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Genome-wide association study of hypersensitivity skin reactions induced by nonionic iodinated contrast media

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Abstract

Background: In Taiwan, nonionic iodinated contrast media (ICMs) are commonly used but can occasionally cause severe side effects. The infrequency of these adverse events, coupled with the complexities in establishing direct causality, poses significant challenges for genetic research.

Objective: To investigate the genetic factors associated with skin reactions mediated by nonionic ICMs on a genome-wide scale.

Methods: A hospital-based cohort from the China Medical University Hospital biobank was utilized to conduct a comprehensive genome-wide association study (GWAS) using PLINK v1.9. The study incorporated two distinct cohorts: one based on adverse drug reaction (ADR) reports, capturing immediate reactions, and the other based on self-reports, which primarily reflected delayed reactions. Known loci were determined by the GWAS catalog. Fine mapping was conducted by FINEMAP to predict causal variants. Pathway enrichment analysis was performed by clusterProfiler to reveal the biological function of the identified genetic signatures.

Results: The ADR-based cohort included 120 cases and 3640 controls. GWAS identified 6 candidate risk loci, namely rs150515068, rs6847491, rs192044153, rs191908641, rs376660317, and rs368821335. The self-report-based cohort, consisting of 275 cases and 8338 controls, revealed 36 additional candidate risk loci. Fine mapping further identified 4 causal variants within each cohort. Pathway analysis showed that immediate HSR-related genes are linked to growth hormone response and signaling, while non-immediate HSR genes are involved in neurotransmission.

Conclusions: This study offers new perspectives on the genetic foundation of nonionic ICM-induced skin reactions within the Taiwanese population, suggesting that the genes contributing to immediate and non-immediate HSRs might have different functional roles.

Key word: Contrast Media, GWAS, ADR, hypersensitivity skin reaction, Pharmacogenomics

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Introduction

Contrast medium is a substance used to enhance the visibility of anatomic structures during medical imaging procedures, such as X-ray, computed tomography, magnetic resonance imaging, and ultrasound. Iodinated contrast media (ICMs) are the most commonly used contrast agents in the world, with over 80 million doses administered annually.[1] However, the administration of these contrast media depends on several variables, such as the indication, the region, and the patient's body weight. The appropriate dose typically ranges from 60 to 150 mL. Ionic and nonionic contrast media differ in terms of their viscosity and osmolality. Currently, nonionic ICMs are more commonly used because of the lower osmolality, which results in less damage to the cell membrane and fewer mild-to-moderate side effects.[1] Although both types of media have a comparable incidence of severe reactions, such reactions are rare.

Among the adverse reactions induced by contrast media are hypersensitivity reactions (HSRs) and chemotoxic reactions. Chemotoxic reactions vary depending on the dose and concentration of the ICM used, and mild symptoms can be managed with oral H1 antihistamine. However, HSRs are more unpredictable and tend to be life-threatening. Generally, HSRs are divided into two categories: immediate (acute) HSRs and nonimmediate (delayed) HSRs.[2] Immediate HSRs typically occur within an hour, while non-immediate HSRs develop gradually, spanning from hours to days or even weeks. Common symptoms of immediate HSRs are erythema, urticaria, and angioedema. The prevalence of immediate ICM-induced hypersensitivity reactions is approximately 0.02-3.1%.[2] In rare and severe cases, life-threatening signs such as anaphylactic shock have been reported. Without proper management, these reactions may result in prolonged illness or even death. Non-immediate HSRs are usually mild to moderate and generally resolve within a week.[3] The prevalence of non-immediate HSRs varies, but it is generally estimated to range from 0.5 to 23%.[2] Consequently, establishing a definitive causal relationship between ICMs and non-immediate HSRs is challenging, since many of these reactions are not directly related to contrast media. However, skin reactions are regarded as well-documented side effects of contrast media and are likely mediated by T-cell responses.[4]

ICM-induced HSRs are associated with multiple risk factors, including poorly controlled bronchial asthma, concomitant medication, rapid administration of ICMs, mastocytosis, autoimmune diseases, and viral infection.[5] Hence, the incidence of ICM-related hypersensitivity may be influenced by the types of ICM as well as a patient's age, sex, and geographical location. Genetic studies have revealed that human leukocyte antigen (HLA) alleles, namely *B*52:01*, *C*12:02*, and *DRB1*15:02*, were associated with an increased risk of ICM-related anaphylaxis in the Korean population.[6] Individuals who experienced anaphylaxis because of ICM exposure exhibited higher frequencies of these alleles compared to healthy individuals.[6] Similar findings were also reported in a study of 61 patients with ICM-induced hypersensitivity and 65 patients with ICM tolerance, where *HLA-B*58:01* was identified as a risk factor for ICM-induced immediate hypersensitivity.[7] Furthermore, Cha et al. reported that both a family history and an individual history of ICM-related HSRs are risk factors for HSRs, indicating potential genetic susceptibility within the Korean population.[8] While several genetic variants have been identified for adverse effects associated with contrast media, the sample size is quite limited. In this study, we conducted a comprehensive genome-wide association study (GWAS) aimed at identifying the genetic risk loci for ICM-induced skin reactions. Here, we examined two cohorts which were selected based on adverse drug reaction (ADR) reports and self-reports (**Figure 1**). ADR reports primarily focus on capturing immediate HSRs because they promptly occur after drug administration, whereas self-reports over three months hold the potential to compile a comprehensive profile of patients' non-immediate HSRs. This study aims to explore the genetic factors and biological mechanisms underlying the two types of ICM-induced HSR, providing insights into treatment strategies for both reactions.

Materials and Methods

Study design and participants

All participants for this study were sourced from China Medical University, utilizing a hospital-based cohort that allowed for the collection of detailed information on therapeutic drug responses and adverse drug reactions (ADRs) from patient medical records. The study focused on investigating skin reactions induced by intravenous nonionic ICMs. At China Medical University Hospital (CMUH), the only nonionic ICMs used are iohexol (V08AB02), ioversol (V08AB07), and iodixanol (V08AB09). Therefore, these three ATC codes were utilized to select individuals for analysis. Leveraging this framework, our study established two cohorts for a pharmacogenomics GWAS. The first cohort was formed based on a rigorous ADR reporting process. Inclusion criteria were: (1). ADR reports submitted within two weeks following the initial prescription of the media; (2). Documentation of skin rash or urticaria in the ADR report; (3). A Naranjo score exceeding 4 and/or a WHO-UMC causality score between 1 and 3. The assessment was performed by at least one clinical pharmacist and one physician at China Medical University Hospital to ensure accuracy and reliability. Controls were identified as subjects not meeting any of the above criteria, with a selection of 30 times the number of cases, matched for sex and age. The second cohort was derived from self-reported incidents, with inclusion criteria as follows: (1). Self-reported

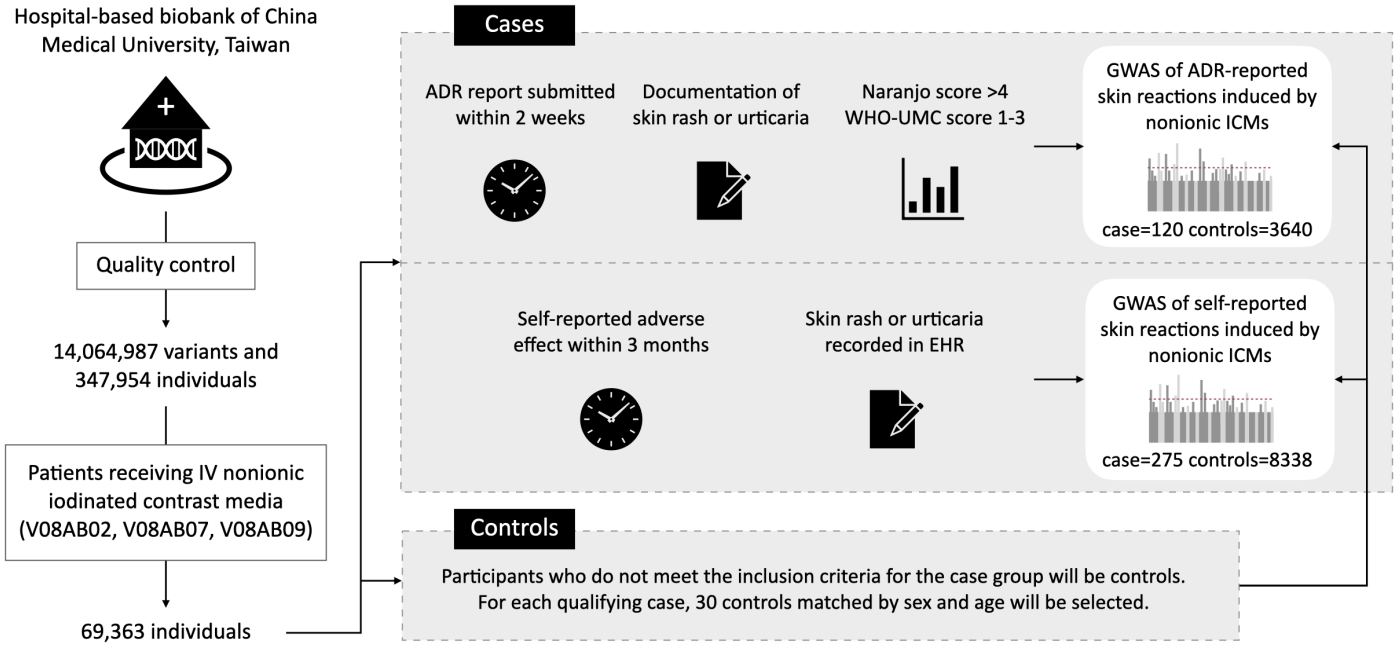


Figure 1. A graphical study design. Subjects who received intravenous (IV) nonionic ICM were initially included in the study. Two cohorts with cases from ADR reports and self-reports were further selected for GWAS. Subjects without adverse effects were defined as controls.

adverse effect noted in medical records within three months of the media's first prescription; (2). Documentation of skin rash or urticaria in the medical record. Similarly, controls for this cohort were subjects not fulfilling any of the above criteria, with a selection of 30 times the number of matched cases based on sex and age. Individuals under 18 years old were excluded from both cohorts.

