PhD thesis

Maria Vølund Heisterberg

Fragrance allergy
Diagnosis, causes and quality of life

Maria Vølund Heisterberg, MD
National Allergy Research Centre
Department of Dermato-Allergology
University of Copenhagen
Gentofte Hospital
Denmark
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The PhD thesis is based on the following manuscripts:

Study Part 1:

I. Fragrance mix II in the baseline series contributes significantly to detection of fragrance allergy. Contact Dermatitis 2010 Nov;63(5):270-6.

II: Deodorants are the leading cause of allergic contact dermatitis to fragrance ingredients. Contact Dermatitis 2011 May;64(5):258-264.

Study Part 2:

III: Fragrance allergy and quality of life – Development and validation of a disease specific quality of life instrument. Submitted to Contact Dermatitis May 2013.

IV: Fragrance allergy and quality of life – A case control study. Submitted to Contact Dermatitis May 2013.

PhD supervisors:
  Jeanne Duus Johansen, Professor, MD, DMSc
  National Allergy Research Centre
  Department of Dermato-Allergology
  Gentofte University Hospital
  University of Copenhagen, Denmark

  Torkil Menné, Professor, MD, DMSc
  National Allergy Research Centre
  Department of Dermato-Allergology
  Gentofte University Hospital
  Copenhagen, Denmark

ABBREVIATIONS

AD   Atopic dermatitis
CI   Confidence interval
DLQI   Dermatology Life Quality index
FM I   Fragrance mix I
FM II   Fragrance mix II
HICC   Hydroxyisohexyl 3-cyclohexene carboxaldehyde
MP   Myroxylon pereirae
SF36   Sort form SF36v2
QoL   Quality of life (health related-quality of life)

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1. Summary

1.1. Summary in Danish

Denne ph.d. afhandling omhandler allergi over for parfumestoffer. Afhandlingens overordnede formål var at undersøge: 1) om den "nye" screeningsmarkør, fragrance mix II (FM II) bidrager til udredningen af allergi, 2) hvilke eksponeringskilder der hyppigst forårsager allergisk kontakteksem over for parfumestoffer og 3) i hvilken udstrækning allergi over for parfumestoffer påvirker livskvaliteten.

Afhandlingen består af 4 studier. De to første studier er databasestudier, der er baseret på epikutan test resultater fra patienter konsekutivt undersøgt for allergi af Den Danske Kontaktdermatitis Gruppe. Hyppigheden af allergi over for FM II blev undersøgt i det første studie. I det andet studie undersøgtes hvilke eksponeringskilder, som havde forårsaget allergi over for parfumestoffer. Det tredje studie er et metodologisk studie, hvor der blev udviklet et sygdomsspecifikt spørgeskema til måling af livskvaliteten blandt individer med parfumeallergi. Det fjerde studie er et matchet case-kontrol studie, som blev anvendt til at validere ovennævnte livskvalitetsspørgeskema, samt til at undersøge livskvaliteten hos individer med parfumeallergi.

Resultaterne viste at FM II er værdifuld i den diagnostiske screening af parfumeallergi. En positiv epikutan test blev fundet hos 4.5% af patienterne og FM II identificerede yderligere 202 (15.6%) patienter, som ellers ikke ville være blevet diagnosticeret med en parfumeallergi. Kosmetiske produkter var den hyppigste årsag til parfumeallergi. Mange forskellige kosmetiske produktgrupper var involveret, de hyppigste var deodoranter, parfumer, cremer og shampoo. Visse produktgrupper synes at være særligt associeret til allergi overfor visse specifikke parfumestoffer. Et nyt simpelt sygdomsspecifikt livskvalitetsspørgeskema blev udviklet og valideret, "det parfumeallergi-specifikke livskvalitets spørgeskema". Samlet set, viste valideringsanalyserne at det var et godt anvendeligt instrument til at måle livskvaliteten hos individer med parfumeallergi.

Ud fra dette livskvalitetsspørgeskema fandt vi, at parfumeallergi påvirker livskvaliteten. Der var en tydelig kønsforskel, idet kvinder i langt højere grad var påvirket, også i forhold til deres kontrol personer, hvilket ikke blev set blandt mændene. Vi fandt desuden, at livskvaliteten var mere nedsat blandt unge kvinder omkring diagnose tidspunktet, samt at antallet af parfumeallergier og sværhedsgraden af allergi var associeret til nedsat livskvalitet blandt alle kvinder. Specielt interessant fandt vi, at nogle specifikke parfumestoffer i særdeleshed var involveret i livskvalitetsforringelse hos kvinder.

Denne ph.d. afhandling bidrager med ny nyttig viden til at optimere diagnosticering af parfumeallergi. FM II indgår nu i standardudredningen for kontaktallergi i hele Danmark. Desuden viser studierne, at visse kosmetiske produktgrupper udgør en særlig risiko for udvikling af allergisk kontakteksem over for parfumestoffer.

Der introduceres i denne ph.d. afhandling et nyt valideret sygdomsspecifikt spørgeskema, som kan bruges til at vurdere livskvaliteten hos individer med parfumeallergi. Endeligt bidrages med ny viden om hvordan parfumeallergi påvirker livskvaliteten.

Disse resultater åbner for mere specifikke forebyggende tiltag på både individuelt- og samfundsmæssigt plan. På det individuelle plan muliggøres mere specifik forebyggelsesvejledning til patienter med parfumeallergi, hvor de faktorer, der har betydning for deres livskvalitet, tages i betragtning. Yderligere vil forbedret diagnostik kunne føre til bedre prognose. På det samfundsmæssige plan kunne de forebyggende tiltag være, at der lovgives om en forbedret mærkning af parfumestoffer på kosmetiske produkter, forbud mod at anvende visse parfumestoffer i høje koncentrationer og evt. helt af forbyde visse parfumestoffer. Disse forebyggende tiltag vil kræve en øget fælles indsats fra patienter, diagnostikere, forskere, myndigheder og parfumeindustri.

1.2. Summary in English

This PhD thesis deals with allergy to fragrance ingredients. The overall objective was to determine: 1) if the "new" screening marker for fragrance allergy, the fragrance mix II (FM II), contributes as a screening marker of fragrance allergy, 2) to determine which exposure sources cause allergic contact dermatitis to fragrance ingredients, and 3) if and how fragrance allergy affects quality of life (QoL).

The thesis consists of 4 studies. The first two studies are epidemiological studies based on patch test results from patients consecutively investigated for allergy by the Danish Contact Dermatitis Group. The prevalence of FM II allergy was determined in the first study and which exposure sources had caused allergic contact dermatitis to fragrance ingredients was investigated in the second study.
The third study is a methodological study where a disease-specific QoL instrument was developed to assess QoL in subjects with fragrance allergy. The fourth study is a matched case-control study which was used in the validation of the above mentioned QoL instrument and furthermore to assess QoL in subjects with fragrance allergy.

Results show that FM II contributes in the diagnostic screening of fragrance allergy. The prevalence of a positive patch test reaction was 4.5%, and it further identified 202 (15.6%) patients, who would otherwise have gone undetected with a fragrance allergy. The most frequent cause of allergic contact dermatitis to fragrance ingredients was cosmetic products. Many different cosmetic product groups were involved. Most frequently listed were deodorants, fine fragrances, lotions and shampoos. An association between certain cosmetic product groups listed as the cause of their allergy and allergy to specific fragrance ingredients/markers was observed.
A new simple disease-specific QoL instrument for fragrance allergic subjects was developed and validated, the Fragrance QoL index. Overall, the validation analyses showed that, it was a good applicable instrument for measuring QoL. From this QoL instrument, we have found that fragrance allergic subjects have impaired QoL. A clear gender difference was found in how fragrance allergy affects QoL, as women had an increased QoL impairment also compared with their controls, which was not found among the men. We also found that young women had increased QoL impairment around time of diagnosis. That the number of fragrance allergies and the severity of the allergy among all women was associated with QoL impairment. Most notably, we found that allergy to certain fragrance ingredients/markers was associated with a reduced QoL among women.

This PhD thesis contributes with new knowledge to optimize the diagnosis of fragrance allergy. The FM II is now a part of the standard screening for contact allergy throughout Denmark. The studies show that certain cosmetic products pose a particular risk for the development of allergic contact dermatitis to fragrance ingredients.
In this PhD thesis a new validated disease-specific QoL instrument is introduced, which can be used to assess QoL among subjects with a fragrance allergy.
These results open up for more specific preventive interventions both at an individual level and at a public health level. At the individual level a more specific guidance in prevention could be established taking the factors that affect their QoL into account. Furthermore, a better diagnosis, could lead to better prognosis. At the public health level the preventive interventions could be through legislation on improvement of the labelling of fragrance ingredients on cosmetic products, prohibit high concentrations of certain fragrance ingredients, and perhaps completely prohibit certain highly allergenic fragrance ingredients. These preventive interventions would require an increased joint effort from patients, healthcare professionals, researchers, government and perfume industry.

2. Background

Fragrance ingredients are the second most frequent cause of contact allergy. Among eczema patients 1 in 10 individuals examined for allergy has an allergy to one or more fragrance ingredients[3-5]. In the general population 1 to 4% has a fragrance allergy[6,7].

2.1. Definition of allergic contact dermatitis

Allergic contact dermatitis is a type IV cell-mediated immunological disease[8]. It manifests as dermatitis, which is redness, scaling, swelling and sometimes blistering of the skin. Contact allergy can develop after direct skin exposure to a sensitizing substance, either after a single exposure or repeated exposures. The pathophysiological mechanism of allergic contact dermatitis can be divided into two phases: an induction phase, where the allergy is developed; and an elicitation phase, where re-exposure to the allergen results in the manifestation of the disease, contact dermatitis. The pathophysiological mechanisms involved in allergic contact dermatitis are not yet fully understood[8]. The allergen has to penetrate the skin barrier where it binds to epidermal molecules and forms a hapten. The hapten is then taken up by dendritic cells located in the skin. They are activated and migrate to the draining lymph node where the immunologic system is further activated. Specific T cell precursors clonally expand and re-circulate through the blood stream and migrate to the tissues, including the skin. The specific T cells are programmed to recognize the allergenic substance and if re-exposed they will be activated and initiate an inflammatory skin reaction (the elicitation phase). Allergy is a life-long condition, where no cure exists[8]. Thus, in case of symptoms the main treatment is symptomatic with topical medicaments, immune suppressive treatments, light treatment and, most importantly, allergen avoidance. When not exposed to the allergenic substance, there is no manifestation of the disease. However, repeated or continuous allergen exposure may lead to chronic eczema, and, eventually, not even allergen avoidance will clear the eczema. Moreover, eczema can spread to areas not in direct contact with the allergenic substance.

Figure 1. Photograf of a lady's neck with allergic contact dermatitis to a fragrance ingredient.

2.2. Epidemiology and exposures to fragrance ingredients

Fragrance ingredients have been used for millennia, and composing a scent is considered by many as an art form. Often a fragrance formula is a blend of not just one fragrance ingredient but up to a 100 or even more different fragrance ingredients. The making of perfumes began in ancient Egypt but was developed and further refined by the Romans, the Persians and the Arabs. Their perfumes were used for more than smelling attractive; they were also used in religious ceremonies and funeral rites, as well as for their medical properties. In the early 14th century, perfumed products came to Europe from the Arabs. France quickly became the European centre of perfume and cosmetic manufacture. Cultivation of flowers for their perfume essence grew into a major industry. The use of perfume in Europe grew steadily and is now an enormous worldwide industry, which is still growing. Because of their widespread use in cosmetic products[9], detergents, cleansing agents, toys and topical medications[10], they are almost impossible to avoid in daily living. While fragrance ingredients are mainly used to give a pleasant odour, they are also used to mask unpleasant smells. In cosmetic products the concentrations of fragrance ingredients vary. In a fine fragrance the concentration can be 15 to 30%, in deodorants 1%, lotions 0.4%, and soaps 0.5 to 2.0%[11]. Thus, consumers are exposed to relatively high doses of fragrance ingredients from the individual cosmetic product and often from many different fragrance ingredients. Furthermore, we are typically exposed not just from one cosmetic product but through many different products during our every day lives (soaps, deodorants etc.). Consequently, the cumulative expose to fragrance ingredients during our daily living is high. Moreover, the perfume industry has some fragrance ingredients, that are particularly frequently used[12,13] and hence the cumulative exposure to these "popular" fragrance ingredients is even higher.

2.3. Definition of fragrance ingredients

A fragrance ingredient is an organic compound with a pleasant odour [14]. According to the European Union's Inventory of Cosmetic Ingredients, the CosIng, 2748 perfuming ingredients/substances currently exist[15] Many of them can cause allergy [16]. Some fragrance ingredients are natural and some are synthetic:

- Natural fragrance ingredients are derived from plants, plant parts or animals. The plant fragrances usually consist of many different organic substances, which may vary over time and location of harvest as their compositions depend on weather conditions, nutrition in the soil and harvest time. Most of the animal fragrances have been substituted with synthetic fragrances.

