Solenopsis geminata (tropical fire ant) anaphylaxis among Thai patients: its allergens and specific IgE-reactivity

Rutcharin Potiwat,[1] Sasipa Tanyaratsrisakul,[2] Santi Maneewatchararangsri,[2] Wiparat Manuyakorn,[3] Ticha Rerkpattanapipat,[4] Yudthana Samung,[1] Chukiat Sirivichayakul,[5] Wanpen Chaicumpa,[6] and Raweerat Sitcharungsi[5]

Abstract

Background: Specific IgE against *Solenopsis invicta* (imported fire ant) remains the current diagnostic tool for allergy to ants worldwide. However, *S. invicta* may not be the only cause of ant anaphylaxis in Thai patients.

Objective: To characterize ant species causing anaphylaxis in Thai patients and to test allergenic reactivity to whole body extracts (WBE) of *S. geminata* (tropical fire ants) in patients with evidence of IgE-mediated ant anaphylaxis.

Methods: Thirty-two patients with ant anaphylaxis were identified. The causative ants collected by the patients were subjected to species identification. Twelve patients with ant anaphylaxis and showed positive skin test or serum specific IgE to *S. invicta* and 14 control subjects were recruited. Whole body extraction from *S. geminata* was performed for protein characterization using SDS-PAGE and protein staining. IgE-immunoblotting and ELISA-specific IgE binding assays were performed on patients' sera and compared with controls.

Results: Of 32 patients with ant anaphylaxis, the most common causative ant identified was *S. geminata* (37.5%). Western blot analysis of crude *S. geminata* revealed 13 refined protein components that bound to patients' serum IgE. Three major allergens with molecular masses of 26, 55 and 75 kDa were identified. All 12 patients gave positive results for specific IgE to *S. geminata* with statistically significant higher absorbance units of 0.390 ± 0.044, compared to healthy control group (0.121 ± 0.010), P < 0.01.

Conclusions: *S. geminata* is identified as the most common causative ant anaphylaxis in Thai patients. Its WBE comprises of 13 IgE-binding components and 3 major allergens (26, 55 and 75 kDa), which supported possible IgE-mediated mechanism.

Keywords: Fire ant, ant allergy, *Solenopsis geminata*, *Solenopsis invicta*, specific IgE-ELISA

From:

[1] Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

[2] Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

[3] Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[4] Department of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[5] Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

[6] Center of Excellence on Therapeutic Proteins and Antibody Engineering, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

Corresponding author:
Raweerat Sitcharungsi
Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand
Email: raweeratsitcharungsi@gmail.com

Abbreviations:
kDa = kilodalton
ELISA = Enzyme-linked immunosorbent assay
WBE = Whole body extract
IFA = imported fire ant
TFA = tropical fire ant

Introduction

Ant hypersensitivity can be fatal, owing to a systemic reaction or anaphylaxis.[1-3] Fire ant anaphylaxis is a serious problem worldwide with reports on fatalites.[2,4] Imported fire ant (IFA), in particular *Solenopsis invicta* (red imported fire ant) and *S. richteri* (black imported fire ant) are well-known for causing life-threatening allergic reactions.[5] The risk of

IFA stings in people living in endemic areas is as high as 30%-60%,[5,6] with a 0.16–16% risk of severe reactions.[7-9] In addition to *Solenopsis* species, *Formica*, *Myrmecia*, *Tetramorium*, *Pogonomyrmex*, *Pachycondyla*, *Odontomachus*, *Rhytidoponera*, *Pseudomyrmex* and *Hypoponera* are ant genera that have also been reported to cause serious hypersensitivity reactions.[10] *Solenopsis geminata* has been reported to be an important stinging ant species causing anaphylaxis in many Asian islands, including Indonesia and Taiwan.[11] Imported fire ants were introduced from South America and spread to Southeastern regions of the United States from 1920 onwards.[12] Currently, IFAs are endemic in some areas of Asia, such as China and Hong Kong, and some parts of Australia.[12]

In Thailand, there are as many as 247 species of ants belonging to nine subfamilies.[13] However, IFAs have not been detected in Thailand. The most common fire ant species found in Thailand is *S. geminata* (tropical fire ant, TFA), but the most common causes of ant anaphylaxis include both *S. geminata* and *Tetraponera* spp.[2,14] However, the confirmation of ant anaphylaxis in suspected patients is currently based on a skin test or specific IgE against *S. invicta* whole body extract (WBE), the only available commercial tests for ant allergy.[7] There have been limited data on cross-reactivity among ant species and only a few studies have reported cross-reactivity between the venom of *Solenopsis* spp.[15,16] and *Pogonomyrmex* spp.[2] Interestingly, a proportion of Thai patients with an obvious history of ant anaphylaxis had negative skin tests and specific IgE against IFAs. This indicated a lack of specific IgE or skin testing reagent to relevant local allergens, as the mechanism of hypersensitivity reaction to ant sting in Thai patients was supposed to be similar to that of IFA sting.