Genotyping, data quality control steps, and imputation

Genotype data were generated from the genetic biobank of CMUH. Ethical approval of the study was granted by the Institutional Review Board of China Medical University (CMUH110-REC3-005 and CMUH111-REC1-176). Genotyping was performed using a TPMv1 array plate (Thermo Fisher Scientific, Santa Clara, CA, USA) designed by the Taiwan Precision Medicine Initiative (TPMI) project and the Academia Sinica, which includes approximately 714,461 probes. Variant calling was performed using Affymetrix Power Tools (Thermo Fisher Scientific). Quality control (QC) was conducted using PLINK 1.9.[9] Samples with ambiguous sex data and a call rate of < 0.9 were excluded. Related individuals (pihat value > 0.1875) and individuals with a heterozygosity rate three standard deviations (SDs) away from the mean were also excluded. All samples passed the ancestry check, with both PC1 and PC2 being within five SDs away from the mean. To conduct genotypic QC, SNPs with a call rate of < 0.9, a Hardy–Weinberg equilibrium p-value of $< 1 \times 10^{-6}$, and a minor allele frequency (MAF) of $< 1 \times 10^{-4}$ were excluded. Following QC, 686,432 variants and 347,954 individuals remained. We used SHAPEIT4 for phasing genotype data, followed by imputation using Beagle 5.2.[10] The accuracy of this imputation approach has been confirmed in our previous study using data from CMUH biobank.[12] The post-imputation variant QC exclusion criteria included an R^2 of < 0.3 and a posterior probability of < 0.9. Therefore, 14,064,987 variants and 347,954 individuals were included in the cohort. Additionally, we performed HLA genotype imputation using the HIBAG tool.[13] HLA alleles were predicted at a four-digit resolution using default settings. We employed next-generation sequencing (NGS)-based HLA typing data from 1012 individuals in the Taiwan Biobank (TWB) as our reference panel. Specifically, alleles of HLA genes associated with hypersensitivity —HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DPA1, and HLA-DPB1—were imputed in this study. Imputations with a posterior probability exceeding 0.9 were considered reliable.

Genome-wide association study (GWAS)

To identify variants associated with ICM-induced skin reactions, PLINK v1.9 was used to conduct a GWAS among the Taiwanese population. Logistic regression was used with an additive genetic model to conduct a case-control association test. The regression was adjusted for sex, age, PC1, PC2, PC3, and PC4 and considered variants with a p-value of $< 5 \times 10^{-8}$ as statistically significant. Manhattan plots and quantile–quantile (Q-Q) plots were constructed by using the R package "ggplot2".

Fine mapping

To refine the genetic associations identified in our GWAS, we performed a fine mapping analysis using FINEMAP with default parameters. This Bayesian statistical approach allowed us to calculate the posterior inclusion probabilities (PIPs) of causality for each SNP within the genomic regions of interest (500kb), aiming to pinpoint the most likely causal variants. CMU biobank-based LD matrix was applied. Variants with PIP exceeding 0.9 were considered causal.

Functional annotation and pathway enrichment analysis

To identify known genetic loci associated with hypersensitivity reactions, we utilized the GWAS catalog, accessed through the R package "gwasrapidd" (accessed on January 30, 2024). Our query focused on identifying variants that have been previously reported to allergic disease, drug allergy, hypersensitivity reaction disease, and drug hypersensitivity syndrome. The Ensembl Variant Effect Predictor (VEP) was used to annotate variant consequences. The R package "clusterProfiler" was applied to conduct GO,[14] KEGG,[15] Reactome,[16] and WikiPathways[17] enrichment analyses. Pathways that achieved a false discovery rate (fdr) of less than 0.05 were considered significant. Finally, data visualization was performed by R version 4.2.

Results

Cohort selection and subject baseline characteristics

The study focused on individuals who developed skin rash or urticaria after being exposed to nonionic ICMs. We analyzed a comprehensive biobank dataset spanning from 1992 to 2020, encompassing a total of 69,363 individuals. We identified two distinct cohorts based on the criteria described. The first cohort was composed of 120 cases with ADR reported, alongside 3,640 controls matched for sex and age. The second cohort consisted of 275 cases based on self-reports, along with 8,338 sex- and age-matched controls. Cases selected from ADR reports revealed a higher occurrence of urticaria (57.5%) compared to rash (42.5%). In contrast, in the cohort of self-reported skin reactions, there was a notably higher incidence of skin rash, observed in 89.8% of cases, while urticaria was less common, comprising only 10.2% of cases (**Table 1**).

Genetic risk loci for ADR-reported skin reactions mediated by ICM

ADR reports capture individuals with a high likelihood of experiencing immediate HSR. In our cohort, we discovered six significant variants, specifically rs150515068 (1p22.1), rs192044153 (4p15.33), rs191908641 (4p15.33), rs6847491 (4p16.3), rs376660317 (14q31.3), and rs368821335 (17p13.1) (**Figure 2A**, **Table 2**). These variants are located in intronic or intergenic regions of the genome. Within the ADR-reported risk variants, four—rs150515068, rs192044153,rs368821335, and rs376660317—were notable, with exceptionally high PIPs surpassing 0.9 (**Table 2, Supplementary Figure 1**). This indicates a strong likelihood that these variants are causal to ICM-induced skin reaction. One of the causal variants, rs368821335, located within the *STX8* gene, emerged as the most significant. According to the GWAS catalog, none of these six variants have previously been reported in association with human diseases. However, it's worth mentioning that rs150515068 is located within the cytoband 1p22.1, a region that has been linked to allergic diseases.[18] A Q-Q plot of this GWAS can be found in **Supplementary Figure 2A**.

Genetic risk loci for self-reported skin reactions mediated by ICM

Self-reported ADR from medical records is more likely to reflect non-immediate HSR. Accordingly, the study applied a second cohort. GWAS identified 36 significant variants located within 13 distinct cytobands, including 5q23.1, 5q23.3, 5q31.1, 6q12, 7p14.3, 7q33, 9q21.1, 9q22.33, 9q31.1, 12p12.3, 15q15.1, 15q24.2, and 19q13.31 (**Figure 2B**, **Table 3**). We observed a significant cluster on chromosome 5, with the top variants being rs768225509 on 5q23.3, chr5:131323533 on 5q31.1, and rs149954748 on 5q23.1 cytoband. Notably, the 5q23.1, 5q31.1, and 15q15.1 cytobands have previously been reported to be associated with allergic diseases.[18,19] These 36 variants were first identified to be associated with ICM-induced skin reactions. In addition, four out of the 36 significant GWAS variants, specifically rs185628691, rs78366360, rs185987258, and rs551686399, also exhibited PIPs above 0.9 (**Table 3**, **Supplementary Figure 3**). Although the significant loci identified in the two cohorts did not overlap, the results suggest distinct genetic factors underlying the two types of HSR. A Q-Q plot of this GWAS can be found in **Supplementary Figure 2B**.

Table 1. Baseline characteristics of the participants included in this GWAS.

	Cohort 1 (ADR report)			Cohort 2 (self-report)		
	Case	Control	*p*-value	Case	Control	*p*-value
Number	120	3640	—	275	8338	—
Age (years, mean ± SD)	58.69 ± 16.75	58.19 ± 16.29	0.999	56.59 ± 13.38	58.69 ± 16.75	0.999
Sex (male, %)	44.3%	52.2%	0.063	57.5%	52.2%	0.3
Rash (%)	42.5%	—	—	89.8%	—	—
Urticaria (%)	57.5%	—	—	10.2%	—	—

p-values were calculated by T-test for continuous variables and Chi-square test for binary variables.

