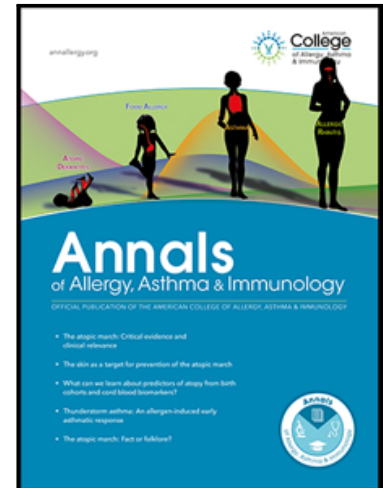


Journal Pre-proof

Genome-wide association study identified a novel genetic variation in HLA DRB1 associated with drug hypersensitivity

So-Young Park MD, PhD , Ah Ra Do BS , Taesung Park PhD ,
Sungho Won PhD , Tae-Bum Kim MD, PhD

PII: S1081-1206(21)01289-8
DOI: <https://doi.org/10.1016/j.anai.2021.11.022>
Reference: ANAI 3782



To appear in: *Annals of Allergy, Asthma Immunology*

Received date: 20 May 2021
Revised date: 8 November 2021
Accepted date: 28 November 2021

Please cite this article as: So-Young Park MD, PhD , Ah Ra Do BS , Taesung Park PhD , Sungho Won PhD , Tae-Bum Kim MD, PhD , Genome-wide association study identified a novel genetic variation in HLA DRB1 associated with drug hypersensitivity, *Annals of Allergy, Asthma Immunology* (2021), doi: <https://doi.org/10.1016/j.anai.2021.11.022>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc.

Genome-wide association study identified a novel genetic variation in HLA DRB1 associated with drug hypersensitivity

Running title: Novel genetic biomarker in drug hypersensitivity

So-Young Park, MD, PhD^{1,2}, Ah Ra Do, BS³, Taesung Park, PhD⁴, Sungho Won, PhD,³ and Tae-Bum Kim, MD, PhD.^{1*}

¹Department of Allergy and Clinical Immunology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

²Department of Internal Medicine, Division of Pulmonary, Allergy and Critical care medicine, Chung-Ang University College of Medicine, Seoul, Korea

³Department of Public Health Science, Seoul National University, Seoul, Korea

⁴Department of Statistics, Seoul National University, Seoul, Korea

Corresponding author: Tae-Bum Kim, MD, PhD

Department of Allergy and Clinical Immunology, Asan Medical Center,

University of Ulsan College of Medicine, 86 Asanbyeongwon-gil,

Songpa-gu, Seoul 138-736, Korea

Tel: 82-2-3010-3280; Fax: 82-2-3010-6969

E-mail: tbkim@amc.seoul.kr

ORCID: Tae-Bum Kim; <https://orcid.org/0000-0001-5663-0640>

Co-corresponding author: Sungho Won, PhD

Department of Public Health Science, Seoul National University,

1, Gwanak-ro, Gwanak-gu, Seoul 151-742, Korea

Tel: 82-2-880-2714; Fax: 82-303-0942-2714

E-mail: sunghow@gmail.com

Footnote: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (2019M3E5D3073365).

Author contributions: SY Park designed this study, acquired data, analyzed data, and drafted the manuscript. AR Do and T Park analyzed data, drafted the manuscript, and revised the manuscript. TB Kim and S won designed this study, analyzed data, drafted the manuscript, and revised the manuscript. All authors read and approved the final manuscript. All authors have accountabilities for all aspects of the work in ensuring that questions related

to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None

Keywords: Adverse drug reaction; Biomarkers; Drug hypersensitivity; Drug allergy; Genome-wide association study

Word count: 997

No. of figures and/or tables: 1 total (1 Table)

Drug hypersensitivity reactions (DHRs) are type B adverse drug reactions (ADRs) and comprise about 15% of all ADRs. They are mediated by an immunological mechanism and are currently unpredictable and heterogeneous.¹ DHRs can be life-threatening, therefore, the development of a biomarker that can predict drug hypersensitivity is very important and genetic biomarkers that predict DHRs are the most studied to date. Several reports indicate that certain HLA types in certain races are associated with DHRs.² However, their genetic biomarkers have been studied mostly in association with specific drugs and specific races. The highly consistent genetic biomarkers that can predict DHRs regardless specific drug, and type of reaction can be used to predict and prevent DHRs.

In this study, we aimed to identify common genetic biomarkers to predict DHRs by including a cohort of patients with DHRs using GWAS.