We aimed to identify the *S. geminata* allergen by preparing crude WBE and study of IgE-binding from patients' sera with evidence of IgE-mediated ant anaphylaxis to *S. invicta*.

Methods

Patients

Thirty-two children and adults with a history of ant anaphylaxis that were seen at allergy clinics at the Faculty of Medicine, Ramathibodi Hospital and the Hospital for Tropical Diseases, and the Faculty of Tropical Medicine, Mahidol University, during 2012–2014, were identified. The inclusion criteria were patients who had a history of anaphylaxis from ant stings and positive results for specific IgE or skin tests using *S. invicta* WBE. The level of specific IgE to IFAs of > 0.35 kAU/L was interpreted as a positive result. The reaction of skin prick test (SPT) was considered positive when a wheal diameter was at least 2 mm larger than negative control with surrounding erythema at 15 minutes. All positive SPT must be at least 3 mm in diameter size. Patients with negative SPT, would proceed to intradermal test, which was considered positive when a wheal diameter was increased at least 3 mm from injection papules with pruritus or surrounding flare after 20 minutes.[17] These are the current methods for diagnosing fire ant anaphylaxis in Thailand because specific investigations for other type of ant, including TFA are not available. Baseline data, including age, gender, underlying diseases, history of stinging hypersensitivity and clinical manifestations, were collected.

Control subjects

Fourteen control subjects were recruited at the Faculty of Tropical Medicine, Mahidol University after obtaining written informed consents. They were healthy volunteers who had history of a fire ant sting without any symptoms or only a small local reaction.

Causative ant identification

All patients were asked to bring the suspected causative ants from the areas where they were stung to the hospital for species identification. The ant species were identified by a taxonomist from The Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University. Because *S. geminata* was found to be the most common ant causing anaphylaxis in the patients, the surveillance teams went to the endemic areas to collect ant samples by direct searching.

Collection of tropical fire ants

Tropical fire ant colonies were collected from patient's houses and were placed into plastic containers with a capacity of $3.14 \times (6.4)^2 \times 9.4$ cm^3, covered with nylon net for air flow. Meat and 10% sugar solution were provided in each container in a cup during transfer of the sample to the laboratory. The ants were washed three times in phosphate buffered saline (PBS; 140 mM NaCl, 10 mM phosphate buffer and 3 mM KCl, pH 7.4 at 25°C; MERCK Millipore), then stored at −80°C before the preparation of crude WBE solution.

Preparation of tropical fire ant allergen

Crude fire ant allergen was prepared from the collected *S. geminata*. Briefly, frozen fire ants were cryogenically grinded in liquid nitrogen until a powder of material appeared. Then, the soluble contents were dissolved in sterile normal saline (NSS) at 4°C for 2 h. Debris was removed by centrifugation at 10,000 g (25°C for 15 min) and the supernatant containing tropical fire ant extract was collected. The total protein concentration was determined using a Pierce™ BCA protein assay kit and bovine serum albumin as the standard (Thermo Fisher Scientific, MA, USA). Allergen was verified by SDS-PAGE and colloidal Coomassie brilliant blue G-250 staining. The extract was stored at -70°C until use. The contamination of *S. geminata* extract was also minimized by using protein-free plastic ware, and sterilizing the cleaned mortar and grinder to prevent the contamination of in-house allergen extract.

SDS-PAGE

Allergens from TFAs-*S. geminata* and IFAs-*S. Invicta* (ALK-Abello Inc., USA), were separated by 13% SDS-PAGE under reducing conditions (20 μg per well) using a Mini-PROTEAN^R Electrophoresis System (Bio-Rad, USA). SDS-PAGE was carried out at 20 mAmp/gel for 45 minutes. Then, separated proteins were stained by colloidal Coomassie brilliant blue G-250, followed by de-staining until the background was clear.

IgE-immunoblot

Tropical and imported fire ant allergens were subjected to 13% SDS-PAGE as described above and then electro-transferred onto PVDF membrane (PALL Corporation,

PA, USA). The membrane was blocked with blocking buffer (5% skim milk in PBS, pH 7.4) for 1 h at 25°C. Then, it was incubated with a 1:100 dilution of individual serum samples in blocking buffer for 2 h at 25°C. Thereafter, it was washed five times, each time for five min with washing buffer (0.05% Tween-20 PBS; PBS-T). Finally, the membrane was incubated with horseradish peroxidase-conjugated mouse anti-human IgE antibody (Southern Biotech, USA) at a dilution of 1:4,000 for 1 h at 25°C, followed by washing as described above. Fire ant allergen-IgE antibody reactive bands were detected by chemiluminescent substrate (Thermo Fisher Scientific (Pierce), USA) and an ImageQuant LAS 4000 mini imager (GE Healthcare Bio-Sciences AB, Sweden). Three experiments were carried out in 12 patients and 14 healthy controls.

