

DR CHUIN YING UNG (Orcid ID : 0000-0001-8487-4589)

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Patch testing with the European baseline series fragrance markers: A 2016 update

Running head: Patch testing for fragrance allergy

C.Y. Ung, J.M.L. White, I.R. White, P. Banerjee and J.P. McFadden

Guy's and St Thomas' University Hospital, St John's Institute of Dermatology, London, UK

Corresponding author: Chuin Ying Ung;

E-mail: chuin.ung06@gmail.com

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What's already known about this topic?

- In Europe, 26 fragrance substances are required to be labelled if present at a concentration of ≥ 10 ppm in leave-on cosmetic products and if present at a concentration of ≥ 100 ppm in rinse-off cosmetic products.
- The markers for fragrance allergy in the European baseline series (Fragrance Mix 1, Fragrance Mix 2, *Myroxylon pereirae* and hydroxyisohexyl 3-cyclohexene carboxaldehyde) contain only half of the 26 fragrance substances.

What does this study add?

- The current commonest fragrance sensitivities in our cohort are to oxidised linalool, oxidised limonene (89, 4.3%) and *Evernia furfuracea*, none of which are represented by the baseline series fragrance markers.
- The majority of contact allergy to fragrances is not identified through history-taking.

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Summary:

Background: Fragrance contact allergy is common and is currently screened for with the European baseline series fragrance markers: Fragrance Mix 1, Fragrance Mix 2, *Myroxylon pereirae* and hydroxyisohexyl 3-cyclohexene carboxaldehyde.

Objectives: To investigate the validity of patch testing with these fragrance markers in detecting fragrance allergy to 26 individual fragrance substances for which cosmetic ingredient labelling is mandatory in the European Union.

Methods: We conducted a retrospective review of the patch test records of all eczema patients who underwent testing with the European baseline series extended with the individual fragrance substances during the period 2015-2016.

Results: 359 patients (17.2%) reacted to one or more allergens from the labelled fragrance substance series and/or a fragrance marker from the European baseline series. The allergens that were positive with the greatest frequencies were oxidised linalool (154; 7.4%, 95% CI 6.3%-8.6%), oxidised limonene (89; 4.3%, 95% CI 3.4%-5.2%) and *Evernia furfuracea* (44; 2.1%, 95% CI 1.5%-2.8%). Of the 319 patients who reacted to any of the labelled fragrance substances, only 130 (40.8%) also reacted to a baseline series fragrance marker. The sensitivity of our history-taking for fragrance allergy was 25.7%.

Conclusions: With the evolving trends in fragrance allergy patch testing with Fragrance Mix 1, Fragrance Mix 2, *Myroxylon pereirae* and hydroxyisohexyl 3-cyclohexene carboxaldehyde are no longer sufficient for screening for fragrance allergy.

Introduction

Fragrance substances are second only to metals as a cause of contact allergy; between 1.1 and 8% of the general population have contact allergy to fragrance substances.^{1,2} Screening for fragrance allergy currently takes the form of patch testing to two mixes of fragrance substances, Fragrance Mix 1 (FM I) and Fragrance Mix II (FM II), a natural substance *Myroxylon pereirae* and the single synthetic fragrance chemical hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), which is also present in FM II. During 2011 and 2012, we compared the reactions of fragrance screen markers to that of the 26 individual fragrance chemicals that are included in EU mandatory labelling.³ We found that reactions to the fragrance screening agents would only detect 57.6% of patients who had reacted to an individual fragrance chemical. Unoxidised linalool and unoxidised limonene were used in this work. Here we similarly compare the reactions for years 2015 and 2016 but used oxidised (ox.) linalool and oxidised (ox.) limonene instead of the unoxidised forms of the two substances. Additionally, we examined the reliability of identifying patients with actual fragrance allergy through a screening history from their first encounter.

Methods

The patch test records of all eczema patients who underwent testing at St John's Institute of Dermatology at Guy's Hospital, London, in 2015-2016 using the European baseline series extended with the 26 individual fragrance substances and trimethylbenzene propanol were reviewed. Each patient in our clinic is asked before patch testing if they have been aware of previously reacting to

perfume or a perfumed product and the result recorded. The results were categorised according to the following: (a) positive, as defined by a definite history of an itchy rash after contact with perfume or perfumed product; (b) uncertain, as defined by a possible reaction to an unidentified perfume or a perfumed product; (c) negative, as defined by no perceived reaction to any perfume or perfumed product.

