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Clinical variables and genetic variants associated with perioperative anaphylaxis in Chinese Han population: A pilot study

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ABSTRACT

Background: Perioperative anaphylaxis (POA) can lead to severe consequences. Identifying clinical risk factors and genetic loci associated with POA through pre-prescription screening may help reduce its incidence.

Methods: Using univariate regression and covariate-adjusted multivariate regression, we retrospectively analyzed the association between clinical characteristics and POA in 72 POA patients and 72 non-POA individuals. The discovery study of whole-exome association relied on whole-exome sequencing of 73 POA cases and 1339 healthy individuals. A replication study involving an independent set of 16 POA cases and 1339 healthy individuals confirmed this association. The accurate typing of human leucocyte antigen through exome sequencing (ATHLATES) algorithm and the whole-exome sequencing data were used for genotyping the human leucocyte antigen G (*HLA-G*) of 73 POA patients. The *HLA-G* of 16 POA cases and 122 non-POA patients were genotyped through Sanger sequencing. We used Fisher's exact probability method to compare the allele and carrier frequencies between POA patients and healthy individuals or non-POA patients. A *Pc* (*P*/Bonferroni correction coefficient) < 0.05 represents statistical significance.

Results: Regression analysis identified female sex, an unconfirmed food allergy label, and a history of prior surgery as clinical variables associated with POA. The whole-exome association discovery study identified a strong signal in the major histocompatibility complex region on chromosome 6, with the rs1130356 being the most significant locus ($P = 1.5E\text{-}10$, OR = 3.4, 95% CI = 2.4–4.9). The replication study verified the association between the rs1130356-T allele and POA cases ($P = 1.0E\text{-}6$, OR = 6.3, 95% CI = 3.1–12.7). Compared with non-POA patients, *HLA-G*01:01* ($Pc = 2.4E\text{-}4$, OR = 2.4, 95% CI = 1.6–3.6) was significantly enriched, while *HLA-G*01:04* ($Pc = 1.2E\text{-}6$, OR = 0.3, 95% CI = 0.2–0.5) was lessened in POA patients.

Conclusion: Our study suggested an association between POA and the risk factors of female sex, an unconfirmed food allergy label, and prior surgery. *HLA-G*, located in the human leucocyte antigen (HLA) region, may act as a surrogate genetic marker for POA. This suggests a causal relationship between this specific genomic region and POA. Our findings shed light on the contribution of human exome genetic variants to the susceptibility to POA.

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INTRODUCTION

Perioperative anaphylaxis (POA) is a rare yet potentially life-threatening systemic hypersensitivity reaction that occurs during the perioperative period, characterized not only by cardiovascular symptoms but also possibly involving cutaneous (such as rash) and respiratory (such as wheezing) manifestations. POA is particularly hazardous for patients under general anesthesia, necessitating prompt cardiopulmonary resuscitation to prevent fatal outcomes. Based on epidemiological data from the World Allergy Organization and the current literature, the incidence of anaphylaxis during anesthesia ranges from 1 in 5000 to 10 000 cases,[1–4] with a mortality rate reaching up to 9%.[3–5] The 6th National Audit Project (NAP6) data reveals that both antibiotics (8–67%) and neuromuscular blocking agents (NMBAs) (11–58%) are the 2 leading culprit drugs that can induce POA,[6] followed by latex (16–19%), hypnotics (0.8–17%), and colloids (2–4%).[3,4,6–9] The perioperative period involves the administration of numerous drugs, complicating the identification of the responsible agent. Identifying potential risk factors for POA is crucial to prevent exposing susceptible individuals to further culprit drugs. NMBAs, indispensable for inducing general anesthesia, currently have no viable substitutes. Pre-medication screening to predict high-risk reactions enables anesthesiologists to recommend preoperative allergy evaluations or opt for alternative anesthesia methods, such as regional or epidural block.

Previous studies have underscored the significance of genetic variants in the development of adverse drug reactions, with notable progress in understanding the relationship between human leucocyte antigen (HLA) alleles and drug hypersensitivity. The majority of risk alleles associated with drug hypersensitivity reactions are concentrated at HLA class I loci, including *HLA-B*15:02* and *HLA-B*58:01* which are closely associated with carbamazepine-induced Stevens-Johnson syndrome or toxic epidermal necrolysis[10] and allopurinol-induced cutaneous adverse drug reactions, respectively.[11] Due to pharmacogenomic research, unpredictable hypersensitivities to drugs have become predictable and preventable. However, our knowledge of genetic predispositions to POA is still in its infancy. Possible explanations include the rarity of Ring and Messmer Grade III or IV cases and the challenges in collecting samples. Previous candidate gene-based studies on immediate hypersensitivity have yielded limited clinical applications.[12,13]

This study aims to bridge this gap by conducting a comprehensive clinical epidemiological and pharmacogenomic investigation to identify both clinical factors and genetic variants associated with POA.

