectively, 231.2 and 85.5 times higher than that of *HLA-DPB2* (Figure S4).

We also examined the eQTL of rs9277630 in the eQTL catalog, NESDA NTR Conditional eQTL Catalog, and JENGER databases (Figure S5 and Table S8). Of 111 independent datasets of the eQTL catalog, the mRNA expression of *HLA-DPA1* and *-DPB1* was detected in all datasets, whereas the mRNA expression of *HLA-DPB2* was detected in only GTEx database. rs9277630 had a positive correlation with the *HLA-DPA1* mRNA expression level in 21 datasets, while a significant negative correlation with *HLA-DPB2* was observed in 35 tissues. No correlation with *HLA-DPB1* was observed in almost all datasets. In the NESDA NTR Conditional eQTL Catalog and JENGER databases, rs9277630 was significantly and positively correlated with the *HLA-DPA1* and *-DPB1* expression levels in peripheral blood. In the JENGER database, rs9277630 was significantly and negatively correlated with the expression of *HLA-DPB2*.

Discussion

This was a GWAS in individuals with WDEIA who were predominantly sensitized to ω-5 gliadin. We identified associations between the HLA-DP locus and WDEIA, which were replicated in an independent Japanese cohort. On the basis

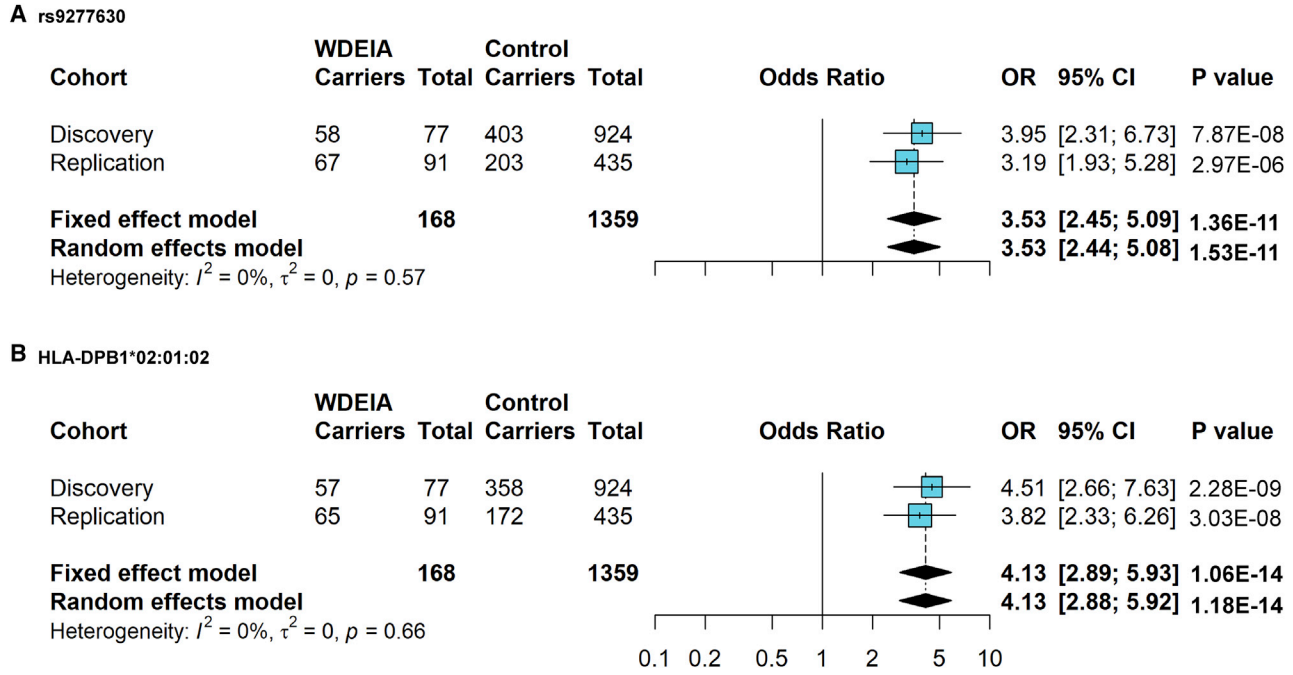

Figure 4. Forest plots and meta-analysis between the discovery and replication sets for a lead SNP (rs9277630) and an HLA-DPB1*02:01:02 allele achieving a genome-wide significance