Positive reactions on day 4 after testing to fragrance markers in the European baseline series (FM I, FM II, *Myroxylon pereirae*, and HICC) or allergens from the fragrance series (the 26 labelled fragrances, with *Evernia prunastri* and *Evernia furfuracea* counting as one of these although identified separately, and trimethylbenzene propanol) were tabulated using Microsoft™ Excel. The concentrations of the fragrance markers are shown in Table 1, and those of the allergens used in the fragrance series are shown in Table 2. Unlike our previous study, limonene and linalool were used in their oxidised forms (Hydroperoxides of limonene 0.3% and Hydroperoxides of linalool 1% respectively). Patch testing was performed with aluminium Finn Chambers® (Bio-Diagnostics® United Kingdom) and allergens were provided by Bio-Diagnostics®, Trolab® (Hermal Almirall, Reinbeck, Germany) and Chemotechnique® (Vellinge, Sweden). Allergens were prepared in petrolatum; FM1, *Myroxylon pereirae* and *Evernia prunastri* contained additional sorbitan sesquioleate 5% as an emulsifier. All allergens were stored, dispensed and used according to the manufacturers' instructions within the recommended stability periods. Reactions were read on days 2 and 4, according to the European Society of Contact Dermatitis guidelines.⁴ Reactions documented as questionable or irritant were considered to be negative.

The sensitivity, specificity, positive predictive value and negative predictive value for diagnosing fragrance allergy, as defined in our study as a positive reaction to at least a fragrance marker or an allergen from the fragrance series, were determined.

Results

There were 2084 records of eczema patients tested with the European baseline series and the fragrance series between January 2015 and December 2016. Of these, 359 (17.2%, 95% CI 15.6% - 18.9%) reacted either to a fragrance marker from the European baseline series or to at least one of the allergens in the fragrance series. Table 1 shows the proportion of positive reactions to the fragrance markers and concomitant reactions with at least one allergen from the fragrance series.

Frequencies of reactions to fragrance series allergens

The allergens that were positive with the greatest frequencies were ox. linalool (154; 7.4%, 95% CI 6.3%-8.6%), ox. limonene (89; 4.3%, 95% CI 3.4%-5.2%), *Evernia furfuracea* (44; 2.1%, 95% CI 1.5%-2.8%), *Evernia prunastri* (36; 1.7%, 95% CI 1.2%-2.4%), cinnamyl alcohol (34; 1.6%, 95% CI 1.1%-2.3%), isoeugenol (31; 1.5%, 95% CI 1.0%-2.1%), HICC (30; 1.4%, 95% CI 1.0%-2.0%), cinnamal (24; 1.2%, 95% CI 0.7%-1.7%), citral (22; 1.1%, 95% CI 0.7%-1.6%) and hydroxycitronellal (22; 1.1%, 95% CI 0.7%-1.6%). Table 2 shows the proportion of positive reactions to the fragrance series and their individual concomitant reactions to the fragrance markers.

Concomitant reactions between fragrance markers and fragrance series allergens

There were 170 (8.2%) patients who reacted to at least one fragrance marker and 319 (15.3%) patients who reacted to at least one fragrance series allergen. Of the patients who reacted to at least one fragrance series allergen, 130 (40.8%) also reacted to at least one fragrance marker (Fig. 1).

Reactions to ox. linalool and ox. limonene

There were 154 (7.4%) and 89 (4.3%) patients with positive reactions to ox. linalool and ox. limonene respectively (Table 3). The majority of patients who reacted to these fragrance terpenes did not react to any of the baseline series fragrance markers (Table 4).

If ox. linalool and ox. limonene were added to the fragrance marker subgroup, 320 (15.3%) patients with allergic reactions would be detected. Of the 178 (8.5%) patients who would react to the remaining allergens in the fragrance series, 139 (78%) would have reacted to at least one fragrance marker (Fig. 2).

Reliability of screening history for fragrance allergy

In our patient cohort 88 patients (15.8%) provided a positive fragrance history, 1697 patients (81.3%) provided a negative patient history and 47 patients (2.2%) were uncertain about their fragrance history (Table 5). Without taking into account the patients with uncertain fragrance history, the sensitivity of our fragrance history for detecting fragrance allergy through patch testing was 25.7% and the specificity was 85.6% whereas the positive predictive value of our fragrance history was 26.7% and the negative predictive value was 85%.

