Clinical and Translational Allergy

ORIGINAL ARTICLE OPEN ACCESS

Identifying Genetic Variants in Patients With Cefaclor-Induced Anaphylaxis Using Human Leukocyte Antigen Typing and Whole-Exome Sequencing

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Received: 19 April 2025 | **Revised:** 27 August 2025 | **Accepted:** 5 September 2025

Funding: This research was supported by a (Grant 21182MFDS295) from the Ministry of Food and Drug Safety from 2021 to 2022. Korean Genome Project data were provided by Korean Genomics Center in Ulsan National Institute of Science and Technology, Republic of Korea. This research was supported by a new faculty research seed money grant of Yonsei University College of Medicine for 2025 (2025-32-0023).

Keywords: anaphylaxis | cefaclor | genetic variation

ABSTRACT

Background: Cefaclor is a commonly prescribed β-lactam antibiotic and a known major cause of immediate-type drug hypersensitivity in Korea. However, its genetic risk factors remain poorly understood. We aimed to identify genetic variants associated with cefaclor-induced anaphylaxis and evaluate their potential clinical implications.

Methods: Whole-exome sequencing and HLA genotyping were performed in 33 patients with cefaclor-induced anaphylaxis and 41 drug-tolerant controls. Associations were assessed using logistic regression. Selected variants were validated in an independent Korean population. Gene set enrichment analysis (GSEA) was performed using association statistics from all variants to investigate relevant biological pathways.

Results: A rare missense variant, rs765144578 in *TPSAB1* was strongly associated with anaphylaxis and remained significant in the validation control group. It was found in 90.91% of patients with hypotension, suggesting a link to reaction severity. Rs192498095 in HLA-DRB5 showed a significant association in the discovery cohort. However, it was not detected in the replication set, likely due to its rarity and polymorphic nature. Co-occurrence of rs765144578 in *TPSAB1* and rs192498095 in *HLA-DRB5* markedly increased risk. GSEA revealed significant enrichment of the TNF-α signaling via NF-κB pathway, reflecting pathway-level immune activation.

Conclusion: Genetic variants in *TPSAB1* and *HLA-DRB5* may contribute to the risk of cefaclor-induced anaphylaxis, and *TPSAB1* may also be associated with severity. These findings may support the development of future screening strategies or individualized risk prediction models in β-lactam allergy.

Sung-Ryeol Kim and Da Eun Lee authors contributed equally to this work and are considered co–first authors.

1 | Introduction

Drugs are the most common triggers of anaphylaxis in adults [1], and drug-induced anaphylaxis carries a higher risk of severe outcomes than other causes [2]. Frequent culprits include antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), and contrast media. Although studies have examined clinical risk factors, predicting drug-induced anaphylaxis in individuals without a prior history of drug allergy remains challenging [3–5].

Cefaclor, a widely prescribed second-generation cephalosporin, is one of the leading causes of drug-induced anaphylaxis in Korea [6, 7]. Notably, cefaclor is associated with a higher incidence of anaphylaxis than other cephalosporins, making it a significant clinical concern [8, 9]. Because antibiotics are widely prescribed, overall exposure is high and may contribute to the observed frequency of hypersensitivity reactions [10] However, prescription volume alone does not fully explain why cefaclor accounts for a disproportionate share of drug-induced anaphylaxis cases in Korea [6].

The high incidence of cefaclor-induced hypersensitivity in Korea may reflect genetic susceptibility, including variations in HLA alleles that affect immune recognition of the drug. Identifying these genetic factors could facilitate the prediction and prevention of severe hypersensitivity reactions in at-risk individuals. Although the intricate interactions among immune cells and signaling pathways in immediate hypersensitivity can limit the practical application of individual genetic variants, research into their genetic basis remains valuable. Such investigations provide key insights into the mechanisms of drug-induced anaphylaxis and may support the development of personalized preventive strategies. Accordingly, we aimed to identify genetic variants associated with cefaclor-induced anaphylaxis by performing whole-exome sequencing (WES) and HLA genotyping.

2 | Methods

2.1 | Study Participants and Data Collection

We enrolled 33 adult patients (≥ 18 years old) with cefaclor-induced anaphylaxis and 41 cefaclor-tolerant controls (Table S1). Patients were recruited from three hospitals in Korea between 2021 and 2022, based on clinical history, oral provocation testing, specific IgE testing to cefaclor, or skin testing. Causality was assessed using the World Health Organization (WHO)-Uppsala Monitoring Center criteria, and only cases classified as "certain" were included [11]. Anaphylaxis was diagnosed according to the World Allergy Organization (WAO) 2020 criteria [12].

Tolerant controls were recruited from the general population via hospital-based posters and included individuals who had taken cefaclor at least twice, with intervals of more than 2 weeks, without developing significant hypersensitivity reactions. Their tolerance status was verified through a review of medical history.

The study protocol was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System (approval number: 9-2020-0124). Written informed consent was obtained from all participants before study enrollment.

2.2 | Sample Preparations and WES

Genomic deoxyribonucleic acid (DNA) was extracted from 74 whole blood samples using the GeneAll Exgene Blood SV mini kit (GeneAll Biotechnology, Seoul, Korea), following the manufacturer's instructions. DNA concentration and quality were assessed using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and purity was verified with A260/280 ratios between 1.5 and 2.2. DNA integrity was confirmed by a DNA integrity number (DIN) > 6.

