









ORIGINAL ARTICLE

Simultaneous patch testing with fragrance markers in the baseline series and the ingredients of fragrance mixes: An update from southern Sweden

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Abstract

Background: Regularly updating the prevalence of fragrance contact allergy (CA) is important. Patch testing with fragrance markers in the baseline series and the ingredients of fragrance mixes (FMs) is still debated.

Objectives: To update the prevalence and clinical characteristics of patients with fragrance CA. To establish the results of patch testing with individual allergens of FMs.

Methods: A retrospective analysis of 3539 patients with dermatitis who were patch tested with the baseline series and FMs ingredients during 2016 to 2020 was performed.

Results: The prevalence of fragrance CA was 13%. About 10% of these patients with fragrance CA would be missed if the individual ingredients were not tested. Unlike hydroxyisohexyl 3-cyclohexene carboxaldehyde, there was no decreasing trend of CA to *Evernia prunastri* (oakmoss) extract after the EU regulation came into force. Patients with CA from only one ingredient of the mixes or having a weak positive reaction to the ingredients were significantly missed when tested with only the fragrance markers in the baseline series.

Conclusions: Patch testing with individual fragrance allergens is crucial for experts to expand knowledge in the fragrance CA field. The concentrations of the allergens in FMs may need to be adjusted to detect patients with fragrance CA, since some were significantly overlooked.

KEYWORDS

epidemiology, *Evernia prunastri* (oakmoss), Finn chamber, fragrance contact allergy, fragrance mix, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC, lyral), *Myroxylon pereirae* resin, patch test, prevalence

1 | INTRODUCTION

Allergic contact dermatitis (ACD) from fragrances is common and has been reported continuously from various countries. Recent studies showed that the prevalence of fragrance allergy varied from 5% to 25% in patch tested patients with dermatitis.¹⁻⁵ However, the different patch test procedures, test preparations, and the number of fragrance allergens included in studies varied.⁵ Regarding the difference of chemical groups of substances among fragrance allergens, including more and diverse test allergens seemed to be a major reason for having a high prevalence of fragrance CA.^{5,6} Fragrance contact allergy (CA) markers that are commonly used in the baseline series include *Myroxylon pereirae* (Balsam of Peru; BOP, 25.0% in pet.), fragrance mix I (FM I, 8.0% in pet.), fragrance mix II (FM II, 14% in pet.), and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC, 5.0% in pet.).^{7,8} Patients with fragrance CA might be missed, as a limited number of allergens are used in the screening process.^{9,10} At the Department of Occupational and Environmental Dermatology, Malmö, Sweden, the ingredients of FM I and FM II have been routinely and simultaneously tested in every patient who visits the clinic for patch testing with the Swedish baseline series.¹⁰ In a previous publication from southern Sweden with retrospective data from 2009 to 2015, FM I tended to be increased, whereas FM II tended to decrease.¹⁰

Because there are many fragrances used and fragrance-related substances, and the exposure to fragrances differs between gender and age in different parts of the world,^{11,12} regularly updating prevalence reports from different regions is important. The factors associated with fragrance CA were also differently reported from different countries.^{2,12} Female sex and older age were clearly related to a fragrance CA; however, the associated clinical characteristics might be different when evaluated among individual fragrance allergens.^{2,12} To improve patient care, investigating patients who are patch tested with the fragrance markers together with fragrance mix ingredients might constitute the basis for further evaluation of how to best diagnose CA resulting from fragrances. This study aimed to update the prevalence and trends of CA to fragrances during 2016 to 2020 in southern Sweden, report the associated factors with fragrance CA, and analyze the benefits and drawbacks of patch testing with individual fragrance mix ingredients.

2 | MATERIALS AND METHODS

2.1 | Study population and patch testing

Dermatitis patients older than 18 years of age who were referred for patch testing from 2016 to 2020 were included in this study. All patients were patch tested as in the previous study with the Swedish baseline series and additional FM I and II ingredients.¹⁰

Fragrance mix allergens in the Swedish baseline series comprised BOP (25.0% in pet.), FM I (8.0% in pet.), FM II (14.0% in pet.), and lichen acid mix (0.3% in pet.) but not HICC (5.0% in pet.). Because BOP, FM I, and FM II are common mixtures of allergens presented in

baseline series worldwide, they were defined as fragrance markers included in the analysis in this study. The constituents of the fragrance mixes used for patch testing are 2% amyl cinnamal, 1% cinnamal, 2% cinnamyl alcohol, 2% eugenol, 2% *Evernia prunastri* (oakmoss) extract, 2% geraniol, 2% hydroxycitronellal, 2% isoeugenol, 20% sorbitan sesquioleate, 2% citral, 1% citronellol, 5% coumarin, 5% farnesol, 10% hexyl cinnamal, and 5% HICC. Patients with incomplete patch test reading of any of these fragrance allergens mentioned were excluded from statistical analysis. If a patient were patch tested on more than one occasion, the patch test results from the only one visit that had completed readings would be included. If the readings were all completed, we would include only the results of the latest occasion.