Discussion

The four fragrance markers detected only 40.8% of patients reacting to any individual fragrance substance. By most definitions this would make the sole use of these four screening agents inadequate as a screening tool for fragrance allergy. When a patient reacted to an individual constituent of FM I concomitant reaction with FMI occurred ranging from 90.9% (eugenol) to 40% (alpha amylcinnamyl alcohol). Similarly, patients who reacted to an individual fragrance constituent of FM II concomitantly reacted to FM II in range from 90% of cases (HICC) to 59.1% cases (citril). Figure 1 demonstrates that to optimally screen for fragrance allergy one should use a combination of fragrance mixes and individual fragrance patch test preparations.

There may be more than one reason for the apparent discrepancy of reactions. These include different concentrations for some fragrances between mix and individual fragrance patch tests. The FM I constituents are mixed at concentration of 1% each but are individually tested at 2%. There may be increased positivity to mixes due to the different chemicals reducing irritant or allergic thresholds.

Although fragrances are classed together as a single group they represent a diverse array of agents. Fragrance chemicals can come from floral plants, citrus, spices and herbs, lichen, trees or be synthetic. Besides increasing the sensitivity of fragrance screening, testing with individual fragrances can also sometimes help in advising patients to avoid specific agents rather than having to avoid all substances containing PARFUM. However, if a patient reacts to one or more of the mixes without also reacting to a specific constituent, or reacts to 3 or more individual fragrances we instruct them to avoid all fragranced products.

Contact allergy to fragrances, as demonstrated here, is not usually identified by the history and there is a diverse array of clinical presentations. One should also bear in mind that fragrance exposure can occur through any of six routes of exposure- i.e. direct contact, airborne, hand to face or body, by proxy, systemic and mucosal.

In summary, testing with the three fragrance mixes and HICC alone has low sensitivity in detecting fragrance allergy. We advocate testing with all 26 individual fragrances that are individually labelled. If this is not practicable for some centres then it is suggested that an additional 9 individual fragrance agents, namely oxidised linalool, oxidised limonene, *Evernia furfuracea*, *Evernia prunastri*, cinnamyl alcohol, cinnamal, citral, hydroxycitronellol and geraniol, should be tested alongside the usual baseline series markers. This may change as HICC and *Evernia* were recently banned in cosmetic products in the EU.⁵

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Table 1. Positive patch test reactions to fragrance markers in the European baseline series and concomitant positive reactions in the fragrance series

Fragrance marker	Concentration in European baseline series (%)	No. of positive reactions (%; 95% CI)	Co-reactions with any fragrance series substance (% of reactions to fragrance marker)
Fragrance mix (FM) I	8	107 (5.1, 4.2-6.2)	93 (86.9)
Fragrance mix (FM) II	14	61 (2.9, 2.2-3.7)	56 (91.8)
<i>Myroxylon pereirae</i>	25	61 (2.9, 2.2-3.7)	45 (73.8)
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)	5	30 (1.4, 1.0-2.0)	21 (70.0)

Table 2. Positive patch test reactions to the fragrance series (in decreasing order of frequency) and concomitant positive reactions to the fragrance markers

