

HLA-DRB1*15:02 Is Associated With Iodinated Contrast Media–Related Anaphylaxis

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Background: The incidence of severe reaction induced by iodinated contrast media (ICM) has increased over the years with an increasing use of imaging modalities. Although ICM anaphylaxis is rare, it can be life-threatening, but currently, there is no biomarker that can identify individuals at risk of ICM anaphylaxis.

Objective: The aim of this study is to investigate the genetic susceptibility of ICM anaphylaxis.

Methods: Patients who had ICM anaphylaxis were enrolled in the study, and their blood samples were collected for genotyping of human leukocyte antigen (HLA)-A, -B, -C, and -DR. The results were compared with those of healthy Korean general population. *MRGPRX2* gene in ICM anaphylaxis group was also sequenced and compared with the Korean standard database of genetic polymorphism.

Results: The frequencies of 3 HLA alleles (B*52:01, C*12:02, and DRB1*15:02) were significantly higher in 47 patients with ICM anaphylaxis. In particular, HLA-DRB1*15:02 was 5 times more frequent in the ICM anaphylaxis group than the Korean general population (34.0% vs 6.6%; odds ratio, 7.306; 95% confidence interval, 3.622–14.740), and this difference was most pronounced in subjects with iohexol-induced anaphylaxis (odds ratio, 16.516; 95% CI, 5.241–52.047; $P < 0.0001$). Eight single nucleotide polymorphisms were identified in *MRGPRX2* gene, but their frequencies were not different in those with ICM anaphylaxis compared with the general Korean population.

Conclusions: HLA-DRB1*15:02 is associated with ICM anaphylaxis in the Korean population.

Key Words: contrast media, anaphylaxis, drug hypersensitivity, HLA allele, genetic polymorphism

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Anaphylaxis is a potentially life-threatening hypersensitivity reaction (HSR) to exogenous stimuli that can lead to rapid multiorgan dysfunction. According to a previous study, 34.8% of anaphylaxis cases in tertiary hospitals were related to drugs, and iodinated contrast media (ICM) was one of the most common causative agents.¹ Hypersensitivity reaction was common with ionic high osmolar ICM, but it became infrequent after the introduction of nonionic low osmolar ICM.² It is estimated that 80% of severe reactions can be prevented by using non-ionic low osmolar ICM, although the death rate by ICM has not changed significantly.³ The studies indicate that the risk of adverse events is not significantly different between low osmolar ICMs.^{4,5} Despite the improvement in the safety of nonionic low osmolar ICM, the incidence of ICM-related adverse reactions has increased in the recent years, likely due to the parallel increase in the use of imaging modalities driven by enhancing technology.⁶ Therefore, an appropriate strategy to manage and prevent ICM HSR is urgently required.

Traditionally, ICM anaphylaxis has been thought to be non-IgE-mediated HSR, but the recent findings suggest the possibility of IgE-mediated allergic reaction, especially in severe anaphylaxis, as more frequent and severe recurrences were observed with repeated exposures to the same causative agent and a higher rate of skin test positivity was observed in severe reactions.^{7,8} The history of prior reaction is a good predictor of subsequent ICM anaphylaxis.⁹ A recent study showed that iopromide HSR was more common after intravenous injection than after intra-arterial injection, suggesting the route of administration may be an important risk factor.¹⁰ Other known risk factors include a history of allergy, asthma, and younger age, although these have limited clinical utility in practice. There is no other clinical tool to identify individuals at risk of ICM anaphylaxis.¹¹

Some drug HSRs are strongly associated with certain HLA alleles,^{12–14} but HLA associations have not been described in ICM anaphylaxis. On the other hand, a mast cell-specific receptor, Mas-related G protein-coupled receptor X2 (*MRGPRX2*), involved in mast cell degranulation, is known to be associated with the drug-induced anaphylactoid reaction.^{15,16} Given most ICM anaphylaxis has been thought to be non-IgE-mediated, *MRGPRX2* may play an important role, but its genetic polymorphisms in ICM anaphylaxis has not been studied. To address this knowledge gap, we analyzed the genetic polymorphisms of HLA and *MRGPRX2* in ICM anaphylaxis patients to see if genetic susceptibility can be identified.