IgE-ELISA assay

ELISA assays were performed to investigate *S. geminata* IgE reactivity in patients' sera. First, TFA allergen (500 ng/well in PBS pH 7.4) was added to an ELISA plate (ExtraGene Inc., Taiwan) and incubated at 4°C overnight. Unbound allergens were washed off with 200 μl of washing buffer (PBS-T) twice, then the wells were blocked with 150 μl of blocking reagent (5% skim milk in PBS) at 37°C for 1 h. After washing, 100 μl of 1:100-diluted serum or blocking reagent alone was added to each well and the plate was incubated at 37°C for 1 h. After washing the ELISA plate five times, 100 μl of a 1:4,000-dilution of horseradish peroxidase-conjugated mouse anti-human IgE secondary antibody (Southern Biotech) were added to each ELISA well and the plate was incubated as described above. After washing, 100 μl of 1-Step™ Ultra TMB substrate (Thermo Fisher Scientific (Pierce), USA) was added to each well. After 15 min incubation, 100 μl of 2 N sulfuric acid was added to each well. The optical density (OD 450 nm) of IgE-ELISA was measured using a microplate reader (Bio-Tek Instruments, VT, USA) at 450 nm (OD_{450nm}). The experiment was performed in duplicate on three independent occasions. For *S. invicta*, the IgE binding was very low and not much different from the background control. This might be the low concentration of *S. invicta* protein from commercial extract and the high viscosity of piperidine solvent, which may affect protein coating efficacy. We thus reported only western blot analysis of *S.invicta*.

Statistical analyses

Median, interquartile range (IQR) or frequency percentage distributions were calculated according to the type of variables for the demographic data. IgE reactivity results analysis was performed by an unpaired t-test using the software GraphPad Prism version 5 (GraphPad Software, La Jolla, CA, USA).

Results

Ant species identification and patients' clinical characteristics

Thirty-two patients with a history of anaphylaxis from ant stings were identified. The suspected causative ants brought in by the patients were identified by the taxonomist and were attributed to the following genera: 12 *S. geminata* (37.5%), 10 *Tetraponera* spp. (31.3%), 2 *Odontoponera* spp. (6.25%), 1 *Pachycondyla* spp. and 1 *Monomorium* spp. Six patients (18.75%) were unable to provide a sample of the causative ants but suspected that they were stung by *S. geminata*.
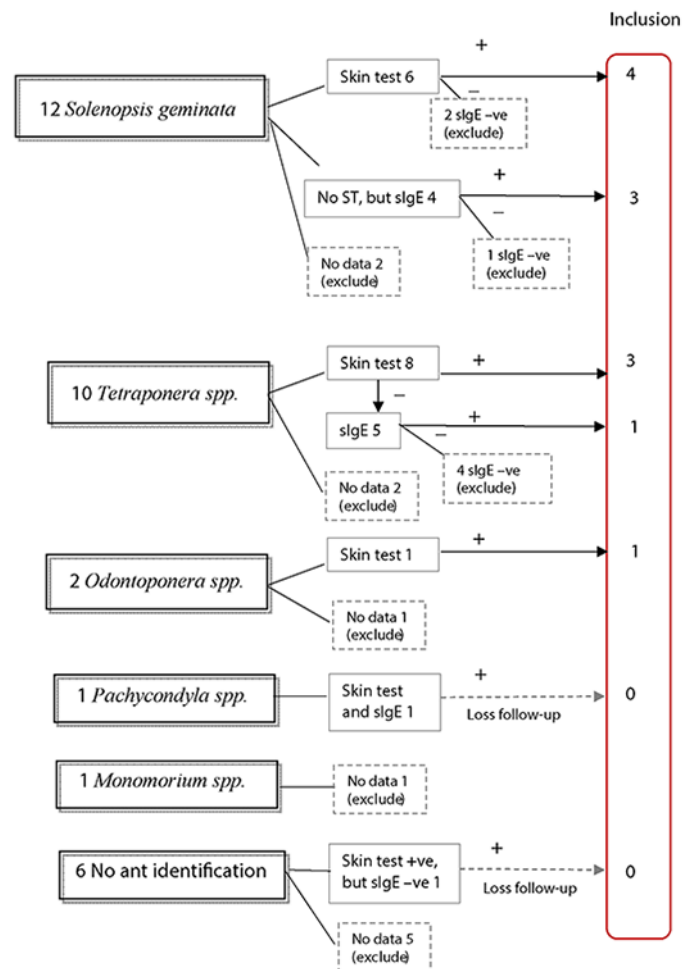
Figure 1. Recruitment diagram of 32 patients.
sIgE: specific IgE to *S.invicta*