Fragrance series allergen	Concentration in European baseline series (%)	No. of patients with positive reactions (% , 95% CI)	Concomitant reactions with FMI (% of reactions to fragrance series allergen)	Concomitant reactions with FMII (% of reactions to fragrance series allergen)	Concomitant reactions with MP (% of reactions to fragrance series allergen)	Concomitant reactions with HICC (% of reactions to fragrance series allergen)
Oxidised linalool †	6	154 (7.4, 6.3 -8.6)	24 (15.6)	17 (11.0)	18 (11.7)	5 (3.2)
Oxidised limonene †	3	89 (4.3, 3.4-5.2)	18 (20.2)	11 (12.4)	10 (11.2)	2 (2.2)
<i>Evernia furfuracea</i> †	1	44 (2.1, 1.5-2.8)	25 (56.8)	12 (27.3)	12 (27.3)	4 (9.1)
<i>Evernia prunastri</i> *	2	36 (1.7, 1.2-2.4)	23 (63.9)	6 (16.7)	11 (30.6)	4 (11.1)
Cinnamyl alcohol*	2	34 (1.6, 1.1-2.3)	20 (58.8)	4 (11.8)	8 (23.5)	1 (2.9)
Isoeugenol*	2	31 (1.5, 1.0-2.1)	24 (77.4)	9 (29.0)	11 (35.5)	3 (9.7)
Hydroxylisohexyl-3-cyclohexene carboxaldehyde**	5	30 (1.4, 1.0-2.0)	15 (50.0)	27 (90.0)	2 (6.7)	30 (100)
Cinnamal*	1	24 (1.2, 0.7-1.7)	16 (66.7)	3 (12.5)	3 (12.5)	1 (4.2)
Citral**	2	22 (1.1, 0.7-1.6)	10 (45.5)	13 (59.1)	2 (9.1)	2 (9.1)
Hydroxycitronellal*	2	22 (1.1, 0.7-1.6)	15 (68.2)	13 (59.1)	2 (9.1)	9 (40.9)
Eugenol*	2	11 (0.5, 0.3-0.9)	10 (90.9)	2 (18.2)	8 (72.7)	0 (0.0)
Geraniol*	2	10 (0.5, 0.2-0.9)	5 (50.0)	5 (50.0)	3 (30.0)	1 (10.0)
Alpha-hexyl cinnamal**	10	7 (0.3, 0.1-0.7)	3 (42.9)	6 (85.7)	1 (14.3)	3 (42.9)
Butylphenyl methylpropional †	10	7 (0.3, 0.1-0.7)	3 (42.9)	4 (57.1)	1 (14.3)	3 (42.9)
Farnesol**	5	5 (0.2, 0.1-0.6)	2 (40.0)	3 (60.0)	0 (0.0)	1 (20.0)
Alpha-amycinnamyl alcohol †	1	5 (0.2, 0.1-0.6)	2 (40.0)	2 (40.0)	0 (0.0)	1 (20.0)
Trimethyl benzene propanol	5	4 (0.2, 0.1-0.5)	1 (25.0)	2 (50.0)	2 (50.0)	1 (25.0)
Benzyl salicylate †	1	3 (0.1, 0.0-0.4)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)
Anise alcohol †	1	3 (0.1, 0.0-0.4)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)
Benzyl benzoate †	1	3 (0.1, 0.0-0.4)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Amyl cinnamal*	2	3 (0.1, 0.0-0.4)	3 (100.0)	2 (66.7)	0 (0.0)	1 (33.3)
Sorbitan sesquioleate	20	3 (0.1, 0.0-0.4)	2 (66.7)	1 (33.3)	2 (66.7)	1 (33.3)
Citronellal**	1	2 (0.1, 0.0-0.3)	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)
Methyl 2-octynoate †	1	2 (0.1, 0.0-0.3)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)
Benzyl alcohol †	10	2 (0.1, 0.0-0.3)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Coumarin**	5	1 (0.05, 0.0-0.3)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)
Benzyl cinnamate	5	1 (0.05, 0.0-0.3)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)

†						
Alpha isomethyl ionone †	1	1 (0.05, 0.0-0.3)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)

* Constituents of FMI, ** Constituents of FMII, † Other fragrance substances not part of fragrance markers that are required for EU labelling

Table 3. Patch test reactions to oxidised linalool and oxidised limonene

	Number of positive reactions (%)				Number of doubtful or irritant reactions (%)
	Total	+	++	+++	
Oxidised linalool	154 (7.4)	139 (6.7)	15 (0.7)	0	32 (1.5)
Oxidised limonene	89 (4.3)	75 (3.6)	14 (0.7)	0	29 (1.4)

Table 4. Positive patch test reactions to oxidised linalool and oxidised limonene and concomitant positive reactions to fragrance markers and other allergens from the fragrance series

	No. of patients with positive reactions	Concomitant reactions to fragrance markers					Co- reactions to any other fragrance series allergen (% of reaction to fragrance terpene)
		FMI (% of reaction to fragrance terpene)	FMII (% of reaction to fragrance terpene)	MP (% of reaction to fragrance terpene)	HICC (% of reaction to fragrance terpene)	One or more fragrance markers (% of reaction to fragrance terpene)	
Oxidised linalool	154	24 (15.6)	17 (11.0)	18 (11.7)	5 (3.2)	42 (27.3)	68 (44.2)
Oxidised limonene	89	18 (20.2)	11 (12.6)	10 (11.2)	2 (2.2)	28 (31.5)	54 (60.7)
Both ox. linalool and ox. limonene	40	12 (30.0)	7 (17.5)	6 (15.0)	2 (5.0)	17 (42.5)	20 (50.0)

Table 5. Patch test reactions to any fragrance marker or fragrance series allergen according to our fragrance screening history

	No. of patients who reacted to any fragrance marker or fragrance series allergen	
	Positive	Negative
No. of patients who reported a positive fragrance history	88	242
No. of patients who reported a negative fragrance history	255	1442
No. of patients who reported an uncertain fragrance history	16	31