Patch test procedures and patch test readings were conducted according to the recommended guidelines of the ICDRG and the European Society for Contact Dermatitis.^{13,14} The allergens (Chemotechnique MB Diagnostics AB, Vellinge, Sweden) were prepared by applying 20 mg (40 mg/cm²) of petrolatum preparations in either 8-mm aluminium Finn Chambers (before 2018) or 8-mm Finn Chambers Aqua (from January 2018) (SmartPractice, Phoenix, AZ, USA). In a minority of cases (about 6%), IQ chambers (Chemotechnique MB Diagnostics AB, Vellinge, Sweden) were used. Allergens in petrolatum preparations (25 mg or 39.06 mg/cm²) were applied in 8 × 8 mm IQ Ultra chambers or 8 × 8 mm IQ Ultimate chambers.¹⁵ The allergens were applied immediately after loading the fragrance allergens in the chambers¹⁶ and left on the upper back for 2 days. Patch test reading was performed on Day (D) 3 or D4 and on D7. The intensity of the reaction was evaluated as negative, doubtful, weakly positive (1+), strongly positive (2+), extremely positive (3+), and irritant reactions. Any positive (1+, 2+, or 3+) reactions on D3/4 or D7 were counted as positive reactions, whereas negative, doubtful, and irritant reactions were concluded as negative for the subgroup statistical analyses.

Patients who had at least one positive reaction to BOP, FM I or its ingredients, or FM II or its ingredients were classified as fragrance CA patients. Patients with a “strong to extreme reaction to the ingredients of the mixes” were the patients who had at least one strong to extreme reaction to any ingredient(s). In addition, patients' demographic data, including age, gender, a history of atopic dermatitis, and site of lesions, were recorded and analyzed. This retrospective study was approved by The Swedish Ethical Review Authority (Ethical Approval number 2020-02190).

2.2 | Statistical analysis

A descriptive analysis was used to report the numbers and percentages of clinical data and prevalences of positive reactions to allergens. Clopper-Pearson (exact) interval was used to report the 95% confidence intervals (CIs) for the prevalences. Two-sided Pearson chi-square test or Fisher exact test was performed to compare the proportion of patients in the two groups. The number of patients with positive and negative reactions to fragrance mixes who had a positive reaction to individual fragrance mix ingredients was compared.

Further comparison was performed in the positive reactions group regarding the intensity of the patch test. We also compared the patients' patch test reactions to fragrance mixes between "weak" and "strong to extreme" reactions to the individual ingredients. According to patients' characteristics, multiple logistic regression was performed to identify statistically significant factors related to fragrance CA. Age was dichotomized into two groups using the cut-off value at 40 years. Every single location of the rash was dichotomized into *having a rash* and *no rash* (reference category). In a multivariable analysis, factors with a *P*-value of less than .2 from the univariable analysis were included to assess the adjusted odds ratios (ORs) with 95% CIs. The mean age differences of the two groups were analyzed using the independent *t* test. A *P*-value of less than .05 was considered statistically significant. PASW Statistics for Windows (version 23.0; SPSS Inc., Chicago, Ill., USA) was used for the statistical analysis.

3 | RESULTS

During the study period, 3663 patients visited our clinic, and 124 patients were excluded. Of all, 3539 patients were included for the analysis; there were 2436 (68.8%) female and 1103 (31.2%) male patients. The mean age was 44.4 ± 17.0 years, with no significant mean age difference between genders (*P*-value = .43). Atopic dermatitis was reported in 27.6% of patients. Common sites of lesions were hands (33.3%), face (21.5%), and upper extremities (9.4%).

3.1 | Prevalence and trend

Of all, 464 patients (13.1%) were diagnosed with CA to fragrance. The 5-year prevalences (95% CI) of CA to BOP, FM I, and FM II were 7.1% (6.2%-8.0%), 6.2% (5.5%-7.1%), and 2.3% (1.8%-2.8%), respectively. The trends of positive reactions to fragrances were not significantly changed during 2016 to 2020 (Figure 1). Figure 2 demonstrates the distribution of positive reactions to tested allergens in fragrance CA patients. Of all fragrance CA patients, 10.3% (48 patients) were additionally detected by patch test with the ingredients of fragrance mixes.

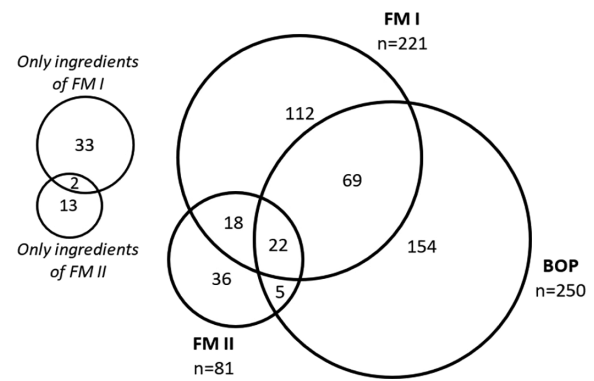


FIGURE 2 All 464 fragrance allergy patients, demonstrating positive reactions to fragrance markers in the baseline series (n = 416) and to only the ingredients of the fragrance mixes (n = 48)