METHODS

Patient screening and enrolment

According to the modified Ring and Messmer scale,[14] all enrolled POA patients were diagnosed based on the Grade IV criteria. These diagnostic criteria included a hypersensitivity within 5 min after the culprit medicine was administered. The hypersensitivity was characterized by cardiac and/or respiratory arrest (including bradycardia and/or malignant arrhythmias). Immediate life-threatening hypotension with multi-organ symptoms such as bronchospasm or cutaneous signs should be accompanied. Additionally, refractory hypotension (unresponsiveness to routine dosage of vasopressor except epinephrine or vasopressin) was present. POA was diagnosed by the senior anesthesiologist in chief charge, and the culprit drugs were verified through intradermal testing (IDT) by 2 designated allergists 4 weeks after the index reaction. Within 15–60 min of onset, serum

acute tryptase levels in POA patients exceeded (1.2 × sBT+2) μg/L, where sBT is the serum baseline tryptase measured at least 24 h after symptoms fully resolved.[15] The serum tryptase assays were performed following the ImmunoCAP kit protocol (Thermo Fisher Scientific Inc., USA). Differential diagnoses such as pulmonary embolism, myocardial infarction, pericardial tamponade, and insufficient capacity were meticulously excluded based on clinical characteristics. For the epidemiological investigation, we employed a matched-pairs case-control design. Non-POA patients, without POA incidents but matching in surgery type, date, age range (within a 5-year difference), medications, and lacking signs of hypersensitivity, were recruited. In the clinical epidemiological study, 72 POA cases and 72 matched non-POA individuals participated.

For the genetic analysis, an additional 17 POA cases were recruited, resulting in a total of 89 POA cases. Through simple randomization, the 89 POA cases were distributed into a discovery cohort of 73 cases and a replication cohort of 16 cases. An additional 50 non-POA patients were also enrolled, matched by surgery type, age, and medication but with a later surgery date. Consequently, 122 non-POA patients (72 absolutely matched and 50 partially matched) were used as the non-POA group in the subsequent genetic analysis (Fig. 1). POA and non-POA patients were unrelated Chinese Han from our institution (decent assessment identified by PLINK 1.9). A total of 1339 in-house healthy individuals with available whole-exome sequencing data from Changhai Hospital, Shanghai, China, formed a healthy population control.

IDT and culprit drug verification

Two designated allergists performed IDT on POA patients at least 4 weeks after POA symptoms were fully resolved. According to the 2019 recommendations of the European Academy of Allergy and Clinical Immunology, the maximum non-irritative concentrations of candidate agents (excluding blood products) were used for IDT.[16] Results were read after 20 min, and a wheal with an increase in diameter of ≥3 mm compared to the original bleb, together with a flare, was considered positive.[16,17] For patients who manifested POA symptoms within 5 min of blood product infusion, anesthesiologists identified the causative agents based on symptoms and tryptase testing.

Whole-exome sequencing

Within 30 min of the POA incident, whole blood samples were taken from the central vein line. The genome DNA extraction and quality assessments followed the TIANamp Genomic DNA Kit