WES was performed using the NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA) with a target mean depth of 100 ×, aiming to capture variants across exons and regulatory intronic regions, including the highly polymorphic HLA region. Raw read quality was assessed using FastQC (v0.11.7) [13], and low-quality bases ($Q < 20$) were trimmed using Trimmomatic (v0.36) [14]. Clean reads were aligned to the human reference genome GRCh37 (hg19), obtained from the UCSC Genome Browser, using Burrows-Wheeler Aligner (BWA) v0.7.12 [15–17].

Variant calling for single nucleotide variants (SNVs) and insertions/deletions (INDELs) was conducted using the Genome Analysis Toolkit (GATK) v4.0.11.0, and functional annotation was performed with SnpEff v4.3 [18, 19].

2.3 | Next-Generation Sequencing (NGS)-Based Typing of HLA Alleles

Following WES, HLA genotyping was performed to identify specific alleles in each participant. The *HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1, -DRA,* and *-DRB1* loci were typed using HISAT-genotype v1.3.3 [20]. *HLA-DRB3, -DRB4, and -DRB5* were genotyped using NGSgo-AmpX v2 (GenDx, Netherlands) following the manufacturer's protocol. Sequence reads were aligned, and alleles were assigned using NGSengine software (GenDx, Netherlands), referencing the IMGT/HLA database [21]. Allele frequencies were calculated for each HLA locus in both the anaphylaxis and tolerant control groups.

2.4 | Statistical Methods for Demographic Characteristics

Statistical analyses were conducted to compare clinical characteristics between patients with cefaclor-induced anaphylaxis and control subjects. Continuous variables (e.g., age, BMI) were analyzed using Student's t-test to evaluate differences between group means. Categorical variables (e.g., sex, underlying diseases) were analyzed using Fisher's exact test or the chi-square test to assess differences in proportions between groups. Statistical significance was set at $p < 0.05$.

2.5 | WES-Based Association Analysis

Prior to association analysis, quality control criteria were applied to both individual-level and variant-level WES data. No individuals were excluded based on genotype call rate, as all participants met the predefined threshold of ≥ 90%. At the variant level, filtering was performed using bcftools (v1.13) on the VCF files [22]. Variants were retained if they had a genotype quality (GQ) score ≥ 30 and a read depth (DP) ≥ 10. The resulting data were converted into binary format using whole-genome analysis toolset (PLINK v2.0) [23, 24]. To minimize false-positive associations, only biallelic single nucleotide variants (SNVs) with a minor allele frequency (MAF) ≥ 0.01 and Hardy–Weinberg equilibrium (HWE) p value > 0.05 were included. After quality control filtering, a total of 197,422 variants across autosomal chromosomes (1–22) remained in the final dataset. To account for potential population stratification, principal component analysis (PCA) was conducted using the SNPRelate package in R (v4.3.1, R Foundation for Statistical Computing, Vienna, Austria).

Genotype–phenotype associations between 33 anaphylaxis patients and 41 tolerant controls were tested using logistic regression under an additive genetic model, implemented in PLINK v2.0. Based on principles of causal inference, none of the clinical variables were considered to temporally or causally precede genetic predisposition. Therefore, we estimated the total effect of genetic variants on the phenotype by adjusting for sex as the sole covariate, without controlling for mediators or downstream clinical traits. Variants were considered statistically significant based on a false discovery rate (FDR)-adjusted p value < 0.01. Among the significant variants, candidates were prioritized as functionally relevant if predicted to be deleterious by SIFT (score < 0.05) or damaging by PolyPhen-2 (HVAR score > 0.5) [25, 26].

To evaluate the reproducibility of the associations identified in the discovery phase, we conducted a validation analysis using an independent control group. Whole-genome sequencing (WGS) data from 232 unrelated Korean individuals—sourced from the Korean Genome Project and provided by the Korean Genomics Center at the Ulsan National Institute of Science and Technology (UNIST), Republic of Korea—served as the independent control cohort [27]. As these WGS data were aligned to the hg38 reference genome, the top functional variants were converted to hg19 coordinates using the lift-over tool available through the UCSC Genome Browser [17]. For the validation analysis, univariate logistic regression assuming an additive genetic model was performed in R to evaluate the association of the top functional variants. Genotype dosage differences were tested between the 33 cases from the discovery analysis and the 232 independent controls. Variants were considered replicated if they demonstrated statistically significant associations ($p < 5 \times 10^{-8}$) in the same direction as in the discovery analysis.

2.6 | Pathway Enrichment Analysis

Pathway-level enrichment was assessed using pre-ranked gene set enrichment analysis (GSEA), implemented via the Broad Institute's GSEA software (v4.3.2) [28]. A ranked gene list was generated by ordering genes based on the lowest p value from the association analysis. In cases where multiple variants mapped to the same gene, only the variant with the smallest p value was used to represent that gene in the ranked list. Enrichment analysis was performed using the Hallmark gene sets from the Molecular Signatures Database (MSigDB v2023.2) [29]. Default parameters were applied, including 1000 permutations, a weighted enrichment statistic, and gene set size filters ranging from a minimum of 3 to a maximum of 500 genes. Nominal p values were used to evaluate enrichment, and gene sets with $p < 0.05$ were considered suggestively enriched.

2.7 | Association Analysis for HLA Genotypes

For HLA association analysis, 15 of 169 typed alleles were selected based on a minimum phenotype count of five and at least a threefold difference in allele frequency between cases and tolerant controls. Associations were tested under a dominant genetic model using Fisher's exact test or the chi-square test in R (version 4.3.1). Statistical significance was defined as $p < 0.05$.