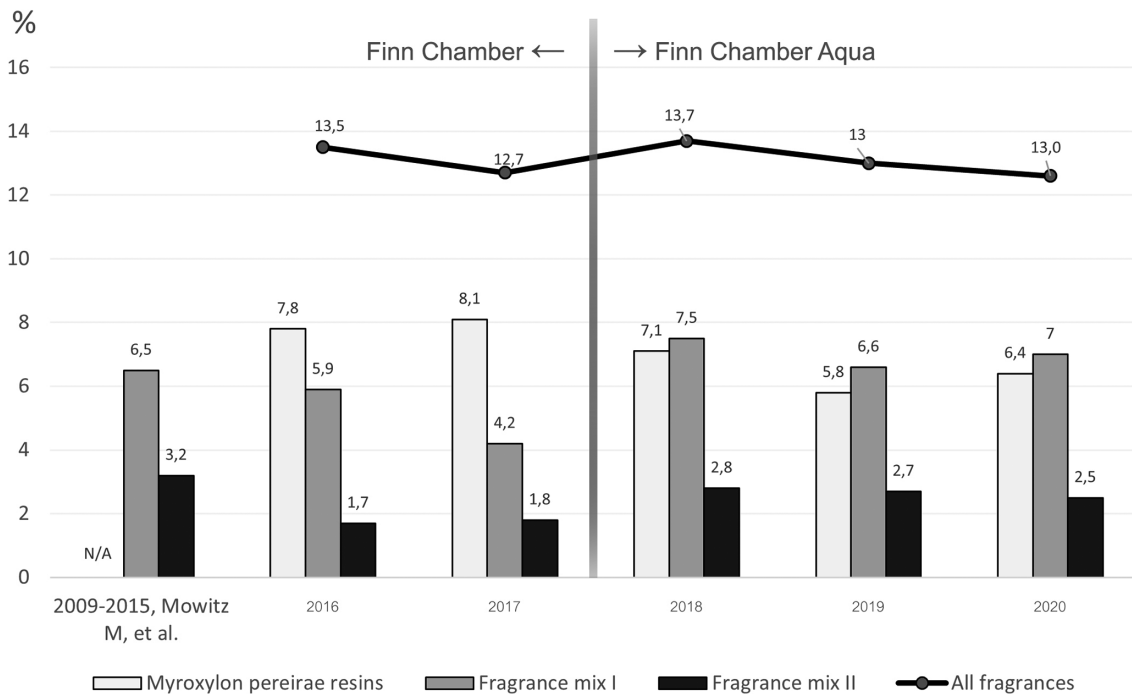


FIGURE 1 Trends of contact allergy to fragrances. All fragrances, patients who tested positive to at least one of the following allergens: *Myroxylon pereirae* resin, fragrance mixes, and ingredients. *P*-values of trends of positive reactions for all fragrances during 2016 to 2020 = 0.97, *M. pereirae* resins = 0.40, fragrance mix I = 0.10, fragrance mix II = 0.49. Prevalences in 2009 to 2015 were reported in Mowitz M, et al., the prevalence of *M. pereirae* resin was not reported¹⁰

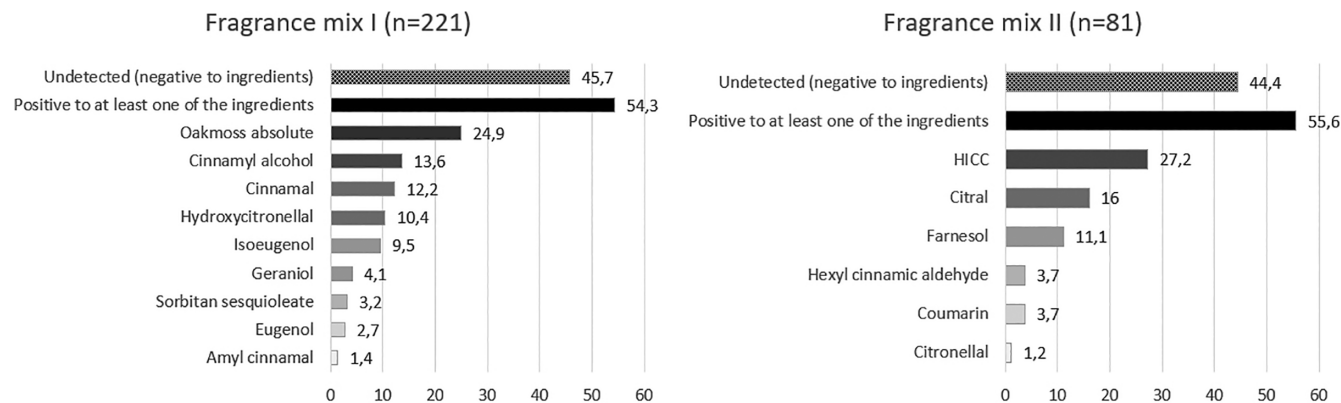


FIGURE 3 Percent of reactions to fragrance mix ingredients in patients with a positive reaction to fragrance mix I and fragrance mix II. HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde

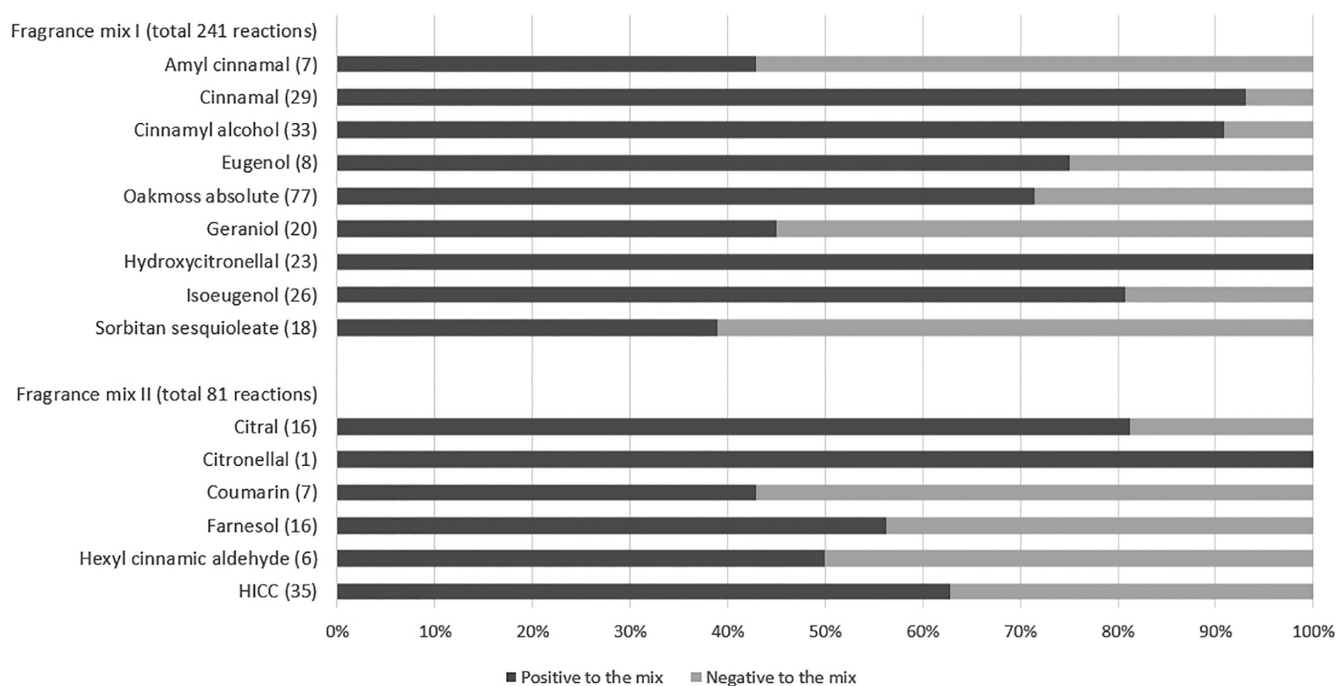


FIGURE 4 Reactions to fragrance mixes I (8.0% in petrolatum) and II (14.0% in petrolatum) in patients with positive reactions to their ingredients. HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde. Numbers in the parentheses represent a total number of reactions to the individual allergens

3.2 | Reactions to fragrance mixes and their ingredients

Figure 3A demonstrates 221 patients with a positive reaction to FM I in the baseline series. The exact culprits remained unidentified in 45.7% of the patients. More than half of them (54.3%) had positive reactions to known ingredients in FM I, including seven patients with positive reactions to sorbitan sesquioleate (20.0% in pet.). Positive reactions to the FM II ingredients were identified in 55.6% of the FM II CA patients (Figure 3B). Figure 4 demonstrates the number of positive reactions to fragrance mix ingredients. Overall, 241 positive reactions to FM I individual ingredients were found in 170 patients, and 81 positive reactions to

FM II individual ingredients were identified in 75 patients. The prevalences of positive reactions to individual FM I and FM II ingredients were mostly less than 1%, except for *Evernia prunastri* extract (2.2%, $n = 77$) and HICC (1.0%, $n = 35$). An additional 49 reactions were detected by testing positive to individual ingredients of FM I but negative to the combined preparation (FM I), and 30 reactions did not have a positive reaction to FM II but were positive to the individual ingredients (Figure 4). Patients with a higher number of positive reactions to the individual ingredients of a mix were more likely to (1) react positively and (2) have a stronger positive reaction to their mixes (Figure 5A, B). In addition, patients with a stronger reaction to the ingredients of the mixes had a significantly higher chance of having a positive reaction to their mix (Figure 6).