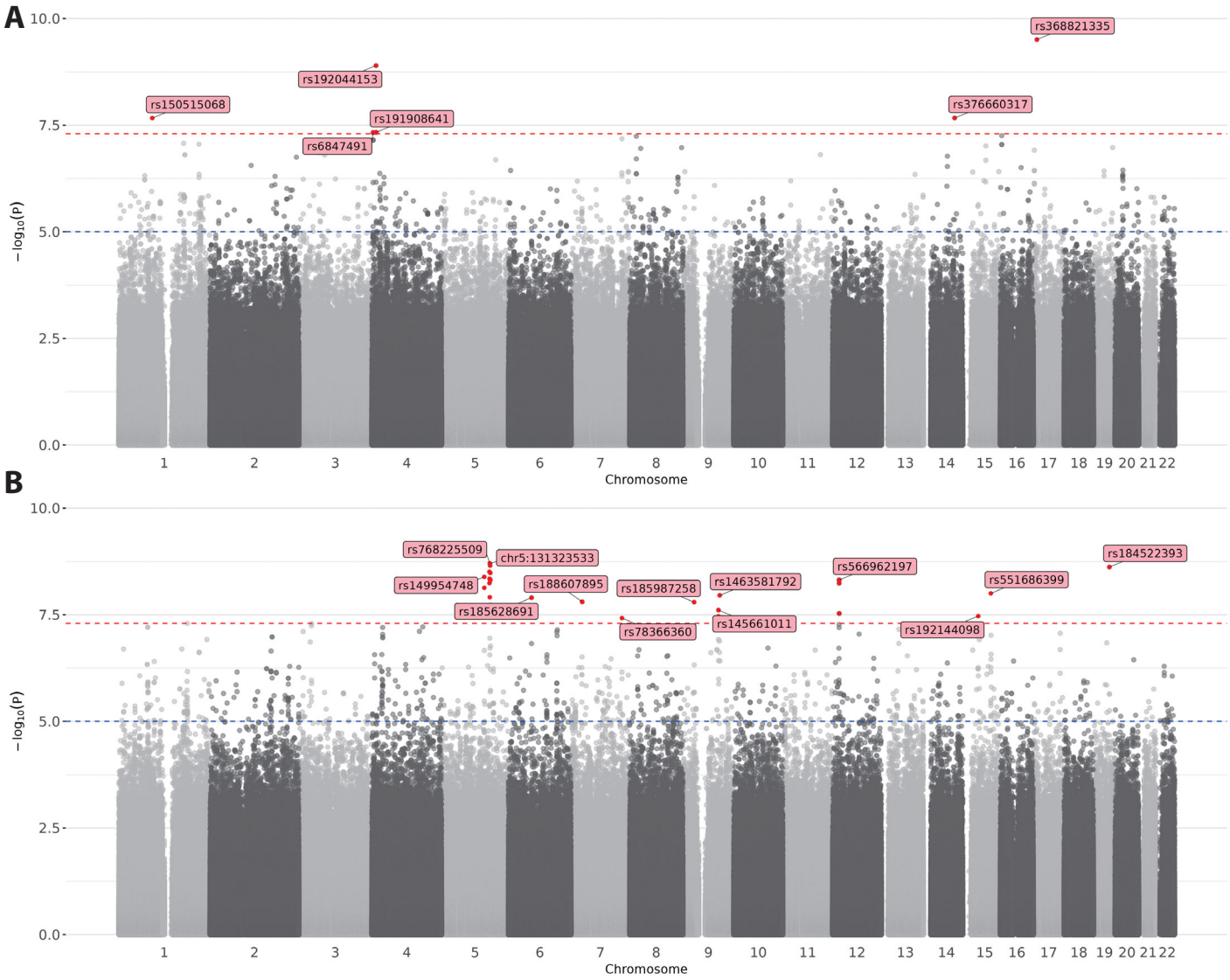


Figure 2. Manhattan plot of nonionic ICM-induced skin reactions based on ADR-reports (A) and self-reports (B). The threshold of genome-wide significance ($p = 5 \times 10^{-8}$) is displayed by the red line, and the threshold of genome-wide suggested significance ($p = 1 \times 10^{-5}$) is displayed by the blue line. In the self-reported cohort, we displayed only the most significant variants identified within each cytoband.

Table 2. Genetic risk loci for ADR-reported ICM-induced skin reactions.

SNP	Chr.	Pos.	Ref.	Eff.	Gene	Region	EAF_cohort	EAF_case	EAF_control	EAF_all	Odds Ratio	*p*-value	PIP
rs368821335	**17**	**9376508**	**C**	**T**	***STX8***	**intronic**	**0.0031**	**0.0333**	**0.0021**	**0.003**	**17.54**	**3.11×10^{-10}**	**0.9997**
rs192044153	**4**	**11563784**	**C**	**T**	-	**intergenic**	**0.0059**	**0.0417**	**0.0047**	**0.0062**	**10.18**	**1.27×10^{-9}**	**0.9582**
rs6847491	4	3482905	G	C	*DOK7*	intronic	0.0024	0.025	0.0017	0.0023	16.11	4.71×10^{-8}	0.3086
rs191908641	4	11682196	C	T	-	intronic	0.0073	0.0424	0.0061	0.0078	7.59	4.66×10^{-8}	0.0219
rs150515068	**1**	**91684469**	**G**	**A**	***TGFBR3***	**intronic**	**0.011**	**0.0551**	**0.0095**	**0.0109**	**5.91**	**2.16×10^{-8}**	**0.994**
rs376660317	**14**	**84788172**	**C**	**T**	-	**intergenic**	**0.0045**	**0.0336**	**0.0035**	**0.0048**	**11.37**	**2.15×10^{-8}**	**0.9873**

Chr., chromosome; Pos., position (GRCh38); Ref., reference allele; Eff., effect allele; EAF_cohort, effect allele frequency in the ADR report cohort; EAF_case, effect allele frequency of cases in the ADR report cohort; EAF_control, effect allele frequency of controls in the ADR report cohort; EAF_all, effect allele frequency in CMUH genetic biobank; PIP, fine mapping posterior inclusion probabilities. Causal variants (PIP > 0.9) were shown in bold.

Table 3. Genome-wide significant variants identified for self-reported ICM-induced skin reactions.

SNP	Chr.	Pos.	Ref.	Eff.	Gene	Region	EAF_cohort	EAF_case	EAF_control	EAF_all	Odds Ratio	*p*-value	PIP
rs551686399	**15**	**76213148**	**G**	**T**	***ETFA***	**downstream**	**0.0034**	**0.0202**	**0.0029**	**0.0037**	**7.5**	**9.95×10^{-9}**	**0.97**
rs189698134	5	116005861	C	A	*LVRN*	intronic	0.0013	0.0128	0.0009	0.0013	15.08	7.41×10^{-9}	0.361
rs573131008	12	18055767	G	A	-	intergenic	0.0062	0.0275	0.0055	0.0048	5.33	5.76×10^{-9}	0.249
rs765282819	5	130114384	A	G	*CHSY3*	intronic	0.0022	0.0165	0.0018	0.0024	9.72	5.71×10^{-9}	0.23
rs761746205	5	132420022	CAT	C	-	intronic	0.0022	0.0167	0.0018	0.0028	9.77	4.89×10^{-9}	0.171
rs566962197	12	18012801	C	T	-	intergenic	0.0061	0.0275	0.0054	0.0047	5.38	4.86×10^{-9}	0.295
rs191549507	12	18016235	G	A	-	intergenic	0.0061	0.0275	0.0054	0.0047	5.38	4.86×10^{-9}	0.294
rs748064964	5	132380091	C	T	*SLC22A5*	intronic	0.0022	0.0167	0.0018	0.0028	9.78	4.73×10^{-9}	0.176
rs539932994	5	130400041	C	T	-	regulatory	0.0022	0.0166	0.0018	0.0027	9.84	4.54×10^{-9}	0.266
rs149954748	5	115980057	A	G	*LVRN*	intronic	0.0012	0.0128	0.0008	0.0013	17.02	4.10×10^{-9}	0.639
rs760982815	5	132495234	C	T	-	intronic	0.0022	0.0167	0.0017	0.0028	10.1	3.30×10^{-9}	0.379
rs182433192	5	130021551	A	G	*CHSY3*	intronic	0.0033	0.0202	0.0027	0.003	7.65	3.10×10^{-9}	0.411
rs189371202	19	43094769	G	A	-	intronic	0.0038	0.0221	0.0032	0.0038	7.17	2.42×10^{-9}	0.331
rs183173099	19	43092293	T	A	-	intronic	0.0038	0.0221	0.0032	0.0038	7.17	2.41×10^{-9}	0.336
rs184522393	19	43060959	T	A	*PSG2*	downstream	0.0038	0.0221	0.0032	0.0038	7.16	2.40×10^{-9}	0.339
rs1299487791	5	131931105	C	T	*MEIKIN*	intronic	0.0021	0.0166	0.0016	0.0027	10.46	2.23×10^{-9}	0.453
rs1031467188	5	131258884	T	C	-	downstream	0.0021	0.0166	0.0016	0.0027	10.51	2.02×10^{-9}	0.29

Table 3. (Continued)

SNP	Chr.	Pos.	Ref.	Eff.	Gene	Region	EAF_cohort	EAF_case	EAF_control	EAF_all	Odds Ratio	*p*-value	PIP
chr5:131323533	5	131323533	C	CCT	-	-	0.0021	0.0166	0.0016	0.0027	10.51	2.01×10^{-9}	-
rs768225509	5	131042958	AT	A	-	intergenic	0.0021	0.0166	0.0016	0.0027	10.52	1.96×10^{-9}	0.324
rs78366360	**7**	**138461267**	**G**	**C**	***TRIM24***	**intronic**	**0.0043**	**0.0208**	**0.0038**	**0.0044**	**5.8**	**3.80×10^{-8}**	**0.905**
rs145183725	9	96643624	C	T	*PRXL2C*	intronic	0.0015	0.0129	0.0012	0.0015	12.23	3.44×10^{-8}	0.355
rs192144098	15	42054702	C	T	*PLA2G4E*	upstream	0.0021	0.0147	0.0016	0.0018	9.49	3.40×10^{-8}	0.831
rs371864045	5	131642570	A	T	-	intronic	0.01	0.0355	0.0092	0.0115	3.98	3.34×10^{-8}	0.133
rs372228929	5	131673691	G	A	-	intronic	0.01	0.0355	0.0092	0.0115	3.98	3.33×10^{-8}	0.12
rs1223694681	12	18144276	GA	G	*RERGL*	intronic	0.0059	0.0256	0.0053	0.0049	5.15	2.94×10^{-8}	0.052
rs987884634	12	18144283	A	G	*RERGL*	intronic	0.0059	0.0256	0.0053	0.0049	5.15	2.94×10^{-8}	0.052
rs145661011	9	96666172	C	G	-	intergenic	0.0015	0.0129	0.0011	0.0015	13	2.45×10^{-8}	0.488
rs761489021	5	130327632	T	C	-	intergenic	0.0025	0.0165	0.002	0.0028	8.52	2.13×10^{-8}	0.058
rs547750106	5	132588472	T	G	*RAD50*	intronic	0.003	0.0184	0.0025	0.0034	7.71	1.86×10^{-8}	0.076
rs185987258	**9**	**30549045**	**A**	**G**	***LINC01242***	**intronic**	**0.0009**	**0.011**	**0.0005**	**0.0007**	**20.99**	**1.60×10^{-8}**	**0.958**
rs140854638	7	30318328	A	G	*ZNRF2*	intronic	0.0009	0.011	0.0005	0.0008	20.88	1.58×10^{-8}	0.483
rs188607895	7	30286136	C	T	*ZNRF2*	intronic	0.0009	0.011	0.0005	0.0008	20.89	1.57×10^{-8}	0.485
rs185628691	**6**	**63223283**	**G**	**C**	***SPTLC1P3***	**upstream**	**0.002**	**0.0149**	**0.0016**	**0.002**	**10.2**	**1.26×10^{-8}**	**0.904**
rs1489438627	5	130939939	T	G	-	intronic	0.0024	0.0167	0.0019	0.003	8.88	1.23×10^{-8}	0.053
rs1463581792	9	100161623	A	C	*INVS*	intronic	0.0013	0.013	0.001	0.001	13.77	1.11×10^{-8}	0.5
rs1484335802	9	100162026	GAAGAA	G	*INVS*	intronic	0.0013	0.013	0.001	0.001	13.77	1.11×10^{-8}	0.499

Chr., chromosome; Pos., position (GRCh38); Ref., reference allele; Eff., effect allele; EAF_cohort, effect allele frequency in the self-report cohort; EAF_case, effect allele frequency of cases in the self-report cohort; EAF_control, effect allele frequency of controls in the self-report cohort; EAF_all, effect allele frequency in CMUH genetic biobank; PIP, fine mapping posterior inclusion probabilities. Causal variants (PIP > 0.9) were shown in bold.