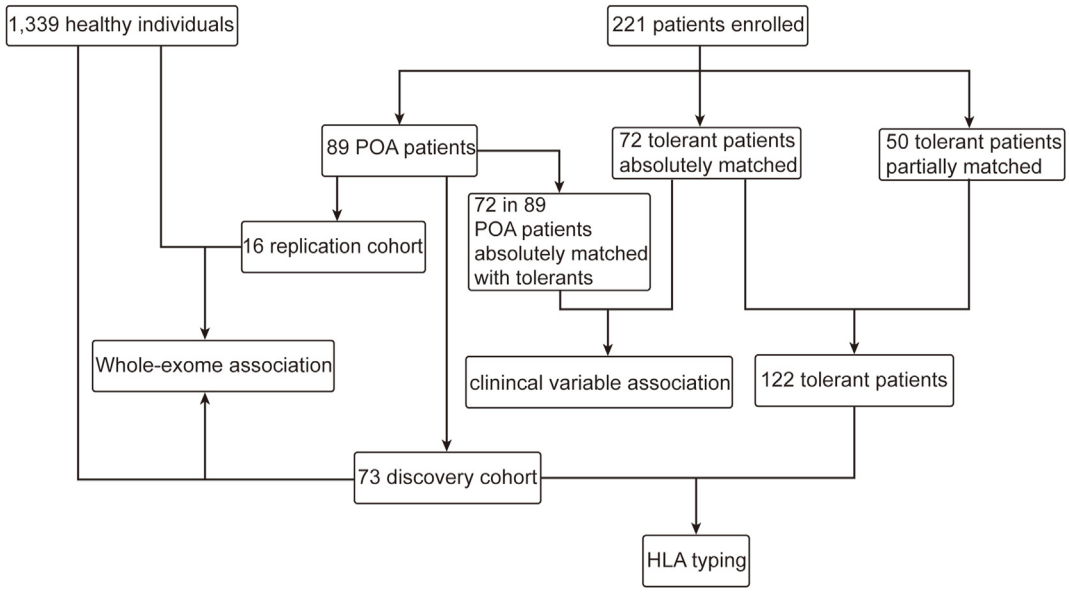

Fig. 1 Flow chart of POA study.

(TIANGEN Biotech Co. Ltd, China) and NanoDropTM One (Thermo Fisher Scientific Inc., USA) protocols, respectively. The Agilent SureSelect V6 (Agilent Inc., USA) was used for exonic fragment capture and the Illumina Novaseq platform for 150bp paired-end sequencing. Reads were trimmed using ultra-fast one-pass FASTQ data preprocessor (fastp 0.20.0) and then aligned to the reference genome (build hg19) using the burrows-wheeler-alignment tool (BWA). Variant identification adhered to the Genome Analysis Toolkit Best Practice.[18]

Plink 1.9, an open-source C/C++ whole-genome association studies toolset, was utilized to execute the quality control and association study. Samples were screened according to sex, individual relatedness (PI_HAT value < 0.25), and genotype completion rate (>95%). In our study, only single nucleotide variants (SNVs) satisfying the following quality control criteria were included for further analysis: (i) read depth $\geq$10, (ii) genotyping quality $\geq$20, (iii) alternative allele read frequency of heterozygotes $\geq$25%, (iv) calling rate $\geq$90% in all samples, (v) minor allele frequency $\geq$0.01 in the genomAD database, and (vi) deviation from the Hardy-Weinberg equilibrium ($P \geq 1 \times 10^{-6}$ in control samples). Principal component analysis (PCA) was performed as follows: (i) data thinned by LD-indep-pairwise 1000 50 0.05; (ii) -pca with default parameter. Logistic regressions with the first and second principal component as covariates were subsequently conducted. The candidate variants were genotyped using Sanger sequencing.

HLA-G genotyping

HLA-G genotyping utilized whole-exome sequencing data and the ATHLATES algorithm for 73 discovery cohort POA patients.[19,20] For 16 replication cohort POA patients and 122 non-POA patients, genotyping proceeded via Sanger sequencing of the *HLA-G* (NM_001363567) fragment, spanning from exon 4 upstream to exon 5 downstream. Forward primer (5′-CTTGTGCTAG GCCAGGC-3′) and reverse primer (5′-AGTGGGA-CAAGAAAACTCAGAC-3′) were designed through Primer Premier 6.0 software and synthesized by Sangon Co. Ltd, Shanghai, China. Alleles carried by more than 3 POA patients were subjected to further investigation to explore the association between *HLA-G* and POA.

Statistical analysis

The minimum sample sizes for the POA group, paired non-POA group, and healthy individual group were calculated using an online tool (www.powerandsamplesize.com) and the design effect of K. J. Lee et al.[21]

The R statistical package (version 4.0.2) facilitated risk effect calculations (OR and 95% CI) for clinical variables on POA occurrence in both univariate and multivariate models. The two-sided *P* value < 0.05 denotes statistically significant, and the statistically significant variable was considered to be associated with POA occurrence.

Fisher's exact probability method was employed to compare the allele and carrier frequencies between POA patients and healthy individuals or non-POA patients. A *P* value < 0.05 was considered indicative of a statistically significant difference in frequencies. To account for multiple comparisons, *P*-values were adjusted using Bonferroni correction method, denoting the corrected *P*-values as *Pc*. Associations with a *Pc* value less than 0.05 were deemed statistically significant. The odds ratio (OR) and 95% confidence interval (CI) were calculated to quantify the strength and precision of the association between the allele or genotype and POA. A total of 148 051 SNVs met quality control criteria for further analysis. Consequently, the exome-wide significance level was set at $P < 3.4E-7$, equivalent to a Bonferroni correction for 148 051 comparisons (0.05/148,051).