- Synthetic fragrance ingredients are generally cheaper and much more chemically well defined than are natural fragrances.

Both synthetic and natural fragrance ingredients can cause contact allergy. What makes some fragrance ingredients more allergenic than others is not fully understood. However, we know that several factors play a role in the state of induction and elicitation of a contact allergy. Of particular relevance is the exposure dose pr. unit area[17-20] and inherent sensitising potential[21,22]. Additionally, exposure location, frequency of exposures, duration of exposure and vehicles play a role in the development of contact allergy. Moreover, a cocktail effect is seen, where a synergistic effect occurs in response to exposure to several different fragrance ingredients [23].

2.4. The diagnosis of fragrance allergy

The golden standard for diagnosing contact allergy is patch testing. In Denmark, when a person is suspected of having a contact allergy they are referred via their general practitioner (or other medical doctor) to a dermatology clinic or directly to a hospital's dermatology department. Patch testing is an investigation where persons are exposed (re-exposed) to a variety of common allergens. International guidelines on the patch testing procedure have been made to ensure reliability [24]. Further, the patch testing materials are standardized in concentration and vehicle. The European Baseline series, which comprises the allergens known to be the most frequent causes of allergy (nickel, fragrances, preservatives, chrome, dyes etc.) is used. The allergens are applied on the patient's back using small aluminium chambers (Finn chambers 8mm) attached by tape. The allergens stay occluded for two days and readings are done on Day 2, Day 3 or 4 and Day 7, according to the recommendation of the International Contact Dermatitis Research Group [25]. If a reaction occurs on the site where an allergen was applied, it is categorized according to its morphology into three different types: allergic reaction (Figure 2), irritant reaction, and doubtful reaction.

Allergic reactions are further graded into: 1) a weak positive plus one (+) reaction, where erythema, infiltration and possible papules are present; 2) a strong positive plus two (++) reaction where there is erythema, homogenous infiltration, papules and vesicles; or 3) an extreme positive plus three (+++) reaction, where there is intense erythema, infiltration and coalescing vesicles [26].
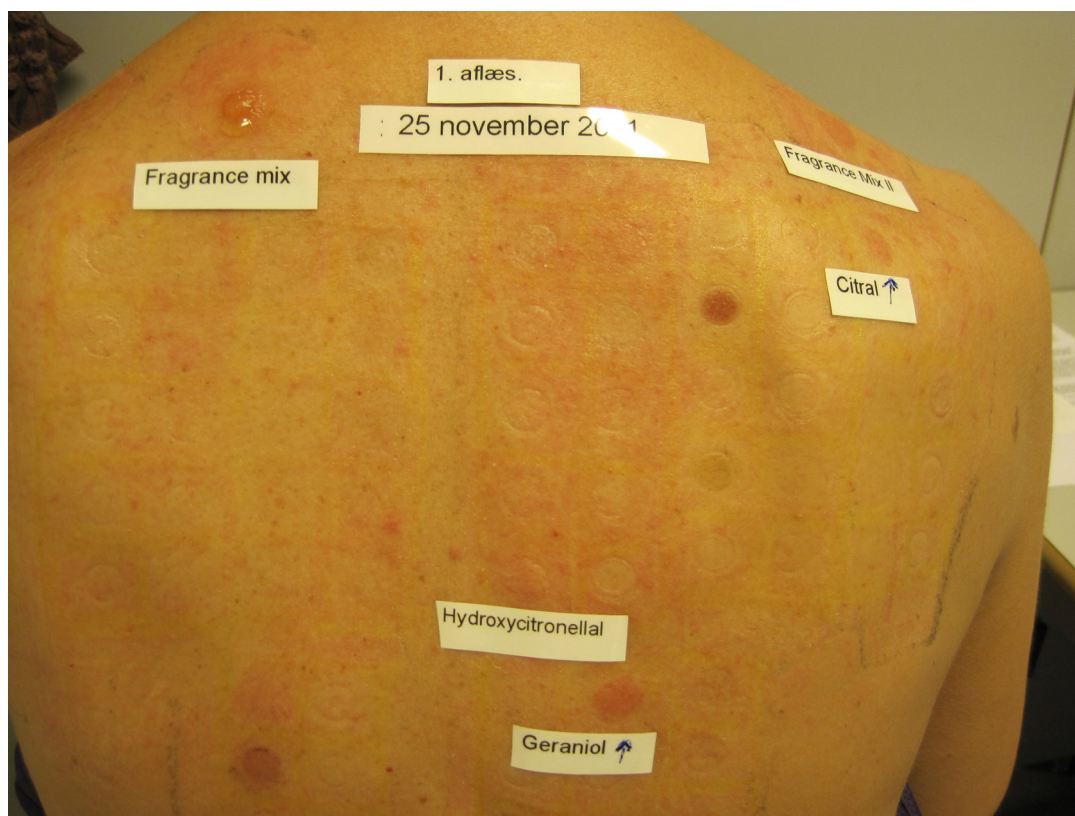
Figure 2. Photograph of a patients back at first patch test reading; displaying several positive reactions to fragrance ingredients/markers.

In case of a positive patch test reaction to a fragrance ingredient/marker, clinical relevance of the reaction is determined by a dermatologist.

Clinical guidelines exist on the evaluation of clinical relevance of a positive patch test reaction:

- Incurred or aggravated eczema as a result of a probable / proven exposure to the substance in question.

- The contact dermatitis can fully or partially be explained by exposure to the substance in terms of temporal correlation, localization and exposure intensity; and, ideally, concentration of the substance.

- It is determined whether the current or previous eczema can be explained by the contact allergy, which will be recorded as a present clinically relevant and/or past clinically relevant positive patch test reaction.

Only when clinical relevance is established, is the diagnosis of allergic contact dermatitis given (Danish Dermatology Society guideline).

The screening markers for fragrance allergy (and other allergens) have changed over time in view of our changing exposures[27], new knowledge about fragrance allergy[28] and because of regulatory restictions[29]. In 1977, a mixture of 8 different fragrance ingredients was introduced[30], the fragrance mix I. This was done because a mixture of fragrance ingredients better mimics real-life exposure, as fragrance ingredients are largely used in combination in consumer products. Fragrance mix I has proven a valuable screening marker of fragrance allergy[4,31]. Another fragrance screening marker which has been used for decades is the Myroxylon Pereirae [32]. It is a natural resin where only 60 to 70% of its constituents have been accurately identified; however, we know it consists of several fragrance ingredients known to be sensitizers[33]. Since 1982 the crude Myroxylon Pereirae has evidently not been used in cosmetic products because of restriction by the International Fragrance Association (IFRA)[34]. However, extracts and distillations of Myroxylon pereirae are still used and it is a frequent cause of allergic contact dermatitis[5]. To comply with the continuous change of fragrance ingredients consumers are exposed to, a new screening marker of fragrance allergy was introduced in 2005, fragrance mix II. It consists of 6 different fragrance ingredients and its contribution as a screening marker of fragrance allergy will be described in this thesis, Part I, Manuscript I.

Fragrance ingredients applied in cosmetic products bought within the EU are regulated by the EU Cosmetic Directive, implemented in Denmark through "Kosmetikbekendtgørelsen"[35]. It states that when a cosmetic product contains fragrance ingredients, this must be labelled on the ingredient list as perfume, aroma or fragrance. In 2005, it was appended that 26 selected fragrance ingredients should also be labelled by their specific name (INCI name) on the ingredient list when applied in concentrations of more than 0.01ppm in leave-on cosmetics and 0.001ppm in rinse-off cosmetic products. These 26 fragrance ingredients were chosen as they are the most frequent causes of fragrance allergy. They became available for patch testing and were included in the standard screening for fragrance allergy at Gentofte University Hospital in 2007. Screening with these 26 fragrance ingredients helps identify subjects who would otherwise have gone undetected [36] and facilitates establishing clinical relevance of a positive patch test. Furthermore, it enables the person with an identified allergy to avoid cosmetic products that contain the fragrance in question, consequently avoiding manifestation of contact dermatitis. However, reading the ingredient list can be difficult[37,38]: the fragrance ingredients can have very long, complicated names, for example hydroxyisohexyl 3-cyclohexene carboxaldehyde, are typed in a front style difficult to read, the list itself is sometimes difficult to locate on the product, and, lastly, the list can be extremely long[39,40].

2.5. Quality of life

The term quality of life (QoL) refers to the general well-being of an individual or a society. The term is used in a wide range of contexts, including the fields of international development, healthcare, and politics. This thesis only concentrates on health-related QoL.

The World Health Organization (WHO) defined (1947-1958) health as: "…a state of complete physical, mental and social well-being and not merely the absence of disease and infirmity", thus when trying to measure the quality of life, all aspects of health should be considered. Over the last decades there has been an increase in the awareness and acknowledgement of the importance of subjective feelings in regard to the impact a disease has on a person. In some regards, QoL measures may be an even better evaluation than clinical findings to assess the impairment caused by a person's disease. As with any situation involving perception, there can be great differences in the rating by different observers, for example doctors, patients etc. Consequently, QoL is often assessed in a questionnaire study. Several different types of questionnaires exist:

- Generic QoL questionnaires (e.g. SF36) are designed to give an overall estimation of the QoL and contain issues relevant to both healthy and sick populations.

- Specialty specific questionnaires (e.g. dermatology life quality index, DLQI) are designed to assess QoL among persons with a skin condition and are relevant for use among those with a skin condition.

- Disease-specific questionnaires are designed to estimate the QoL in persons with a specific disease (e.g. psoriasis). Thus, they are primarily intended for use in that particular population with that condition (e.g. in persons with psoriasis).

Often, the different types of QoL questionnaires are used together so as to give the best estimate of QoL[41,42].

In the dermatology field, the SF-36 is considered the reference instrument of choice among the generic QoL instruments, supplemented by either a disease-specific or specialty-specific QoL instrument[43]. The QoL in eczema populations has been assessed in many studies by various different QoL instruments. They all point in the same direction: that having eczema impairs QoL [41,42,44,45]. Different factors have shown to be important in regard to how affected their QoL is by their eczema. The aggravating factors include the eczema location (e.g. hand[46,47] or face[44]), having occupational eczema[48], age (young people)[49], gender differences (women)[42], and time of diagnosis (early diagnosis [49,50]). Thus many factors influence how eczema affects QoL[51]. Contact allergy has also been found to be associated with an increased impairment of QoL[47,50,52-54]. Eczema tends to be a fluctuating disease, which would also be reflected in the QoL. Likewise, contact allergy is a fluctuating disease and also potentially avoidable if not exposed to the allergen. Consequently, as allergy is a lifelong condition, the behavioural changes in the effected persons' daily lives would also have to be lifelong. For some, the consequences mean vast changes in their daily behaviour. However, this depends on the allergen and their lifestyle. The more wide spread exposure, the more changes need to be implemented to avoid the allergen. Because fragrance ingredients are widely used in daily consumer products, avoidance of fragrance ingredients can be difficult [55] and for some, such avoidance has a significant effect on their daily living[37]. However, little is known about the impairment of QoL in fragrance-allergic individuals and no disease-specific QoL instrument exists for those with fragrance allergy.

3. Aims of the studies

3.1 Study Part I. Manuscript I and II

- To determine whether the new screening marker of fragrance allergy, the fragrance mix II, contributes as a screening marker of fragrance allergy by identifying more subjects with a fragrance allergy than do the standard fragrance screening markers.

- To determine which exposure sources cause allergic contact dermatitis to fragrance ingredients in eczema patients in Denmark.

3.2 Study Part II. Manuscript III and IV

- To develop and validate a disease-specific quality of life instrument for persons with a fragrance allergy.

- To determine if fragrance allergy affects quality of life.

- To investigate possible factors that influence quality of life in persons with fragrance allergy compared with other eczema patients.

4. Materials and methods

This thesis is based on an eczema population in Denmark. It is separated into two parts: the first part consists of two epidemiological register-based studies concerning persons patch tested for allergies at either a dermatology clinic or in a dermatology department at a university hospital; the second part consists of a methodological study where a new instrument to assess quality of life in persons with fragrance allergy was developed. Moreover, the second part also consists of a matched case-control study based on a questionnaire survey among 1650 individuals with or without a fragrance allergy.

4.1. The patch test

All patch tests were done according to international guidelines [56] using Finn Chambers® (8mm) applied on the back with Scanpore tape and left for 2 days. Readings were done on Days 2, 3 or 4 and 7 according to the recommendation of the International Contact Dermatitis Research Group [25]. All participants had been consecutively patch tested with at least the European Baseline Series.

In Study I, consecutively patch test results with the FM II were compared with the results of the other standard screening markers of fragrance allergy (8% FM I, 25% MP, 5% HICC and/or colophonium). The FM II had been included in the baseline series in 2005 and consists of 6 different fragrance ingredients: 2.5% HICC, 1% citral, 2.5% farnesol, 2.5% coumarin, 0.5% citronellol and 5% α-hexyl cinnamal in pet.