Figure 1 showed recruitment diagram of 32 patients. 7 of 12 patients stung by *S.geminata*, 4 of 10 stung by *Tetraponera spp.*, and 1/2 stung by *Odontopenera* with positive tests to *S.invicta* were included in the study. Unfortunately, we have 2 positive patients who loss to follow-up (1 stung by *Pachycondyla* and another with no ant identification). Testing data were missing in 11 patients. Overall skin test to *S.invicta* was performed in 17 patients: positive in 10 and negative in 7 patients. And specific IgE to *S.invicta* was done in 13 patients: positive in 5 and negative in 8 patients.

A total of 12 patients who showed positive results with the specific IgE or skin test using *S. invicta* WBE were recruited into the patient group and 14 healthy volunteers were recruited into the control group. The clinical characteristics of the patients and control subjects are shown in **Table 1**. Seven patients (7/12; 58.3%) were female with a median age (IQR) of 12.5 (5–63) years, while 11 of 14 (78.6%) control subjects were female, and a median (range) age of 28 (23–39) years. Nine patients had a history of atopic diseases including allergic rhinitis (50%), allergic rhinoconjunctivitis (8.3%) and asthma (33.3%). All atopic diseases were under-controlled during the study. Two subjects (14.3%) in the control group had a history of allergic rhinitis.

Table 1. Clinical characteristics of 15 patients with fire ant anaphylaxis and 14 control subjects

Subject No.	Gender	Age (yr)	Atopy	Symptoms	Causative ant from ant identification	Serum gEIFA	Skin test IFA
P1	M	8	AR, ASTHMA	S, RS	*S. geminata*	9.33	+
P2	F	10	ARC	S, RS, GI	*S. geminata*	1.57	+
P3	F	37	N	S, RS	*S. geminata*	<0.35	+
P4	F	27	AR	S, RS, CVS	*S. geminata*	13.4	+
P5	M	12	AR, ASTHMA	S, RS	*S. geminata*	11.0	ND
P6	M	7	ASTHMA	S, CVS	*Odontoponera denticulata*	22.4	+
P7	M	5	ASTHMA	S, RS	*Tetraponera rufoniara*	0.74	-
P8	F	63	N	S, CVS	*Tetraponera rufoniara*	<0.35	+
P9	M	16	AR	S, RS	*Tetraponera rufoniara*	5.62	+
P10	F	13	N	S, RS, CVS	*Tetraponera rufoniara*	21.6	+
P11	F	11	AR	S, RS	*S. geminata*	1.84	ND
P12	F	18	AR	S, RS	*S. geminata*	3.37	ND
C1	F	29	AR	N	ND	<0.35	ND
C2	F	23	N	N	ND	<0.35	ND
C3	F	24	N	N	ND	<0.35	ND
C4	F	39	N	N	ND	<0.35	ND
C5	M	32	N	N	ND	<0.35	ND
C6	F	36	N	N	ND	<0.35	ND
C7	F	25	N	N	ND	<0.35	ND
C8	F	27	N	N	ND	<0.35	ND
C9	M	28	N	N	ND	<0.35	ND
C10	F	28	N	N	ND	<0.35	ND
C11	M	27	N	N	ND	<0.35	ND
C12	F	37	N	N	ND	<0.35	ND
C13	F	27	N	N	ND	<0.35	ND
C14	F	29	AR	N	ND	<0.35	ND

P, patient; C, control; M, male; F, female; AR, allergic rhinitis; ARC, allergic rhino-conjunctivitis; S, skin manifestations such as urticaria, angioedema, and swelling of eyes and mouth; RS, symptoms of respiratory system such as wheeze and bronchospasm; CVS, symptoms of cardiovascular system such as low blood pressure, shock; GI, gastrointestinal symptoms such as vomiting; IFA, imported fire ant extract; N, none; +, positive; -, negative; ND, not determined.

The level of serum-specific IgE to IFAs was positive in 10 patients (83.3%), while the skin test was positive in 8 of 9 patients (88.9%). All control subjects gave negative results for serum-specific IgE to IFAs.