RESULTS

Baseline characteristics of POA and non-POA patients

Clinical characteristics and variables for POA cases and non-POA groups were summarized in Table 1. The ages of both groups followed normal distribution with coefficients of kurtosis and skewness less than 1.

Logistic regression analysis for clinical variables

Univariate regression and covariate-adjusted multivariate regression for clinical variables are demonstrated in Table 2. In univariate analysis, female sex ($P = 0.0028$, OR = 2.8, 95% CI = 1.4–5.6), unconfirmed food allergy label ($P = 5.0E-4$, OR = 4.8, 95% CI = 2.0–11.5), and prior surgery

	POA (n = 72)	Non-POA (n = 72)
Age (years)	54.0 ± 15.2	54.4 ± 15.2
Female sex	41 (56.9)	23 (31.9)
Chinese Han	72 (100)	72 (100)
ASA ≥III	13 (18.1)	8 (11.1)
Unconfirmed drug allergy label	14 (19.4)	13 (18.1)
Unconfirmed food allergy label	27 (37.5)	8 (11.1)
Prior surgery	27 (37.5)	11 (15.3)
Immune disorder	12 (16.7)	8 (11.1)
Hypertension	14 (19.4)	9 (12.5)
Coronary heart disease	9 (12.5)	4 (5.6)
Antihypertensive medicine	11 (15.3)	7 (9.7)
Surgery type		
Abdomen	29 (40.2)	29 (40.2)
Orthopedics	18 (25.0)	18 (25.0)
Cardiothoracic	7 (9.7)	7 (9.7)
Neurosurgery	10 (13.9)	10 (13.9)
Otolaryngology	8 (11.1)	8 (11.1)
Suspected culprit allergen		
SG	28 (38.9)	0 (0.0)
NMBAs	24 (33.3)	0 (0.0)
Antibiotics	13 (18.1)	0 (0.0)
Blood products	7 (9.7)	0 (0.0)

Table 1. Characteristics and variables in POA and non-POA groups. *Data are mean ± SEM or number (%); ASA: American Society of Anesthesiologists; SG: succinylated gelatin; NMBAs: neuromuscular blocking agents (rocuronium and cisatracurium); antibiotics (cephalosporin, vancomycin, and quinolone); immune disorders: asthma, urticaria, allergic rhinitis included; antihypertensive medicine: angiotensin receptor blocker, angiotensin-converting enzyme inhibitor, beta-blocker, calcium channel blocker, diuretic; blood products: suspended red blood cells, plasma; unconfirmed food/drug allergy label: patient's self-reporting history of food/drug allergy reaction without laboratory test confirmation*

(P = 0.0031, OR = 3.3, 95% CI = 1.5–7.4) were identified as clinically associated variables for POA. In the multivariate regression model, adjusted by covariate factors, the odd ratios of unconfirmed food allergy label and prior surgery increased by 1.1 (5.9 vs. 4.8) and 0.7 (4.0 vs. 3.3), respectively. In contrast, the odd ratio for females remained nearly unchanged (2.8 vs. 2.7). Specifically, the risk of POA was 2.7 times higher in female patients than in male patients. Patients bearing a label of unconfirmed food allergy or with a history of prior surgery exhibited a 5.9- and 4.0-fold increased risk of POA, respectively, compared to their counterparts without any record of food allergy label or history of prior surgery.

Whole-exome association analysis

In the discovery phase, we conducted an exome-wide association analysis using 148 051 single variants on autosomal chromosomes that passed the quality control. The association analysis of the differentiation among 148 051 SNVs between POA patients and healthy individuals is presented in Fig. 2. When the threshold was set at the $P < 1.0E-4$ significance level threshold, 259 variants were associated with POA occurrence. Remarkably, 141 of these variants were located on chromosome 6, specifically in the HLA locus region. When the threshold was elevated to the whole-exome significance level of $P < 3.4E-7$, significant associations were observed with 36