In Study II, all persons had been consecutively patch tested with at least the baseline screening markers for fragrance allergy, which comprised FM I, FM II, HICC and MP.

In Study III and IV, all persons had likewise been consecutively patch tested. However, the screening markers for fragrance allergy varied over the 10 years the inclusion criteria spanned (2000-2010). Thus, persons from 2000 to 2005 were patch tested with the fragrance screening markers FM I and MP. In 2005, FM II and HICC were added, and in 2007 the 26 individual fragrance ingredients, which are to be labelled on cosmetic products, were included.

4.2. Database

Data were retrieved from a database of patients patch tested by members of the Danish Contact Dermatitis Group (DCDG). At the time of the study Part 1, the DCDG comprised three dermatology departments at university hospitals (Gentofte University Hospital, Odense University Hospital and Århus University Hospital), and 7 dermatology clinics located in Rødovre, Aalborg, Herning, Vejle, Bagsværd, Hørsholm and Kalundborg. The database is managed by the National Allergy Research Centre at the Department of Dermato-Allergology at Gentofte University Hospital. It contains information on patch test date, reactions, relevance of patch test (past or present) and demographic characteristics. Additionally, all relevant exposure sources causing fragrance allergy are routinely registered. The cosmetic exposures are registered in two general categories ("leave-on" and "rinse off") and in specific cosmetic product groups (lotions, fine fragrances, deodorants etc.). If a cosmetic product cannot be categorized into a specific group, either because it is unknown or it does not match any of the predetermined categories, it is registered as "unspecified leave-on" or "unspecified rinse-off". More than one cosmetic product can be recorded as the cause of contact dermatitis. The results are entered into the database manually at each participating clinic or hospital. The members of the DCDG are located throughout Denmark and represent the Danish eczema population.

4.3. Part 1. Study design of manuscript I and II

The studies were designed as epidemiological register-based studies. The results are based on data obtained from subjects investigated for allergy by the DCDG. Some individuals contributed to both studies.

4.3.1. Study population, manuscript I

The study population had been consecutively patch tested with the fragrance mix II (FM II) in the baseline series during 1 January 2005 − 31 December 2008. In all, 12302 subjects had been patch tested with the FM II: 8063 women and 4239 men, mean age 44.8 years (SD18.2).

4.3.2. Study population, manuscript II

All subjects included had been consecutively patch tested with fragrance markers of the baseline during 1 January 2005 − 31 June 2009. In all, 17716 subjects had been patch tested: 11610 women and 6106 men. The mean age was 44 years (SD 18.3).

4.3.3. Data analysis and management

Data were retrieved from the database and checked for outliers and missing data and any inconsistencies were double checked with hospital records. Some subjects had been patch tested several times and the patch test with the strongest patch test reaction was included.

4.3.4. Statistical methods

The statistical analyses were performed in Statistical Product and Service Solutions (SPSS$^{®}$ Inc., Chicago, IL, USA) software for windows Version 15 and OPENEPI (www.openepi.com). Prevalences of allergy to the different patch test ingredients were shown as crude percentages of positive reactions of those patch tested. For comparison of categorical variables, for example concomitant reactions between FM II and other fragrance markers, a chi square test was performed. To describe the strength of the concomitant association odds ratios (OR), 95% confidence intervals (CI) were used. *P* values below 0.05 were considered significant.

4.4. Part 2. Study design in manuscript III & IV

Study Part 2 consists of two studies. The first study was based on data from a methodological study among 68 persons with a fragrance allergy. Their narratives formed the basis for the development of the Fragrance QoL index, which was validated in a Danish eczema population in the questionnaire survey and in a retest. The second study relies on data from a questionnaire survey among 1650 consecutively patch tested persons. It was performed to evaluate the Fragrance QoL index and to investigate the QoL in those with a fragrance allergy.

4.4.1. Study design in development of the Fragrance QoL index, manuscript III

A disease specific QoL instrument for individuals with a fragrance allergy, the Fragrance QoL index, was developed by the chief investigator together with the supervisors. It was designed much in the same manner as one of the most frequently used QoL instruments for dermatology patients was developed, the Dermatology Life Quality index (DLQI) by Finlay in the 1994[57]. In all, 122 persons had been selected randomly from all those with a positive patch test reaction investigated at Gentofte University Hospital (2000−2010). The selection was done using Microsoft Sql Server 2008 (NewID()-function) via the National Contact Dermatitis Database. The participants were posted a letter asking them to write down all aspects in their lives affected by their fragrance allergy. A stamped, addressed return envelope was included. A second letter was sent to increase the response rate. Of the 122 recipients, 68 responded (55.7%) with detailed narratives of how their fragrance allergy affected their QoL: 4 replied they did not wish to participate and 50 did not respond. The elaboration given by the 4 declining participation was in one recipient misunderstanding the question, and 3 not finding participation relevant, as they had no discomfort worth writing about. The responder's narratives formed the basis for the development of 13 items via a content analysis. The process involved in the development of the Fragrance QoL index is illustrated in Figure 3.

4.4.2. Validation of the Fragrance QoL index

The Fragrance QoL was validated in a questionnaire survey among 1650 eczema patients and in a retest conducted approximately 3−6 months after the questionnaire survey. Convergent validity of the Fragrance QoL index was assessed by the correlations with two other QoL instruments and self-estimated disease severity. The two QoL instruments chosen for the validation were a generic questionnaire (SF36v2) and a dermatology-specific QoL questionnaire (DLQI). They were chosen because they are widely used, well validated, translated to Danish[58-65], and have often been used in studies investigating an eczema population[66]. Self-estimation of eczema has been validated in previous studies[67,68] and considered to be a satisfactory method to determine eczema severity.

- The Dermatology Life Quality index (DLQI): consists of 10 items concerning respondents' QoL regarding dermatology-specific conditions during the previous week. Each item is scored from 0 to 3. A total DLQI score can be calculated with a minimum score of 0 and a maximum score of 30. The higher the score, the greater the QoL impairment. Permission to use the DLQI was granted by Dr Mohammad Khurshid Azam Basra, Department of Dermatology, Cardiff University School of Medicine, Heath Park, Cardiff, CF14 4XN, United Kingdom.

- The Short Form 36 version 2 (SF36v2): consists of 36 items concerning general well-being during the last 4 weeks. It yields 8 dimensions of functional health and well-being scores as well as psychometrically-based physical (PCS) and mental (MCS) health summary scores, all

are scored between $0-100$. The lower the score, the greater the QoL impairment. The SF36v2 was licensed through www.qualitymetric.com.

- Disease severity was assessed on a visual analogue scale (VAS). The question was phrased: "How do you assess the degree of your eczema on a scale from 0 to 10, where 0 corresponds to no eczema and 10 correspond to very severe eczema?". Respondents were asked to answer this in relation to current eczema and in relation to when the eczema was the worst.

Validation analyses

Several different validation analyses were performed: 1) convergent validity in relation to other QoL instruments, which was done to test whether the Fragrance QoL index correlated with other QoL instruments; 2) convergent validity in relation to disease severity, which was done to test whether the Fragrance QoL index correlated with self-estimated disease severity; 3) reproducibility of the Fragrance QoL was investigated by comparing the responses from the questionnaire and the retest, described by the intra-item correlations (ICC). To ensure no interference from improvement and/or worsening of disease, an analysis of only "stable" patients was conducted. "Stable" patients were defined as those with no change in the eczema severity measured on the VAS. A difference of more than 1 point (VAS_{test}-VAS_{retest}) was considered a change in disease severity. In all, 71 patients were "stable" regarding their eczema and were included in the intra-item correlations disease as unchanged (ICC_{DU}); 4) responsiveness to change was investigated via data from the test-retest; 5) a Rasch analysis was done to give a range of details for assessing whether summarizing of the item scores into the Fragrance QoL index score was justified; 6) finally, a factor analysis was done to test how much of the variation could be described by the major linear contrasts.

4.4.3. Study design in re-test

The re-test consisted of a questionnaire survey comprising the Fragrance QoL index and a question on disease severity enclosed with a stamped, addressed return envelope. It was posted to 193 individuals (fragrance positive n= 72 and non-fragrance positive n=121) randomly selected using an inbuilt SPSS function among those who had answered the postal questionnaire survey. The re-test was sent once and the response rate was 72.5% (n=140).

4.4.4. Translation of the Fragrance QoL index

The Fragrance QoL index was originally developed in Danish. It was translated into English according to standardized methods [69]. The first translation was done by two native English-speaking professional translators from Danish to English. Then it was translated back to Danish by a Danish expert in English and expert on the dermatology field. The back-translation was compared with the original Fragrance QoL index and discussed among the chief investigator and the translators. Minor revisions were made to the English translated version and it was once more translated back to Danish and compared with the original Danish version of the Fragrance QoL index. The translation was primarily conducted for this study and to enable application in English-speaking individuals.

Figure 3. Schematic illustration of the development of the Fragrance QoL index

122 individuals with a fragrance allergy were randomly selected to participate in a postal survey. All had been patch tested at Gentofte University Hospital (2000-2010) and all had a positive reaction to at least one fragrance marker. A questionnaire was sent twice with a stamped, addressed return envelope to increase the response rate.

4 recipients did not wish to participate and 50 did not respond

68 recipients responded to the questionnaire with detailed narratives of how their fragrance allergy affected their quality of life (59 women and 9 men).

Their narratives were categorized and formed the basis for 13 items comprising the Fragrance QoL index and were discussed in a panel of experts on contact allergy to ensure relevance.

1. Pilot study: a semi-structured telephone interview or person-to-person interview testing each item for clarity, relevance and comprehension in subjects with a fragrance allergy (n=3) and without a fragrance allergy (n=7). Furthermore, a panel discussion was held among experts in the field of contact allergy.

Revision of the questionnaire according to comments from the interviews and discussion.

2. Pilot study: a semi-structured telephone interview or person-to-person interview testing each item for clarity, relevance and comprehension among those with a fragrance allergy (N=4) and individuals who were not fragrance allergic (N=6).

Minor revisions to the questionnaire according to comments from the interviews and discussion

The Fragrance QoL index was tested in a postal questionnaire survey together with SF36v2, DLQI and disease severity questions among 550 persons with fragrance allergy and 1100 without a fragrance allergy; the response rate was 66%.

A retest of the Fragrance QoL index was done in 193 persons; the response rate was 72.5%.

Data were manually entered into a database and checked for typing errors. Statistical analyses were made in SPSS and quality metric health outcome scoring software. Rasch test was done in Winstep Software.

The Fragrance QoL index was translated into English according to standardized methods.

4.4.5. Study design in the case-control study, manuscript IV

A questionnaire survey was designed as a matched case-control study. The case group had fragrance allergy and the control group did not have fragrance allergy. The questionnaire package was posted to the participants, in all 1650, who had been consecutively patch tested at Gentofte University hospital during 1 January 2000 − 31 December 2010. A stamped, addressed return envelope was included. A reminder postcard was sent after approximately 1−2 months in order to increase the response rate. To further increase the response rate, the questionnaire was sent a second time including a stamped, addressed envelope. The relatively long study-inclusion period was deliberately chosen because we wanted to investigate the effect of receiving the diagnosis of a fragrance allergy over time. Data on the participant's patch test reaction, date of patch testing, and demographic characteristics were retrieved from the National contact dermatitis database by the computer expert managing and routinely handling the database.

4.4.6. The questionnaire survey

The questionnaire survey consisted of the Fragrance QoL index and two other widely used, well-validated instruments of QoL (DLQI and SF36v2). Further, the questionnaire included baseline questions on eczema and diseases other than eczema. These baseline questions on eczema were adopted from well validated questions used in previous Danish studies[37,70,71]. To define atopic dermatitis among respondents, questions from the UK Working Party's diagnostic criteria were used [1]. To define respondents with multiple chemical sensitivity syndrome (MSC) questions on were asked to determine whether La Cour's criteria for MCS were fulfilled [2].

Each questionnaire item was tested for clarity, relevance and comprehension in two separately performed pilot studies and also via panel discussions among experts on the field of contact allergy. The development and validation of the questionnaire is schematically illustrated in Figure 3.

4.4.7. Matching of the case-control participants

The participants of the questionnaire survey consisted of a case group and a control group. To assure comparability, both the case and the control group had been patch tested for allergy at the same hospital, Gentofte University Hospital in the same period. The inclusion criterion for the case group was: that participants had at least one positive patch test reaction to a fragrance marker/ingredient during the study period; for the control group, that participants had been patch tested in the same period without any positive patch test reactions to a fragrance marker/ingredient. In all, 550 cases were randomly selected from all persons with a positive patch test reaction to a fragrance ingredient/marker from the National Contact Dermatitis Database. Two control persons were matched on age (±1 year), sex and patch test year (±1 year) to each of the cases. The computer expert routinely handling the database randomly selected individuals from the National Contact Dermatitis Database among all who matched the criteria. The response rate of the questionnaire was 65.7% (1084/1650).