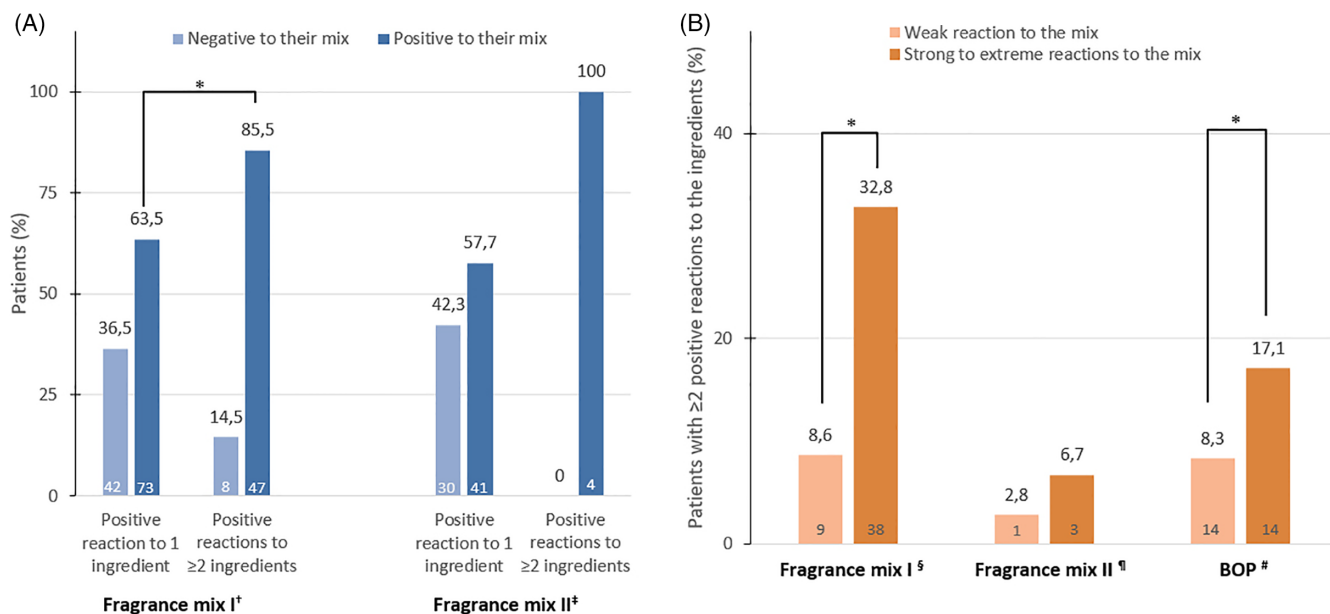


FIGURE 5 Percentage of patients. (A) Patients with positive and negative reactions to fragrance mix I and fragrance mix II according to the number of reactions to the ingredients and (B) patients with two or more reactions to the ingredients of fragrance mixes. BOP, *Myroxylon pereirae* resins; *P*-value less than .05 indicates statistically significant difference. Odds ratio (95% confidence interval), OR (95% CI) are demonstrated below when *P*-values < .05. †*P*-value = .003, OR (95% CI) = 3.38 (1.46-7.83); ‡*P*-value = .145; § *P*-value < .001, OR (95% CI) 5.20 (2.37-11.40); ¶ *P*-value = .625; # *P*-value = .040, OR (95% CI) = 2.27 (1.02-5.01) when compared to number of positive reactions the ingredients of fragrance mix I with intensity of positive reactions to *M. pereirae* resin (n = 250)

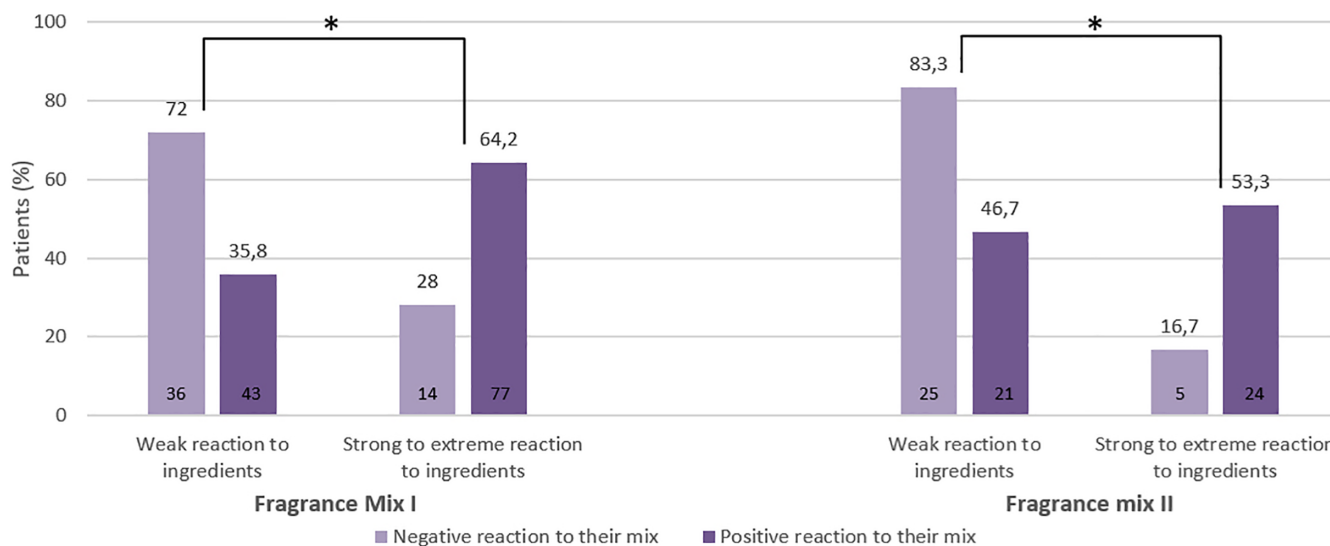


FIGURE 6 Intensities of positive reactions to individual ingredients and patch test reactions to their fragrance mix in the baseline series. *A *P*-value less than .05 indicates a statistically significant difference. Odds ratio (95% confidence interval), OR (95% CI) are demonstrated below when *P*-values < .05. Fragrance mix I, *P*-value < .001, OR (95% CI) = 4.61 (2.24-9.47); fragrance mix II, *P*-value = 0.001, OR (95% CI) = 5.71 (1.86-17.60)

3.3 | Characteristics of fragrance contact allergy patients

Significant associated factors with fragrance CA were patients with age equal to or older than 40 years and having a history of atopic

dermatitis (Table 1). Tables S1-S3 demonstrate comparisons of characteristics of patients in different subgroups: patients with BOP, FM I, or FM II CA. There were significantly more patients in the age group >40 years among BOP-positive patients than among BOP-negative patients (adjusted odds ratio [aOR] 2.14, 95% confidence interval

[CI] 1.55-2.94). Patients with FM I CA were significantly older than 40 (aOR 1.85, 95% CI 1.33-2.57); female (aOR 1.53, 95% CI 1.07-2.19), or had a history of atopic dermatitis (aOR, 1.61, 95% CI 1.16-2.40). The mean age was significantly higher in the group with

CA to FM II (P -value = .03). There was no specific location of the rash that significantly associated with CA to any fragrance markers. Percentages of positive reactions to FM I and FM II ingredients were compared between genders and age groups (Figure 7).