2.8 | Subgroup Analysis

To further explore phenotype-specific genetic associations, two targeted subgroup analyses were carried out. The first assessed the association between the top variant in the tryptase gene, rs765144578 in *TPSAB1*, and hypotension, a severe clinical manifestation of anaphylaxis. Patients with anaphylaxis who presented with hypotension were classified into the hypotension subgroup. The second analysis evaluated the risk of anaphylaxis among individuals carrying both rs765144578 in *TPSAB1* and rs192498095 in *HLA-DRB5*, compared to those carrying neither variant. All association analyses were performed using logistic regression models adjusted for sex.

3 | Results

3.1 | Clinical Characteristics of Participants

The mean age of the patients was 46.7 years, and 26 patients (78.8%) were female. Significant differences were observed between the anaphylaxis and control groups in terms of age and history of food allergy. The most common clinical manifestations among patients were cutaneous symptoms (93.3%), followed by respiratory (66.7%), cardiovascular (42.4%), and gastrointestinal (30.3%) symptoms. Serum-specific IgE (sIgE) to cefaclor was measured in 32 patients, all of whom tested positive (> 0.35 kUA/L). One additional patient, who had positive results on both the skin test and oral provocation test, did not undergo sIgE testing, resulting in no available sIgE data for that case. Detailed clinical features of the enrolled patients and controls are summarized in Table S1.

3.2 | Identification of Genetic Risk Variants via WES

Figure 1 presents a PCA of 197,422 genetic variants in 33 patients with cefaclor-induced anaphylaxis and 41 cefaclor-tolerant controls. PC1 and PC2 explained 1.98% and 1.95% of the total genetic variance, suggesting minimal separation along the major axes of variation and indicating limited evidence of population stratification. Although the anaphylaxis group showed slightly greater dispersion compared to controls, the substantial overlap between groups and broad confidence ellipses indicate no clear genetic differentiation. Therefore, PCA-derived principal components were not included as covariates in the association analysis.

During the discovery phase, genetic association analysis identified multiple clusters of variants across several genomic regions (Figure 2). Association analysis, conducted via logistic regression adjusted for sex, revealed 164 candidate variants across chromosomes 1 to 22 that met the significance threshold (FDR-adjusted $p < 0.01$; Table S2). The Manhattan plot highlighted prominent peaks, prompting further investigation into functionally relevant variants that may influence gene expression or phenotype.

Among these 164 variants, six single nucleotide polymorphisms (SNPs) were prioritized as functionally significant based on predicted pathogenicity (SIFT score < 0.05 or PolyPhen-2 HVAR score > 0.5) (Table 1). Notably, rs765144578 variant in the *TPSAB1* locus demonstrated the strongest association with cefaclor-induced anaphylaxis (adjusted $p < 0.001$; risk allele frequency [RAF] 0.44 vs. 0.05; odds ratio [OR] 98.96; 95% confidence interval [CI], 17.65–555.04).

Other functionally relevant variants included:

- rs779277004 variant in *MUC4* (RAF 0.38 vs. 0.05; OR 45.87; 95% CI, 9.24–227.81),
- rs61388923 variant in *MUC4* (RAF 0.39 vs. 0.05; OR 35.11; 95% CI, 9.20–133.99),
- rs192498095 variant in *HLA-DRB5* (RAF 0.33 vs. 0.04; OR 25.84; 95% CI, 6.44–103.74),