We recruited 378 patients reporting DHRs who visited the Asan Medical Center from 2012 to 2017. Patients reporting DHRs were evaluated by allergist and after that, they were categorized by certain or probable according to WHO-UMC causality analysis system. The study was approved by the Institutional Review Board of Asan Medical Center (2011-0939). Blood samples were collected from each subject and genotyped using the K-CHIP (<http://kogo.or.kr>). Data from the Severance Hospital Health Examination Center (SHHC) in Korea were used as the control group data (N=3,780) and genotyped using the K-CHIP. The SNP genotype was called by using the K-medoids clustering-based calling approach to minimize the batch effect.³ The EIGENSTRAT approach was applied to pooled data and principal component score (PC score) was calculated between the control group and the patient group (to match subjects with similar genetic information); a total of 10 controls per patient were matched with similar scores.⁴

Then, the genotyping analysis was replicated with an additional 185 patients with DHRs. These patients were genotyped using an Axiom Asian chip (Affymetrix). The control subjects were enrolled at the SHHC (N=2,312) and genotyped using a K-CHIP. We pooled all the data and performed a target imputation. We evaluated the quality of SNPs and subjects. We excluded SNPs for which the P-values for the Hardy-Weinberg equilibrium (HWE) test were less than 1×10^{-5} , minor allele frequencies (MAFs) were less than 0.05, or genotype call rates were less than 0.95. GWAS with discovery data was conducted using generalized logistic regression (GLR). Age and sex were included as covariates. In addition, the LR model was used to predict binary responses (control vs. DHR). Both GLR and LR were

applied using the R statistical software (www.r-project.org). We imputed SNPs located within $\pm 5,000$ base pairs using the Sanger Imputation Service (<https://imputation.sanger.ac.uk>). The mean age of DHR patients was 42.03 ± 14.82 years, and 247 patients accounted for female gender. Of these, 295 cases had immediate hypersensitivity reactions and 83 had delayed reactions. The most common causative drugs that triggered immediate hypersensitivity were NSAIDs followed by antibiotics. In the delayed reaction group, antibiotics were the most common followed by anticonvulsants. The mean age of control group was 43.07 ± 9.85 years and a total of 1236 patients accounted for female gender. In the replication analysis, the mean age was 50.38 ± 16.58 years, and 105 females were included. 80 patients had immediate reactions, whereas 105 patients had delayed reactions. The control group comprised total of 921 females, and the mean age was 46.96 ± 10.72 (**Table 1**). In the replication group, NSAIDs were the most common causative drugs followed by antibiotics. A total of 265,878 SNPs were passed through quality control (call rate > 98%, MAF > 0.05). First, we analyzed the relationship between the two groups of DHRs (immediate and delayed reactions). Only one SNP is close to the genome-wide significance level (rs35372932, $p=7.99E-08$), which is an upstream region of the HLA-DRB1 gene on chromosome 6 (**Table 1**). Second, we divided the immediate and delayed reactions independently and repeated the analysis. A total of five genome-wide significant SNPs (rs11206477, rs62225078, rs73202933, rs72622187, and rs139141104) were compared between the delayed hypersensitivity and control groups. Among them, the significant SNP rs139141104 is an intron region of the *MUC22* gene on chromosome 6 (**Table 1**). We performed a replication analysis using target imputation. Of the SNPs identified as significant in discovery data analysis, only rs35372932, which is an upstream region of HLA-DRB1 on chromosome 6 ($p=0.008$, **Table 1**), was found to be statistically significant.

In this study, we identified rs35372932 SNPs related to DHRs, which are common markers not limited to a specific drug hypersensitivity phenotype or a causative drug. The rs35372932 SNP was associated with HLA-DRB1. In many studies so far, HLA-DRB1 has been reported in relation to drug hypersensitivity; for example, HLA-DRB1*0101 is associated with nevirapine hypersensitivity reaction.⁵ In addition, HLA-DRB1*1302 is related to aspirin-induced urticaria/angioedema in a Korean population.⁶ HLA-DRB1 polymorphism has been reported to be associated with various autoimmune diseases, in addition, viral or bacterial infection is known as one of the trigger factors, and polymorphisms that occur in response to these infections lead to unexpected reactions.⁷ Similarly, viral infection could be a trigger factor for hypersensitivity reactions in drug-induced hypersensitivity syndrome.⁸ Some cases have shown similar clinical features, and autoimmune diseases have been reported to be closely related to DHRs.⁹ In this study, total of 5 out of all patient groups exhibited autoimmune disease. This study has several limitations. First, the sample size is relatively small. Second, it was not possible to conduct a functional study. Finally, Correlations between phenotypes and genotypes of HSR have not been clearly defined. Nevertheless, this study could provide important clues regarding the immune mechanisms of DHRs that have not yet been clearly elucidated. If possible, functional research studies with other groups should be conducted in the future. Unlike previous studies, we report the associated genes that may be involved in DHR, in general, and is not limited to DHR specific to race, drug type, or ethnicity, and this may have great significance in the clinical field in the efficient diagnosis and prediction of DHRs.