All patients reported severe systemic reactions after a clearly identified ant sting. The most frequently involved symptom was urticaria, which was reported in all patients. Ten patients had respiratory symptoms such as wheezing, dyspnea and a spasmodic cough. Three patients had syncope, two patients suffered shock and one patient had gastrointestinal symptoms, which included vomiting. Most patients had been stung only once. Finally, among 12 patients recruited, 7 were stung by *S.geminata*, 4 by *Tetraponera rufoniara*, and 1 by *Odontoponera denticulata*.

### Fire ant allergen profile and specific IgE reactivity

The WBE of TFA allergen was prepared from crude extract of *S. geminata*. Its protein contents showed a significantly different profile on an SDS-PAGE gel compared to IFA allergen (*S. invicta*). While, TFA extract revealed more than 20 bands of protein ranging from 10 to 200 kDa, IFA allergens appeared to have only two major bands between 25 and 35 kDa. (**Figure 2**). To verify background binding, mock allergen extract without fire ant was included in western blotting and nothing was detected with serum from two randomly selected patients (data not shown).

### Determination of specific IgE against *S. geminata* and *S. invicta*

Specific IgE to *S. geminata* and *S. invicta* was detected by western blot analysis and 13 reactive bands were detected in patients' serum (**Figure 3** and **Table 2**). The common IgE-specific reactive bands of *S. geminata* (presented in >80% of ant allergic patients' serum) had estimated molecular masses of 26, 55 and 75 kDa, which showed reactivity in 100%, 83.3% and 91.7% of patients, respectively. (Arrows, **Figure 3**). More

than 50% of patients also had IgE-reactive band to *S.geminata*'s protein at molecular weight of 85 and 95 kDa. (**Table 2**) For *S. invicta* allergen, only weak IgE reactive bands at 26 and 55 kDa were identified. The serum of control subjects (n=14) did not show any reactive bands for either *S. geminata* or *S. invicta* allergens, as shown in **Figure 3**.
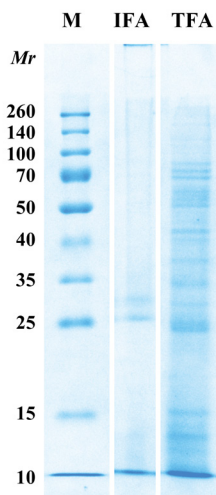
Figure 2. SDS-PAGE showing protein compositions of imported fire ant (IFA) and tropical fire ant (TFA) extracts according to relative molecular mass (Mr).
Mr: relative molecular masses ranging from 10 to 260; M: molecular weight marker
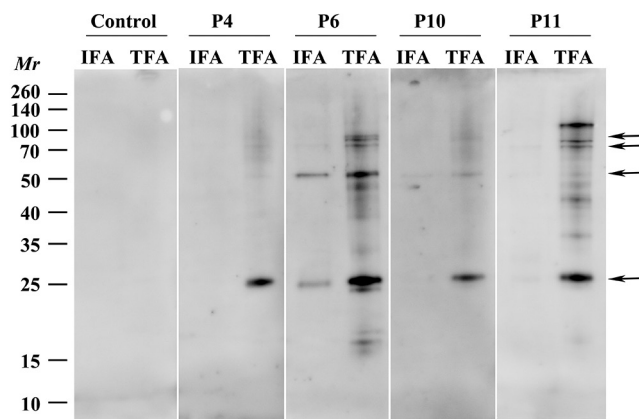
Figure 3. Immunoblot of IgE-reactive components of *S. invicta* (IFA) and *S. geminata* (TFA) extracts among patients and healthy controls
IgE-reactive patterns of 4 allergic patients (P4, P6, P10 and P11) and one healthy control are shown. Arrows indicate common IgE-reactive allergens of tropical fire ants that reacted with more than 80% of allergic patients. The healthy control did not show any IgE-reactive bands.
Mr: relative molecular masses of the proteins; IFA: imported fire ant; TFA: tropical fire ant

Table 2. The IgE reactivity profile of tropical fire ant allergens in patients with evidence of IgE-mediated to *S. invicta*

MW (kDa)	Patient number												% of patients reacted to each reactive band
	1	2	3	4	5	6	7	8	9	10	11	12	
115	●	●			●						●		33.3
95		●			●	●	●	●	●	●			58.3
85		●			●	●	●	●	●		●		58.3
75	●	●	●	●	●	●	●	●	●		●	●	91.7
55	●	●	●	●	●	●			●	●	●		83.3
48			●			●					●	●	33.3
44		●									●	●	25.0
38		●	●		●	●							33.3
36											●		8.3
33						●		●					16.7
26	●	●	●	●	●	●	●	●	●	●	●	●	100.0
24			●			●			●				25.0
17			●			●		●			●		33.3

Filled circles (●) represent protein bands that reacted with serum IgE from each patient, as determined by western blot analysis.
MW: molecular weight of IgE-binding protein, kDa: kilodalton