MATERIALS AND METHODS

Study Subjects

Patients who experienced anaphylaxis to nonionic low osmolar ICM between March 2017 and October 2017 were identified from the

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Korean registry of ICM adverse reaction, which includes 7 regional tertiary hospitals. After obtaining consent from the patients, blood samples were collected. Demographic data such as age, sex, number of ICM exposures, and skin test results were retrieved from electronic medical records. Anaphylaxis was diagnosed when 1 of the following 3 WAO clinical criteria was met: (1) acute skin or mucosal involvement accompanied by respiratory compromise, hypotension, and/or organ dysfunction; (2) 2 or more of skin-mucosal involvement, respiratory compromise, hypotension, and/or persistent gastrointestinal symptoms; or (3) hypotension after exposure to known allergen.¹⁷ The severity of anaphylaxis was determined using the grading system by Brown et al.¹⁸ To compare the allele frequency, previously reported HLA frequencies of 485 healthy Korean individuals were used as a control.¹⁹

HLA Typing

Genomic DNA was extracted from peripheral blood mononuclear cells of patients. Sequence-based typing of HLA-A, -B, -C, and -DRB1 genes was performed using the AVITATM HLA-SBT kits (Biowithus Inc, Seoul, South Korea). In accordance with the manufacturer's instructions, exons 2 to 4 of class I and exon 2 of HLA-DRB1 were amplified using a locus specific primer set in the kit. Polymerase chain reaction (PCR) products were sequenced directly in both forward and reverse directions of each exon on an ABI3730 Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequenced results were analyzed using BOWITHUS SBT analyzer software ver. 2.7.5 (Biowithus Inc).

MRGPRX2 Sequencing

We sequenced *MRGPRX2* gene in ICM anaphylaxis group.²⁰ DNA was extracted from 200 µL of whole blood in ACD tubes using QuickGene DNA whole blood kit (Kurabo, Osaka, Japan). One exon of genomic DNA was amplified by PCR. The primers of the exon were designed according to the published sequence (NCBI Genbank: NC_000011.10, no. 5188–7160). Gene Runner Version 6 (Gen-Bank, Bethesda, MD) was used to design PCR primer pairs. Four forward primers and 4 reverse primers were identified, and primer sequencing information was described in the Table E1 in the Online Repository (PCR using HelixAmp Ready-2x-Go [premium pfu] PCR mix; Nanohelix, Daejeon, South Korea). Reactions were carried out using ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA). The PCR fragments were analyzed on 2% agarose/0.5X tris-borate EDTA gels. Direct sequencing was performed with a BigDye Terminator Kit (Applied Biosystems, Foster City, CA) on ABI 3730 DNA Analyzer (Applied Biosystems).

Skin Test With ICM

Skin tests were performed in some individuals as a part of routine clinical care using 7 nonionic ICM (iopamidol, iohexol, ioversol, iopromide, iomeprol, iobitridol, and iodixanol). Skin prick tests were performed with undiluted ICM, and intradermal tests were performed with 1:10 dilutions. Skin prick test was considered positive if the size of the wheal was at least 3 mm in diameter with surrounding erythema after 15 minutes. Intradermal test was considered positive if the size of the initial wheal increased by at least 3 mm in diameter with surrounding erythema after 20 minutes.

Statistical Analysis

We compared HLA genotypes of ICM anaphylaxis patients with those of the Korean general population. Pearson χ^2 test or Fisher exact test was performed to evaluate the differences in HLA allele frequencies between the ICM anaphylaxis group and the control group. *P* values less than 0.05 were considered significant. All statistical analyses were performed using PASW Statistics 24.0 (SPSS Inc, Chicago, IL). Multiple comparisons were corrected using Bonferroni correction.

Ethical Review and Approval

This study was approved by the institutional review board of each hospital (IRB no. 1705-012-851), and written informed consent was obtained from all participants after a detailed explanation of the study.

RESULTS

Baseline Characteristics of the Study Subjects

Forty-seven patients (22 males and 25 females) with the diagnosis of ICM anaphylaxis were recruited (Table 1). The mean $\pm$ standard deviation (SD) age of subjects was 61.0 $\pm$ 12.8 years. All patients had cardiovascular involvement with hypotension. Cutaneous and respiratory manifestations occurred in 42.6% (20/47) and 38.3% (18/47), respectively.

The median number of ICM exposure at the time of initial ICM anaphylaxis was 2 (interquartile range [IQR], 1.00–5.25). Records of the previous ICM exposures were available in 40 patients; anaphylaxis occurred with the first exposure to ICM in 47.5% (19/40) of patients, whereas 52.5% (21/40) of patients had anaphylaxis on repeated exposures to ICM.

Six different ICM were identified as causative agents of anaphylaxis; iohexol was the most common (13/47, 27.7%), followed by iopamidol (12/47, 25.5%), iopromide (9/47, 19.1%), iomeprol (4/47, 8.5%), iobitridol (3/47, 6.4%), and iodixanol (2/47, 4.3%). The culprit ICM was unknown in 4 patients (4/47, 8.5%).