References

1. Dykewicz MS, Lam JK. Drug Hypersensitivity Reactions. *The Medical clinics of North America*. 2020;104:109-128.
2. Pirmohamed M, Ostrov DA, Park BK. New genetic findings lead the way to a better understanding of fundamental mechanisms of drug hypersensitivity. *The Journal of allergy and clinical immunology*. 2015;136:236-244.
3. Seo S, Park K, Lee JJ, Choi KY, Lee KH, Won S. SNP genotype calling and quality control for multi-batch-based studies. *Genes & genomics*. 2019;41:927-939.
4. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nature genetics*. 2006;38:904-909.
5. Martin AM, Nolan D, James I, et al. Predisposition to nevirapine hypersensitivity associated with HLA-DRB1*0101 and abrogated by low CD4 T-cell counts. *AIDS (London, England)*. 2005;19:97-99.
6. Kim SH, Choi JH, Lee KW, et al. The human leucocyte antigen-DRB1*1302-DQB1*0609-DPB1*0201 haplotype may be a strong genetic marker for aspirin-induced urticaria. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*. 2005;35:339-344.
7. Arango MT, Perricone C, Kivity S, et al. HLA-DRB1 the notorious gene in the mosaic of autoimmunity. *Immunol Res*. 2017;65:82-98.
8. Shiohara T, Kano Y. A complex interaction between drug allergy and viral infection. *Clin Rev Allergy Immunol*. 2007;33:124-133.
9. Michels AW, Ostrov DA. New approaches for predicting T cell-mediated drug reactions: A role for inducible and potentially preventable autoimmunity. *J Allergy Clin Immunol*. 2015;136:252-257.

Table 1. Demographic information and genome-wide significant SNPs with discovery and replication analysis

Discovery analysis

	Age, years	Female,N(%)	Immediate reaction, N(%)	Delayed reaction, N(%)
Drug hypersensitivity (N=378)	42.03 ± 14.82	247 (65.34 %) (78.04%)	295 (78.04%)	83 (21.96%)

Control (N=3,780)	43.07 $\pm$ 9.85	1,236 (32.70 %)		
--------------------------	------------------	-----------------	--	--

Significant SNPs with discovery data

Ch r	SNP	Nt	Logistic regression (Control vs ADR (Immediate +Delayed))		Generalized logistic regression (Control vs Immediate)		Generalized logistic regression (Control vs Delayed)		AIC
			OR [95% CI]	P-value	β	P-value	β [95% CI]	P-value	
6	rs35372932	32597208	1.77 [1.44, 2.18]	7.99×10^{-8}	-	-	-	-	2361.1
6	rs139141104	31021244	-	-	-0.80	0.0189	-2.04 [-1.30, -2.77]	2.90×10^{-8}	2723.73

SNP information with discovery data

Chr	SNP	Nt	Alt / Ref	Minor allele frequency					East Asian population from gnomAD	HWE	Rsq	Genotype	Missing Rate	Gene
				All	Control	Immediate	Delayed	Korean reference dataset						
6	rs35372932	32597208	T / C	0.29	0.28	0.37	0.40	0.27	0.29	0.91	-	genotype	0	HLA-DRB1
6	rs139141104	31021244	G / A	0.09	0.09	0.08	0.05	-	0.008	0.37	0.92	imputed	0	MUC22

Replication analysis

	Age, years	Female,N(%)	Immediate reaction, N(%)	Delayed reaction, N(%)
Drug hypersensitivity	50.38 $\pm$ 16.58	102 (55.14 %)	105 (56.76 %)	80 (43.24 %)

(N=185)				
Control (N=2,312)	46.96 ± 10.72	921 (39.84 %)		

Significant SNPs with discovery data

Chr	SNP	Nt	Logistic regression (Control vs ADR (immediate +delayed))		Generalized logistic regression (Control vs Immediate)		Logistic regression (Immediate vs Delayed)		Generalized logistic regression (Control vs delayed)	
			β [95% CI]	P-value	β	P-value	β	P-value	β [95% CI]	P-value
6	rs35372932	32597208	0.32 [0.19,0.45]	0.008	-	-	-	-	-	-
6	rs139141104	31021244	-	-	-0.32	0.5158	-0.70	0.4282	-0.16 [-0.57,0.25]	0.6512

SNP information with replication data

Chr	SNP	Nt	Alt /Ref	Minor allele frequency				HWE	Missing Rate	Rsqr	genotype
				All	Control	Immediate	Delayed				
6	rs35372932	32597208	T/C	0.25	0.2469	0.3143	0.3038	0.4897	0.0008	-	genotyped
6	rs139141104	31021244	G/A	0.05386	0.05277	0.09048	0.0375	0.4331	0	0.966	imputed

Chr: Chromosome; Nt: Nucleotide; Alt: alternative allele; Ref: Reference allele; HWE: Hardy-Weinberg equilibrium;