	Univariate		Multivariate	
	P	OR (95% CI)	*P*	OR (95% CI)
Age	0.56	1.0 (1.0–1.0)		
<34 years		1.0		
34–60 years	0.98	1.0 (0.4–2.2)		
>60 years	0.76	0.9 (0.4–2.0)		
Female sex	**0.0028**	**2.8(1.4–5.6)**	**0.0087**	**2.7(1.3–5.5)**
ASA ≥ III	0.24	1.8 (0.7–4.6)		
Unconfirmed drug allergy label	0.83	1.1 (0.5–2.5)		
Unconfirmed food allergy label	**5.0E-4**	**4.8(2.0–11.5)**	**3.0E-4**	**5.9(2.3–15.1)**
Prior surgery	**0.0031**	**3.3(1.5–7.4)**	**0.0012**	**4.0(1.7–9.4)**
Immune disorder	0.34	1.6 (0.6–4.2)		
Hypertension	0.26	1.7 (0.7–4.2)		
Coronary heart disease	0.16	2.4 (0.7–8.3)		
Antihypertensive medicine	0.32	1.7 (0.6–4.6)		

Table 2. Regression analysis of POA clinical variables. *P < 0.05 is shown in bold. Variables with P values less than 0.05 in univariate regression analyses were involved in multivariate regression analyses and received covariate-adjusted P values*

SNVs on chromosome 6, located within the HLA region (chr6:29.36–31.80 Mb). The most substantial associations were observed at rs1130356 (NM_001384290:c.C351T, p.H117H) (P = 1.5E-10, OR = 3.4, 95% CI = 2.4–4.9), followed by rs1610644 (P = 3.3E-9, OR = 3.3, 95% CI = 2.3–4.7) and rs1611208 (P = 3.6E-9, OR = 3.2, 95% CI = 2.3–4.7). Across the whole exome, no SNVs in other regions reached the whole-exome significance level. To substantiate the association between rs1130356 and POA, and to exclude interferences from other diseases, a replication study was conducted using an independent set of 16 POA cases and 1339 healthy individuals, which verified the significant enrichment of the rs1130356-T allele in POA patients (P = 1.0E-6, OR = 6.3, 95% CI = 3.1–12.7).

In addition to the variant rs1130356, identified as the most significant variant and located within the gene body of *HLA-G*, 36 additional variants achieving the whole-exome significance (P < 3.4E-7) were detected, predominantly clustering around *HLA-G*. Given the crucial role of *HLA* loci in the pathogenesis of POA, we focused on investigating whether the locus-associated signal was driven by any of the *HLA-G* alleles. Our cohort comprised 5 distinct *HLA-G* alleles, including *HLA-G*01:01, HLA-G*01:05 N, HLA-G*01:04*, and *HLA-G*01:06*. Upon conducting association analyses, the common alleles *HLA-G*01:01* and *HLA-G*01:04* both surpassed the established threshold for significance, as detailed in Table 3.

After Bonferroni correction, the frequency of *HLA-G*01:01* allele remained higher in the POA group compared to the non-POA group (58.3% vs. 36.9%, Pc = 2.4E-4, OR = 2.4, 95% CI = 1.6–3.6). In contrast, the *HLA-G*01:04* allele frequency was significantly lower in the POA group than in the non-POA group (18.5% vs. 43.9%, Pc = 1.2E-6, OR = 0.3, 95% CI = 0.2–0.5).

Furthermore, 61 of 73 cases (83.6%) carried *HLA-G*01:01* with 37 heterozygotes and 24 homozygotes, and only 73 of 122 (59.8%) non-POA patients carried *HLA-G*01:01* with 56 heterozygotes and 17 homozygotes. Carriers of the *HLA-G*01:01* allele were 3.4 times more likely to develop POA than non-carriers (Pc = 2.9E-3, OR = 3.4, 95% CI = 1.7–7.0). Conversely, 26 of 73 cases (35.6%) carried *HLA-G*01:04* with 25 heterozygotes and 1 homozygote, and 79 of 122 (64.8%) non-POA patients carried *HLA-G*01:04* with 51 heterozygotes and 28 homozygotes