HLA alleles associated with hypersensitivity allergic reactions

To better understand the role of the HLA gene in allergic reactions, we performed HLA genotype imputation at four-digit resolution using Taiwanese-specific reference panels. We conducted chi-square tests to evaluate the association between HLA allele types and case/control status in both cohorts. This additional analysis identified significant associations for multiple HLA alleles, including *HLA-A*31:01*, *HLA-DPB1*09:01*, *HLA-C*03:04*, *HLA-DQB1*06:09*, and *HLA-DRB1*13:02* (**Supplementary Figure 4**). It's noteworthy that these findings remained significant at a false discovery rate (FDR) of 0.05.

Functional annotations

To investigate the functionality of loci associated with ICM-induced skin reactions, we compiled a list of variants and performed annotations for genetic consequences. For the two cohorts, we annotated 620 and 952 variants, respectively, with genome-wide suggestive association signals ($p < 1 \times 10^{-5}$; **Supplementary Figure 2**). Consequently, we identified 53 overlapping suggestive variants within 46 genes between the two cohorts (**Supplementary Figure 5A-B**). Most of the suggestive variants were in intronic and intergenic regions (80.6% for Cohort 1 and 86.7% for Cohort 2; **Supplementary Figure 5C-D**).

Pathway enrichment analysis

To investigate the biological function of genes associated with ICM-induced skin reactions, we converted genes with GWAS suggestive variants from both cohorts into Entrez gene IDs for pathway enrichment analysis. Accordingly, 229 genes from cohort 1 and 390 genes from cohort 2 were evaluated for enrichment in the Gene Ontology Biological Process (GOBP), KEGG Pathway, Reactome Pathway, and WikiPathways databases. Key findings for ADR-reported cases include: GOBP analysis highlighted genes' involvement in growth hormone response and signaling (**Figure 3A**). KEGG analysis showed enrichment in pathways related to long-term depression and nutrient metabolism (**Figure 3B**). For self-reported cases, GOBP results revealed enrichment in neurotransmission (**Figure 3E**), with KEGG pathways highlighting cortisol synthesis and secretion, glutamatergic synapse, phospholipase D signaling pathway, and Cushing syndrome (**Figure 3F**). Reactome pathway showed that SARS-CoV-2 modulates autophagy was the top pathway with the largest rich factor (**Figure 3G**). However, after correcting for false discovery rates, none of these findings remained statistically significant. Nevertheless, the results implied that the biological mechanisms behind immediate and non-immediate HSR could involve distinct pathways.

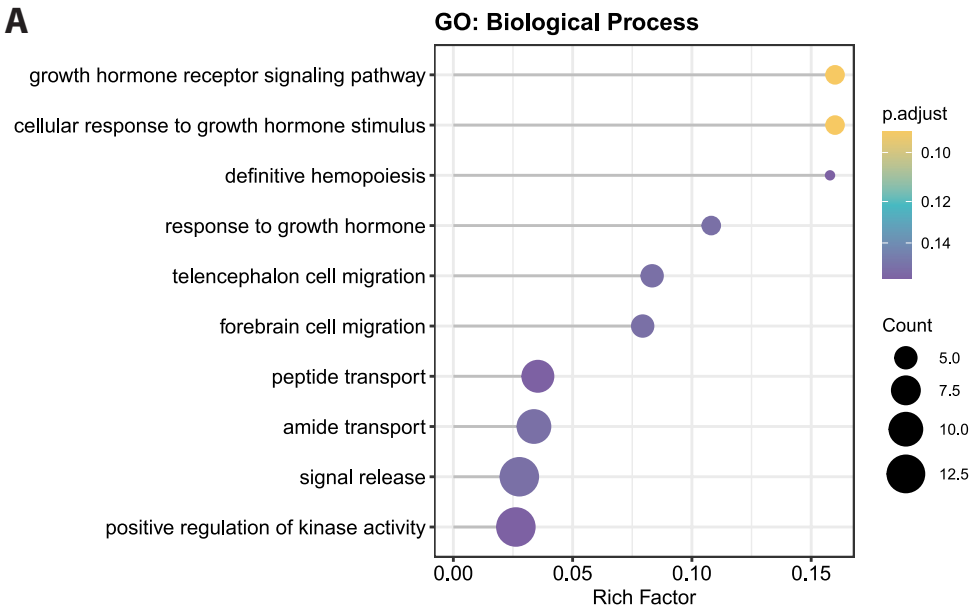


Figure 3. Pathway enrichment analysis of GWAS suggestive genes for ICM-induced skin reactions based on ADR reports (A–D) and self-reports (E–H). The top 10 enriched results were listed for each database and displayed by the order of rich factors. The Rich factor is defined as the ratio of input genes that are annotated in a term to all genes that are annotated in the same term.

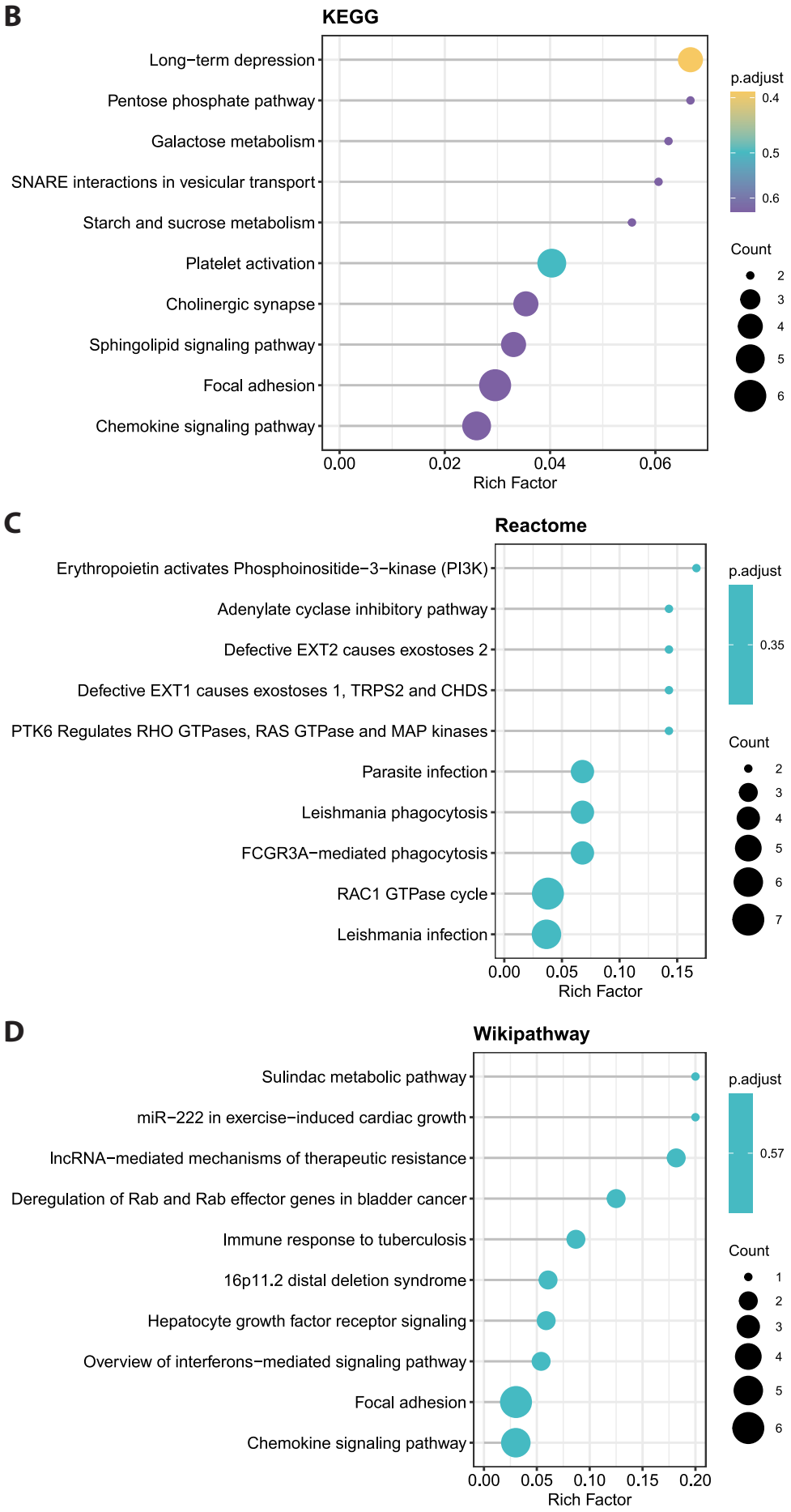
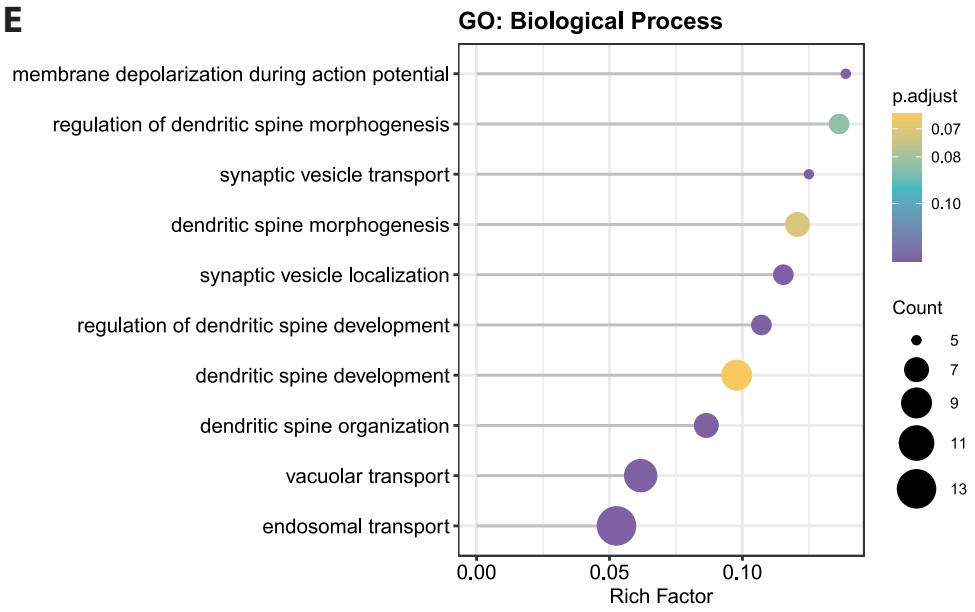
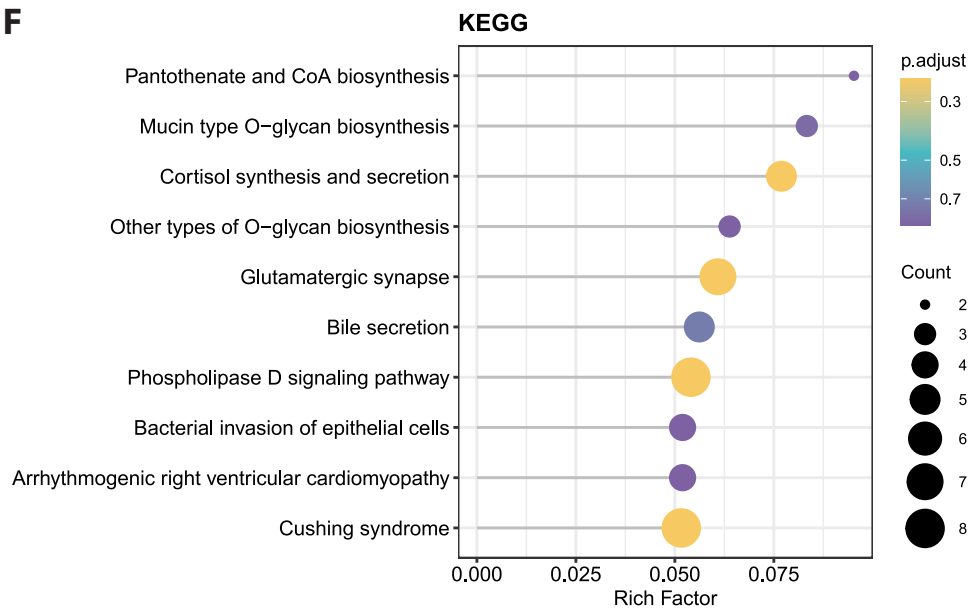