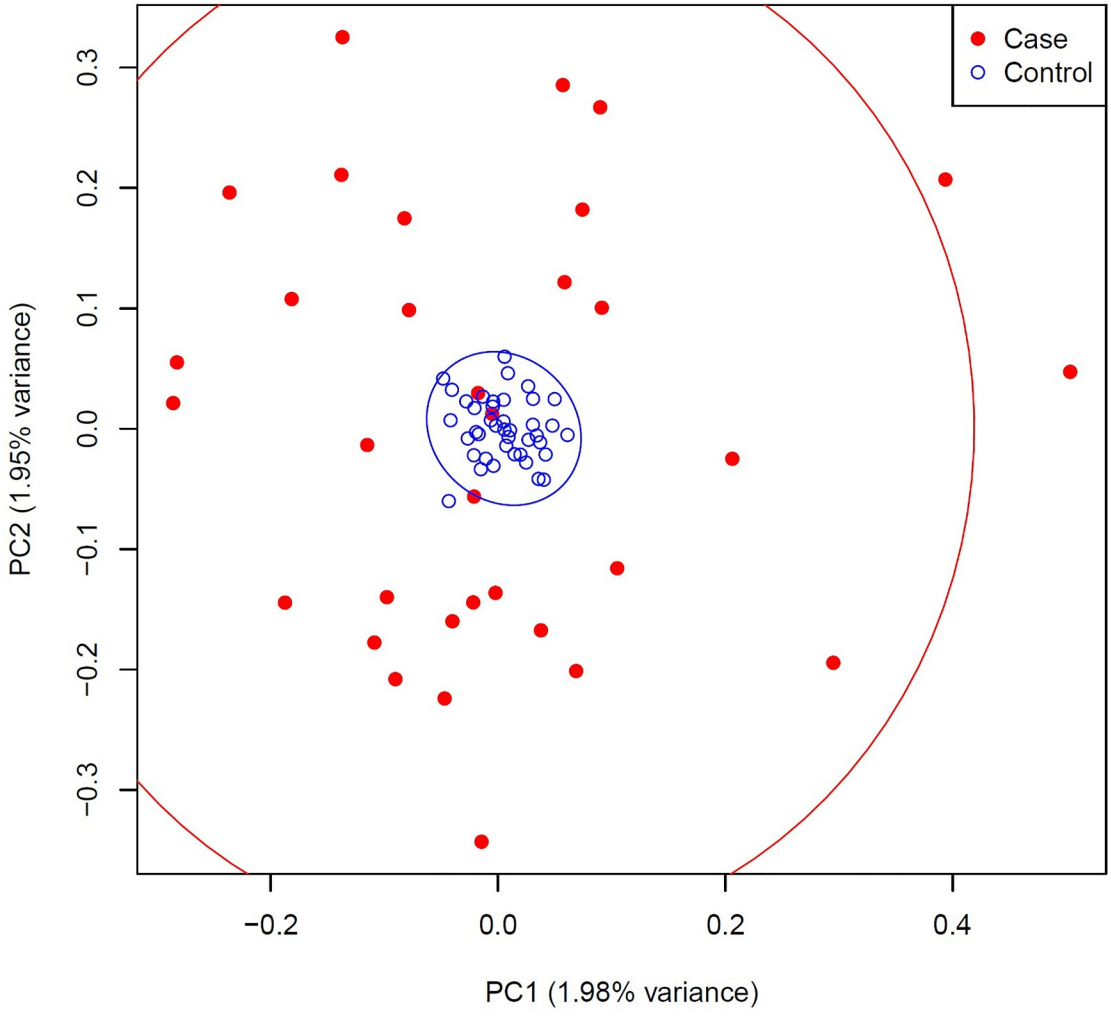

FIGURE 1 | Principal component analysis of genetic variants in patients with cefaclor-induced anaphylaxis and tolerant controls. PCA was performed using 197,422 variants derived from 33 patients with cefaclor-induced anaphylaxis (red dots) and 41 tolerant controls (blue circles), plotted against the first two principal components (PC1 and PC2). The large dispersion and substantial overlap of confidence ellipses demonstrate limited population differentiation.

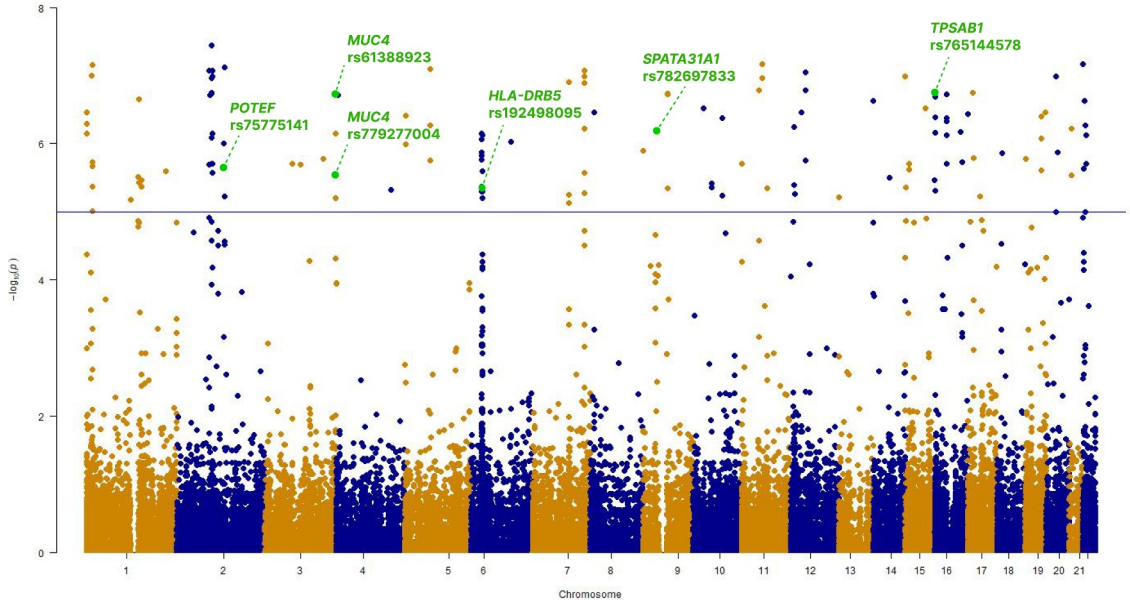

FIGURE 2 | Manhattan plot of genetic association study in cefaclor-induced anaphylaxis. Each dot represents the negative $\log_{10}P$ of 197,422 variants derived from a logistic regression model adjusted for sex, comparing 33 patients with cefaclor-induced anaphylaxis and 41 tolerant controls. The blue horizontal line indicates the significance threshold, which was set at a false discovery rate (FDR)-corrected $p < 0.01$. Green dots highlight the top six functionally annotated single nucleotide polymorphisms (SNPs), annotated with corresponding gene name and rsIDs.

- rs75775141 variant in *POTEF* (RAF 0.71 vs. 0.12; OR 14.89; 95% CI, 4.86–45.65),
- rs782697833 variant in *SPATA31A1* (RAF 0.96 vs. 0.15; OR 14.64; 95% CI, 5.09–42.11).

Validation analysis using genotype data from 232 individuals in the general population confirmed the association of rs765144578 in *TPSAB1* with cefaclor-induced anaphylaxis ($p = 9.51 \times 10^{-11}$; RAF 0.44 vs. 0.08; OR 38.20; 95% CI, 12.68–115.11). While rs75775141 in *POTEF* and rs779277004 in *MUC4* showed associations in the same direction, they did not reach statistical significance in the validation dataset. The remaining three variants could not be evaluated due to their low allele frequencies.