4.4.8. Data entering and validation of data

The data entry programme was constructed in SPSS® Data Entry Builder (SPSS Inc., Illinois, USA) by the chief investigator. All variables were set up in the exact same order as in the questionnaire items. Data from the questionnaires were entered manually.

In the matched case-control study (n=1084) data entering was done by the chief investigator and two assistants trained in data entering. In the retest (n=140) data entering was solely done by the chief investigator. To ensure validity of the data entering a detailed instruction on how the entry

should be conducted was made by the chief investigator and reviewed with the assistants. The instructions included information on how to handle any discrepancy for each of the questions, for example if a respondent had answered a question by ticking off two boxes, where only one was allowed, this was considered a misunderstanding of the question and left blank. To further ensure the validity of the data, the data entering of the matched case-control questionnaires was done over a short period (three weeks), were the chief investigator was involved at all time, thus, any problems arising was addressed immediately and ensured consistency. Data from both questionnaires were checked for typing errors by the chief investigator, where 10% of all questionnaires were retyped, giving a discrepancy of < 1%. Additionally, each item in the questionnaire was checked for outliers and missing data. Any inconsistencies were double checked with the original questionnaire.

4.4.9. Statistical methods, manuscript III

Statistical analysis of correlations, crude percentages, mean and standard deviation were performed using the Statistical Product and Service Solution package (SPSS® Inc., Chicago, IL, USA) for windows version 19. Calculations on the SF36v2 dimensions were done in Quality Metric Health Outcomes™ Scoring Software 4.5 and the Rasch analysis was performed in Winstep Software (www.winstep.com)

The different validation tests consisted of:
1. Analysis of correlations where the Spearman rho (rS) was calculated. The Spearman correlation is used as the responses were given on an ordinal scale and were not necessarily expected to be linear. The correlation between the Fragrance QoL index and the DLQI was also illustrated in a scatter plot.
2. Analysis of internal consistency of the Fragrance QoL index score was tested with Cronbach's alpha coefficient. A rule of interpretation the Cronbach's alpha coefficient is shown in Table 1.

Table 1. Interpretation of Cronbach's alpha

Cronbach's alpha	Internal consistency
$\alpha \geq 0.9$	Excellent
$0.8 \leq \alpha < 0.9$	Good
$0.7 \leq \alpha < 0.8$	Acceptable
$0.6 \leq \alpha < 0.7$	Questionable
$0.5 \leq \alpha < 0.6$	Poor
$\alpha < 0.5$	Unacceptable

3. Analysis of reproducibility of the Fragrance QoL index was done by calculation of the intra-item correlations (ICC) in the test-retest study.
4. Analysis of reproducibility of the Fragrance QoL index in individuals who did not vary in their self-estimated severity of eczema in the test and re-test was done by calculation of the intra-item correlations where the disease was unchanged (ICC_{DU}) in the test-retest study.
5. Analysis of test-retest reliability was illustrated in a Bland and Altman plot, a graphical method to compare two measurements.
6. Analysis of the Fragrance QoL index's responsiveness to change with self-estimated disease severity was performed as it should be able to discriminate changes in disease severity. This is illustrated visually in a scatter plot and tested with Pearson's correlation.

7. Rasch analysis was performed as the Rasch model is the only item response theory (IRT) model in which the total score across items characterizes a person totally, which is what is done in the calculation of the Fragrance QoL index score and why this model was chosen.
8. Factor analysis was performed to search for joint variations in response to unobserved latent variables. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy tested whether the partial correlations among variables were small. A KMO value above 0.05 is satisfactory for factor analysis to proceed. The Bartlett's test of sphericity was used to test the null hypothesis, if the variables in the population correlation matrix were uncorrelated.

In all the statistical analyses a p value of < 0.05 was considered significant.

4.4.10. Statistical methods, manuscript IV

Statistical analyses were performed using the Statistical Product and Service Solution package (SPSS$^{®}$ Inc., Chicago, IL, USA) for windows version 19. Standard methods were used for the descriptive statistics, crude percentages, mean and standard deviation. The analyses for differences between the case group and the control group were done accounting for the matching in: 1) a conditional logistic regression model, which is a model designed for analysing responses in a case-control setting where one or several controls are matched to one case; 2) univariate analyses performed accounting for the matching. Several logistic regression analyses and Mann Whitney tests were preformed stratified by case group and control group to test for differences and confounding factors within each group. The binary logistic regression model was checked by Hosmer-Lemeshow goodness of fit test.

In all the statistical analyses p values of < 0.05 was considered significant.

5. Results

5.1. Results study Part I, manuscript I & II

5.1.1. Description of study population

The study population in study Part 1 comprised of subjects consecutively patch tested by the Danish Contact Dermatitis Group. In manuscript I, a total of 12302 were included. All had been tested with the FM II in the baseline series between 2005 and 2008. In manuscript II, all individuals included had been patch tested with at least one of the screening markers of fragrance allergy (FM I, FM II, MP or HICC) between 2005 and 2009. A total of 17716 subjects were included. Their descriptive data can be described via the MOAHLFA index, which is an initialization of M: male; O: occupational causation of dermatitis; A: atopy; H: hand dermatitis; L: leg dermatitis; F: face affected by dermatitis; and AA: age ≥40 years.

Table 2. MOAHLFAA index of study population in manuscript I

| Index | Tested subjects | |
	Numbers	%
M	4239	34.5
O	1468	11.9
A	2109	17.1
H	4619	37.5
L	625	5.1
F	2287	18.6
AA	7389	60.1
Total	12 302	100

Table 3. MOAHLFAA index of study population in manuscript II

| Index | Tested subjects | |
	No.	%
M	6106	34.5
O	2067	11.7
A	3115	17.6
H	6625	37.4
L	815	4.6
F	3370	19.0
AA	10465	59.1
Total	17716	100

The sex distribution of the patch tested subjects was the same in both manuscripts (65.5% women and 34.5% men). Many of the other demographic characteristics were likewise similar, see Table 2 and Table 3. The mean age in both studies was 44.8 years and in manuscript I standard deviation 18.2; in manuscript II, 18.3 years.

5.1.2. Allergy to fragrance mix II

A positive patch test reaction to the FM II was observed in 4.5 % of the 12302 subjects tested. This was the second highest prevalence of the fragrance screening markers, after the fragrance mix I. A significantly higher frequency of FM II allergy was observed among women compared with men and among subjects with facial dermatitis. The majority of the 553 positive patch test reactions to FM II were of clinical relevance (72.2%). Concomitant reactions between the other screening markers of fragrance (FM I, MP and HICC) allergy occurred: 202 subjects (15.6%) would not have been identified with a fragrance allergy if they had not been tested with the FM II, see Figure 4.

Figure 4. Illustration of concomitant reactions between fragrance mix II and the other screening markers of fragrance allergy (fragrance mix I, Myroxylon pereirae and /or HICC)
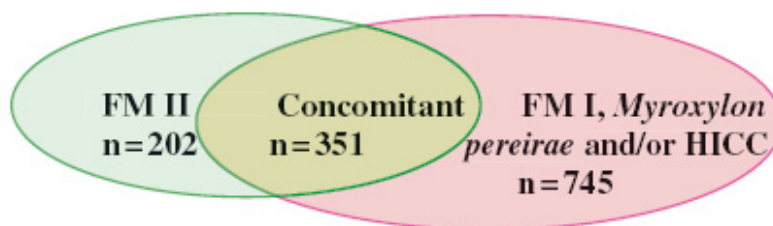
Thus, including FM II as a screening marker of fragrance allergy contributed significantly in identifying more subjects with a fragrance allergy, who otherwise would have gone undetected.

5.1.3. Cosmetic exposure sources causing fragrance allergy

Cosmetic products were the most frequent exposure source causing allergic contact dermatitis to fragrance ingredients/markers (42.1%). It was found that a wide range of cosmetic products were the cause. The majority were leave-on products (74.3%), which cover products that are left on the skin for example fine fragrances, deodorants, lotions etc. Rinse-off cosmetic products which are products washed off the skin after use, for example soaps and shampoos, were listed in 248 subjects as having caused their fragrance contact dermatitis. The cosmetic products most frequently listed as having caused fragrance allergy were deodorants, scented lotions, fine fragrances and shampoos (Figure 5).

Figure 5. Prevalence of specific cosmetic cosmetic product groups listed as having caused fragrance allergy (N=576)
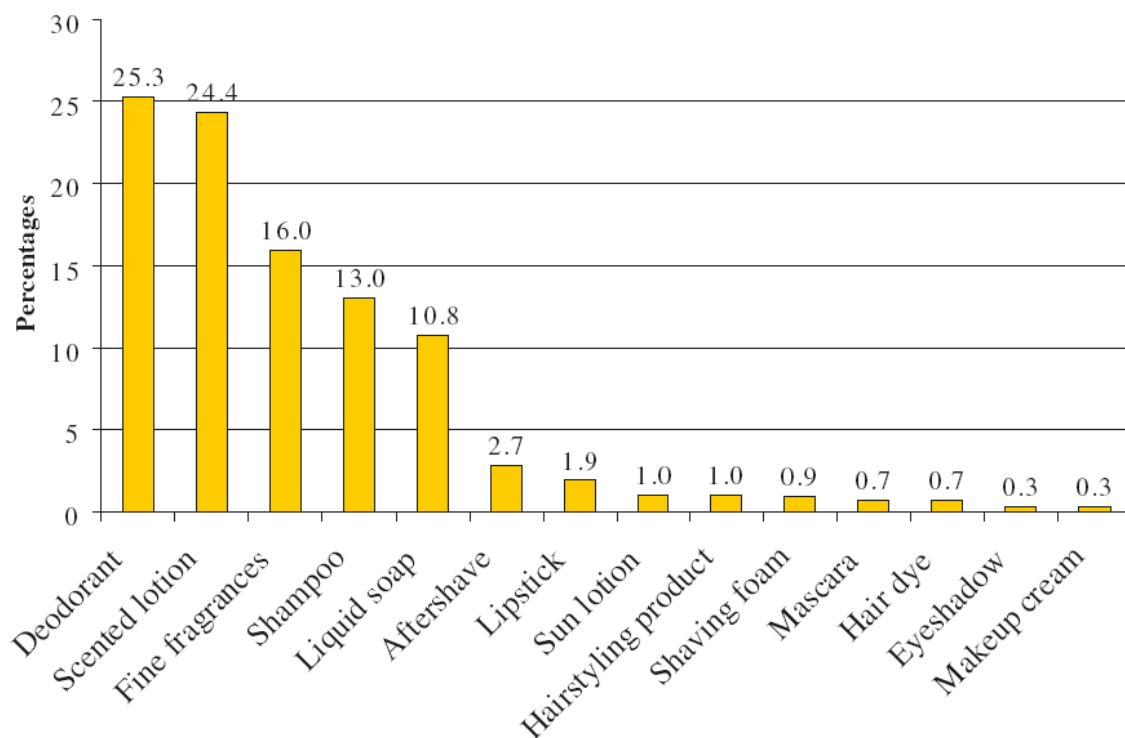
A clear gender difference in the distribution of cosmetic products listed as causing fragrance allergic contact dermatitis was observed. Deodorants were 2.3 times more frequently listed as the cause of their allergy (p<0.001) in men compared with women. Scented lotion and fine fragrances were significantly more frequently listed in women compared with men as a cause of their contact allergy. No gender difference was observed with shampoo (Figure 6).