Forest plots for each marker associated with WDEIA at a genome-wide level of significance were generated according to the Mantel-Haenszel method. Odds ratio (OR) and 95% confidence interval (CI) are displayed on the x axis. The number of carriers and total individuals with each marker of each cohort (discovery and replication sets) and the combined analysis are shown. The diamond shows the final ORs and 95% CIs for fixed-effect and random-effect meta-analyses of two cohorts, and we used heterogeneity (I^2) and between-study variance (τ^2) to assess the heterogeneity of random-effect model in effect sizes between cohorts.

of association data obtained by HLA imputation and targeted sequencing of *HLA-DPA1*, *-DPB1*, and *-DPB2*, the HLA-DPB1*02:01:02 allele displayed the most significant association with WDEIA. Although previous studies found significant associations between other food allergies, including those caused by shrimp, peaches, milk, eggs, and peanuts, and the HLA-DQ locus,[26–28] our study did not detect significant WDEIA-related associations with the HLA-DQ locus (Table S5). Therefore, the mechanism of ω-5 gliadin-induced allergy may differ from that of other food allergies. Because these previous association studies examined allergies without inducing exercise, it is possible that the HLA-DP locus is specifically associated with WDEIA. Further studies should elucidate *HLA* involvement in the mechanism of WDEIA.

The HLA-DPB1*02:01:02 allele frequency was 0.432 and 0.220 in the groups of affected individuals and control individuals, respectively (Table S6). Our control group allele frequency was comparable with that (0.228) in a different general Japanese population cohort (n = 1,120),[29] indicating that our targeted sequencing approach was appropriate. The HLA-DPB1*02:01:02 allele frequency is 0.140, 0.138, 0.103, and 0.361, in Chinese,[30] European American,[31] Mexican,[32] and Spanish populations,[33] respectively. In almost all populations, this allele has the second or third highest frequency, suggesting that it may be used globally as a WDEIA-risk biomarker.

A previous GWAS aimed to identify a biomarker for wheat allergies following the use of a soap containing hydrolyzed wheat protein (HWP) by participants.[34] In 2012–2014, a study found that 2,111 Japanese subjects suffered from allergic urticaria, anaphylaxis, and/or WDEIA after using this soap, causing public and social concerns.[35] The major allergen of this soap is Glupearl 19S, produced by the acid treatment of gluten at a high temperature for a short time.[36,37] The allergy to this HWP-containing soap was significantly associated with the HLA-DQ locus. Specifically, amino acid position 34 of HLA-DQα1 (OR = 0.45 [95% CI, 0.39–0.53], $p = 2.96 \times 10^{-24}$) had the most significant association in the GWAS set. In contrast to the findings of these previous studies, our study detected no significant association of the amino acid position 34 with WDEIA (OR = 1.2 [95% CI, 0.7–2.1], p = 0.477; data not shown), indicating differences in the major allergens between WDEIA and HWP allergy. Gluten from fractionated wheat protein contains glutenins and gliadins.[38] Noguchi et al. reported that the major HWP allergen might be glutenin recognized by the amino acid residue 34 of HLA-DQα1.[39]

In this study, HLA-DPB1*02:01:02 showed the most significant association with WDEIA, but HLA-DPB2*01:01:02 was also suggestive of an association with WDEIA. Although *HLA-DPB2* is a pseudogene, its mRNA expression might upregulate *HLA-DPB1* expression.[40] Moreover, allele-specific expression in HLA and other autoimmune loci is known to change dynamically during T cell activation.[41]

The expression level of HLA-DPB2*01:01:02 might control that of HLA-DPB1*02:01:02. The GTEx database indicated high *HLA-DPA1* and *HLA-DPB1* expression, along with low *HLA-DPB2* expression, in various tissues (Figure S4). On the basis of the three eQTL databases, the lead SNP rs9277630 was positively associated with *HLA-DPB1* expression and negatively associated with *HLA-DPB2* expression (Table S7). In the future, it would be necessary to clarify the relationship between *HLA-DPB1* and *HLA-DPB2* in the pathogenesis of WDEIA.