3.3 | Pathway Enrichment Analysis via GSEA

GSEA revealed signaling pathways potentially contributing to the pathogenesis of cefaclor-induced anaphylaxis (Table 2). Among the analyzed gene sets, TNF-α signaling via the NF-κB pathway was significantly enriched (188 genes; normalized enrichment score [NES] = 1.20; nominal $p = 0.017$). Other pathways, including IL-6 JAK STAT3 signaling (NES = 1.14; $p = 0.147$) and oxidative phosphorylation (NES = 1.10; $p = 0.143$), also exhibited positive enrichment trends but without statistical significance. Detailed gene lists and enrichment scores for the TNF-α signaling via NF-κB pathway are provided in Table S3.

3.4 | HLA Alleles Associated With Anaphylaxis

NGS-based HLA typing revealed specific alleles associated with cefaclor-induced anaphylaxis (Table 3). The overall distribution of HLA alleles observed in the study population is shown in Figure S1. Among these, the HLA-B*07:02 was significantly associated with an increased risk of anaphylaxis, being present in 5 out of 33 patients but absent in all 41 tolerant controls ($p = 0.015$; OR = 7.15; 95% CI, 1.22–353.85). In contrast, HLA-C*01:02 appeared to have a protective effect, being present in 4 anaphylaxis cases and 14 tolerant controls ($p = 0.033$; OR = 0.27; 95% CI, 0.06–0.99). Although not statistically significant, several HLA class II alleles including DQB1*03:03, DQB1*05:01, and DRB5*01:01 showed notable differences in frequency between cases and controls.

3.5 | Evaluation of Top Variants in Clinical Subgroups

Subgroup analysis revealed a strong association between the rs765144578 variant in *TPSAB1* and hypotension as a manifestation of anaphylaxis (Table 4). Among the 11 patients who experienced hypotension, 10 (90.91%) carried the variant, compared with 4 of 41 tolerant controls (9.76%), yielding an OR of 130.71 ($p = 1.13 \times 10^{-4}$). To further investigate this association, the frequency of the rs765144578 variant was compared between patients with hypotension and individuals from the general Korean population, in which 16.0% (37/232) carried the variant. This comparison yielded an

TABLE 1 | Top six functional variants associated with cefaclor-induced anaphylaxis identified by genetic association analysis.

						Discovery phase ($N = 74$)					Validation control ($N = 232$)		
						RAF							
Chr	Position	Variant	Gene	REF	RA	Case ($N = 33$)	Tolerant ($N = 41$)	Number of risk allele carriers (patients/controls)	OR (95% CI)	$P_{adjusted}$[a]	RAF	OR (95% CI)	p value
2	130832292	rs75775141	*POTEF*	T	A	0.71	0.12	33/6	14.89 (4.86–45.65)	0.004	0.54	7.78 (3.41–17.77)	1.13×10^{-6}
3	195507475	rs779277004	*MUC4*	G	C	0.38	0.05	25/4	45.87 (9.24–227.81)	0.004	0.24	3.35 (1.45–7.73)	4.65×10^{-3}
3	195508010	rs61388923	*MUC4*	C	A	0.39	0.05	26/4	35.11 (9.20–133.99)	< 0.001	—	—	—
6	32489852	rs192498095	*HLA-DRB5*	A	G	0.33	0.04	22/3	25.84 (6.44–103.74)	0.006	—	—	—
9	39890309	rs782697833	*SPATA31A1*	G	A	0.96	0.15	32/6	14.64 (5.09–42.11)	0.002	—	—	—
16	1291623	rs765144578	*TPSAB1*	C	T	0.44	0.05	29/4	98.96 (17.65–555.04)	< 0.001	0.08	38.21 (12.68–115.11)	9.51×10^{-11}[b]

Abbreviations: Chr, chromosome; CI, confidence interval; OR, odds ratio; RA, risk allele; RAF, risk allele frequency; REF, reference allele.
[a]FDR-adjusted p values were derived from logistic regression analyses adjusted for sex, comparing 33 patients with cefaclor-induced anaphylaxis and 41 tolerant controls.
[b]Statistically significant at $p < 5 \times 10^{-8}$.

TABLE 2 | Gene set enrichment analysis of variants associated with cefaclor-induced anaphylaxis.

Identifier	Gene set pathway annotation	Gene set size[a]	Normalized enrichment score	*p* value
M5890	TNF-α signaling via NF-κB	188	1.20	0.017[b]
M5897	IL-6 JAK STAT3 signaling	78	1.14	0.147
M5928	MYC targets V2	54	1.14	0.198
M5936	Oxidative phosphorylation	174	1.10	0.143
M5926	MYC targets V1	180	1.08	0.214
M5932	Inflammatory response	191	1.07	0.238

Note: Gene sets were tested for enrichment using pre-ranked gene set enrichment analysis (GSEA) with Hallmark gene sets from Molecular Signatures Database (MSigDB).
[a]Gene set size refers to the number of genes in the pathway that were present in the pre-ranked input list.
[b]Gene sets with nominal $p < 0.05$ were considered suggestively enriched.

TABLE 3 | HLA alleles associated with cefaclor-induced anaphylaxis.