Figure 6. Gender distribution of the four most frequently listed causes of fragrance allergy

■ Cosmetic products listed as having caused fragrance ACD in males n=145
■ Cosmetic products listed as having caused fragrance ACD in females n=431
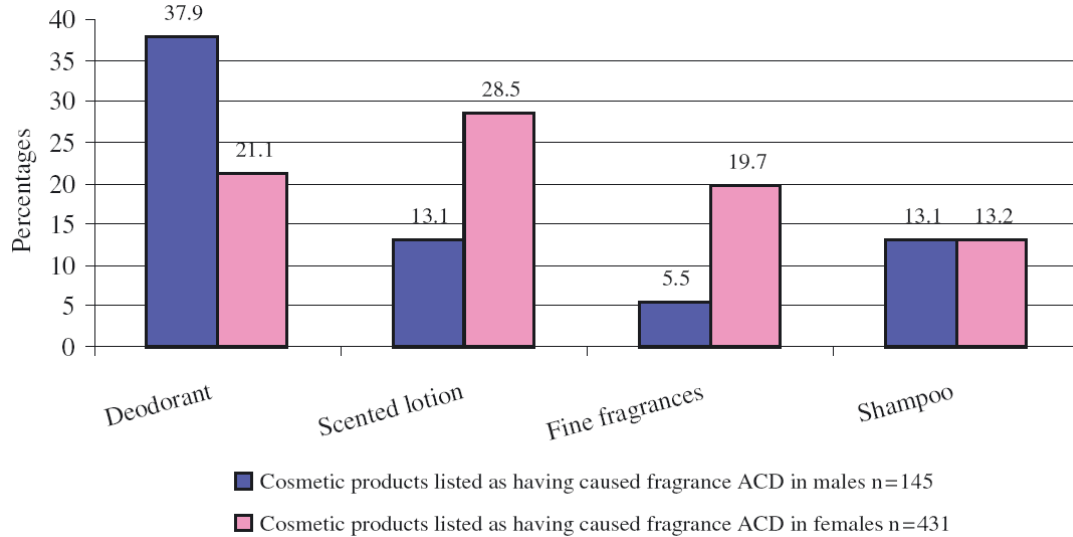
Investigation of which of the cosmetic product groups were listed as the cause of contact allergy to each of the fragrance ingredients/markers is described in Table 4. There was an overrepresentation among some product groups and allergy to specific fragrance ingredients/markers. Thus, when looking at the cosmetic products, which most frequently are the cause of fragrance allergy, deodorants were listed as the cause of allergy to FM II or hydroxyisohexyl 3-cyclohexene carboxaldehyde more frequently, than the other screening markers of fragrance allergy, Figure 7.

Figure 7. The prevalence of a positive patch test reaction to the screening markers of fragrance allergy among the 4 most frequent causes of fragrance allergy
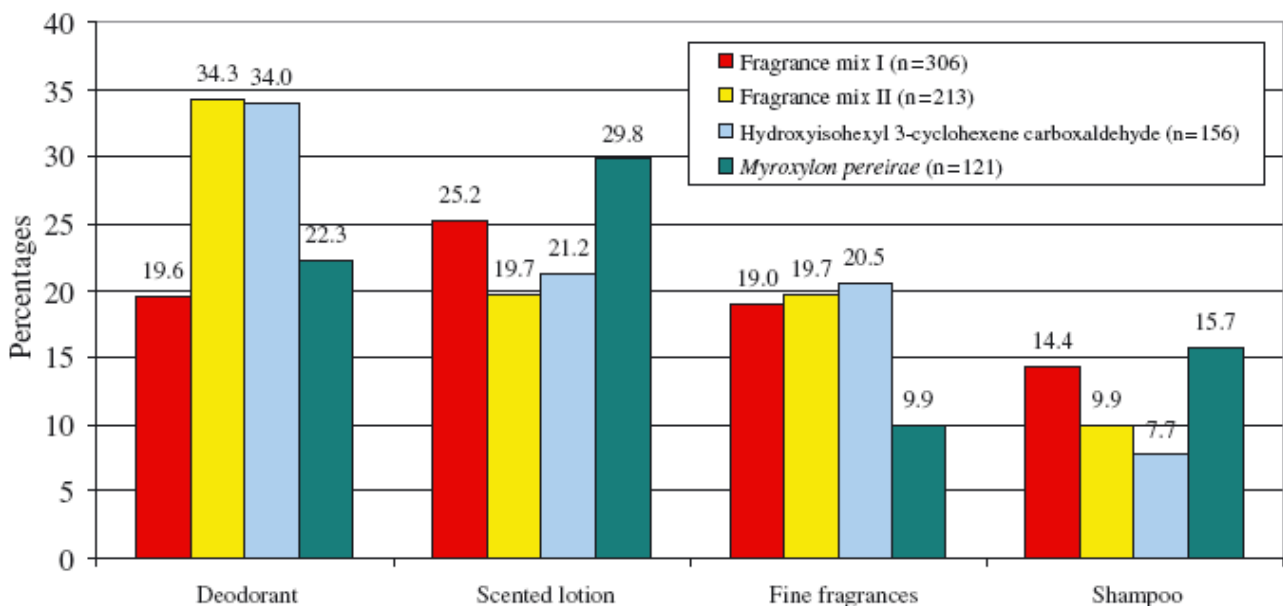
Table 4. The prevalence of cosmetic product groups listed as the cause of fragrance allergic contact dermatitis

Fragrance screening markers of the baseline series

Product	n	Fragrance mix I		Fragrance mix II		Hydroxyisohexyl 3-cyclohexene carboxaldehyde		Myroxylon pereirae	
		n	%	n	%	n	%	n	%
Deodorant	213	60	28.2	73	34.3	53	24.9	27	12.7
Scented lotion	188	77	41.0	42	22.3	33	17.6	36	19.1
Fine fragrances	144	58	40.3	42	29.2	32	22.2	12	8.3
Shampoo	96	44	45.8	21	21.9	12	12.5	19	19.8
Liquid soap	84	37	44.0	17	20.2	16	19.0	14	16.7
Aftershave	23	9	39.1	6	26.1	3	13.0	5	21.7
Lipstick	12	4	33.3	5	41.7	0	0.0	3	25.0
Sun lotion	10	4	40.0	2	20.0	3	30.0	1	10.0
Hairstyling product	6	3	50.0	2	33.3	1	16.7	0	0.0
Shaving foam	6	4	66.7	1	16.7	0	0.0	1	16.7
Mascara	4	2	50.0	0	0.0	1	25.0	1	25.0
Hair dye	4	3	75.0	0	0.0	0	0.0	1	25.0
Eyeshadow	5	2	40.0	1	20.0	1	20.0	1	20.0
Makeup cream	3	1	33.3	1	33.3	1	33.3	0	0.0

A cosmetic product could be listed as the cause of allergic contact dermatitis resulting from more than one fragrance marker.

5.2. Study Part II, manuscript III

Narratives from 68 subjects with a fragrance allergy formed the basis in the development of the disease-specific QoL instrument, the Fragrance QoL index. It was validated in a questionnaire survey including other QoL instruments and estimations on disease severity. Additionally, it was validated in a re-test.

5.2.1. Description of study population in the development of the Fragrance QoL index

Fragrance allergic individuals had received a letter asking them to describe in detail the effect fragrance allergy had on their lives. The demographic characteristics of the 68 respondents and the 54 non-respondents can be described via the MOAHLA index, see Table 5.

Table 5. Description of respondents and non-respondents		
	Respondents n=68	NON-respondents n=54
M	13.0%*	28.3%
O	21.7%	11.3%
A	21.7%	20.8%
H	42.0%	45.3%
L	0.0%	3.8%
F	29.0%	17.0%
AA	72.5%	56.6%

* Fisher's exact test; significant difference between the respondents versus non-respondents $p < 0.05$
Chi^2 tests were also performed: no significant difference between the respondents and non-respondents $p < 0.05$ was observed.

There was a significant gender difference among the respondents (Table 5); moreover, there was a strikingly low number of males selected to participate, despite a randomly selection from the total number registered with a fragrance allergy in the National Contact Dermatitis Database (Table 2 and Table 3). The respondents had a noticeably higher prevalence of occupational dermatitis, facial dermatitis and age ≥40 years than did the non-respondents; however, it was not statistically significant.

5.2.2. The Fragrance QoL index

The Fragrance QoL index consists of 13 items answered on a visual analogue scale from 0 to 10 (Figure 8). The time frame covered by the items was set to "currently", as in this present moment. A summarization of all the scores, when more than 11 items are answered, can be calculated. In item 6, there was the possibility of answering "not applicable", which was scored as 0. In item 12, the score was reversed (10=0, 9=1,......, 0=10). This Fragrance QoL index score has a minimum score of 0 and maximum score of 130, and the higher the score, the greater the QoL impairment.

5.2.3. Study population in the validation of the Fragrance QoL index

The Fragrance QoL index was validated in the questionnaire study among 1084 eczema patients. Those with a fragrance allergy accounted for 35%, and those who were non-fragrance positive accounted for 65.0%. Bear in mind they were matched 1:2. Thus, there was no significant difference in the response rates. Overall, the two groups were quite similar regarding demographic characteristics (Table 6).

Figure 8. The Fragrance QoL index

Your own evaluation of your rash

The following statements concern how you currently feel about your rash. *Please put a cross on the line that best agree with how you feel. The numbers range from 0 to10, where 0 is completely disagree, 5 is partly agree and 10 is strongly agree.*

1. Your rash has had a negative effect on your quality of life.

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

2. You must take special measures in your daily life to avoid situations that could provoke your rash.

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

3. You are often bothered by fissures and/or cracked skin.

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

4. You are often bothered by itchy skin.

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

5. You are often bothered by pain or smarting.

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

6. Your rash has caused you to take special measures in order to do your work/studies, *e.g. use gloves, be exempt from certain duties, change job/studies, stop working, or other similar measures to avoid provoking your rash.*

☐ Not applicable

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

7. You restrict physical contact with your family/friends in order to avoid provoking your rash.

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

8. Your rash often makes you irritable or stressed.

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

9. You are often worried about being exposed to things that can provoke your rash.

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

10. You feel less attractive because of your skin disease.

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

11. You miss being able to smell nice.

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

12. You know what provokes your rash. *This means that you have a clear idea of what provokes/exacerbates your rash.*

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

13. You feel people should show more consideration towards your illness.

Completely disagree   0   1   2   3   4   5   6   7   8   9   10
                       Partly   Strongly agree

Table 6. Demographic and disease burden of the study population

Respondents

| | Fragrance positive | | | | | | Non-fragrance positive | | | | | | Total | |
| | Women | | Men | | Women and men | | Women | | Men | | Women and men | | Women and men | |
	n (mean)	% (SD)	n (mean)	% (SD)	n (mean)	% (SD)	n (mean)	% (SD)	n (mean)	% (SD)	n (mean)	% (SD)	n (mean)	% (SD)
Responders	290	76.5%	89	23.5%	379	35.0%	551	78.2%	154	21.8%	705	65.0%	1084	-
Age	(45.8)	(12.6)	(48.9)	(11.9)	(46.5)	(12.5)	(46.6)	(12.3)	(49.7)	(11.0)	(47.3)	(12.1)	(47.1)	(12.2)
Prevalence of Eczema														
1 year prevalence	215[a]	81.7%	54	68.4%	269	78.7%	357	75.3%	99	76.3%	456	75.5%	725	76.7%
Point prevalence	160	56.7%	42	47.7%	202	54.6%	271	50.0%	81	53.3%	352	50.7%	554	52.1%
Duration of eczema														
Once, less than 2 weeks	9[b]	3.5%	4	5.2%	13	3.9%	18	3.9%	2	1.5%	20	3.3%	33	3.5%
Once, longer than 2 weeks	11[b]	4.2%	3	3.9%	14	4.2%	40	8.6%	12	9.1%	52	8.7%	66	7.1%
Many times	135[b]	51.9%	42	54.5%	177	52.5%	249	53.4%	65	49.2%	314	52.5%	491	52.5%
(almost) all the time	105[b]	40.4%	28	36.4%	133	39.5%	159	34.1%	53	46.2%	212	35.5%	345	36.9%
Severity of eczema VAS														
Eczema presently	(3.6)	(2.8)	(3.1)[c]	(2.5)	(3.5)	(2.7)	(3.6)	(3.0)	(3.9)	(3.0)	(3.7)	(3.0)	(3.6)	(2.9)
Eczema when worst	(7.8)	(2.4)	(7.2)	(2.7)	(87.6)	(2.5)	(7.7)	(2.5)	(7.6)	(2.3)	(7.6)	(2.5)	(7.6)	(2.5)
Other skin diseases														
Atopic dermatitis*	62	21.4%	16	18.0%	78	20.6%	98	17.8%	22	14.3%	120	17.0%	198	18.3%
Other skin disease	118	40.7%	24	27.0%	142	37.2%	214	38.5%	38	24.7%	250	35.5%	395	36.1%
Other allergies ** (other than fragrance)	183[d]	63.1%	44[e]	49.4%	227[f]	59.9%	222	40.3%	54	35.1%	276	39.1%	503	46.4%

n: Number of respondents

mean: Mean of age, and severity of eczema measured on the visual analogue scale for eczema currently and when worst.

SD: Standard deviation

%: Crude percentages of all respondents who answered the item. If respondents had not answered the item they were counted as missing in the analysis. Statistical tests were performed accounting for the matching for each of the items (univariate analyses and logistic regression analyses)

* Atopic dermatitis as defined by Hanifin et al. One major, at least 3 minor criteria.

** Positive patch test reaction to a marker of the baseline series other than a fragrance screening marker (Fragrance mix I, fragrance mix II, hydroxyisohexyl 3-cyclohexene carboxaldehyde and Myroxylon pereirae.

[a] Fragrance positive women compared with control women in a logistic regression analyses accounting for the matching, p= 0.042

[b] Fragrance positive women had a significantly different duration of eczema compared with control women in a logistic regression analyses accounting for the matching, p= 0.041

[c] Fragrance positive men score significantly lower on the visual analogue scale on self estimated eczema severity (3.1) currently compared with non-fragrance positive men (3.9); Univariate analysis accounting for the matching, p=0.048.