Not only genetic factors but also environmental factors may affect the WDEIA risk. For example, administering aspirin facilitates the absorption of non-digested gliadin from the intestine into blood circulation[17,42] and augments the allergic reaction in WDEIA by lowering the threshold and increasing the severity of the adverse reaction.[43] Furthermore, the gut microbiome was previously assessed to identify other environmental factors,[43] but the microbial diversity did not differ between 25 individuals with WDEIA and 25 healthy control individuals owing to the small sample size. Hence, an integrative analysis, including genetic and environmental factors, will be useful in elucidating the WDEIA mechanism.

Although association studies using an additive model are common,[44] HLA was associated in a dominant manner with disease in a previous report,[45] and highly significant, non-additive dominance effects within HLA loci were observed in rheumatoid arthritis, type 1 diabetes, psoriasis vulgaris, and celiac disease.[46] Therefore, we performed an association study by using three common genetic models to determine the best inheritance model, finding that the lead SNP rs9277630 of the HLA-DPB1*02:01:02 allele in the dominant model showed the highest OR and minimum p value compared with the other models (Table S4). Therefore, we selected the dominant model for the subsequent analysis.

Although we detected a statistically significant association between HLA-DPB1*02:01:02 and the WDEIA risk, the underlying causal mechanisms remain to be elucidated. In addition to genetic studies in a variety of populations, functional assessment of the underlying mechanisms will provide deeper insights into the pathogenesis of WDEIA.

The HLA-DPB1*02:01:02 allele is significantly associated with WDEIA in the Japanese population. If validated in additional populations, this may have broad implications for risk assessment, diagnosis, and treatment of WDEIA.

Data and code availability

All data are contained in the paper and its supplemental information are available upon request to the corresponding author. The genotyping datasets are not publicly available because of institutional ethics restrictions. The R code used is publicly available and cited in the subjects and methods.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.ajhg.2021.06.017.

Acknowledgments

We appreciate the participants of this study. We would like to thank Editage (see web resources) for English language editing. This work was supported by the Practical Research Project for Allergic Diseases and Immunology (Research on Allergic Diseases and Immunology) from the Japan Agency for Medical Research and Development; AMED (JP17ek0410020); and the Clinical Research Support Program from the Japanese Society of Allergology. This work was also partially supported by JSPS KAKENHI grant number JP17K00938, Japan Agency for Medical Research and Development; AMED (JP20ek0410076); and a grant from Nipponham Foundation for the Future of Food.

Declaration of interests

The authors declare no competing interests.

Received: March 19, 2021
Accepted: June 15, 2021
Published: July 9, 2021

Web resources

Editage, https://www.editage.com/
GTEx Portal database, https://gtexportal.org/home/
JBIC, https://www.jbic.or.jp/english/
JENGER databases, http://jenger.riken.jp
NESDA NTR Conditional eQTL Catalog, https://eqtl.onderzoek.io/index.php?page=info
OMIM, https://www.omim.org/
PSC, http://www.jpma.or.jp/information/research/psc/e02psc/about.html

References

1. Scherf, K.A., Brockow, K., Biedermann, T., Koehler, P., and Wieser, H. (2016). Wheat-dependent exercise-induced anaphylaxis. Clin. Exp. Allergy *46*, 10–20.
2. Kushimoto, H., and Aoki, T. (1985). Masked type I wheat allergy. Relation to exercise-induced anaphylaxis. Arch. Dermatol. *121*, 355–360.
3. Morita, E., Chinuki, Y., Takahashi, H., Nabika, T., Yamasaki, M., and Shiwaku, K. (2012). Prevalence of wheat allergy in Japanese adults. Allergol. Int. *61*, 101–105.
4. Arámburo-Gálvez, J.G., Beltrán-Cárdenas, C.E., Geralda André, T., Carvalho Gomes, I., Macêdo-Callou, M.A., Braga-Rocha, E.M., Mye-Takamatu-Watanabe, E.A., Rahmeier-Fietz, V., Figueroa-Salcido, O.G., Vergara-Jiménez, M.J., et al. (2020). Prevalence of Adverse Reactions to Gluten and People Going on a Gluten-Free Diet: A Survey Study Conducted in Brazil. Medicina (Kaunas) *56*, 163.
5. Nwaru, B.I., Hickstein, L., Panesar, S.S., Roberts, G., Muraro, A., Sheikh, A.; and EAACI Food Allergy and Anaphylaxis Guidelines Group (2014). Prevalence of common food allergies in Europe: a systematic review and meta-analysis. Allergy *69*, 992–1007.
6. Vierk, K.A., Koehler, K.M., Fein, S.B., and Street, D.A. (2007). Prevalence of self-reported food allergy in American adults and use of food labels. J. Allergy Clin. Immunol. *119*, 1504–1510.