TABLE 1 Characteristics of patients with fragrance allergy

	All patients N = 3539 N	Fragrance allergy		Univariable analysis ^a		Multivariable analysis ^a			
		YES (n = 464)		NO (n = 3075)		P-value	OR (95% CI)	P-value	OR (95% CI)
		N	%	N	%				
Age, y (mean ± SD)	44.36 ± 16.99	49.86 ± 16.46	43.53 ± 16.92	<.001					
Age over 40 y	1963	315	67.9	1648	53.6	<.001 ^b	1.83 (1.49-2.25)	<.001	1.96 (1.54-2.50)
Female gender	2436	341	73.5	2095	68.1	.020 ^b	1.30 (1.04-1.62)	.072	1.26 (0.98-1.61)
Atopic dermatitis	976	141	30.4	835	27.2	.146 ^b	1.17 (0.95-1.45)	.003	1.45 (1.14-1.85)
Localization (n = 2905)									
Hands and fingers	1179	143	39.5	1036	40.7	.654	0.95 (0.76-1.19)		
Face	762	101	27.9	661	26.0	.440	1.10 (0.86-1.41)		
Arms and armpits	333	41	11.3	292	11.5	.930	0.99 (0.70-1.39)		
Generalized	164	20	5.5	144	5.7	.915	0.97 (0.60-1.58)		
Trunk	192	17	4.7	175	6.9	.117 ^b	0.67 (0.40-1.11)	.065	0.62 (0.37-1.03)
Legs	88	14	3.9	74	2.9	.320	1.34 (0.75-2.40)		
Head and neck	78	9	2.5	69	2.7	.802	0.91 (0.45-1.85)		
Genitalia and groin	51	6	1.7	45	1.8	.879	0.94 (0.40-2.21)		
Feet	43	6	1.7	37	1.5	.765	1.14 (0.48-2.72)		
Oral cavity	15	5	1.4	10	0.4	.030 ^b	3.55 (1.21-10.44)	.078	2.67 (0.90-7.93)

Abbreviations: CI, confidence interval; OR, odds ratio; SD, standard deviation.

^aThe reference categories were age ≤40 years, male, having no history of atopic dermatitis, and having no rash on the individual location.

^bVariables with a P -value < .2 were included in multivariate analysis; P -value < .05 indicates a statistically significant difference.

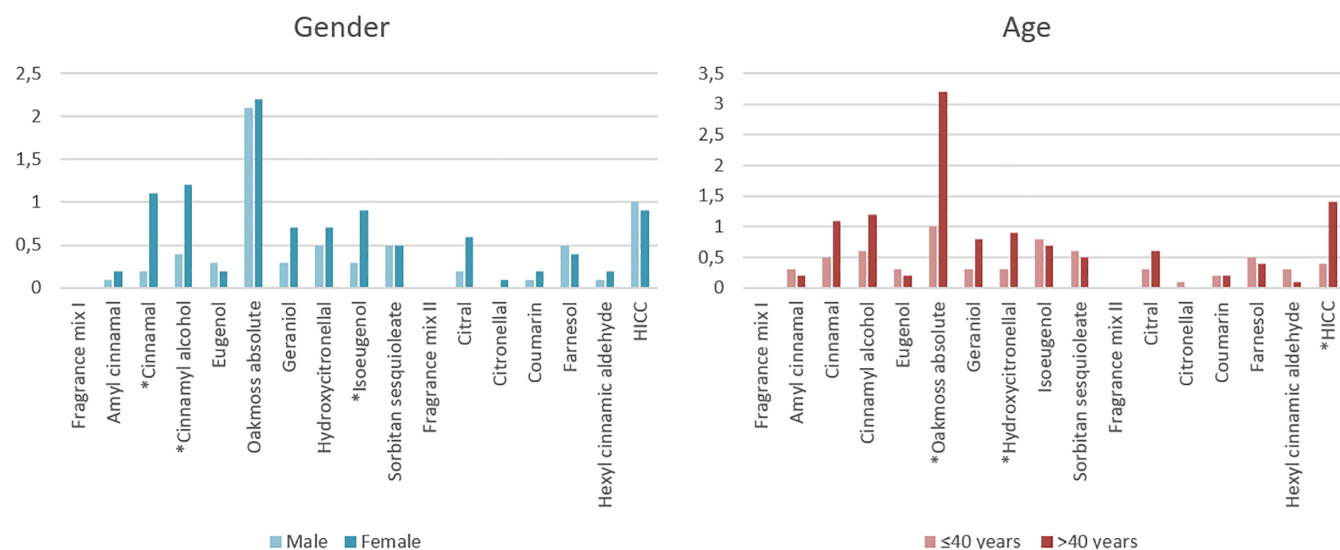


FIGURE 7 Comparisons of percentages of positive reactions to fragrance mix materials between gender and age in all tested patients (n = 3539). HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde; * P -value < .05 indicates statistical significance with the odds ratios (95% confidence intervals) between genders for cinnamal = 6.17 (1.47-26.00), cinnamyl alcohol 3.31 (1.16-9.44), and isoeugenol = 3.50 (1.05-11.67), and between age groups for *Evernia prunastri* (oakmoss) extract = 3.39 (1.92-5.99), hydroxycitronellal = 2.91 (1.08-7.85), and HICC = 3.24 (1.41-7.45)

4 | DISCUSSION

4.1 | Prevalences, trends, and characteristics of patients with fragrance contact allergy