Figure 3. (Continued)





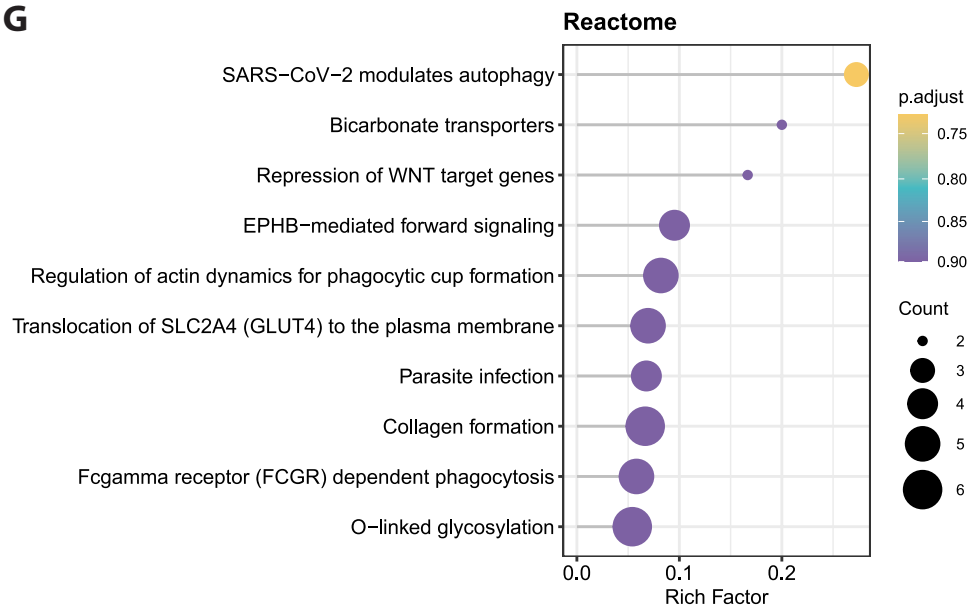


Figure 3. (Continued)

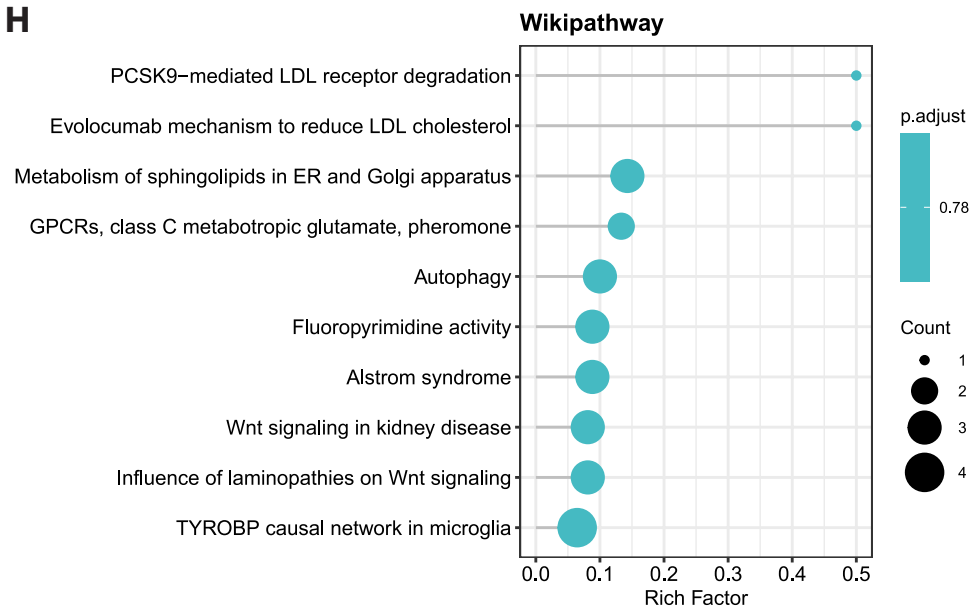


Figure 3. (Continued)

Discussion

Establishing the causal relationship between nonionic ICMs and skin reactions poses a challenge due to the scarcity of such adverse events. To overcome this, we utilized a powerful hospital-based biobank of CMUH that linked the genetic data to the electronic medical records of the participants. In this study, our assessment of causality relied on both ADR reports and self-reports. Given the variance in the immediacy of skin reaction reporting between the two cohorts, it is posited that the findings from the ADR-reported cohort more accurately reflect the underlying mechanisms of immediate HSR, whereas those from the self-reported cohort are indicative of non-immediate HSR. Research has demonstrated that immediate HSRs to contrast media are linked to histamine release, likely via an IgE-mediated pathway.[20] An increase in activation of mast cells and basophils has been observed in immediate HSRs.[21] This activation may result from IgE-dependent pathway, complement-dependent pathway, or direct membrane effect of ICM, suggesting a mechanically heterogeneous nature of the disease. On the other hand, the pathogenesis of nonimmediate reactions to ICM is linked to T-cell involvement, evidenced by clinical similarities to other T-cell-mediated drug allergies and an increased incidence of allergic reactions upon re-exposure to ICM.[22] Immunohistological studies and flow cytometry experiments have confirmed the presence of T-cell infiltration in the skin following exposure to ICM.[23]

Defining an ADR case is a rigorous process that involves multiple procedures to ensure accuracy and reliability. It requires comprehensive data collection and a careful review of previous pharmacological reports. In addition, multiple factors should be considered during this process, such as the duration between reaction and drug intake, patient characteristics, drug interactions, and patient compliance. Therefore, both the Naranjo algorithm and/or the WHO-UMC causality scale were employed to ensure the ADR assessment reached at least the 'possible' level of causality in our ADR-reported cohort. Each of the confirmed cases was validated by at least one clinical pharmacist and one physician. In our cohort, the prevalence of ADR-reported HSR was 0.123% (120/97281), while the prevalence of self-reported HSR was 0.283% (275/97281). The prevalence of adverse reactions in both cohorts was much lower than the reported prevalence for ionic ICMs, which is approximately 5% to 12%.[24] Despite the rarity of such cases, we identified six candidate variants associated with ICM-induced skin reactions, namely rs368821335 in *STX8*, rs6847491 in *DOK7*, rs150515068 in *TGFBR3*, rs192044153, rs191908641, and rs376660317. According to the GWAS catalog, none of these variants are previously associated with human disease. *STX8* is a member of the syntaxin family that has been linked to endosomal protein trafficking.