	Dominant genotype frequency			
HLA types	Cases	Controls	OR[a] (95% CI)	*p* value
B * 07:02	0.15	0	7.15 (1.22–353.85)	0.015[b]
B * 46:01	0.06	0.15	0.38 (0.04–2.33)	0.286
B * 54:01	0.03	0.12	0.23 (0–2.20)	0.216
B * 58:01	0.06	0.15	0.38 (0.04–2.33)	0.286
C * 01:02	0.12	0.34	0.27 (0.06–0.99)	0.033[b]
C * 03:02	0.06	0.15	0.38 (0.04–2.33)	0.286
DQA1 * 01:01	0.18	0.05	4.25 (0.69–46.14)	0.128
DQA1 * 01:03	0.09	0.24	0.31 (0.05–1.38)	0.125
DQB1 * 03:03	0.09	0.27	0.28 (0.05–1.19)	0.074
DQB1 * 05:01	0.21	0.05	5.14 (0.89–54.43)	0.069
DQB1 * 06:02	0.09	0.24	0.31 (0.05–1.38)	0.125
DRB1 * 01:01	0.18	0.05	4.25 (0.69–46.14)	0.128
DRB1 * 08:03	0.06	0.17	0.32 (0.03–1.84)	0.283
DRB1 * 15:01	0.09	0.24	0.31 (0.05–1.38)	0.125
DRB5 * 01:01	0.09	0.27	0.28 (0.05–1.19)	0.074

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio.
[a]Odds ratios and 95% confidence intervals were calculated under a dominant model using Fisher's exact test, with Haldane's correction applied where necessary.
[b]Statistically significant at $p < 0.05$.

TABLE 4 | Association between the rs765144578 variant in TPSAB1 and hypotension in patients with cefaclor-induced anaphylaxis.

Clinical symptoms	Cases with symptoms GF	Tolerant controls			Validation controls		
		GF	OR (95% CI)	$P_{adjusted}$[a]	GF	OR (95% CI)	*p* value
Hypotension	10/11 (90.91%)	4/41 (9.8%)	130.71 (11.02–1550.47)	1.13×10^{-4}	37/232 (16.0%)	52.70 (6.55–424.16)	1.94×10^{-4}

Abbreviations: CI, confidence interval; GF, genotype frequency (Number of risk allele carriers); OR, odds ratio.
[a]*p* values from the sex-adjusted additive logistic regression model.

OR of 52.70 ($p = 1.94 \times 10^{-4}$), further supporting its potential involvement in severe presentations of immediate hypersensitivity reactions.

Furthermore, the co-occurrence of rs765144578 variant in *TPSAB1* and rs192498095 variant in *HLA-DRB5* demonstrated a markedly stronger association with anaphylaxis than either variant alone. Individuals carrying both variants were significantly more frequent in the anaphylaxis group than in tolerant controls (57.6% vs. 2.4%), with an OR of 657.38 (95% CI, 38.87–11,119; $p < 0.001$), suggesting a potential synergistic effect between the two loci.

4 | Discussion

Previous genetic studies on drug hypersensitivity have predominantly focused on delayed-type reactions. However, an increasing number of genetic factors have also been associated with immediate hypersensitivity. For example, polymorphisms in cytokine-related genes—such as TNF-α, IL-4, IL-13, IL-10, and IL-18—as well as in the FcεRI receptor on mast cells, have been linked to immediate hypersensitivity reactions to β-lactam antibiotics [30–34]. In addition, genome-wide association studies (GWAS) have reported associations with various HLA genes, including *HLA-B, HLA-DRA, HLA-DRB1*04:03, HLA-DRB1*10:01, HLA-DRB1*14:54,* and *HLA-B*55:01* [35–38]. Although these genetic markers have not yet proven to be practically useful for predicting drug-induced hypersensitivity, they have provided valuable insights into the immunogenetic mechanisms underlying anaphylaxis. Building on this background, we aimed to elucidate the genetic mechanisms specific to cefaclor-induced anaphylaxis and explore their potential clinical relevance.

This study explores genetic predispositions to cefaclor-induced anaphylaxis at both the exome-wide and HLA region levels, focusing specifically on patients with confirmed anaphylaxis rather than general hypersensitivity. Through genetic association study, we identified 164 variants meeting the significance threshold (FDR-adjusted $p < 0.01$). Among these, six variants were prioritized based on predicted pathogenicity using PolyPhen-2 and SIFT algorithms. The genes harboring these variants included *POTEF, MUC4, SPATA31A1, HLA-DRB5*, and *TPSAB1*.

Three of these genes—*POTEF, MUC4*, and *SPATA31A1*—showed higher variant frequencies in patients compared to tolerant controls. However, based on their known biological roles—*POTEF* in retinal homeostasis [39], *MUC4* in mucin production [40], and *SPATA31A1* in cell differentiation and spermatogenesis [41]—it is unlikely that these genes are directly involved in the pathogenesis of cefaclor-induced anaphylaxis.

In contrast, the *HLA-DRB5* and the *TPSAB1* are more plausibly implicated in drug induced anaphylaxis. *HLA-DRB5*, a class II HLA gene, has previously been associated with IgE sensitization [42], while *TPSAB1* encodes α- and β-tryptases. β-tryptase, the most abundant mediator stored in mast cell granules, plays a central role in allergic responses by amplifying mast cell degranulation and enhancing inflammatory signaling [43].