ICM Skin Test Results in Patients With ICM Anaphylaxis

Twenty-seven patients underwent ICM skin test, and 20 patients (74.1%) were positive to at least one ICM. Most positive results were observed in intradermal test except for one patient who had skin prick test positivity. When skin test positivity was assessed according to the known culprit ICM, iopromide (4/4) and iobitridol (2/2) showed 100%

TABLE 1. Demographics and Clinical Characteristics of Study Subjects (n = 47)

Category	n (%)
Age, y	61.00 $\pm$ 12.75
Male-to-female ratio	22:25
Median no. previous exposures to ICM, median (IQR)	2 (1.00–5.25)
Hypersensitivity symptoms according to organ systems	
Skin	20 (42.6%)
Respiratory	18 (38.3%)
Cardiovascular	47 (100%)
Gastrointestinal	8 (17.0%)
Underlying allergic diseases	6 (12.8%)
Drug allergy	4 (10.6%)
Pollen-food allergy	1 (2.1%)
Culprit ICM	
Iohexol	13/47 (27.7%)
Iopamidol	12/47 (25.5%)
Iopromide	9/47 (19.1%)
Iomeprol	4/47 (8.5%)
Iobitridol	3/47 (6.4%)
Iodixanol	2/47 (4.3%)
ICM skin test (n = 27)	
Positive response to at least one ICM	20 (74.1%)
Positive response to culprit ICM	17 (63.0%)

IQR, interquartile range; ICM, iodinated contrast media.

positivity, followed by iohexol (6/7; 85.7%), iopamidol (7/9; 77.8%), and iomeprol (1/4; 25%). The skin test positivity between different ICM types was not statistically significant ($P = 0.062$).

Seventeen (85%) of the 20 patients with positive skin tests were positive to the culprit ICM, whereas 3 patients were only positive to ICM other than the culprit ICM. When assessing the cross-reactivity based on the skin test findings, 5 patients (25%) were positive to single ICM agent, whereas 15 patients (75%) showed positivity to at least 2 ICM agents (Fig. E1 in the Online Repository, <http://links.lww.com/RLI/A515>).

The median time interval between ICM anaphylaxis and the skin test was 8 weeks (IQR, 3–60.5 weeks). The skin test positivity rates according to the timing of ICM skin tests were as follows; 78.6% was positive within 2 months ($n = 14$), 100% between 2 to 6 months ($n = 4$), and 55.6% after 6 months ($n = 9$).

HLA Allele and Phenotype Frequencies

The frequencies of 9 HLA alleles (HLA-A*24:01, B*51:01, B52*01, C*03:03, C*07:01, C*07:02, C*08:03, C*12:02, and DRB1*15:02) in patients with ICM anaphylaxis were statistically different from those of Korean general population (Table 2). Out of these 9 HLA alleles, HLA-B*52:01, C*12:02, and DRB1*15:02 remained significant after Bonferroni correction for multiple comparisons. HLA-DRB1*15:02 was most frequently (34.0%) observed in patients with ICM anaphylaxis, whereas its frequency was only 6.6% in Korean general population (odds ratio [OR], 7.306; 95% confidence interval [CI], 3.622–14.740; $P = 3.20 \times 10^{-7}$). It remained significant after Bonferroni correction for multiple comparisons ($P_c = 2.78 \times 10^{-5}$). HLA-DRB1*15:02 frequency varied according to the culprit ICMs (Fig. 1). HLA-DRB1*15:02 frequency was highest in subjects with iohexol-induced anaphylaxis (53.8%), whereas its frequency was 26.5% of subjects with anaphylaxis to other ICM (OR, 3.833; 95% CI, 0.964–15.239, $P = 0.077$). In iohexol-induced anaphylaxis, the presence of HLA-DRB1*15:02 increased the OR of developing ICM anaphylaxis to 16.516 (95% CI, 5.241–52.047; $P < 0.0001$).

Thirteen patients (27.7%) with ICM anaphylaxis had both HLA-B*52:01 and HLA-C*12:02, and their frequencies were significantly higher than 5.6% and 5.0% found in Korean general population, respectively (OR, 6.486; 95% CI, 3.071–13.700; $P = 8.00 \times 10^{-6}$ and OR, 7.344; 95% CI, 3.436–15.697; $P = 3.00 \times 10^{-6}$, respectively). These remained significant after Bonferroni correction for multiple comparisons ($P_c = 6.96 \times 10^{-4}$ and 2.61×10^{-4} , respectively). These 13 patients (27.7%) shared the same 4 loci of HLA-A*24:02-B*52:01-C*12:02-DRB1*15:02. However, as there was no available data on the

frequency of 4 loci in the Korean general population, frequencies of 3 loci (HLA-A*24:02-B*52:01-C*12:02, B*52:01-C*12:02-DRB1*15:02, and A*24:02-B*52:01-DRB1*15:02) were compared with those of Korean general population. All of these had higher rates compared with those of the Korean general population, and they remained significant after Bonferroni correction for multiple comparisons (Table 3).