Golebiewska et al. showed that platelet dense granule secretion involves the participation of *STX8*, and the *STX8*-mediated pathway plays a role in enhancing thrombus stabilization.[25] The variant rs6847491 is an intronic variant on *DOK7*, which encodes a protein that plays an essential role in neuromuscular synapse formation. Mutations in the gene are the major cause of congenital myasthenia.[26] Furthermore, *DOK7* inhibits the proliferation, migration, and invasion of breast cancer cells through the PI3K/PTEN/AKT pathway.[27] However, the role of *DOK7* in the pathogenesis of ICM-induced skin rash is unclear. Our research highlighted rs150515068 as a risk variant with a high probability of being causal (PIP = 0.994). This variant is located on the *TGFBR3* gene, which encodes the TGF-β type III receptor. TGF-β pathway regulates immune responses, cell proliferation, and inflammation. Genetic variants of *TGFBR3* have been linked to Marfan syndrome,[28] Vogt–Koyanagi–Harada disease, and Behçet's disease.[29] Kim et al. reported that *TGFBR3* variation is associated with airway inflammation and hyperreactivity.[30] Since immediate hypersensitivity reactions are considered to be driven by histamine release, TGFBR3 may regulate the immune system to maintain immune response homeostasis. Variations in TGFBR3 could potentially disrupt this balance. This connection is particularly relevant in the context of skin reactions to nonionic ICM, as the underlying mechanisms may involve similar dysregulated inflammatory and immune response pathways.

To access the genetic factors underlying non-immediate HSR, we selected a second cohort of patients with self-reported reactions. This larger cohort identified 36 significant variants. The majority of these variants are located on chromosome 5, encompassing genes such as *LVRN*, *CHSY3*, *MEIKIN*, *SLC22A5*, and *RAD50*. FFine mapping of the association predicted another four causal variants on chromosome 6, 7, 9, and 15. Notably, we identified *PLA2G4E* as a risk gene. *PLA2G4E*, along with *PLA2G2F* and *PLA2G4D*, plays a significant role in the pathophysiology of hypersensitivity skin reactions, as observed in conditions like psoriasis and Pityriasis Rubra Pilaris (PRP).[31] These phospholipases, primarily expressed in the epidermis are key contributors to proinflammatory processes. Gene silencing experiments targeting these three PLA2 enzymes have demonstrated their pivotal roles in modulating immune responses and epidermal thickness, thereby underscoring the importance of PLA2s in regulating epidermal barrier homeostasis and inflammation. Additionally, *PSG2* is known to encode glycoproteins that play roles in modulating immune responses, particularly in the context of pregnancy. These proteins, synthesized by placental trophoblasts and released into the maternal bloodstream, can potentially regulate maternal immunity by influencing the expression of cytokines and chemokines.[32] Further studies are required to investigate the roles of these genes in the development or modulation of hypersensitivity skin reactions. It is noteworthy that none of the significant genetic variants were found to be shared between the two cohorts. Pathway analysis of the two cohorts also showed distinct results, suggesting that ADR-reported skin reactions and self-reported skin reactions might be regulated through different immune responses.

Previous studies have identified certain HLA alleles, such as *HLA-B*52:01*, *HLA-C*12:02*, *HLA-DRB1*15:02*, and *HLA-B*58:01*, to be associated with hypersensitivity reactions to contrast media.[6,7] However, these findings were primarily derived from South Korean populations with limited sample sizes. In contrast, our study boasts the largest sample size to date. Our investigation revealed several HLA alleles linked to contrast media hypersensitivity, including *HLA-A*31:01*, *HLA-C*03:04*, *HLA-DPB1*09:01*, *HLA-DQB1*06:09*, and *HLA-DRB1*13:02*. Notably, *HLA-A*31:01* and *HLA-C*03:04*, classified under MHC class I, have previously been associated with drug hypersensitivity reactions, particularly to carbamazepine.[33,34] Although MHC class II alleles, such as *HLA-DPB1*09:01*, *HLA-DQB1*06:09*, and *HLA-DRB1*13:02*, have not been commonly reported in association with contrast media hypersensitivity or drug reactions, they are strongly linked to immune disorders like systemic lupus erythematosus.[35] Given the limited replication of prior studies and the potential for differences in HLA allele types across populations, our findings may represent a novel avenue for further exploration.

There are several limitations in this study. The primary limitation is the relatively small number of cases involving ICM-induced skin reactions. The availability of cases was directly tied to the incidence rates observed in practice, reported to be as low as 0.02%.[2] Moreover, ADR reports primarily capture severe cases, with moderate to mild cases likely underreported. Similarly, the self-reported cases may also be underestimated, as patients do not always return for follow-ups to document their conditions. Consequently, inflation factors were calculated to be 0.664 for the ADR-reported cohort and 0.773 for the self-reported cohort, which may suggest false negatives due to limited power. To address this limitation, we performed a post-hoc power analysis using the G*Power software, applying logistic regression with an assumed odds ratio of 1.3 and a type I error rate of 0.05. This yielded a power of 0.99 for the self-reported cohort and 0.79 for the ADR-reported cohort, revealing the smaller ADR cohort size resulted in lower power. Nonetheless, both groups achieved or approached a power of 0.8, suggesting our study had adequate power to detect significant effects. Secondly, we were unable to rule out the potential influence of drug interactions, which may have introduced bias, especially in the self-reported data. Self-reported data, while valuable, may introduce variability due to subjective interpretations of symptoms or recall biases, potentially affecting the consistency of genetic associations identified.

In conclusion, the key strengths of this study include access to detailed therapeutic drug response data from medical records and a rigorous case selection process based on ADR reports. This approach ensures that the cases examined were truly indicative of the reactions to ICM. Additionally, the study investigated genetic risk variants on a genome-wide scale, extending the findings beyond previous research that primarily focused on the HLA region. We successfully identified six candidate genome-wide significant variants associated with hypersensitivity skin reactions triggered by nonionic ICMs, namely rs150515068, rs6847491, rs368821335, rs192044153, rs191908641, and rs376660317. Furthermore, the inclusion of a secondary cohort based on self-reported cases was crucial in identifying an additional 36 genome-wide significant variants associated with ICM-induced skin reactions. We found that the genes involved in immediate and delayed HSRs have different functions. This study offers fresh insights into the genetics behind skin reactions caused by nonionic ICMs in the Taiwanese population.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Informed consent

This study obtained approval from the Institutional Review Board (IRB) for human experimentation. Deidentified genetic and clinical data were obtained with informed consent from the patients. Ethical approval of the study was granted by the Institutional Review Board of China Medical University Hospital (CMUH110-REC3-005 and CMUH111-REC1-176).

Acknowledgments

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Data availability

The data that support the findings of this study are available from the China Medical University Genetic Biobank but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the China Medical University Genetic Biobank. GWAS summary statistics for the ADR-reported cohort are available on LocusZoom: https://my.locuszoom.org/gwas/94849/?token=b665dbfd3b6c4749877125942b9beffb. GWAS summary statistics for the self-reported cohort are available on LocusZoom: https://my.locuszoom.org/gwas/306470/?token=ec97db1b2d0e48f19024053ffedbebb0

Code availability

The underlying code for this study is not publicly available for proprietary reasons.

Contributions

- M.R.L., T.Y.L., W.C.C., and F.J.T. contributed to the design of this study.
- T.Y.L. organized patients' medical information and performed the GWAS. M.R.L. performed the post-GWAS analyses and was a major contributor to writing the manuscript.
- H.Y.H. and Y.W.H. collected and organized the adverse drug reaction (ADR) report data.
- W.H.C. provided support in statistical coding.
- P.D.P. and P.P.L. contributed to composing the introduction section.
- W.C.C. and F.J.T. supervised and supported this project.
- W.C.C. and F.J.T. reviewed the manuscript and provided valuable feedback.
- All authors approved the submission of this manuscript.