Specific IgE ELISA reactivity to S. geminata allergen

The specific IgE reactivity to TFA allergen was determined by ELISA using patients' and control subjects' sera at a dilution of 1:100. The absorbance unit of IgE reactivity was significantly higher in patients compared with healthy controls (0.390 ± 0.044 and 0.121 ± 0.010, respectively; P value <0.01), with a greater than 99% confidence interval (**Figure 4**). Using the cut-off value of 0.151 (mean of control + 3×standard deviation (3SD)), 100% (12/12) of patients gave a positive IgE-specific result to the crude extract of TFAs.
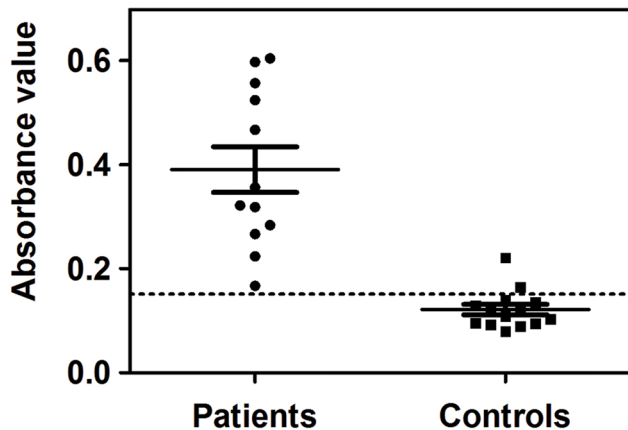
Figure 4. Specific IgE ELISA reactivity to *S. geminata* allergens among patients and healthy controls
The specific serum-IgE binding reactivity is shown at an absorbance value (OD 405 nm) for individual subjects. Dashed line indicates the cut-off value at 0.151 (mean of control + 3SD). The data are averages from three independent experiments.

Discussion

Our study is the first and the largest case series of ant anaphylaxis in Thai population. It is the first report to demonstrate that IgE antibody from *S. invicta* allergic patients with a history of ant anaphylaxis, recognized allergenic components present in *S. geminata* allergen extracted in-house, differentially from that of IFA allergen.

All patients in our study displayed anaphylaxis, with 100% showing cutaneous manifestations and 83% showing respiratory symptoms. One limitation of our study was the small number of patients since we enrolled only patients who presented with severe allergic reactions referred to the Medical Centers in Bangkok, and most therefore lived in Bangkok or in nearby provinces. Some cases of ant anaphylaxis that resulted in death in the southern and northern parts of Thailand would have been reported in the newspaper, but the provision of medical details was limited. From 32 patients identified, only 12 of these were recruited because of the requirement of evidence of specific IgE to fire ant, and the limitation of available test allergen. We did not include patients who had anaphylaxis to *S.geminata*, but had negative testing to *S.invicta*, and thus we could have missed some cases of mono-sensitized to *S.geminata*. Despite the possibility of uncertainty regarding the species of the causative ants because some patients might not be sure that they had brought in the correct species. We demonstrated that our patients with presence of specific IgE

to *S.invicta*, actually reacted to various type of ants, that were *S. geminata*, *Tetraponeera rufoniara*, *Odontoponera denticulata* and *Pachychondyla spp*. This finding suggested the possibility of cross-reactivity between *S.invicta* and other genera of ants, other than *S.geminata*.

Of the 12 patients included in our study, positivity of specific IgE levels to IFA extract was found in 83.3% (10/12), while skin test was positive in 88.9% (8/9). Two patients (16.7%) who had negative results of specific IgE to IFA, both had positive skin test. A practice parameters for stinging insect hypersensitivity had revealed that 20% of patients with positive skin test results for hymenoptera venom allergy showed negative results for serum-specific IgE.[17] Another study demonstrated that serum *S. invicta*-specific IgE had lower sensitivity than the skin test for the diagnosis of fire ant allergy.[18] These evidences were all consistent with our findings. However, our finding might also indicate the limitation of using *S. invicta*-specific IgE for the diagnosis of ant hypersensitivity possibly caused by *S. geminata* and *Tetraponera spp*. among the Thai population.

In the current study, majority of patients' serum IgE bound to the allergens of WBE of crude *S. geminata*, and it suggested that hypersensitivity reaction to *S. geminata* could be a type I, IgE-mediated mechanism.