As HLA-A*24:02, B*52:01, and C*12:02 showed linkage disequilibrium with DRB1*15:02 in patients with ICM anaphylaxis, these alleles did not improve the detection rate of ICM anaphylaxis.¹⁹ In contrast, the frequencies of HLA-B*51:01 and HLA-C*03:03 were lower in ICM anaphylaxis group at 2.1% and 6.4% compared with 16.3% and 22.7% of the Korean general population, respectively. However, these differences were not significant after Bonferroni correction.

The frequency of HLA-DRB1*15:02 allele was not different between patients who experienced anaphylaxis at the first exposure to ICM and those patients who had on repeated exposures (31.6% vs 35.7%, $P = 0.769$).

MRGPRX2 Sequencing

Eight single nucleotide polymorphisms (SNPs) (rs11024970, rs10833049, rs11024969, rs7112398, rs7112385, rs7126647, rs7126647, and rs7126062) were found in patients with ICM anaphylaxis. Their frequencies were compared with those of KOVA (Korean Variant Archive), which includes 1055 healthy Korean individuals.²⁰ Three SNPs (rs11024970, rs10833049, rs11024969) were also identified in KOVA, but there were no statistically significant differences (Table 4). There were no significant differences in these SNPs in those who developed ICM anaphylaxis after the first exposure compared with those who developed anaphylaxis after repeated exposures ($P = 0.721$).

DISCUSSION

This study explored genetic susceptibility of ICM anaphylaxis for the first time and presented HLA-DRB1*15:02 as a novel risk factor. This suggests that HLA-DRB1*15:02 may play a role in inducing IgE-mediated response to ICM and points to the possibility that ICM anaphylaxis may be preventable with genetic screening.

Iodinated contrast media anaphylaxis may occur as a result of complex interactions between genetic and environmental factors, much like other allergic diseases. Therefore, in this complex interaction, HLA allele is likely to play an important role. The study by Jung et al⁹ showed that the greatest risk factor for ICM anaphylaxis was the history of previous severe reaction (OR, 8.884; 95% CI, 2.108–37.442). A similar finding was also noted for HSR to ICM, where the history of the

TABLE 2. Association of HLA-A, -B, -C, and -DRB1 With ICM Anaphylaxis

HLA Allele	ICM Anaphylaxis (n = 47)	Korean General Population (n = 485)	OR (95% CI)	P*	P _c †
A*24:02	27 (57.4)	188 (38.8)	2.133 (1.163–3.911)	0.013	1.131
B*51:01	1 (2.1)	79 (16.3)	0.112 (0.015–0.822)	0.010	0.870
B*52:01	13 (27.7)	27 (5.6)	6.486 (3.071–13.700)	8.00×10^{-6}	6.96×10^{-4}
C*03:03	3 (6.4)	110 (22.7)	0.232 (0.071–0.763)	0.010	0.870
C*07:01	3 (6.4)	3 (0.6)	10.955 (2.147–55.899)	0.010*	0.870
C*07:02	14 (29.8)	73 (15.1)	2.394 (1.222–4.693)	0.009	0.783
C*08:03	4 (8.5)	6 (1.2)	7.426 (2.018–27.333)	0.008*	0.696
C*12:02	13 (27.7)	24 (5.0)	7.344 (3.436–15.697)	3.00×10^{-6}	2.61×10^{-4}
DRB1*15:02	16 (34.0)	32 (6.6)	7.306 (3.622–14.740)	3.20×10^{-7}	2.78×10^{-5}

P values and ORs were calculated from phenotype frequency.
*P value by Fisher exact test.
†P_c: corrected P value after Bonferroni correction (multiplied by 87; 87 loci).
ICM indicates iodinated contrast media; CI, confidence interval; OR, odds ratio.