To validate the associations identified in the initial case–control analysis, we conducted a secondary analysis using whole-genome sequencing data from healthy unrelated Korean individuals. The rs765144578 variant in *TPSAB1* remained significantly associated with cefaclor-induced anaphylaxis, reaching genome-wide significance ($p < 1 \times 10^{-8}$). In contrast, the variants in *HLA-DRB5, MUC4,* and *SPATA31A1* were not detected in the validation dataset, possibly due to low allele frequencies, differences in sequencing depth, or quality control filtering. Taken together, *TPSAB1* and *HLA-DRB5* emerge as meaningful genetic contributors to cefaclor-induced anaphylaxis based on both biological plausibility and case–control association signals. However, the lack of replication for *HLA-DRB5* in the validation cohort warrants cautious interpretation of its role. The rarity and polymorphic nature of the variant, along with differences in analytic conditions between the discovery and validation datasets, may have contributed to the absence of rs192498095 in *HLA-DRB5* in the replication analysis.

HLA association analysis identified *HLA-B*07:02* as a potential risk allele and *HLA-C*01:02* as a possible protective allele in cefaclor-induced anaphylaxis. Although a direct mechanistic link between HLA class I alleles and allergen sensitization has not been well established, several studies have reported associations between HLA class I alleles and drug-induced immediate hypersensitivity reactions. For instance, beta-lactam-induced hypersensitivity has been associated with *HLA-B*48:01, HLA-C*04:06,* and *HLA-C*08:01* [44]. Similarly, *HLA-B*46:01* has been implicated in asparaginase-induced hypersensitivity, while *HLA-B*38:02* and *HLA-B*58:01* have been associated with contrast media-induced hypersensitivity [45, 46]. These precedents support the plausibility of associations between *HLA-B*07:02, HLA-C*01:02*, and cefaclor-induced immediate hypersensitivity.

A recent study identified *HLA-DRB1*04:03* and *HLA-DRB1*14:54* as risk factors for cefaclor-induced immediate hypersensitivity [36]. In our cohort, *HLA-DRB1*04:03* was observed in three patients but not in any tolerant controls, while *HLA-DRB1*14:54* was found in one patient and three tolerant controls, with no statistically significant difference. Given the small number of individuals carrying these alleles, a reanalysis in a larger cohort is warranted to clarify their potential roles. Additionally, a separate study recently reported *HLA-DRB3*02:02* as a risk factor for penicillin hypersensitivity [47]. In our cohort, this allele was present in nine patients and eleven tolerant controls, again showing no significant difference. It is noteworthy that the previous association was observed in the context of delayed hypersensitivity, whereas our study focused on immediate hypersensitivity. This difference in immunopathological context may partly explain the lack of concordant findings.

We identified an association between the rs192498095 variant in *HLA-DRB5* and cefaclor-induced anaphylaxis based on WES data. However, HLA genotyping did not reveal a specific *HLA-DRB5* allele significantly associated with the phenotype. Among the alleles examined, *HLA-DRB5∗01:01* showed the greatest frequency difference—being more common in controls than in patients—but this difference was not statistically significant (OR = 0.28, $p = 0.074$). Linkage disequilibrium analysis also showed low correlation ($r^2 = 0.13$) between *HLA-DRB5∗01:01* and the reference allele A of rs192498095, suggesting a weak relationship between the SNP and this classical allele. Since classical HLA alleles are typically determined by combinations of multiple variants, the observed discrepancy suggests that rs192498095 may reflect a regulatory or non-classical signal not directly tied to a specific *HLA-DRB5* allele.

Genetic variability in *TPSAB1* has previously been associated with the severity of hypersensitivity reactions [48, 49]. In our subgroup analysis, the rs765144578 variant was identified in 90.91% of patients with hypotension yielding an OR of 130.71 (Table 4), which exceeded the overall OR of 98.96 observed in the total cohort. These findings suggest a strong association between this variant and the severity of cefaclor-induced immediate hypersensitivity reactions. Given the known role of *TPSAB1* in mast cell activation and tryptase production, it is possible that this rare missense variant may potentially affect tryptase protein stability or expression, thereby enhancing mast cell degranulation and amplifying allergic inflammation [50]. The variant demonstrated consistent associations across both the discovery and validation cohorts. However, further prospective and functional studies are warranted to clarify its mechanistic role and assess its clinical relevance.

Immediate drug hypersensitivity is known to be influenced by various genetic factors [51], and the presence of multiple susceptibility variants may act synergistically to increase the likelihood of reaction [52]. In our subgroup analysis, the co-occurrence of the *TPSAB1* variant rs765144578 and the *HLA-DRB5* variant rs192498095 was strongly associated with cefaclor-induced anaphylaxis (Table 5; OR = 657.38), suggesting a combined contribution of pathways involving IgE sensitization and tryptase-mediated mast cell activation to the pathogenesis of this condition. In addition, TNF-α signaling may also play a critical role in the development of cefaclor-induced anaphylaxis. Both GSEA and genetic association analysis revealed significant enrichment of the TNF-α signaling via the NF-κB pathway in affected individuals. The top six functional variants identified by GWAS were not mapped to any of the significantly enriched pathways, including the TNF-α signaling set. This suggests that these variants may exert their pathogenic effects through independent or complementary mechanisms. Although no single GWAS-identified variant directly overlapped with the TNF-α pathway, the GSEA findings point to an aggregated immune activation signal at the pathway level. This finding aligns with previous reports linking TNF-α gene variants to immediate hypersensitivity reactions to β-lactam antibiotics [30]. TNF is produced during the early stages of allergen sensitization and is essential for the production of antigen-specific IgE [53]. It also plays a key role in mast cell activation [54], wherein NF-κB serves as a central transcription factor mediating TNF-α signaling [55]. The observed enrichment of this pathway raises the possibility that polymorphisms in the TNF promoter region may modulate TNF-α expression, thereby contributing to amplified allergic inflammation.