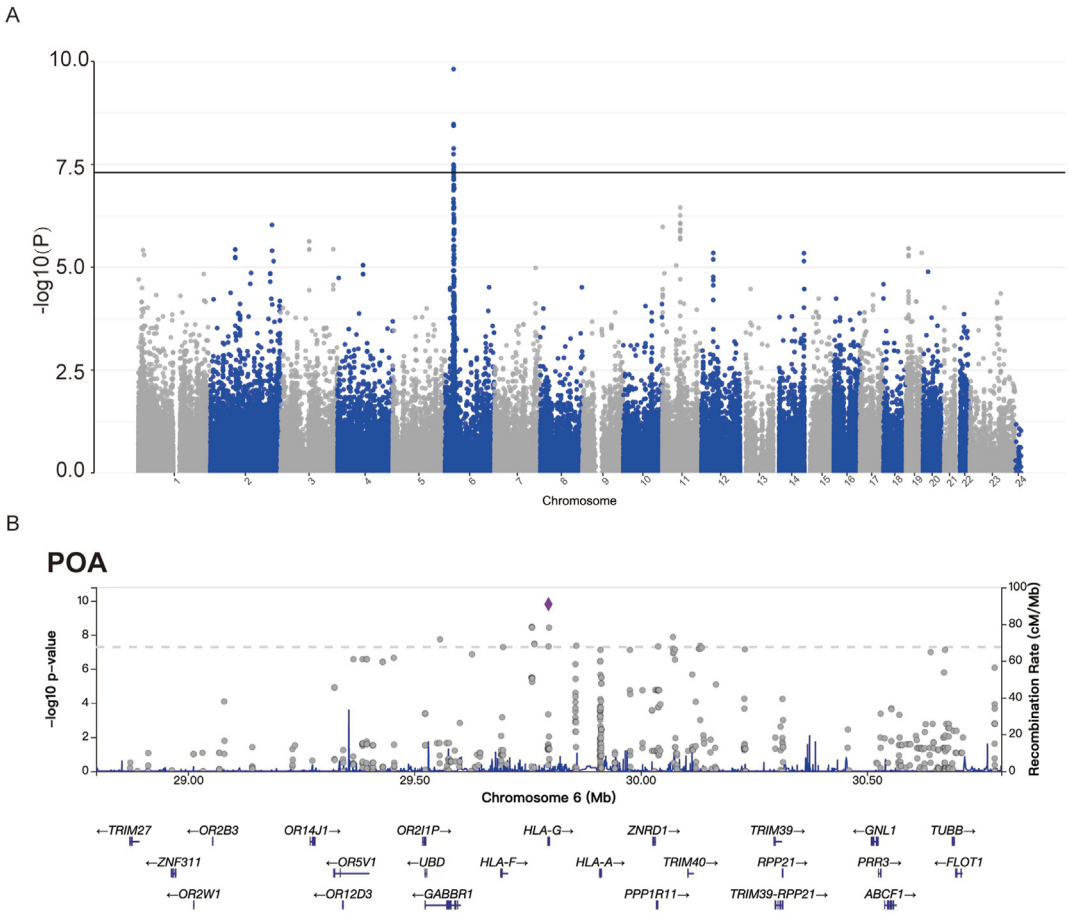

Fig. 2 Association plots of whole-exome sequencing. (A) The upper horizontal line denotes the observed *P* value < 3.4E-7 at the whole-exome level. (B) Variants among the *HLA* locus region on the short arm of chromosome 6. The purple diamond represents rs1130356 (*P* = 1.5E-10, OR = 3.4, 95% CI = 2.4–4.9). The figure was produced using the LocusZoom software (http://locuszoom.sph.umich.edu/locuszoom/).

HLA alleles	POA (n = 146) allele frequency %		Non-POA (n = 244) allele frequency%		*P*	*Pc*	OR	95% CI
G*01:01	85	58.3	90	36.9	6.0E-5	2.4E-4	2.4	1.6–3.6
G*01:05 N	23	15.8	39	16.0	1.0		0.9	0.6–1.7
G*01:04	27	18.5	107	43.9	3.1E-7	1.2E-6	0.3	0.2–0.5
G*01:06	10	6.8	6	2.6	0.06		2.9	1.0–8.2

Table 3. *HLA-G* allele frequencies between POA and non-POA groups. Pc = P × 4; *Pc < 0.05 is shown in bold*

(*Pc* = 4.2E-4, OR = 0.3, 95% CI = 0.2–0.6). Moreover, the homozygous *HLA-G*01:04* presented stronger associations with POA (1.4% vs. 23.0%, *Pc* = 3.9E-5, OR = 0.05, 95% CI = 0.006–0.4), implying that the risk of POA in homozygotes of *HLA-G*01:04* was only one-twentieth of that in non-homozygotes. According to *HLA-G* genotyping data, *HLA-G*01:01* was associated with a higher incidence of POA, while *HLA-G*01:04* was a protective factor against POA (Table 4).