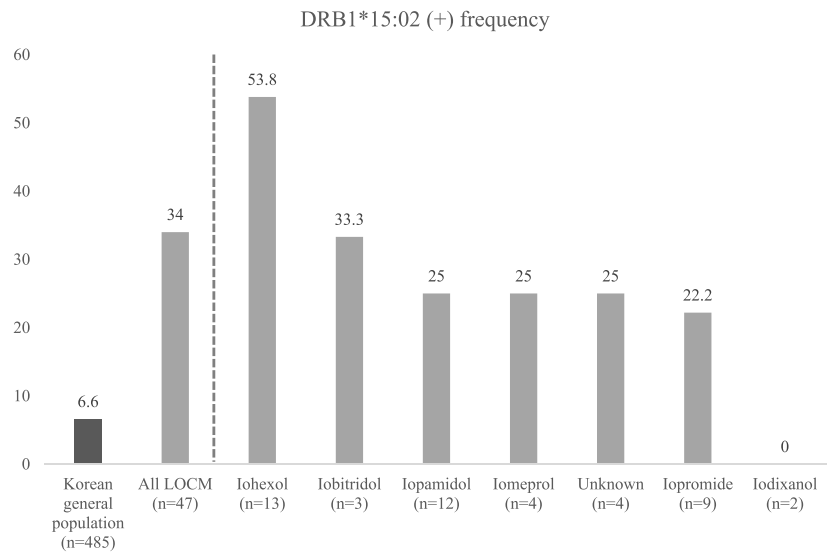


FIGURE 1. The proportion HLA-DRB1*15:02 in the Korean general population and subjects with ICM anaphylaxis. The positive rate of HLA-DRB1*15:02 was 34.0% in general but increased to 53.8% in cases with iohexol-induced anaphylaxis.

previous reaction was the most significant risk factor (OR, 15.9; 95% CI, 7.8–32.3).²¹ However, this is only helpful for patients who have already experienced ICM anaphylaxis. Although several other risk factors such as history atopy, asthma, or female sex were described, these do not increase risks substantially and are not practically useful.²² In this context, a different approach is needed to screen patients at increased risk of developing ICM anaphylaxis. Our findings show that HLA-DRB1*15:02 was found in almost one third of patients with ICM anaphylaxis, whereas its frequency was only 6.6% in Korean general population and its frequency was much higher in iohexol-induced anaphylaxis (53.8%). This finding is particularly striking given the calculated OR in those who carry HLA-DRB1*15:02 is similar to the OR described for patients with the history of previous reaction.

Human leukocyte antigen associations and genetic risk factors have been extensively studied in other drug HSRs. For example, HLA-B*15:02 allele was highly associated with carbamazepine-induced severe cutaneous adverse reactions (SCARs) in Han Chinese,¹² but it was not replicated in Europeans and Koreans, likely due to its low allele frequency in the latter populations.¹³ Other reported HLA associations in SCARs include HLA-B*58:01 and allopurinol-induced SCARs,^{13,14,23} HLA-B*57:01 and abacavir hypersensitivity syndrome,²⁴ HLA-B*59:01 and methazolamide-induced Stevens-Johnson syndrome/toxic epidermal necrolysis,²⁵ and HLA-B*13:01 and dapsone hypersensitivity syndrome.²⁶ However, these strong associations were only observed in T cell-mediated HSR.^{12–14}

There are other studies that showed significant associations between certain class II HLA alleles and IgE-mediated reactions, although with much lower relative risk. In one study, the frequencies of HLA-DQ7 and DR11 were higher in patients with IgE-mediated pyrazolone HSR than normal controls.²⁷ Another study reported that patients with asparaginase HSR including severe anaphylaxis had a higher proportion of HLA-DRB1*07:01 than normal controls.²⁸ Moreover, the presence of specific SNPs have been reported in the interregions of HLA-DRA and DRB5 in patients with immediate beta-lactam antibiotic allergy.²⁹

Although ICM anaphylaxis has been traditionally regarded as “anaphylactoid reaction” rather than “IgE-mediated allergy,” ICM anaphylaxis presents with similar clinical features observed in other typical IgE-mediated anaphylaxis. Recently, it has been suggested that some ICM anaphylaxis caused by nonionic low osmolar ICM is true IgE-mediated allergic reaction. This is supported by the presence of positive skin test results in the previous studies.^{7,30–34} In our previous study, positive skin test was reported to be 64.7% in those with anaphylaxis and 81.8% in those with anaphylactic shock.⁸ However, it is unknown how specific variants of HLA class II alleles could be a risk factor for IgE-mediated response. There are a few possible hypotheses; first, ICM could be better presented on certain HLA class II molecules due to drug-specific HLA binding as described in the “p-i concept.”³⁵ In this study, those with iohexol-induced anaphylaxis had the highest HLA-DRB1*15:02 frequency compared with those with anaphylaxis