7. Ontiveros, N., Rodríguez-Bellegarrigue, C.I., Galicia-Rodríguez, G., Vergara-Jiménez, M.J., Zepeda-Gómez, E.M., Arámburo-Galvez, J.G., Gracia-Valenzuela, M.H., and Cabrera-Chávez, F. (2018). Prevalence of Self-Reported Gluten-Related Disorders and Adherence to a Gluten-Free Diet in Salvadoran Adult Population. Int. J. Environ. Res. Public Health *15*, 786.
8. Cabrera-Chávez, F., Dezar, G.V., Islas-Zamorano, A.P., Espinoza-Alderete, J.G., Vergara-Jiménez, M.J., Magaña-Ordorica, D., and Ontiveros, N. (2017). Prevalence of Self-Reported Gluten Sensitivity and Adherence to a Gluten-Free Diet in Argentinian Adult Population. Nutrients *9*, 81.
9. Kraft, M., Dölle-Bierke, S., Renaudin, J.M., Ruëff, F., Scherer Hofmeier, K., Treudler, R., Pföhler, C., Hawranek, T., Poziomkowska-Gęsicka, I., Jappe, U., et al. (2021). Wheat Anaphylaxis in Adults Differs from Reactions to Other Types of Food. J. Allergy Clin. Immunol. Pract., S2213-2198(21)00382-2.
10. Ebisawa, M., Ito, K., Fujisawa, T.; Committee for Japanese Pediatric Guideline for Food Allergy, The Japanese Society of Pediatric Allergy and Clinical Immunology; and Japanese Society of Allergology (2020). Japanese guidelines for food allergy 2020. Allergol. Int. *69*, 370–386.
11. Pico, J., Bernal, J., and Gómez, M. (2015). Wheat bread aroma compounds in crumb and crust: A review. Food Res. Int. *75*, 200–215.
12. Thongngarm, T., Wongsa, C., Pacharn, P., Piboonpocanun, S., and Sompornrattanaphan, M. (2020). Clinical Characteristics and Proposed Wheat-Cofactor Challenge Protocol with a High Diagnostic Yield in Adult-Onset IgE-Mediated Wheat Allergy. J. Asthma Allergy *13*, 355–368.
13. Kennard, L., Thomas, I., Rutkowski, K., Azzu, V., Yong, P.F.K., Kasternow, B., Hunter, H., Cabdi, N.M.O., Nakonechna, A., and Wagner, A. (2018). A Multicenter Evaluation of Diagnosis and Management of Omega-5 Gliadin Allergy (Also Known as Wheat-Dependent Exercise-Induced Anaphylaxis) in 132 Adults. J. Allergy Clin. Immunol. Pract. *6*, 1892–1897.
14. Palosuo, K., Varjonen, E., Kekki, O.M., Klemola, T., Kalkkinen, N., Alenius, H., and Reunala, T. (2001). Wheat omega-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. J. Allergy Clin. Immunol. *108*, 634–638.
15. Morita, E., Matsuo, H., Mihara, S., Morimoto, K., Savage, A.W., and Tatham, A.S. (2003). Fast omega-gliadin is a major allergen in wheat-dependent exercise-induced anaphylaxis. J. Dermatol. Sci. *33*, 99–104.
16. Asaumi, T., Yanagida, N., Sato, S., Shukuya, A., Nishino, M., and Ebisawa, M. (2016). Provocation tests for the diagnosis of food-dependent exercise-induced anaphylaxis. Pediatr. Allergy Immunol. *27*, 44–49.
17. Matsuo, H., Morimoto, K., Akaki, T., Kaneko, S., Kusatake, K., Kuroda, T., Niihara, H., Hide, M., and Morita, E. (2005). Exercise and aspirin increase levels of circulating gliadin peptides in patients with wheat-dependent exercise-induced anaphylaxis. Clin. Exp. Allergy *35*, 461–466.
18. Morita, E., Chinuki, Y., and Takahashi, H. (2013). Recent advances of in vitro tests for the diagnosis of food-dependent exercise-induced anaphylaxis. J. Dermatol. Sci. *71*, 155–159.
19. Lehto, M., Palosuo, K., Varjonen, E., Majuri, M.L., Andersson, U., Reunala, T., and Alenius, H. (2003). Humoral and cellular responses to gliadin in wheat-dependent, exercise-induced anaphylaxis. Clin. Exp. Allergy *33*, 90–95.
20. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., and Sham, P.C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. *81*, 559–575.
21. Patterson, N., Price, A.L., and Reich, D. (2006). Population structure and eigenanalysis. PLoS Genet. *2*, e190.
22. Kanai, M., Akiyama, M., Takahashi, A., Matoba, N., Momozawa, Y., Ikeda, M., Iwata, N., Ikegawa, S., Hirata, M., Matsuda, K., et al. (2018). Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. Nat. Genet. *50*, 390–400.
23. Okada, Y., Momozawa, Y., Ashikawa, K., Kanai, M., Matsuda, K., Kamatani, Y., Takahashi, A., and Kubo, M. (2015). Construction of a population-specific HLA imputation reference panel and its application to Graves' disease risk in Japanese. Nat. Genet. *47*, 798–802.
24. Jia, X., Han, B., Onengut-Gumuscu, S., Chen, W.M., Concannon, P.J., Rich, S.S., Raychaudhuri, S., and de Bakker, P.I. (2013). Imputing amino acid polymorphisms in human leukocyte antigens. PLoS ONE *8*, e64683.
25. Pruim, R.J., Welch, R.P., Sanna, S., Teslovich, T.M., Chines, P.S., Gliedt, T.P., Boehnke, M., Abecasis, G.R., and Willer, C.J. (2010). LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics *26*, 2336–2337.
26. Khor, S.S., Morino, R., Nakazono, K., Kamitsuji, S., Akita, M., Kawajiri, M., Yamasaki, T., Kami, A., Hoshi, Y., Tada, A., et al. (2018). 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. *8*, 1069.
27. Dimitrov, I., and Doytchinova, I. (2016). Associations between Milk and Egg Allergens and the HLA-DRB1/DQ Polymorphism: A Bioinformatics Approach. Int. Arch. Allergy Immunol. *169*, 33–39.
28. Madore, A.M., Vaillancourt, V.T., Asai, Y., Alizadehfar, R., Ben-Shoshan, M., Michel, D.L., Kozyrskyj, A.L., Becker, A., Chan-Yeung, M., Clarke, A.E., et al. (2013). HLA-DQB1*02 and DQB1*06:03P are associated with peanut allergy. Eur. J. Hum. Genet. *21*, 1181–1184.
29. Hirata, J., Hosomichi, K., Sakaue, S., Kanai, M., Nakaoka, H., Ishigaki, K., Suzuki, K., Akiyama, M., Kishikawa, T., Ogawa, K., et al. (2019). Genetic and phenotypic landscape of the major histocompatibilty complex region in the Japanese population. Nat. Genet. *51*, 470–480.
30. Lind, A., Akel, O., Wallenius, M., Ramelius, A., Maziarz, M., Zhao, L.P., Geraghty, D.E., Palm, L., Lernmark, Å., and Larsson, H.E. (2019). HLA high-resolution typing by next-generation sequencing in Pandemrix-induced narcolepsy. PLoS ONE *14*, e0222882.
31. Creary, L.E., Gangavarapu, S., Mallempati, K.C., Montero-Martín, G., Caillier, S.J., Santaniello, A., Hollenbach, J.A., Oksenberg, J.R., and Fernández-Viña, M.A. (2019). Next-generation sequencing reveals new information about HLA allele and haplotype diversity in a large European American population. Hum. Immunol. *80*, 807–822.
32. González-Quezada, B.A., Creary, L.E., Munguia-Saldaña, A.J., Flores-Aguilar, H., Fernández-Viña, M.A., and Gorodezky, C. (2019). Exploring the ancestry and admixture of Mexican Oaxaca Mestizos from Southeast Mexico using next-generation sequencing of 11 HLA loci. Hum. Immunol. *80*, 157–162.
33. Montero-Martín, G., Mallempati, K.C., Gangavarapu, S., Sánchez-Gordo, F., Herrero-Mata, M.J., Balas, A., Vicario, J.L., Sánchez-García, F., González-Escribano, M.F., Muro, M., et al. (2019). High-resolution characterization of allelic and haplotypic HLA frequency distribution in a Spanish population