HLA allele	POA (n = 73) carrier frequency%		Non-POA (n = 122) carrier frequency%		P	*Pc*	OR	95% CI
G*01:01	61	83.6	73	59.8	7.2E-4	**2.9E-3**	3.4	1.7–7.0
G*01:05 N	21	28.8	36	29.5	1.0		0.9	0.5–1.8
G*01:04	26	35.6	79	64.8	1.0E-4	**4.2E-4**	0.3	0.2–0.6
G*01:06	10	13.7	6	4.9	0.06		3.1	1.1–8.8

Table 4. *HLA-G* allele carrier frequencies between POA and non-POA groups. Pc = P × 4; *Pc < 0.05 is shown in bold*

DISCUSSION

Consistent with the current literature, our investigation between clinical variables and POA demonstrates that being female and having a label of unconfirmed food allergy are associated with an increased risk of POA.[22] Additionally, we observed that patients with a history of prior surgery had a fourfold increase in the risk of POA compared to those without such a history. Moreover, we identified a significant signal associated with POA within the HLA region on chromosome 6, with the strongest association occurring at rs1130356 in *HLA-G*. In addition to the most significant variant rs1130356 (P = 1.5E-10), 36 *HLA-G* variants achieved exome-wide significance (P < 3.4E-7) and exhibited clustering. Consequently, analysis of *HLA-G* alleles in POA cases and non-POA controls might enable us to determine the specific *HLA-G* allele implicated in a POA event.

In our cohort, the *HLA-G*01:04* allele was more prevalent in the non-POA group, while the *HLA-G*01:01* allele was enriched in the POA group, indicating a significant association between *HLA-G* alleles and POA susceptibility. Furthermore, a stronger association between *HLA-G*01:04* homozygotes and POA was observed compared to *HLA-G*01:04* heterozygotes (OR: 0.05 vs. 0.3), suggesting an allele dosage effect influencing genetic predisposition to POA. These observations raised the hypothesis that *HLA-G* might be in linkage disequilibrium with unidentified POA-causative loci or gene regulatory elements. The possible linkage disequilibrium enabled the detection of a significant association between *HLA-G* and POA. Although the *HLA-G*01:04* allele was negatively correlated with POA susceptibility (0 < OR < 1) in statistical analysis, it remains unclear if this allele confers protection against POA. Future research is required to elucidate the molecular mechanisms underlying its influence on POA. Although traditional immunological theory primarily implicates HLA class II molecules in immediate hypersensitivity reactions, we cannot entirely dismiss a causal association between HLA-G, a class I molecule, and POA. Therefore, *HLA-G* may serve as a genetic marker to further explore the genetic etiology of POA across the genome.

The higher incidence of POA in females may be attributed to increased exposure to cleaning or skincare products containing quaternary ammonium compounds, a fundamental building block of NMBAs. This is reflected in our finding that 19 of 24 NMBA-induced POA patients were female. Furthermore, female sex could constitute a risk factor for POA, potentially due to sex-specific disparities in adaptive immune responses. This is evidenced by comparatively larger B cell populations and higher antibody production in females than in males.[23] In contrast, Francuzik et al. reported that males aged 13–65 years had a higher likelihood of experiencing severe anaphylactic shock compared to age-matched females.[24] The discrepancy between our study and Francuzik's findings warrants further investigation.

Patients with a history of previous surgical procedures were identified as being at an elevated risk for POA, potentially due to immunological memory of prior operative interventions, resulting in an intensified response upon re-exposure. As stated in NAP6,[25] the severity of anaphylaxis is proportional to the specificity of the allergen. In terms of surgical types, abdominal surgery had the highest percentage of POA cases, with 29 out of

72, or 40.2%. Taking into account different medical specialties, abdominal surgeries encompass gastroenterology, gynecology and obstetrics, hepatology, and more. Compared to any of the above specialties, orthopedic surgery exhibited the highest incidence of POA cases. The elevated incidence in orthopedic surgery may be ascribed to the prevalent use of diluted rehydration, specifically the infusion of succinylated gelatin, alongside intraoperative analgesia and deep muscle relaxation. These practices, while aiming to minimize blood loss and enhance surgical visibility, potentially heighten the risk of exposure to allergens. Besides, orthopedic surgeries are frequently preceded by excruciating conditions such as fractures, stress, and intraoperative use of prostheses and cements, further increasing the likelihood of exposure to offending medications and culprit drugs. In contrast, enhanced recovery after surgery (ERAS) protocols, which advocate for restricted or goal-directed fluid therapy and simplified prescriptions in abdominal, cardiothoracic, neurologic, and otolaryngologic procedures, may reduce allergen exposure in these procedures.