TABLE 3. Association of Three-Locus HLA Haplotypes With ICM Anaphylaxis

Three-Locus HLA Haplotypes	ICM Anaphylaxis (n = 47)	Korean General Population (n = 485)	OR (95% CI)	P*	P _c †
A*24:02-B*52:01-C*12:02	13 (27.7)	20 (4.1)	8.890 (4.074–19.398)	5.96 × 10 ^{−7}	4.89 × 10 ^{−5}
B*52:01-C*12:02-DRB1*15:02	13 (27.7)	23 (4.7)	7.680 (3.577–16.490)	2.00 × 10 ^{−6}	1.64 × 10 ^{−4}
A*24:02-B*52:01-DRB1*15:02	13 (27.7)	19 (3.9)	9.378 (4.270–20.595)	3.85 × 10 ^{−7}	3.16 × 10 ^{−5}

*P value by Fisher exact test.
†P_c: corrected P value after Bonferroni correction (multiplied by 82; 82 three-locus haplotypes).
CI indicates confidence interval; OR, odds ratio.

TABLE 4. Comparison of SNPs of MRGPRX2 in ICM Anaphylaxis Patients and KOVA

MRGPRX2	ICM Anaphylaxis (n = 47)	Korean General Population (n = 1055)	P
rs11024970	7/94 (8.0)	252/2106 (13.6)	0.183
rs10833049	11/94 (13.3)	399/2104 (23.4)	0.077
rs11024969	7/94 (8.0)	253/2112 (13.6)	0.182

P values were calculated from genotype frequency.
KOVA indicates Korean Variant Archive.¹⁶

due to other ICM although it did not reach statistical significance, possibly due to the limited sample size. If this is the case, then a specific ICM structure could contribute to an increased risk of ICM anaphylaxis by stronger drug-HLA binding. Alternatively, a specific class II HLA variant may allow better presentation of ICM due to its intrinsic ability to present a larger breadth of peptides and other nonpeptide antigens such as ICM. One study suggested that specific HLA-DRB1 variants could present a larger breadth of viral peptides, resulting in stronger CD4⁺ T cell activity and lower viremia in HIV infections.³⁶ Another study showed that HLA-DRB1*07:01 variant had an altered structure of HLA-DR, resulting in increased asparaginase binding affinity and a higher risk of asparaginase hypersensitivity.²⁸ Finally, HLA-DRB1*15:02 may allow better presentation of ICM-haptenated peptide on antigen presenting cells resulting in subsequent T cell activation. Ultimately, we speculate that increased presentation of ICM to T cells by HLA-DRB1*15:02 is responsible for B cells to undergo class-switching to produce ICM-specific IgE. Further studies will be required to assess the validity of these hypotheses.

In addition to HLA-DRB1*15:02, we found a higher frequency (27.7%) of HLA-C*12:02 and HLA-B*52:01 in ICM anaphylaxis patients. However, HLA-C*12:02 and HLA-B*52:01 were both present in HLA-DRB1*15:02 carriers due to linkage disequilibrium and their presence is unlikely to be significant on their own.¹⁹ As a potential screening risk marker, HLA-DRB1*15:02 is the most promising candidate in Koreans. However, this allele is present in less than 1% of European populations.³⁷ Although HLA-DRB1*15:02 had low sensitivity (34.0%) for ICM anaphylaxis in our cohort, its frequency was higher in those who had iohexol-induced anaphylaxis (53.8%). However, given that ICM anaphylaxis is rare, the positive predictive value is likely to be low. Nonetheless, it may still have a limited clinical utility in risk stratification and in deciding the choice of ICM. Therefore, further studies will be required to verify its clinical utility.

MRGPRX2 is a protein coding gene for MAS-related GPR family member X2, which is a subtype of human G-protein coupled receptor of MRGPRX1 to 4 and only MRGPRX2 is expressed in human mast cells.³⁸ Recent studies have shown that activation of this receptor promotes degranulation of various mast cell lines, which leads to cytokine responses and HSR. This process is distinct from IgE-mediated mast cell activation mediated by FcεR1.³⁹ One study reported 4 natural SNPs of MRGPRX2 in human cell line refractory to mast cell activation.⁴⁰ Another study reported a total of 13 MRGPRX2 SNPs in diverse races, but there was no association with phenotypes.⁴¹ In our study, a total of 8 SNPs were found, but there was no significant difference when compared with the healthy Korean individual database. This suggests non-IgE-mediated mast cell activation via MRGPRX2 polymorphism may not play a significant role in ICM anaphylaxis. This hypothesis is also in keeping with positive skin test results in a majority of patients.