The present study had several limitations. First, completely ruling out sequencing or variant calling errors was difficult due to the highly polymorphic nature of the HLA region. To mitigate this, we employed a target depth of 100 × and implemented stringent quality control procedures. Second, the rs765144578 variant in *TPSAB1* may be indirectly linked to hereditary alpha tryptasemia (HαT), potentially contributing to increased severity of hypersensitivity. However, we were unable to assess serum tryptase levels or determine *TPSAB1* copy number, which limits our ability to definitively exclude this possibility. Nevertheless, none of the patients had a clinical history suggestive of HαT, and the involvement of this single nucleotide variant in HαT pathogenesis appears unlikely. Lastly, the relatively small sample size may limit the generalizability and statistical power of some findings. Despite these limitations, the associations identified—particularly those involving *TPSAB1*—remain compelling and biologically plausible. These findings not only enhance our understanding of the genetic basis of cefaclor-induced anaphylaxis but may also inform future clinical applications. Although routine pre-prescription screening is not currently feasible, variants such as rs765144578 could help guide risk stratification in individuals requiring re-administration of β-lactam antibiotics. Furthermore, the markedly increased risk observed in individuals harboring both TPSAB1 and HLA-DRB5 variants highlights the potential utility of polygenic risk models. Such models may offer improved predictive accuracy over single-marker approaches and warrant validation in larger, multi-center cohorts.

4.1 | Conclusion

Cefaclor-induced anaphylaxis appears to involve multiple genetic factors, with rs765144578 in *TPSAB1* showing consistent associations with both risk and severity. Although rs192498095 in HLA-DRB5 showed a significant association in the patient cohort, it was not detected in the validation dataset, possibly due to its rarity. In addition, The *HLA-B*07:02* demonstrated a significant association, though further validation is needed. These findings provide new insight into the genetic architecture of immediate hypersensitivity to cefaclor. The strong association of TPSAB1, and its potential interaction with HLA-DRB5, may support the future development of predictive strategies for identifying individuals at increased risk. Further validation and

TABLE 5 | Synergistic effect of rs192498095 and rs765144578 variants on the risk of cefaclor-induced anaphylaxis.

Variants	Cefaclor anaphylaxis ($N = 33$)	Tolerant control ($N = 41$)	OR (95% CI)	$P_{adjusted}$[a]
rs192498095 (+)/rs765144578(+)	19 (57.6%)	1 (2.4%)	657.38 (38.87–11119)	6.91×10^{-6}
rs192498095 (−)/rs765144578(−)	1 (3.0%)	35 (85.4%)		

Abbreviations: CI, confidence interval; OR, odds ratio.
[a]*p*-values from the sex-adjusted additive logistic regression model.

functional studies are warranted to explore the clinical utility and mechanistic basis of these associations.

Author Contributions

Conceptualization: Jae-Hyun LEE, Jung-Mi Oh, Sung-Ryeol Kim. Methodology: Sung-Ryeol KIM, Jae-Hyun LEE, Jung-Mi Oh, Jung-Won Park. Software: Da Eun Lee. Data curations: Sung-Ryeol Kim, Hye-Ryun Kang, Kyung Hee Park. Investigation: Da Eun Lee, Hyun Young Jung, In-Wha Kim. Validation: Da Eun Lee, Hyun Young Jung, In-Wha Kim, Kyung Hee Park. Formal analysis: Da Eun Lee, Hyun Young Jung. Supervision: Jae-Hyun LEE, Jung-Mi Oh. Funding acquisition: Jae-Hyun LEE, Jung-Mi Oh. Visualization: Da Eun Lee, Hyun Young Jung. Project administration: Jae-Hyun LEE, Jung-Mi Oh. Resources: Jae-Hyun LEE, Hye-Ryun Kang, Jung-Mi Oh, Jung-Won Park. Writing – original draft: Sung-Ryeol KIM, Da Eun Lee. Writing – review and editing: Jae-Hyun LEE, Jung-Mi Oh.

Acknowledgements

This research was supported by a (Grant 21182MFDS295) from the Ministry of Food and Drug Safety from 2021 to 2022. Korean Genome Project data were provided by Korean Genomics Center in Ulsan National Institute of Science and Technology, Republic of Korea. This research was supported by a new faculty research seed money grant of Yonsei University College of Medicine for 2025 (2025-32-0023).

Ethics Statement

The present study was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System (approval number: 9-2020-0124). Written informed consent was obtained from all participants prior to their participation in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The genome metadata supporting the findings of this study have been deposited in the European Genome-phenome Archive (EGA) under the accession ID EGAD50000001660 (https://ega-archive.org/datasets/EGAD50000001660). Access to these data will be granted upon reasonable request and following data access committee approval, in accordance with ethical and privacy considerations. In addition, the analysis code and command-line workflows used in this study are openly available on GitHub at https://github.com/DAEUN-LEE94/cefaclor-anaphylaxis to support reproducibility and transparency.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Figure S1: Distribution of HLA Class I and Class II genotypes in 33 patients with cefaclor-induced anaphylaxis. **Figure S2:** Distribution of HLA Class I and Class II genotypes in 41 tolerant controls. **Table S1:** Demographic and baseline clinical characteristics of patients with cefaclor-induced anaphylaxis and tolerant controls. **Table S2:** One hundred sixty-four candidate variants associated with cefaclor-induced anaphylaxis identified by exome-wide association study. **Table S3:** Detailed gene lists and enrichment scores for the TNF-α signaling via NF-κB (M5890) pathway.