Using ELISA, all 12 patients displayed serum IgE specific to *S. geminata* allergens, and significantly higher IgE reactivity was observed in the patients compared with the healthy controls. This might indicate that TFA extract is more sensitive than IFA extract for the identification and diagnosis of patients suffering from ant hypersensitivity in Thailand. However, this was limited by the findings that two healthy subjects showed slightly higher specific IgE levels, eventhough their IgE-bindings were not detected by western blot analysis. Thus, false positive of specific IgE-ELISA system could be suspected with the rate of 14.3% (2/14). Anyway, the specificity of crude WBE of TFA was supported by the western blot data as well, in which serum IgE from all patients reacted to various TFA proteins, whereas none of the healthy controls showed reactivity. Although we found more than 10 proteins recognized by the IgE from patients' sera, the proteins with relative molecular weights of 26, 55 and 75 kDa showed more than 80% recognition, which suggested that these three proteins might be the major allergens of TFAs.

Surprisingly, only weak IgE binding to *S. invicta* extract was observed in 33% of patients by immunoblotting, despite that majority of patients had specific IgE level to *S.invicta* at baseline. All patients in our study received IFA immunotherapy, which might explain a decrease the serum IgE binding against *S. invicta*, as detected by immunoblotting as well as very low binding of IgE to *S.invicta* allergens in IgE-ELISA system. Moreover, the serum from patients at the time of positive test to specific IgE to *S. invicta* were not available, and blood sample to perform immunoblotting were drawn separately. However, the major reactive proteins were found to have similar molecular weights of 26 and 55 kDa to the major allergens of *S. geminata*. This indicated the possibility of cross-reactivity of patients' serum-specific IgE, with IgE induced by *S. geminata* allergens cross-reacting to that of *S. invicta*.

Owning to the high number of protein bands of crude WBE of TFA, the contamination of *S. geminata* extract was also

concerned. We have multiple steps in preparation of crude extract to ensure the purification: washing the collected ant with PBS before use, using protein-free plastic ware, and sterilizing the cleaned mortar and grinder. Moreover, we have performed western blot analysis of mock allergen extract without fire ant, and nothing was detected with serum from two randomly selected patients.

The venom of IFAs is unique because it has a low protein content and a high concentration of piperidine toxin that consists of 90–95% water-soluble alkaloids, 2-methyl-6 -alkylpiperidines.[7,19] Currently, four important allergens have been isolated and characterized from *S. invicta* including Sol i 1 (37 kDa), Sol i 2 (26 kDa), Sol i 3 (24 kDa) and Sol i 4 (13 kDa).[19-21]

The Sol i 4 allergen has 35% identity with the Sol i 2 allergen but does not immunologically cross-react.[22] Similarly, *S. geminata* venom harbors four allergenic proteins designated Sol gem 1, Sol gem 2, Sol gem 3 and Sol gem 4. After evaluating Sol gem 2 under non-reducing and reducing conditions, its native form was identified as a dimer with molecular weights of 28 and 15 kDa and allergenic properties similar to Sol i 2 of *S. invicta*.[23]

A previous study demonstrated that the standard dosage of 0.5 ml of 1:100 wt/vol of *S. invicta* used as immunotherapy for ant anaphylaxis Thai patients demonstrated only 60% efficacy for preventing a field ant re-sting. However, after increasing the dosage to 0.5 ml of 1:50 wt/vol of *S. invicta*, the efficacy increased to 100%.[24] Since there is no *S. invicta* in Thailand and the majority of ant in Thailand is *S. geminata* and *Tetraponera spp*. This study may be an indirect evidence of the possibly cross-reactivity between *S. invicta* and other ants found in Thailand, particularly *S. geminata* and *Tetraponera spp*. on clinical outcome.

Information found in this study may be crucial for future studies using recombinant major allergens from TFAs as diagnostic and, possibly, therapeutic agents for ant allergic patients. The inclusion of patients from various regions in Thailand and patients negative for specific IgE or skin tests to *S. invicta* is important to confirm this hypothesis. In patients who had negative testing to both TFA extract and *S.invicta*, *Tetraponera spp*. seems to be another important ant causing anaphylaxis in Thai patients and worth studying. The properties and functions of targeted allergens should be explored to design effective diagnostic and therapeutic strategies. Whole body extractions of TFAs are producible as in-house allergens and might be applied for skin prick tests or immunotherapy in the future.

Conclusion

S. geminata was identified as the most common causative ant in Thai patients with ant anaphylaxis. Whole body extraction of *S. geminata*, prepared in-house for allergenic studies, revealed 13 IgE-binding components using patients' sera, with three major allergens (26, 55 and 75 kDa). Moreover, all studied patients harbored specific IgE antibody recognizing the allergenic components present in *S. geminata* supported the possible IgE-mediated mechanism to *S. geminata*. The 26 and 55 kDa allergens may induce the production of cross-reactive

IgE that can recognize allergens from both *S. geminata* and *S. invicta*.