There are several limitations in this study. First, we did not use ICM tolerant controls. However, given the incidence of severe HSR to ICM is low at less than 0.1%, using HLA frequency data from the general Korean population is highly unlikely to affect the findings. Second,

we had a limited number of patients with ICM anaphylaxis due to its rarity.⁴² Third, this study only confirmed the association of HLA alleles and ICM anaphylaxis in Korean population. Further studies are needed to confirm the genetic susceptibility of ICM anaphylaxis in other ethnic groups since the distribution of HLA alleles varies according to ethnicity. Finally, only exons and introns near the splice site and not the whole introns of MRGPRX2 gene were analyzed in this study, whereas several SNPs were previously reported in the intronic region of MRGPRX2.⁴¹ Therefore, whole-genome sequencing using next-generation sequencing method may be helpful to rule out the association of MRGPRX2 gene with ICM anaphylaxis.

In conclusion, HLA-DRB1*15:02 is strongly associated with ICM anaphylaxis, particularly in iohexol-induced anaphylaxis. This suggests a potential role of HLA-DRB1*15:02 in inducing IgE-mediated response to ICM. Further detailed studies will be required to assess its diagnostic utility in preventing ICM anaphylaxis and its role in driving allergic immune mechanism.

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REFERENCES

1. Yang MS, Lee SH, Kim TW, et al. Epidemiologic and clinical features of anaphylaxis in Korea. *Ann Allergy Asthma Immunol.* 2008;100:31–36.
2. Segal AJ, Bush WH Jr. Avoidable errors in dealing with anaphylactoid reactions to iodinated contrast media. *Invest Radiol.* 2011;46:147–151.
3. Caro JJ, Trindade E, McGregor M. The risks of death and of severe nonfatal reactions with high- vs low-osmolality contrast media: a meta-analysis. *AJR Am J Roentgenol.* 1991;156:825–832.
4. Suh YJ, Yoon SH, Hong H, et al. Acute adverse reactions to nonionic iodinated contrast media: a meta-analysis. *Invest Radiol.* 2019;54:589–599.
5. Lenhard DC, May E, Morgenroth C, et al. Adverse skin reactions to iodinated x-ray contrast agents in healthy rats. *Invest Radiol.* 2014;49:779–787.
6. Christiansen C. X-ray contrast media—an overview. *Toxicology.* 2005;209:185–187.
7. Yoon SH, Lee SY, Kang HR, et al. Skin tests in patients with hypersensitivity reaction to iodinated contrast media: a meta-analysis. *Allergy.* 2015;70:625–637.
8. Kim MH, Lee SY, Lee SE, et al. Anaphylaxis to iodinated contrast media: clinical characteristics related with development of anaphylactic shock. *PLoS One.* 2014; 9:e100154.
9. Jung JW, Choi YH, Park CM, et al. Outcomes of corticosteroid prophylaxis for hypersensitivity reactions to low osmolar contrast media in high-risk patients. *Ann Allergy Asthma Immunol.* 2016;117:304–309.e301.
10. Endrikat J, Michel A, Kolbach R, et al. Risk of hypersensitivity reactions to Iopromide after intra-arterial versus intravenous administration: a nested case-control analysis of 133,331 patients. *Invest Radiol.* 2019;1.
11. Kim SH, Jo EJ, Kim MY, et al. Clinical value of radiocontrast media skin tests as a prescreening and diagnostic tool in hypersensitivity reactions. *Ann Allergy Asthma Immunol.* 2013;110:258–262.
12. Ferrell PB Jr, McLeod HL. Carbamazepine, HLA-B*1502 and risk of Stevens-Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations. *Pharmacogenomics.* 2008;9:1543–1546.
13. Kang HR, Jee YK, Kim YS, et al. Positive and negative associations of HLA class I alleles with allopurinol-induced SCARs in Koreans. *Pharmacogenet Genomics.* 2011;21:303–307.
14. Jung JW, Song WJ, Kim YS, et al. HLA-B58 can help the clinical decision on starting allopurinol in patients with chronic renal insufficiency. *Nephrol Dial Transplant.* 2011;26:3567–3572.
15. Liu R, Che D, Zhao T, et al. MRGPRX2 is essential for sinomenine hydrochloride induced anaphylactoid reactions. *Biochem Pharmacol.* 2017;146:214–223.
16. McNeil BD, Pundir P, Meeker S, et al. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature.* 2015;519:237–241.
17. Simons FE, Arduoso LR, Bilo MB, et al. World allergy organization guidelines for the assessment and management of anaphylaxis. *World Allergy Organ J.* 2011;4: 13–37.
18. Brown SG. Clinical features and severity grading of anaphylaxis. *J Allergy Clin Immunol.* 2004;114:371–376.
19. Lee KW, Oh DH, Lee C, et al. Allelic and haplotypic diversity of HLA-A, -B, -C, -DRB1, and -DQB1 genes in the Korean population. *Tissue Antigens.* 2005;65: 437–447.