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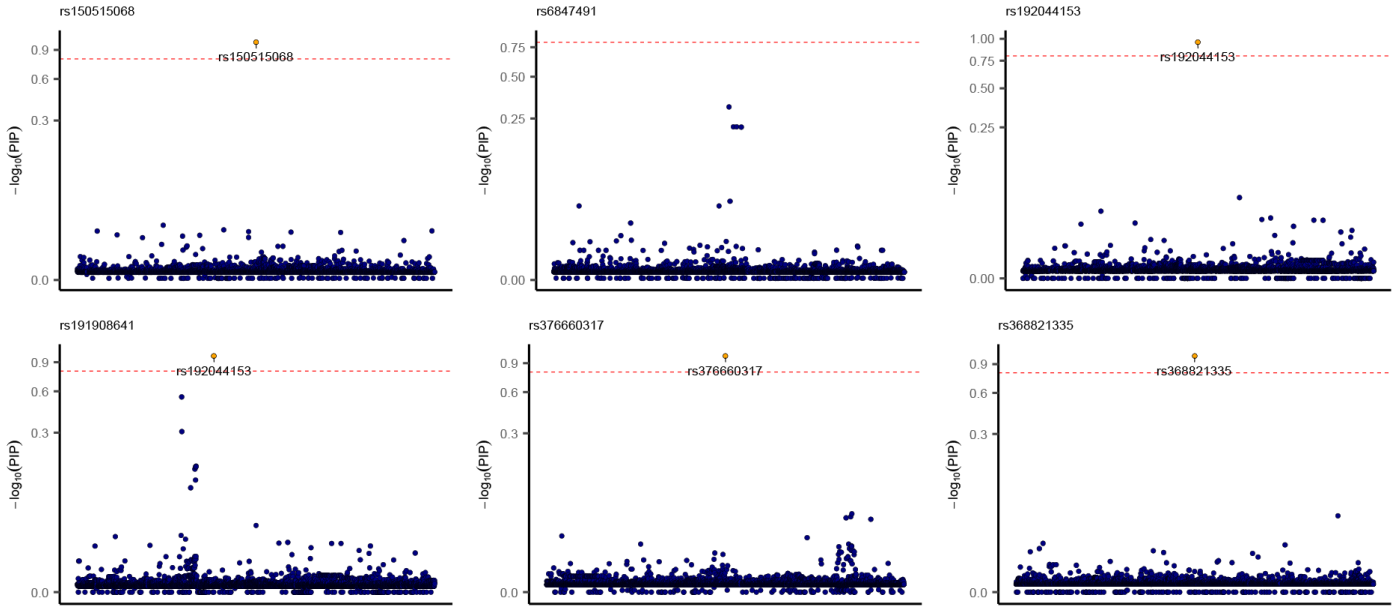
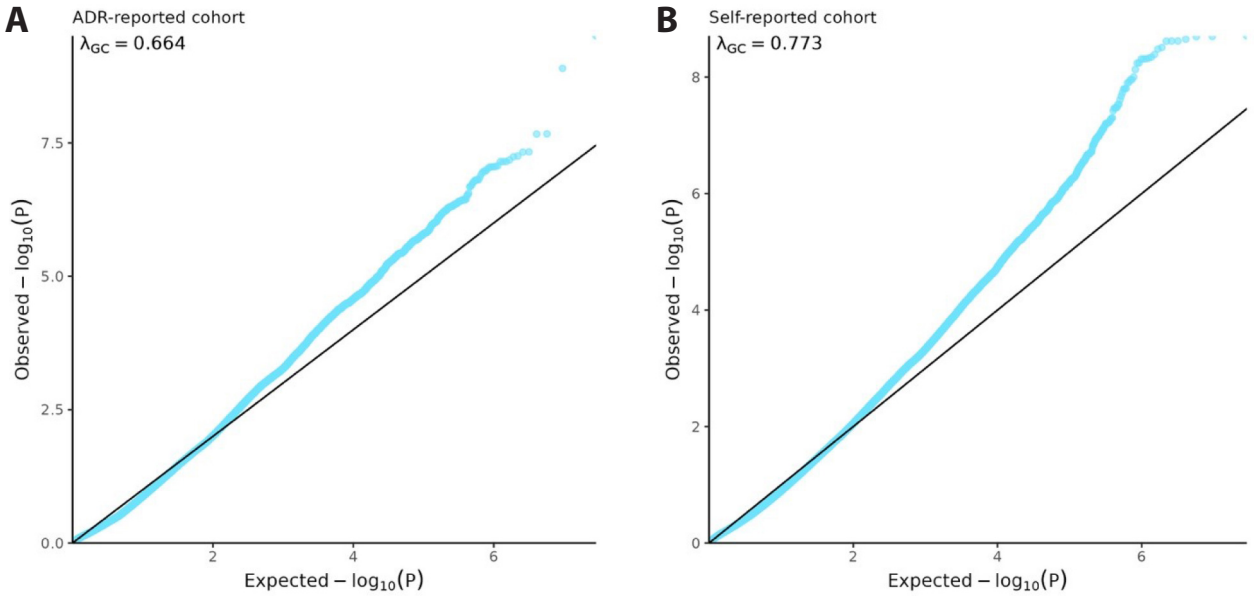
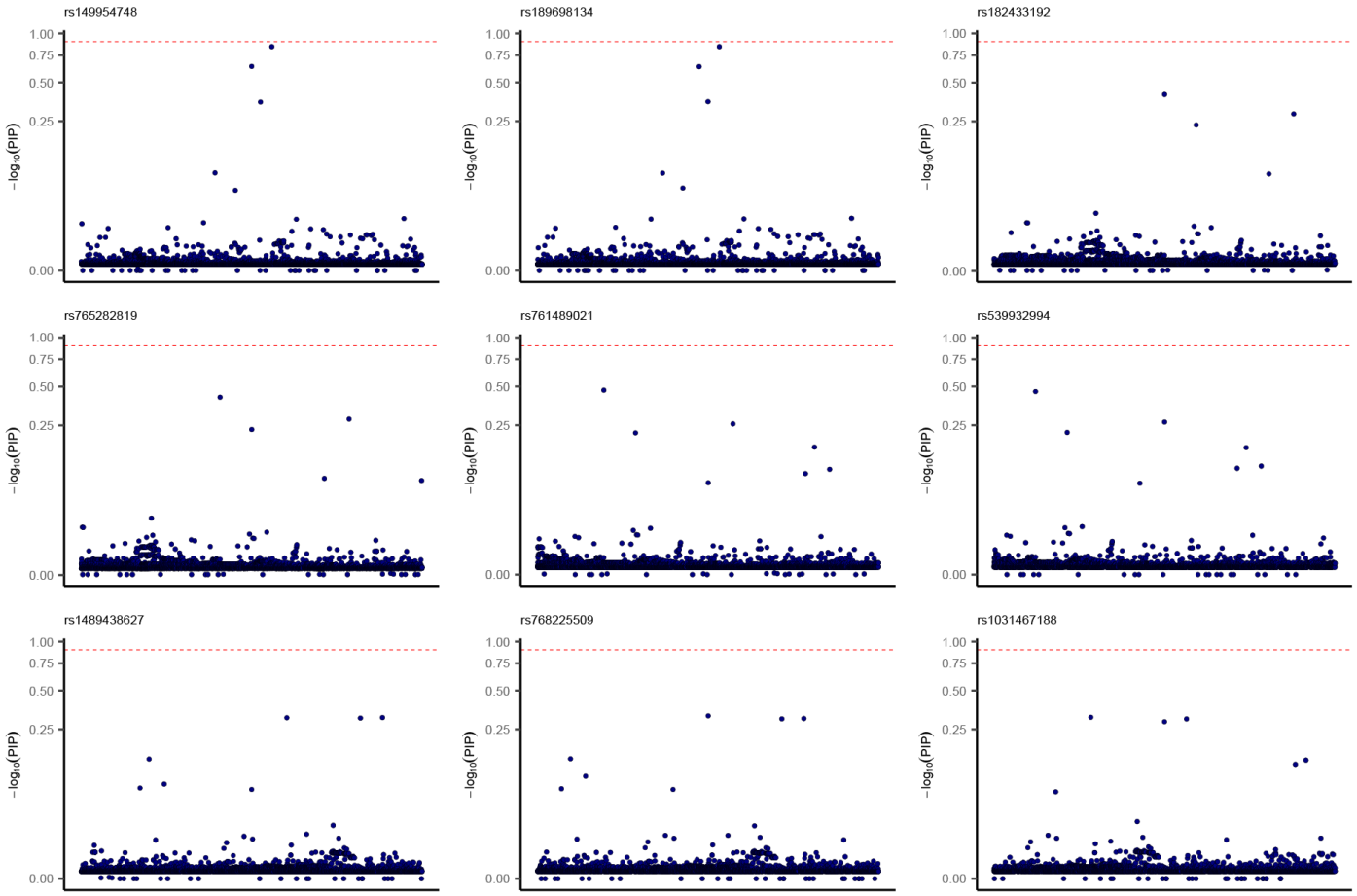
Supplementary material



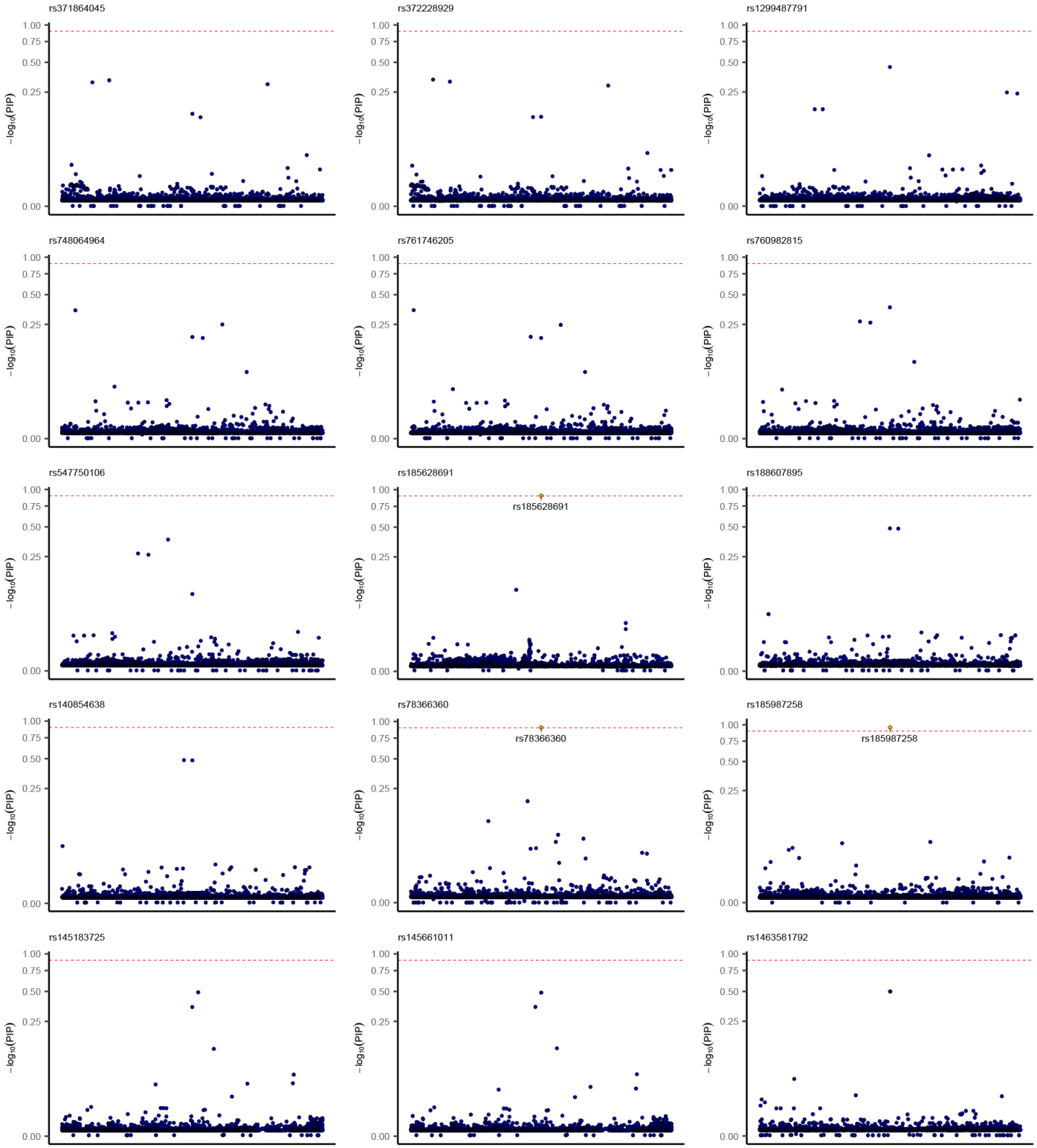
Supplementary Figure 1. Reginal plot of the GWAS significant variants for ADR-reported HSR after fine mapping.