[d] Fragrance positive women compared with control women in a logistic regression analyses accounting for the matching, p= 0.001

[e] Fragrance positive men compared with control men in a logistic regression analyses accounting for the matching, P=0.028

[f] Fragrance positive women and men compared with controls in a logistic regression analyses accounting for the matching, P= 0.001.

5.2.4. Validation of the Fragrance QoL index

Results from the validation analyses of the Fragrance QoL index are shown in the following tables and figures.

5.2.4.1. Convergent validity in relation to the other fragrance QoL instruments

The results of the correlations between the Fragrance QoL index and the DLQI and the SF36v2 are shown in Table 7. A good significant correlation was found to the DLQI (r_S=0.70). However, the correlation to the SF36v2 showed only weak to moderate inverse correlations to both the mental component summary score (r_S=-0.22) and the physical component summary score (r_S=-0.31). The non-linear correlation between the Fragrance QoL index and the DLQI is illustrated in Figure 9.

Table 7. Spearman correlations between the Fragrance QoL index and SF36v2 and DLQI

| | The Fragrance index | | | | | | DLQI | |
| | Fragrance positive | | Non-fragrance positive | | All respondents | | All respondents | |
	r_S	P value	r_S	P value	r_S	P value	r_S	P value
DLQI	0.70	<0.001	0.74	<0.001	0.72	<0.001	-	-
SF36 MCS	-0.22	<0.001	-0.30	<0.001	-0.28	<0.001	-0.19	<0.001
SF36 PCS	-0.31	<0.001	-0.31	<0.001	-0.31	<0.001	-0.23	<0.001

DLQI: The dermatology quality of life index.
SF36 MCS: Mental component summary score of the SF36 questionnaire.
SF36 PCS: Physical component summary score of the SF36 questionnaire.
r_S: Spearman correlation coefficient.
Fragrance positive: a positive patch test to at least one fragrance marker.
Non-fragrance positive: no positive patch test to a fragrance marker.

Figure 9. Scatter plot of the correlation between the Fragrance QoL index and the DLQI

Fragrance positive: at least one positive patch test reaction to a fragrance screening marker/ingredient
Non-fragrance positive: no positive patch test reaction to any fragrance screening marker/ingredient.
Each line represents an estimate (kernel) of the relation between the means for the fragrance positive (red) and the non-fragrance positive (green). A significant nonlinear correlation is observed between the DLQI and the Fragrance QoL index scores (Spearman correlation, p<0.001).

5.2.4.2. Convergent validity in relation to disease severity

The convergent validity of the QoL instruments in relation to disease severity for the Fragrance QoL index and the DLQI showed good correlations. However, in the SF36v2 both the mental component summary score and the physical component summary score had weak correlations to self-estimated disease severity.

Figure 10. Correlation between self-estimated disease severity in the current moment and QoL
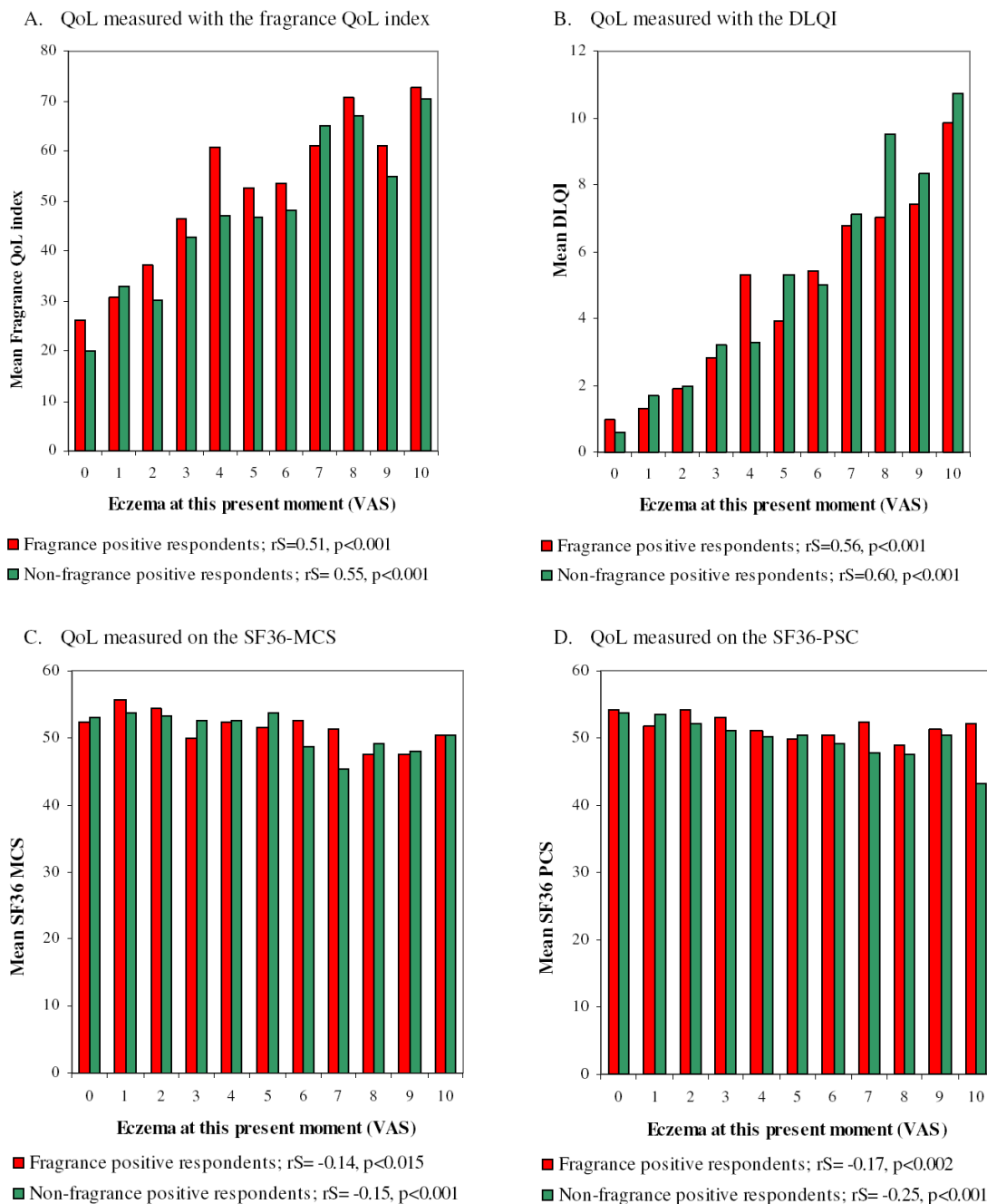
A. QoL measured with the fragrance QoL index

- Fragrance positive respondents; rS=0.51, p<0.001
- Non-fragrance positive respondents; rS= 0.55, p<0.001

B. QoL measured with the DLQI

- Fragrance positive respondents; rS=0.56, p<0.001
- Non-fragrance positive respondents; rS=0.60, p<0.001

C. QoL measured on the SF36-MCS

- Fragrance positive respondents; rS= -0.14, p<0.015
- Non-fragrance positive respondents; rS= -0.15, p<0.001

D. QoL measured on the SF36-PSC

- Fragrance positive respondents; rS= -0.17, p<0.002
- Non-fragrance positive respondents; rS= -0.25, p<0.001

Self-estimated disease severity was measured on a visual analogue scale (VAS), where 0 is no eczema and 10 is very severe eczema. The quality of life was measured with (A) the Fragrance QoL index , (B) DLQI , (C) SF36v2: mental component summary score (MCS) and (D) SF36v2: physical component summary score (PCS).
rS: Spearman correlation coefficient.
Non-fragrance positive: patch test negative reaction to all fragrance screening markers/ingredients.
Fragrance positive: at least one positive patch test reaction to a fragrance screening marker/ingredient.

5.2.4.3. Reliability of the Fragrance QoL index

The reproducibility of the fragrance QoL showed significant intra-item correlations (ICC) in the test-retest study and also in the ICC $_{disease\ unchanged.}$ Internal consistency of the Fragrance QoL index score was tested with Cronbach's alpha coefficient, (α =0.92), which can be interpreted as an excellent consistency.

Table 8. Correlation between the Fragrance QoL index in the test and re-test

Item	Test		Re-test		Intraclass coefficient	
	mean	(SD)	mean	(SD)	ICC	ICC $_{DU}$
1	3.88	(3.02)	3.66	(3.12)	0.77	0.86
2	4.60	(3.78)	5.37	(3.74)	0.78	0.80
3	4.93	(3.49)	4.29	(3.04)	0.77	0.77
4	5.47	(3.20)	4.74	(3.14)	0.72	0.66
5	4.03	(3.40)	3.71	(3.13)	0.72	0.72
6	3.49	(4.06)	3.73	(3.98)	0.81	0.89
7	0.54	(1.36)	0.76	(2.08)	0.68	0.72
8	2.63	(2.74)	2.59	(2.95)	0.83	0.91
9	2.74	(3.02)	2.58	(3.38)	0.72	0.79
10	2.26	(3.02)	2.64	(3.22)	0.76	0.99
11	2.49	(3.46)	3.16	(3.90)	0.82	0.89
12	6.02	(3.57)	6.55	(3.45)	0.75	0.82
13	1.54	(2.60)	1.46	(2.44)	0.63	0.61
Disease severity	3.47	(2.88)	3.17	(2.82)	0.56	-
Fragrance QoL index score	41.82	(25.62)	43.07	(26.31)	0.86	0.92

All ICC values were highly significant, p<0.001.
ICC: Intraclass coefficient.
ICC$_{DU}$: Only including respondents with no change in disease severity (n=71), a change of > 1 point on the visual analogue scale (VAS$_{test}$-VAS$_{retest}$) was considered a change in disease severity.
Mean: mean of the VAS.
SD: Standard deviation.

The test-retest reliability is also illustrated in a Bland and Altman plot, which is a graphical method to compare two measurements. It illustrates a good agreement between the test and retest.

Figure 11. Bland & Altman plot illustration of the correlation of the test-retest
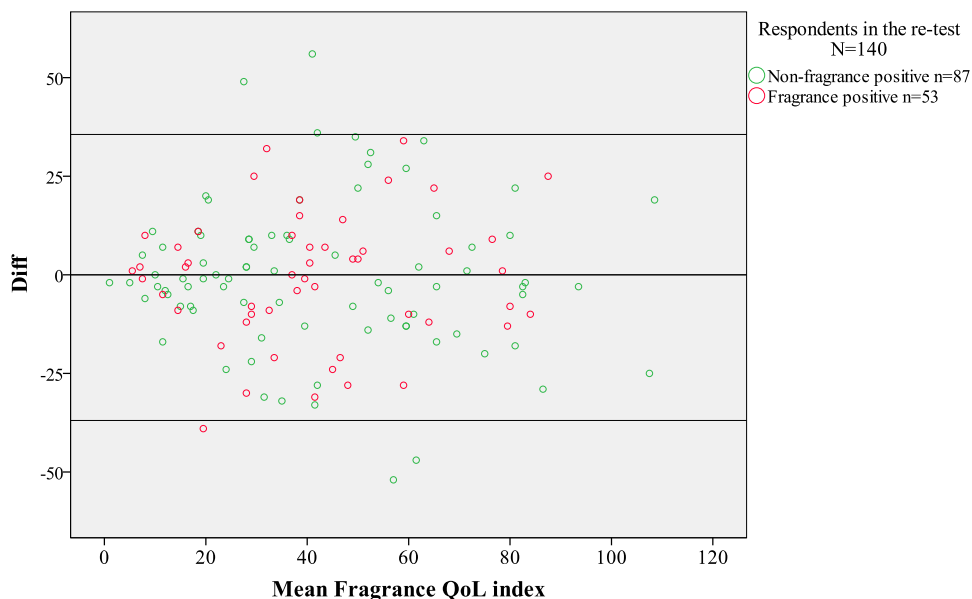
The plot illustrates the difference (Diff) of the mean of the Fragrance QoL index score in the test and re-test compared with the difference of the mean of the self-estimated disease severity measured on the VAS in both the test and re-test. Horizontal lines are drawn at the mean difference, and at the limits of agreement, which are defined as the mean difference ±1.96 times the standard deviation of the differences.

5.2.4.4. Responsiveness to change

Results of the Fragrance QoL index's ability to discriminate between individuals with change in disease severity in the test and re-test is illustrated visually in a scatter plot, Figure 12. Pearson's test for correlation showed a strong statistically significant correlation, r=0.76.