HLA-G, first reported to be expressed in trophoblast cells in 1990, plays a role in mediating fetal tolerance to maternal immunity.[26–28] Beyond its expression in immune-exempted cells and tissues in a normal physiological state, HLA-G is also involved in transplantation, tumor immune escape, and autoimmune diseases under pathological conditions.[29–31] The secreted form of HLA-G*01:04 has been proposed to be artificially processed as a precursor molecule for treating recurrent miscarriage.[32,33] Detecting the secreted HLA-G*01:04 in POA patients is crucial for understanding the potential effects of *HLA-G* variants on protein function. In a large US cohort study by Alexei Gonzalez-Estrada and colleagues, obstetrics/gynecology procedures were observed to have the lowest incidence of POA.[34] Moreover, there is an indication that pregnancy might confer a protective effect against immediate drug hypersensitivity reactions. Our data, however, showed that all female POA patients, except for 1 8-year-old girl, had a history of pregnancy, highlighting the need for further research to re-exam the potential link between pregnancy history and immune tolerance of HLA-G to POA.

Recent evidence has implicated the MRGPRX2 protein as a receptor for various pharmaceuticals and cationic proteins, with the potential to directly induce mast cell degranulation and anaphylactic reactions.[35–38] It has been proposed that POA is caused by abnormal activation of this receptor because variants in *MRGPRX2* can render the receptor more sensitive to drugs like rocuronium.[39] Transmembrane domain missense variants (M196I, L226P, and L237P) of *MRGPRX2* are believed to result in a gain-of-function phenotype for rocuronium-induced mast cell degranulation.[40] Nevertheless, cells transiently expressing M196I, L226P, and L237P variants of *MRGPRX2* did not exhibit an enhanced degranulation in response to rocuronium compared to the wild-type receptor,[39] suggesting a multifactorial and complex pathology of POA. Seven variants were found in 73 POA cases, including 3 nonsynonymous variants (NM_054030 c.T195G: p.S65S, c.A185G: p.N62S, and c.A46C: p.N16H), and 2 synonymous variants (NM_054030 c.*315A>T, c.*299A>G). However, the detailed association analysis of *MRGPRX2* gene with POA did not detect any significant effect of *MRGPRX2* variants. Our results indicate that variants in either the coding or the regulatory regions of *MRGPRX2* are unlikely to substantially affect POA susceptibility.

This study has several limitations. First, an insufficient number of non-POA patient samples attenuated the association between clinical variables and POA. POA patients in this study did not include non-IgE-mediated cases without tryptase increase. This factor warrants careful consideration in the participant selection for our subsequent research. Second, additional clinical variables associated with POA risk were not investigated, such as antiseptics, ongoing infections, hormonal therapy, pregnancy, NSAIDs, statins, bone cement in orthopedic surgery, latex exposure, mastocytosis, and others.[41] Constrained by the criteria of our matched-pairs case-control design, we were unable to incorporate gender as a criterion, a limitation that, if addressed, would reduce the available pairs to fewer than 72. Therefore, gender-based group bias in outcomes is inevitable. Third, in alignment with classical immunological principles, our study was unable to identify any variants associated with, or in linkage disequilibrium with,

HLA class II loci, which play a crucial role in immediate hypersensitivity reactions. Fourth, genetic differences between racial groups prevented us from extrapolating our Chinese population findings to other populations. Finally, the underlying genetic pathogenic effect of *HLA-G* on POA remains unknown and necessitates further investigation, which was absent in our study.

In conclusion, our study determined that female sex, unconfirmed food allergy label, and prior surgery were associated with POA, and we provided the novel insights into the genetic predispositions linked to POA. Furthermore, *HLA-G*01:01* was identified as a risk factor and *HLA-G*01:04* as a protective factor for POA. These findings highlight the *HLA-G* locus as a potential candidate region for POA association signaling. However, future research is imperative to uncover causal genes and underlying molecular mechanisms that influence the susceptibility to POA induced by perioperative drugs.

Abbreviations
POA, perioperative anaphylaxis; NMBA, neuromuscular blocking agent; HLA, human leucocyte antigen; ASA, American Society of Anesthesiologists; MRGPRX2, MAS-related G protein receptor X2; SNV, single nucleotide variant.

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Data availability statement
Data relevant to the study are available from the corresponding author upon reasonable request.

Authors' contributions
ZQ and YC: Conception of the work, analysis of data, and drafting the work. YS, JZ, YMQ, JJY: Acquisition of data for the work. QHX: Supervision of the project, and final approval of the version to be published.

Ethics approval
This study was approved by Ethics Committee of Scientific Research and Trial of XX Hospital (2021-KY-0378-002) and written informed consent was obtained from patients' relatives soon after the PAS occurrence.

Authors' consent for publication
All authors have approved the final manuscript and approved of the submission to World Allergy Organization Journal.

Patient and public involvement
Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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Declaration of competing interest
The authors declare that they have no conflicts of interest.

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