Supplementary Figure 2. Quantile-quantile (Q-Q) plots for the GWAS analyzing skin reactions induced by nonionic ICMs. (A) The Q-Q plot based on ADR reports. (B) The Q-Q plot based on self-reported data.



Supplementary Figure 3. Regional plot of the GWAS significant variants for self-reported HSR after fine mapping.



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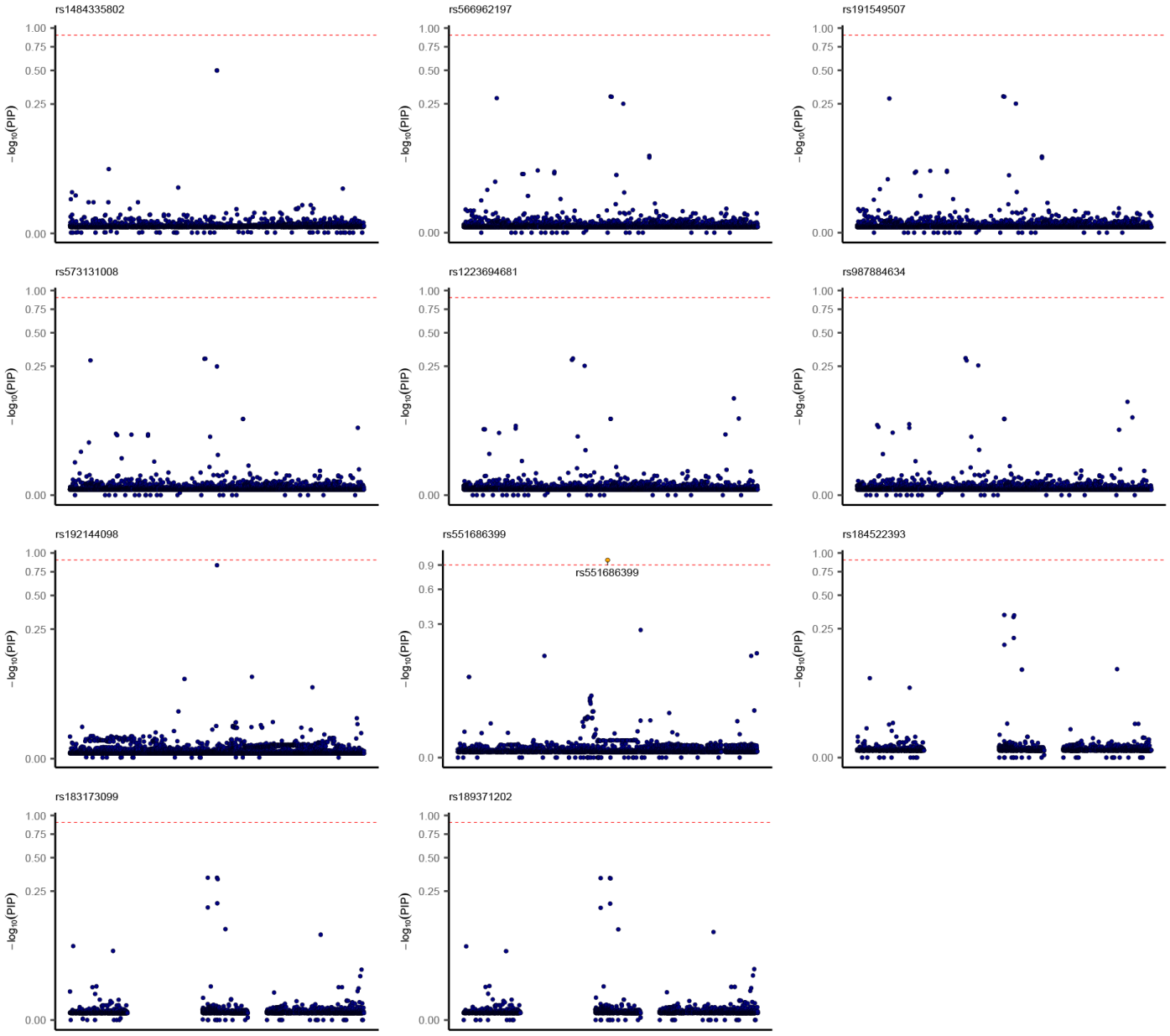

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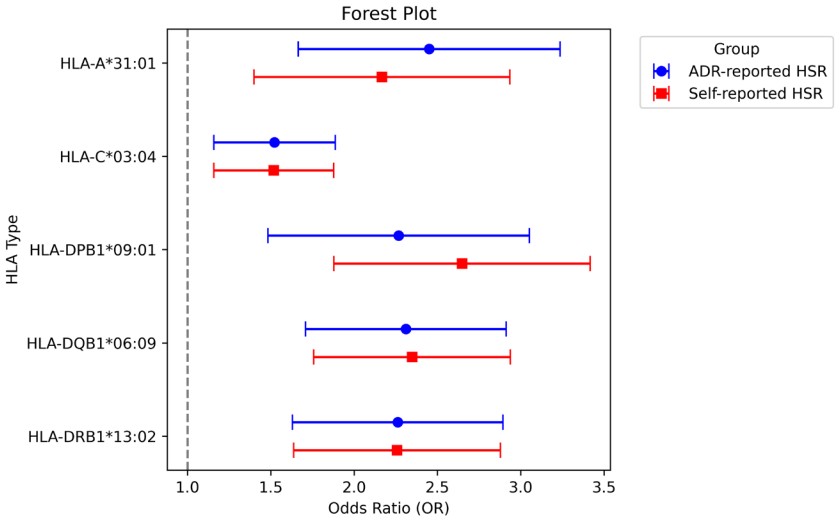
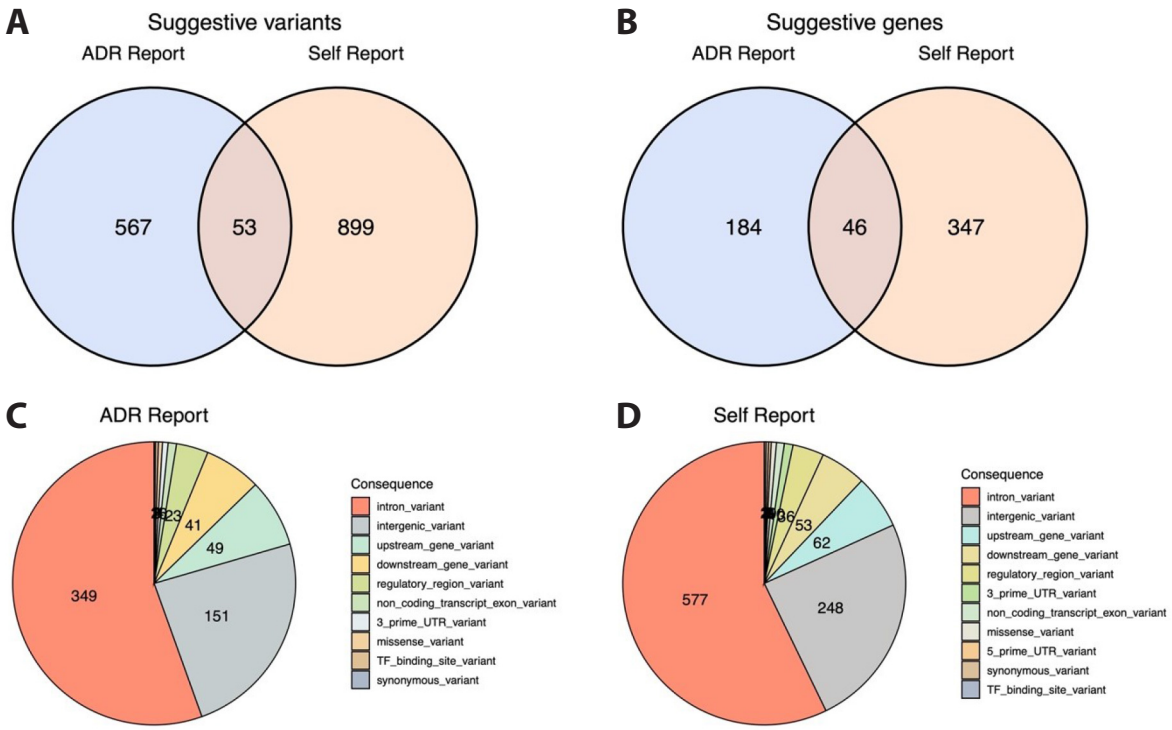


Supplementary Figure 4. Association analysis of imputed HLA alleles with hypersensitivity skin reaction. The results from ADR-reported cases are depicted in red, while those from self-reported cases are shown in blue. Significant associations are denoted by asterisks.



Supplementary Figure 5. Suggestive variants for self-reported ICM-induced skin reactions and those reported as ADRs. Overlapping suggestive variants (A) and genes (B) between the two cohorts. Consequences of suggestive variants for ICM-induced skin reactions reported as ADRs (C) and self-reported ICM-induced skin reactions (D).