Figure 12. Scatter plot of the Fragrance QoL index responsiveness to change with changes in disease severity (test-retest)
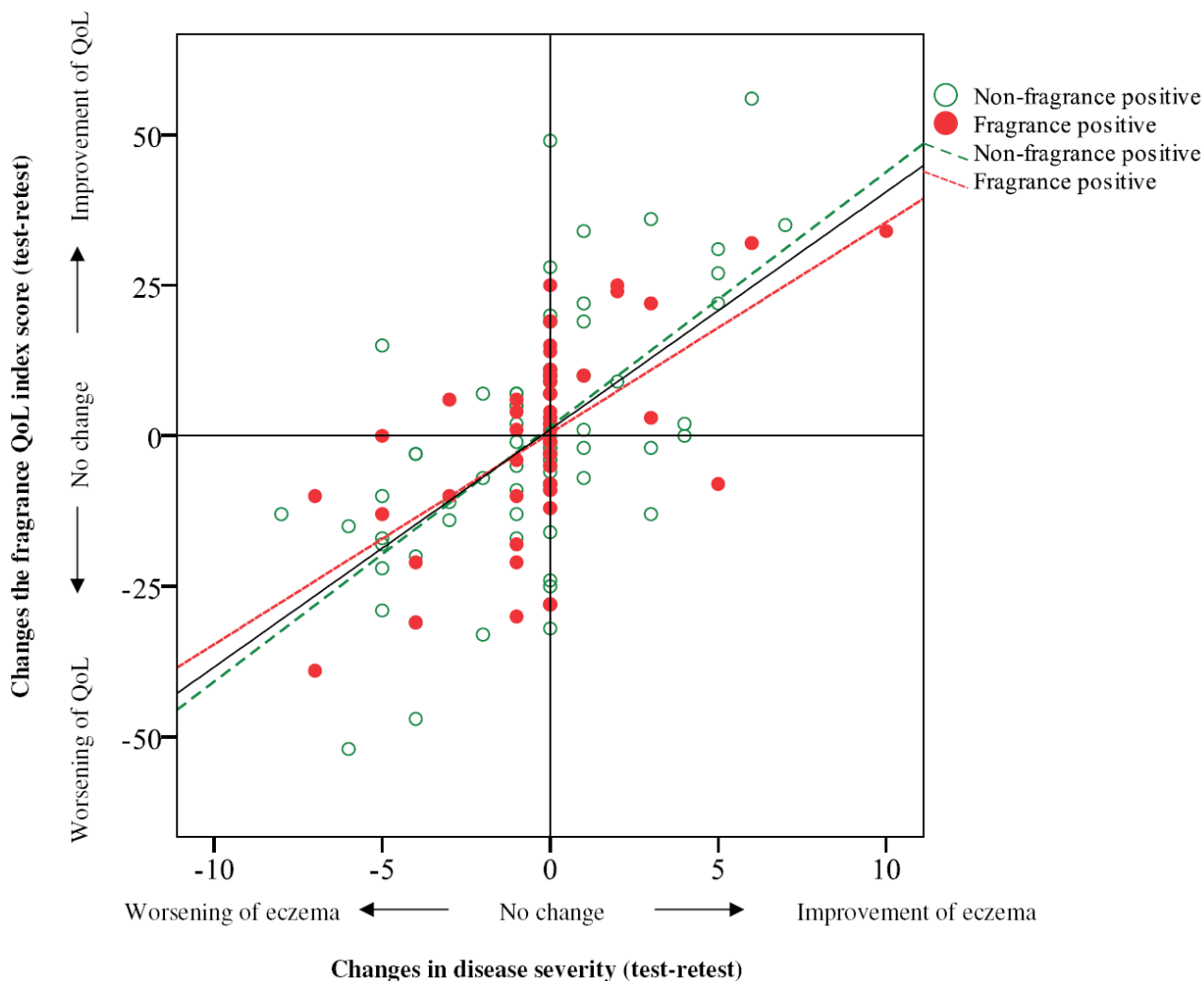
Self-estimated disease severity was measured on a visual analogue scale in the test (VAS_{test}) and in the retest (VAS_{retest}). Changes in disease severity was calculated VAS_{test} - VAS_{retest} and positive scores indicate improvement of disease, 0 indicate no change in disease, and negative scores indicate worsening of disease. Likewise, the difference in QoL was calculated, where the Fragrance QoL index score in the re-test was subtracted from the score from the test. Pearsons test; r=0.76, p<0.001.

5.2.4.5. Rasch analysis

The results from the Rasch test showed that all the items fit the model well for women and men with and without fragrance allergy. A one dimensional measure of QoL without indications of heterogeneity or disturbance of outliers was seen.

5.2.4.6. Factor analysis

The factor analysis was performed separately on the fragrance positive and non-fragrance positive; and on women and men; and in the total number of respondents. All analyses showed high KMO values (>0.9). A highly significant values of Bartlett's test of sphericity and scree plots indicated that the first factor described 40−50% of the variation, while the following three factors accounted for 5−10% of the variation. This means that the Fragrance QoL index constructed from the 13 items captures a very high fraction of the variability in the scoring.

Overall, the validation analyses showed that the Fragrance QoL index was a good instrument for measuring QoL. It showed a good significant convergent validity to both DLQI and self-estimated disease severity. The Cronbach's alpha indicated an excellent correlation and the intra-item correlations were satisfactory. A significant strong correlation in the responsiveness to change with changes in disease severity was found ($r=0.76$; $p<0.001$). The factor analyses and Rasch analyses reveal that summation of Fragrance QoL index score is justified, that there are no other major underlying factors. Thus, sub scaling of the Fragrance QoL index would also be redundant.

5.3. Study Part II, manuscript IV

5.3.1. Study population - description

Clinical and demographic characteristics are described in Table 6 and complementary description of the participants in Table 9.

5.3.2. Response rate

The questionnaire was posted to 1650 individuals and 1084 responded (65.7%). There was no difference in the response rate of the case group (fragrance positive) and the control group (non-fragrance positive) or between men (p=0.07) and women (p=0.22). Furthermore, there was no change in the response rate regarding when they had been patch tested, see Figure 13. A significant age difference in the response rate was observed, trend analysis p<0.001. Figure 14 illustrates that the higher the age (years), the higher the response rate.

Figure 13. Response rate stratified by patch test year
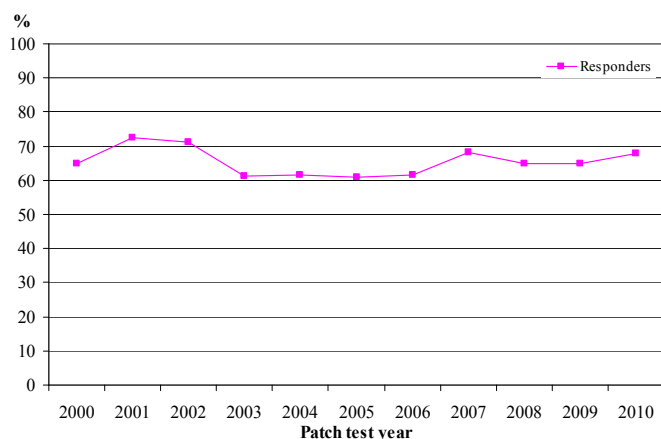
Figure 14. Response rate of participants stratified by age
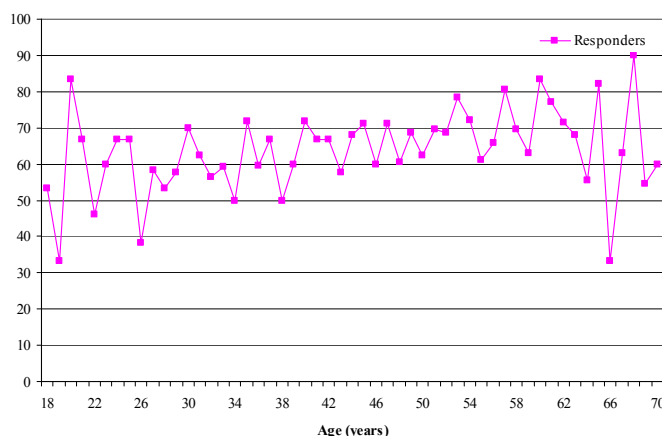
5.3.3. Quality of life and fragrance allergy

Results from QoL measured with the Fragrance QoL index are described in the following tables and figures, manuscript IV.

QoL and gender differences for each of the items comprising the Fragrance QoL index are described in Table 10. There were clearly gender differences in many aspects regarding QoL and fragrance allergy. A statically significant difference between the fragrance positive case group and the non-fragrance positive control group (p=0.042) was seen for the Fragrance QoL index score. However, this difference was seen only among the women, where a significant QoL impairment was observed (p=0.014). No significant difference in QoL was seen among the men with a fragrance allergy and their controls in QoL (p=0.732). When performing a multiregression analysis separately for women and men including parameters showing statistically significant differences in disease burden/conditions (Table 9), we found that QoL impairment was still significantly associated with having a fragrance allergy among the women (p=0.042). For the fragrance allergic

men and their controls we again found no significant association between QoL and fragrance allergy (p=0.163).

Within each of the 13 items of the Fragrance QoL index, there were some similarities among men and women. Those with a fragrance allergy more frequently felt they had to take precautions to avoid triggering exposures, they all missed smelling nice and they better understood what provokes their eczema compared with their respective control groups. Fragrance positive women worried about being exposed to things that could provoke their eczema significantly more than their control group. Furthermore, they felt people should show more consideration towards their condition and felt more restricted in having physical contact with family/friends compared with their controls.

In general, men were not as affected as women by fragrance allergy in their QoL. We did not find any significant impact in QoL regarding any of the following investigations for the men.

The top ten fragrance markers of allergy among those who answered the questionnaire are shown in Table 11 together with the impairment of QoL. The prevalence of a positive patch test reaction to a fragrance marker was the highest for fragrance mix I (n=174), followed by *Myroxylon pereirae* (n=94) and fragrance mix II (n=54). A significant impairment in QoL was detected among the women with an allergy to fragrance mix I (FM I), fragrance mix II (FM II), *Myroxylon pereirae* (MP), and the strongest significance was for hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), which also showed the greatest impairment of all the individual fragrance markers among women.

Impairment of QoL for women with fragrance allergy was not significantly correlated with age (Pearson's test; p=0.24). However, for the non fragrance positive women a significant inverse correlation was observed as they were less affected in their QoL with higher age (Pearson's test; p=0.02). Impairment of QoL for each of the age groups stratified by time of patch test showed a significant difference in the youngest age group. This difference is shown in Figure 15. It illustrates the QoL impairment for different age groups over time. Thus, the more recently women in the youngest age group had been diagnosed with a fragrance allergy, the greater QoL impairment (trend test, p=0.03.

Figure 15. Quality of life in women at different age groups stratified by patch test year

Scatter plot with trend lines illustrating QoL in women in different age groups stratified by patch test year. Each line represents the linear association between the QoL during different patch test years for each age group: the green line (age group 18-29; trend test, p=0.03); orange line (age group 30-39; p= 0.95); blue line (age group 40-49; p= 0.20); pink line (age group 50-59; p=0.39) and black line (age group 60-70; p=0.32).
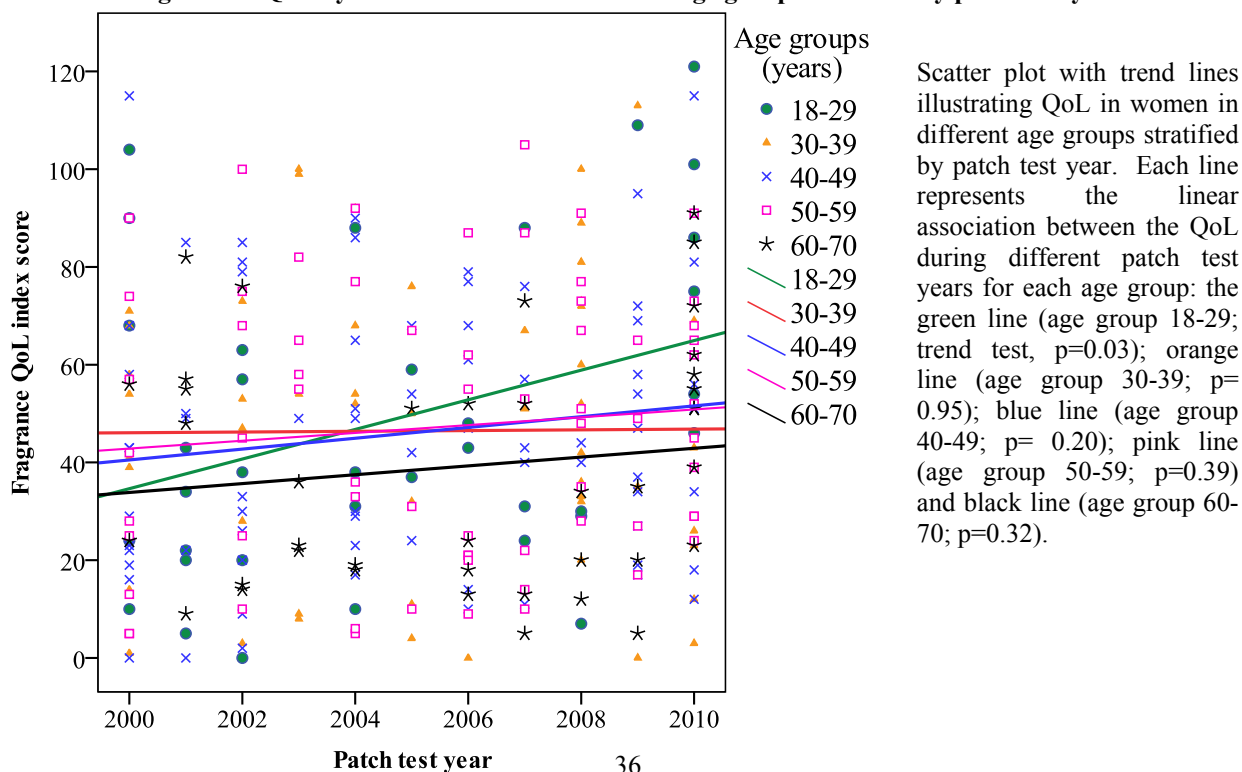
Table 9. Clinical and demographic characteristics of fragrance positive and non-fragrance positive respondents in the questionnaire study N=1084