This study showed that the prevalence of CA from fragrances was about twofold in overall dermatitis patients compared to those in the general population.¹¹ Older age group (≥ 40 years) and a history of atopic dermatitis were significant factors for overall fragrance CA. Even though the relationship between atopic dermatitis and CA has not been concluded,¹⁷ atopic dermatitis has sometimes been found to be an associated factor with ACD, or the patients with atopic dermatitis tended to have a higher number of patch test reactions, especially to fragrances.^{18,19} Concerning fragrance CA, there have also been divergent opinions on atopic dermatitis and contact sensitization. First, patients with atopic dermatitis might be sensitized at an earlier age by applying moisturizers that might contain fragrances. In contrast, some patients might have been recommended to use fragrance-free products. In addition, patients with atopic dermatitis might be able to induce immunological tolerance to some fragrances through gastrointestinal exposure more effectively than nonatopic dermatitis patients.²⁰ Therefore, the association between atopic dermatitis and fragrance CA might not be convincingly proven, depending on skin barrier defect, genetic predisposition, and individual's skin exposure to allergens.^{17,21}

It has been reported previously that women have a tendency to be exposed to scented products more than men in a general population.¹¹ Dermatitis patients in this study represent patients who were referred because of having clinical eczema, which were primarily selected. Men were less admitted for patch testing, given that gender was not a significant factor associated with fragrance CA by using multivariate analysis in this study. According to the skin exposure, fragrances are not limitedly used only in products applied to specific body areas,¹¹ the site of skin lesions and exposure are unpredictable in patients with a fragrance CA.

The recent prevalences of BOP CA have been reported to be 2.5% to 6.6% in dermatitis patients who underwent patch testing,^{1,2,12,22-25} which were slightly lower than this study (7.1%). The exposure through the oral mucosa might be sensitized by overlooked exposure.²⁶ This study found that the prevalence of concurrent reactions between BOP and FM I was 2.6% in overall dermatitis patients (Figure 2), which was higher than the previous reports.^{2,23} Correspondingly, the concurrent reactions BOP were found in 41.2% of FM I CA patients. The mutual components rated to have high frequencies of concurrent positive reactions to BOP were isoeugenol, eugenol, and cinnamyl alcohol, which are ingredients in FM I; whereas the reaction to farnesol (a component of FM II) was low.^{12,25} Because there were similar prevalences of positive reactions to BOP and FM I in this study and others, a higher prevalence of concurrent reactions between BOP and FM I found in this study could suggest a higher rate of sensitivity to the mutual compounds or a higher parallel exposure to other materials containing these two fragrance markers, or a combination of both. Regarding the patients' characteristics, the BOP CA patients were significantly older but with uncertain differences between genders.²⁵

When comparing with data sets from the same time period, FM I has often been ranked the highest rate of CA to fragrances compared to other fragrance markers.^{1,2,12,22,24} CA to FM I has become higher than to BOP in the last few years (Figure 1). Using different types of patch test chambers in our clinic during the study period is one of our major concerns. Finn chamber and Finn Chamber Aqua, which were routinely used for patch testing in 2016 to 2017 and 2018 to 2020, respectively, could affect the patch test results for FM I and BOP.²⁷ It is possible that more patients would react to BOP when applied in Finn Chamber, and more patients would react to FM I when tested with Finn Chamber Aqua.²⁷ However, only using different chambers could not affect the changing trends regarding our further statistical analysis. Therefore, the changing trends of CA to BOP and FM I could be from the increased chance of skin sensitization to the allergens in addition to the effects from the patch test system itself.

The prevalence of FM II CA has usually been lower than FM I.^{1,2,12,22,24} The wide gap of prevalences of FM I (3.0%-9.4%) and II (2.7%-6.9%) CA in different countries during recent years might be an effect of multiple factors, which the chance of skin exposure to different scents could be the most plausible explanation. Female, older age group, and a history of atopic dermatitis were associated with CA to FM I, but not to FM II. In another recent study, where patch testing with individual fragrance mix ingredients was performed in selected patients, geraniol was found to have more positive reactions in women, whereas *Evernia prunastri* extract had a higher prevalence in men.¹² Unlike in our study, in which the test was routinely performed in all dermatitis patients, significantly higher prevalence of CA to cinnamal, cinnamyl alcohol, and isoeugenol were found in women (Figure 7). A difference in skin exposure to fragrances between populations might be another explanation of the different results. In concordance with the report of the skin exposure to fragrances,¹¹ the results from this study strongly support skin sensitization to the fragrance materials in FM I in women. Regarding atopic dermatitis, the result of this study is similar to reports from the United States and Europe that FM I was the only fragrance marker in the baseline series that has been related to patients with atopic dermatitis.^{19,28} Scented product avoidance, especially products containing ingredients of FM I, should be recommended for patients with atopic dermatitis.

4.2 | *Evernia prunastri* (oakmoss) extract and HICC contact allergy after the ban

Prevalences of positive reactions to *Evernia prunastri* extract and HICC as individual ingredients of fragrance mixes in this study were the highest among all and significantly more in patients in the older age group (Figure 7). This study found that the prevalence of HICC CA had decreased compared to 2009 to 2015.¹⁰ This corresponds with the reports that the rate has gradually decreased worldwide, especially in European countries, in which patch testing with separated preparations of HICC might not be needed in the baseline series.²⁹⁻³¹ This was the result of the ban from the market of HICC in August 2017, announced by the European Commission (Regulation