20. Lee S, Seo J, Park J, et al. Korean variant archive (KOVA): a reference database of genetic variations in the Korean population. *Sci Rep*. 2017;7:4287.
21. Pradubpongsa P, Dhana N, Jongjareamprasert K, et al. Adverse reactions to iodinated contrast media: prevalence, risk factors and outcome-the results of a 3-year period. *Asian Pac J Allergy Immunol*. 2013;31:299–306.
22. Sánchez-Borges M, Aberer W, Brockow K, et al. Controversies in drug allergy: radiographic contrast media. *J Allergy Clin Immunol Pract*. 2019;7:61–65.
23. Jung JW, Kim JY, Yoon SS, et al. HLA-DR9 and DR14 are associated with the allopurinol-induced hypersensitivity in hematologic malignancy. *Tohoku J Exp Med*. 2014;233:95–102.
24. Mallal S, Phillips E, Carosi G, et al. HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med*. 2008;358:568–579.
25. Kim SH, Kim M, Lee KW, et al. HLA-B*5901 is strongly associated with methazolamide-induced Stevens-Johnson syndrome/toxic epidermal necrolysis. *Pharmacogenomics*. 2010;11:879–884.
26. Zhang FR, Liu H, Irwanto A, et al. HLA-B*13:01 and the dapsone hypersensitivity syndrome. *N Engl J Med*. 2013;369:1620–1628.
27. Kowalski ML, Woszczek G, Bienkiewicz B, et al. Association of pyrazolone drug hypersensitivity with HLA-DQ and DR antigens. *Clin Exp Allergy*. 1998;28:1153–1158.
28. Fernandez CA, Smith C, Yang W, et al. HLA-DRB1*07:01 is associated with a higher risk of asparaginase allergies. *Blood*. 2014;124:1266–1276.
29. Gueant JL, Romano A, Cornejo-Garcia JA, et al. HLA-DRA variants predict penicillin allergy in genome-wide fine-mapping genotyping. *J Allergy Clin Immunol*. 2015;135:253–259.
30. Laroche D, Aimone-Gastin I, Dubois F, et al. Mechanisms of severe, immediate reactions to iodinated contrast material. *Radiology*. 1998;209:183–190.
31. Trecka J, Schmidt C, Seitz CS, et al. Anaphylaxis to iodinated contrast material: nonallergic hypersensitivity or IgE-mediated allergy? *AJR Am J Roentgenol*. 2008;190:666–670.
32. Lerondeau B, Trechot P, Waton J, et al. Analysis of cross-reactivity among radiocontrast media in 97 hypersensitivity reactions. *J Allergy Clin Immunol*. 2016;137:633–635.e634.
33. Morales-Cabeza C, Roa-Medellin D, Torrado I, et al. Immediate reactions to iodinated contrast media. *Ann Allergy Asthma Immunol*. 2017;119:553–557.
34. Schrijvers R, Breynaert C, Ahmedali Y, et al. Skin testing for suspected iodinated contrast media hypersensitivity. *J Allergy Clin Immunol Pract*. 2018;6:1246–1254.
35. Pichler WJ, Adam J, Watkins S, et al. Drug hypersensitivity: how drugs stimulate T cells via pharmacological interaction with immune receptors. *Int Arch Allergy Immunol*. 2015;168:13–24.
36. Ranasinghe S, Cutler S, Davis I, et al. Association of HLA-DRB1-restricted CD4 (+) T cell responses with HIV immune control. *Nat Med*. 2013;19:930–933.
37. Gonzalez-Galarza FF, Takeshita LY, Santos EJ, et al. Allele frequency net 2015 update: new features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. *Nucleic Acids Res*. 2015;43:D784–D788.
38. Fujisawa D, Kashiwakura J, Kita H, et al. Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria. *J Allergy Clin Immunol*. 2014;134:622–633.e629.
39. Cildir G, Pant H, Lopez AF, et al. The transcriptional program, functional heterogeneity, and clinical targeting of mast cells. *J Exp Med*. 2017;214:2491–2506.
40. Alkanfari I, Gupta K, Jahan T, et al. Naturally occurring missense MRGPRX2 variants display loss of function phenotype for mast cell degranulation in response to substance P, hemokinin-1, human β -defensin-3, and icatibant. *J Immunol*. 2018;201:343–349.
41. Yang S, Liu Y, Lin AA, et al. Adaptive evolution of MRGX2, a human sensory neuron specific gene involved in nociception. *Gene*. 2005;352:30–35.
42. Kim MH, Park CH, Kim DI, et al. Surveillance of contrast-media-induced hypersensitivity reactions using signals from an electronic medical recording system. *Ann Allergy Asthma Immunol*. 2012;108:167–171.