References

1. Tankersley MS, Ledford DK. Stinging insect allergy: state of the art 2015. J Allergy Clin Immunol Pract. 2015;3:315-22.
2. Potiwat R, Sitcharungsi R. Ant allergens and hypersensitivity reactions in response to ant stings. Asian Pac J Allergy Immunol. 2015;33:267-75.
3. Srisong H, Daduang S, Lopata AL. Current advances in ant venom proteins causing hypersensitivity reactions in the Asia-Pacific region. Mol Immunol. 2016;69:24-32.
4. Haddad Junior V, Larsson CE. Anaphylaxis caused by stings from the Solenopsis invicta, lava-pés ant or red imported fire ant. Anais Brasileiros de Dermatologia. 2015;90:22-5.
5. deShazo RD, Butcher BT, Banks WA. Reactions to the stings of the imported fire ant. N Engl J Med. 1990;323:462-6.
6. deShazo RD, Williams DF, Moak ES. Fire ant attacks on residents in health care facilities: a report of two cases. Ann Intern Med. 1999;131:424-9.
7. Stafford CT. Hypersensitivity to fire ant venom. Ann Allergy Asthma Immunol. 1996;77:87-95.
8. Adams CT, Lofgren CS. Red imported fire ants (Hymenoptera: Formicidae): frequency of sting attacks on residents of Sumter County, Georgia. J Med Entomol. 1981;18:378-82.
9. Triplett RF. The imported fire ant: health hazard or nuisance? South Med J. 1976;69:258-9.
10. Klotz JH, deShazo RD, Pinnas JL, Frishman AM, Schmidt JO, Suiter DR, et al. Adverse reactions to ants other than imported fire ants. Ann Allergy Asthma Immunol. 2005;95:418-25.
11. Hoffman DR. Ant venoms. Curr Opin Allergy Clin Immunol. 2010;10: 342-6.
12. La Shell MS, Calabria CW, Quinn JM. Imported fire ant field reaction and immunotherapy safety characteristics: the IFACS study. J Allergy Clin Immunol. 2010;125:1294-9.
13. Jaitrong W, Nabhitabhata J. A list of known ant species of Thailand (Formicidae: Hymenoptera). Thailand Natural History Museum J. 2005;1(1):9-54.
14. Puranitee P, Vilaiyuk S, Kamchaisatian W, Manuyakorn W, Teawsomboonkit W, Sasisakunporn C, et al. Reported ant re-sting reaction in Thai children with ant anaphylaxis receiving immunotherapy. J Allergy Clin Immunol. 2012;115:AB228.
15. Hoffman DR, Smith AM, Schmidt M, Moffitt JE, Guralnick M. Allergens in Hymenoptera venom. XXII. Comparison of venoms from two species of imported fire ants, Solenopsis invicta and richteri. J Allergy Clin Immunol. 1990;85:988-96.
16. Pinnas JL, Strunk RC, Wang TM, Thompson HC. Harvester ant sensitivity: In vitro and in vivo studies using whole body extracts and venom. J Allergy Clin Immunol. 1977;59:10-6.
17. Golden DBK, Moffitt J, Nicklas RA, Freeman T, Graft DF, Reisman RE, et al. Stinging insect hypersensitivity: A practice parameter update 2011. J Allergy Clin Immunol. 2011;127:852-4.e1-23.

18. Stafford CT, Moffitt JE, Bunker-Soler A, Hoffman DR, Thompson WO. Comparison of in vivo and in vitro tests in the diagnosis of imported fire ant sting allergy. Ann Allergy. 1990;64:368-72.
19. Hoffman DR. Reactions to less common species of fire ants. J Allergy Clin Immunol. 1997;100:679-83.
20. Hoffman DR. Allergens in Hymenoptera venom XXIV: the amino acid sequences of imported fire ant venom allergens Sol i II, Sol i III, and Sol i IV. J Allergy Clin Immunol. 1993;91:71-8.
21. Hoffman DR, Dove DE, Jacobson RS. Allergens in Hymenoptera venom: XX. Isolation of four allergens from imported fire ant (Solenopsis invicta) venom. J Allergy Clin Immunol. 1988;82:818-27.
22. Hoffman DR. Fire ant venom allergy. Allergy. 1995;50:535-44.
23. Sukprasert S, Uawonggul N, Jamjanya T, Thammasirirak S, Daduang J, Daduang S. Characterization of the allergen Sol gem 2 from the fire ant venom, Solenopsis geminata. J Venom Anim Toxins Trop Dis. 2012;18: 325-34.
24. Manuyakorn W, Benjaponpitak S, Kamchaisatian W, Sasisakulporn C, Jotikasthira W. Safety and efficacy of ant rush immunotherapy in children. Asian Pac J Allergy Immunol. Forthcoming 2016.