EU 2017/1410).³² In this study, only 13 patients (0.37% of tested patients) with CA to HICC would be missed if testing with only FM II (Figure 4), similar to the results of an ICDRG study in 2012 to 2016 (0.3%).³⁰ Therefore, the inclusion of HICC in the baseline series should be withdrawn. At the same time, atranol and chloroatranol, two natural components of *Evernia prunastri* extract, were also banned.³² The frequency of *Evernia prunastri* extract used in personal products was low compared to other fragrances in the European market.³³ However, the CA rate did not rapidly correspond with the ban as it could be seen for HICC. It remained similar to previous studies,^{5,34} and was slightly increased (2.2%) compared to our study before 2016 (1.8%).¹⁰ The concentrations of atranol and chloroatranol in the patch test preparation were the same even after the ban. This finding brings a significant issue to the public's attention about the ban and its use in cosmetics. The regulation was amended in 2017 declaring that *cosmetic products containing atranol and chloroatranol shall not be "placed" and "made available" on the Union market from August 2019 and August 2021, respectively.* Following up on the prevalence after the ban and further investigations to explain the non-decreasing trend would be interesting in the near future. Another issue that we should be aware of is that, although lower levels of atranol and chloroatranol in *Evernia prunastri* extract could reduce the chance of skin sensitization, the other components might still be able to induce skin reactions.^{35,36}

4.3 | Patch testing with individual ingredients of the fragrance mixes

Patch testing with additional individual fragrance allergens seems to be needed to detect fragrance CA in patients with negative reactions to the fragrance markers in the baseline series.⁹ In total, 1.4% (48/3539) of overall dermatitis patients or 10.3% (48/464) of fragrance CA patients would be missed if they were tested with only fragrance markers in the baseline series. As expected, the patch test results of fragrance mixes and their ingredients in this study were not precisely consistent with each other (Figures 3 and 4). This has been observed in some studies and remained a concerning problem.^{10,12,37} The possible explanations were (1) false reactions³⁷ (either to the mixes or to their ingredients), (2) different test concentrations of the individuals in the mixes and their ingredients' test preparations^{38,39}; (3) some materials in the mixes might have a mild irritation effect and enhance absorption of other substances by disrupting the skin barrier when they are mixed; (4) a combination effect—a positive reaction to a mix might be a combination of multiple doubtful reactions to more than one ingredient, which might be read as doubtful or negative when tested with individual ingredients. This is supported by the results in Figure 5B. Patients with a strong to extreme reaction to the mix were more likely than the patients with a weak reaction to have a simultaneous positive reaction to more than one of the ingredients. Finally, the divergent results might be seen when testing with the mixes and ingredients from different batches.^{40,41}

The aims of using fragrance mixes have been discussed frequently by experts. Although FM I and FM II have been used comprehensively

for screening in baseline series for decades, the concentrations of their preparations have not been intensely studied or standardized as in other individual fragrances. Fragrance mixes should detect almost all patients with a fragrance CA to help the patients avoid scented products. This study emphasizes that the fragrance markers in the baseline series could mainly detect the patients with "multiple allergies" or "stronger patch test reactions" to the ingredients of the mixes.

About half of patients with positive reactions to fragrance mixes did not react to any of their constituents (Figure 3). It has been suggested that the test preparations of individual fragrance allergens might need to be increased if the patients reacted to the mixes but did not react to their ingredients' preparations.³⁷ Because the concentrations in the preparations of individual ingredients were higher than in the mixes, additional cases could unquestionably be detected (Figure 4). From the figure, 30 of 81 positive reactions were further detected because the concentrations of the individual ingredient preparations in FM II are twice comparing to themselves in the mix. Furthermore, the patients with hydroxycitronellal CA were all detected, whereas less than half of amyl cinnamal, geraniol, coumarin, and hexyl cinnamic aldehyde CA cases were identified by fragrance mixes (Figure 4). This could be explained by the theory of the different dose-response relationship of an allergic contact reaction for different allergens,³⁹ in which some allergens might need a much higher concentration to elicit a stronger patch test reaction, whereas others might need only a slightly higher concentration. Consequently, patch testing with a double concentration for hydroxycitronellal might not increase the positive reaction rate like other allergens. More investigations aiming at adjusting the test concentrations of fragrance mixes and their ingredients are beneficial before modification in the preparations for patch testing.

4.4 | Limitation

This study was set to focus on the main fragrance allergens widely used in the baseline series. Including other fragrance markers such as colophonium, sesquiterpene lactone mix, lichen acid mix, ox. linalool and ox. limonene might affect the prevalence of overall fragrance CA.⁴² The authors concerned the use of different types of patch testing chambers as discussed. However, the test procedure was standardized, as mentioned in Materials and Methods.

5 | CONCLUSIONS

The overall fragrance CA prevalence is still common. Dermatitis patients with older age were significantly related to fragrance CA. Being female was a significantly associated factor with FM I CA, of which cinnamal, cinnamyl alcohol, and isoeugenol were found to be related culprits. CA to HICC was rapidly decreased, whereas CA to *Evernia prunastri* extract remained high after the ban came into force at the same time, suggesting that 5% HICC in pet. should be

withdrawn from the baseline series. BOP, FM I, and FM II are valuable for detecting most patients with a fragrance CA. However, the patients with a weak positive reaction and who had CA to only one mix ingredient will risk being missed when only patch testing with the mixes. To improve the investigation for diagnosing missing cases, the concentrations of individual ingredients in FM I and FM II may be needed to increase to detect undiagnosed fragrance CA patients while not causing irritation or active sensitization effects.

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CONFLICT OF INTEREST

Magnus Bruze is a member of the Expert Panel for Fragrance Safety - <http://fragrancesafetypanel.org/> advisory to fragrance industry. The other authors have no conflict of interest to declare.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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