using high-throughput next-generation sequencing. Hum. Immunol. *80*, 429–436.
34. Noguchi, E., Akiyama, M., Yagami, A., Hirota, T., Okada, Y., Kato, Z., Kishikawa, R., Fukutomi, Y., Hide, M., Morita, E., et al. (2019). HLA-DQ and RBFOX1 as susceptibility genes for an outbreak of hydrolyzed wheat allergy. J. Allergy Clin. Immunol. *144*, 1354–1363.
35. Yagami, A., Aihara, M., Ikezawa, Z., Hide, M., Kishikawa, R., Morita, E., Chinuki, Y., Fukutomi, Y., Urisu, A., Fukushima, A., et al. (2017). Outbreak of immediate-type hydrolyzed wheat protein allergy due to a facial soap in Japan. J. Allergy Clin. Immunol. *140*, 879–881.e7.
36. Chinuki, Y., and Morita, E. (2012). Wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat protein in soap. Allergol. Int. *61*, 529–537.
37. Fukutomi, Y., Itagaki, Y., Taniguchi, M., Saito, A., Yasueda, H., Nakazawa, T., Hasegawa, M., Nakamura, H., and Akiyama, K. (2011). Rhinoconjunctival sensitization to hydrolyzed wheat protein in facial soap can induce wheat-dependent exercise-induced anaphylaxis. J. Allergy Clin. Immunol. *127*, 531–533.e1-3.
38. Urade, R., Sato, N., and Sugiyama, M. (2018). Gliadins from wheat grain: an overview, from primary structure to nanostructures of aggregates. Biophys. Rev. *10*, 435–443.
39. Lyu, L., Yao, J., Wang, M., Zheng, Y., Xu, P., Wang, S., Zhang, D., Deng, Y., Wu, Y., Yang, S., et al. (2020). Overexpressed Pseudogene *HLA-DPB2* Promotes Tumor Immune Infiltrates by Regulating *HLA-DPB1* and Indicates a Better Prognosis in Breast Cancer. Front. Oncol. *10*, 1245.
40. Gutierrez-Arcelus, M., Baglaenko, Y., Arora, J., Hannes, S., Luo, Y., Amariuta, T., Teslovich, N., Rao, D.A., Ermann, J., Jonsson, A.H., et al.; NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium (2020). Allele-specific expression changes dynamically during T cell activation in HLA and other autoimmune loci. Nat. Genet. *52*, 247–253.
41. Kohno, K., Matsuo, H., Takahashi, H., Niihara, H., Chinuki, Y., Kaneko, S., Honjoh, T., Horikawa, T., Mihara, S., and Morita, E. (2013). Serum gliadin monitoring extracts patients with false negative results in challenge tests for the diagnosis of wheat-dependent exercise-induced anaphylaxis. Allergol. Int. *62*, 229–238.
42. Christensen, M.J., Eller, E., Mortz, C.G., Brockow, K., and Bindslev-Jensen, C. (2019). Wheat-Dependent Cofactor-Augmented Anaphylaxis: A Prospective Study of Exercise, Aspirin, and Alcohol Efficacy as Cofactors. J. Allergy Clin. Immunol. Pract. *7*, 114–121.
43. Du, Z., Gao, X., and Yin, J. (2020). Gut microbiome alterations in patients with wheat-dependent exercise-induced anaphylaxis. Int. Immunopharmacol. *84*, 106557.
44. Clarke, G.M., Anderson, C.A., Pettersson, F.H., Cardon, L.R., Morris, A.P., and Zondervan, K.T. (2011). Basic statistical analysis in genetic case-control studies. Nat. Protoc. *6*, 121–133.
45. Terao, C., Yoshifuji, H., Matsumura, T., Naruse, T.K., Ishii, T., Nakaoka, Y., Kirino, Y., Matsuo, K., Origuchi, T., Shimizu, M., et al. (2018). Genetic determinants and an epistasis of *LILRA3* and HLA-B*52 in Takayasu arteritis. Proc. Natl. Acad. Sci. USA *115*, 13045–13050.
46. Lenz, T.L., Deutsch, A.J., Han, B., Hu, X., Okada, Y., Eyre, S., Knapp, M., Zhernakova, A., Huizinga, T.W., Abecasis, G., et al. (2015). Widespread non-additive and interaction effects within HLA loci modulate the risk of autoimmune diseases. Nat. Genet. *47*, 1085–1090.