	Respondents										
	Women					Men					
	Fragrance positive		Non-fragrance positive		p	Fragrance positive		Non-fragrance positive		p
	n	(%)	n	(%)		n	(%)	n	(%)	
Participants	424	(33.3)	848	(66.7)	-	126	(33.3)	252	(66.7)	-
Responders	290	(68.4)	551	(65.0)	0.22	89	(70.6)	154	(61.1)	0.07
Eczema										
Point prevalence	160	(56.7)	271	(50.0)	0.07	42	(47.7)	81	(52.6)	0.41
Atopic dermatitis[i]	62	(21.4)	98	(17.8)	0.21	16	(18.0)	22	(14.3)	0.47
Occupational eczema	45	(15.5)	80	(14.5)	0.70	14	(15.7)	34	(22.1)	0.21
Other allergies[ii]	183	(63.1)	222	(40.3)	0.001	44	(49.4)	54	(35.1)	0.03
Other skin diseases	118	(40.7)	214	(38.5)	0.52	24	(27.0)	38	(24.7)	0.60
psoriasis	17	(5.9)	53	(9.6)	0.06	4	(4.5)	10	(6.5)	0.52
acne	26	(9.0)	41	(7.4)	0.44	7	(7.9)	8	(5.2)	0.41
urticaria	43	(14.8)	65	(11.8)	0.21	5	(5.6)	6	(3.9)	0.51
skin diseases not mentioned above	52	(17.9)	89	(16.2)	0.51	11	(12.4)	19	(12.3)	0.97
Other disease[iii]										
Rhinitis	94	(34.3)	159	(30.6)	0.28	34	(41.5)	35	(24.1)	0.006
Food allergy	70	(25.5)	104	(20.2)	0.08	12	(14.6)	22	(15.4)	0.89
Asthma	52	(19.0)	102	(19.6)	0.84	18	(21.7)	22	(15.1)	0.22
Depression	52	(19.0)	102	(19.5)	0.87	10	(12.0)	23	(16.2)	0.40
Diabetes	17	(6.2)	32	(6.1)	0.94	14	(16.7)	11	(7.6)	0.037
Anxiety	16	(5.9)	53	(10.2)	0.04	2	(2.4)	9	(6.3)	0.17
Heart disease	11	(4.0)	35	(6.7)	0.13	8	(9.6)	14	(9.7)	0.99
COLD[iv]	8	(3.0)	25	(4.8)	0.22	1	(1.2)	8	(5.6)	0.14
Stroke	4	(1.5)	18	(3.4)	0.11	5	(6.0)	10	(7.0)	0.79
Heart attack	2	(0.7)	9	(1.7)	0.27	6	(7.1)	7	(4.9)	0.49
MCS[v]	14	(4.8)	10	(1.8)	0.016	0	-	1	(0.6)	-

N: Numbers

%: Crude percentages. If respondents had not answered the item they were counted as missing in the analysis.

Logistic regression accounting for the matching; P- values <0.05 considered statistically significant are highlighted red. Fishers exact test was performed with small samples, but no statistical significant differences were found.

[i] Atopic dermatitis, UK criteria [1]

[ii] A positive patch test to a marker of the baseline series other than a fragrance marker/ingredient.

[iii] The diagnosis was established by the question "Has your doctor ever told you that you have/have had ……"?.

[iv] COLD: Chronic Obstructive Lung Disease

[v] MCS: Multiple Chemical Sensitivity syndrome; La Cour's criteria [2]

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Respondents

Table 10. Quality of life measured with the Fragrance QoL index

| QoL instruments | Women and men | | | | | Women | | | | | Men | | | | | Fragrance positive women vs. fragrance positive men Mann-Whitney test |
| | Fragrance positive N=379 | | Non-fragrance positive N=705 | | Univariate analysis accounting for the matching | Fragrance positive N=290 | | Non-fragrance positive N=551 | | Univariate analysis accounting for the matching | Fragrance positive N=89 | | Non-fragrance positive N=154 | | Univariate analysis accounting for the matching | |
Fragrance QoL index	Mean	SD	Mean	SD	p	Mean	SD	Mean	SD	p	Mean	SD	Mean	SD	p	p
Item 1. Eczema and QoL	4.1	3.2	3.9	3.2	0.156	4.3	3.3	3.8	3.3	0.059	3.7	2.9	4.0	3.0	0.518	0.165
Item 2. Avoid situations	5.2	3.6	4.0	3.7	<0.001	5.3	3.6	4.2	3.8	<0.001	4.5	3.4	3.3	3.4	0.010	0.075
Item 3. Fissures and cracks	4.7	3.5	4.7	3.7	0.862	4.8	3.5	4.6	3.7	0.446	4.4	3.6	5.0	3.6	0.284	0.388
Item 4. Itching	5.6	3.4	5.3	3.6	0.218	5.6	3.4	5.2	3.6	0.128	5.4	3.3	5.5	3.3	0.761	0.590
Item 5. Pain and smarting	4.1	3.4	4.1	3.6	0.774	4.2	3.4	4.0	3.7	0.444	3.8	3.2	4.2	3.5	0.391	0.333
Item 6. Work and school	3.4	4.0	3.2	4.0	0.371	3.6	4.1	3.2	4.0	0.175	2.7	3.9	3.1	3.9	0.514	0.228
Item 7. Impaired physical contact	0.9	2.1	0.5	1.5	0.001	1.0	2.3	0.5	1.5	<0.001	0.5	1.2	0.4	1.4	0.755	0.639
Item 8. Irritation and stress	2.8	3.0	2.7	3.1	0.627	2.8	3.1	2.7	3.2	0.468	2.5	2.8	2.6	3.0	0.743	0.568
Item 9. Worried	3.3	3.2	2.5	3.1	<0.001	3.5	3.4	2.5	3.1	<0.001	2.6	2.6	2.2	2.7	0.353	0.093
Item 10. Less attractive	2.6	3.2	2.6	3.3	0.861	2.8	3.4	2.7	3.4	0.636	1.8	2.5	2.0	3.0	0.614	0.082
Item 11. Miss to smell nice	3.7	3.6	1.9	3.1	<0.001	4.1	3.7	2.0	3.1	<0.001	2.6	3.1	1.4	2.7	0.004	0.002
Item 12. Understand triggers	7.2	3.1	5.3	3.8	<0.001	7.3	3.0	5.5	3.8	<0.001	6.6	3.3	4.4	3.7	<0.001	0.102
Item 13. Consideration from people	2.0	2.9	1.4	2.4	<0.001	2.2	3.0	1.5	2.5	0.001	1.4	2.3	0.9	1.8	0.105	0.150
Fragrance QoL index score	44.0	27.3	40.4	27.0	0.042	45.7	28.2	40.5	27.5	0.014	38.4	23.6	39.6	25.0	0.732	0.05

N: number of respondents

Mean: The items were answered on a visual analogue scale (0-10). The higher the score the more affected the respondents are.

SD: Standard deviation

P values with statistical significant difference (p<0.05) are highlighted red.

Fragrance positive: at least one positive patch test reaction to a screening marker/ingredient of fragrance allergy.

Non-fragrance positive: no positive patch test reaction to a screening marker/ingredient of fragrance allergy.

Table 11. The prevalence of a positive patch test reaction to the top 10 fragrance markers/ingredients and quality of life to these specific fragrance ingredients/markers compared to non-fragrance positive in women and men

Positive patch test reactions

Fragrance QoL index score

Patch test markers of fragrance allergy	Women		Men		Women			Men		
	Numbers positive/tested	%	Numbers positive/tested	%	Fragrance positive Mean (SD)	Non fragrance positive Mean (SD)	p-value	Fragrance positive Mean (SD)	Non fragrance positive Mean (SD)	p-value
Fragrance mix I	174/825	21.1	51/239	21.3	46.0 (28.0)	41.4 (27.7)	0.03	39.2 (23.2)	39.1 (25.0)	0.89
Myroxylon pereirae	94/838	11.2	22/241	9.1	48.2 (28.0)	41.7 (27.8)	0.02	30.8 (20.7)	40.1 (24.8)	0.13
Fragrance mix II	54/524	10.3	17/160	10.6	50.8 (27.3)	43.0 (28.2)	0.04	36.9 (26.3)	42.1 (25.4)	0.49
Oxidized linalool	7/68	10.3	1/23	4.3	53.6 (32.7)	49.5 (25.8)	0.39	54.0 (-)	43.0 (30.1)	0.81
Oxidized limonene	6/67	9.0	3/22	13.6	53.5 (41.4)	49.3 (25.0)	0.38	47.3 (22.8)	42.9 (31.1)	0.99
Evernia furfuracea	25/281	8.9	10/90	11.1	48.6 (22.3)	45.9 (28.7)	0.44	39.1 (23.9)	45.8 (25.9)	0.53
Hydroxycitronellol	49/669	7.3	7/193	3.6	34.8 (26.5)	43.6 (27.8)	0.18	29.5 (20.6)	40.5 (24.9)	0.27
Evernia prunastri	48/760	6.3	20/219	9.3	48.4 (24.8)	42.4 (27.9)	0.07	37.4 (18.1)	39.6 (24.9)	0.64
HICC	32/671	4.8	14/194	7.2	56.6 (26.3)	42.2 (27.6)	0.002	29.5 (23.6)	41.2 (24.9)	0.13
Isoeugenol	25/670	3.0	6/193	3.1	45.1 (30.0)	42.9 (27.7)	0.41	47.4 (30.7)	39.9 (24.6)	0.61

QoL: quality of life.
HICC: hydroxyisohexyl 3-cyclohexene carbosaldehyde.
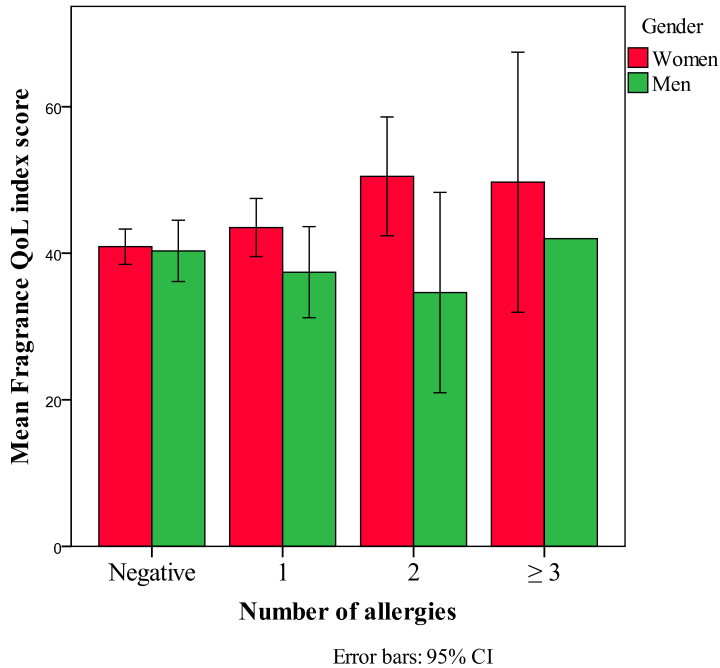Mean: mean of the Fragrance QoL index score; high score indicate greater QoL impairment.
SD: Standard deviation
The screening for fragrance allergy comprises of 31 markers/ingredients: The 10 listed above and methyl 2-octynoate, cinnamal, cinnamal, citral, eugenol, cinnamyl alcohol, α-hexyl cinnamal, butylphenyl methylpropional, amyl cinnamal, geraniol, benzyl salicylate, coumarin, amyl cinnamyl alcohol, majantol, citronellol, and the following did not have any positive reactions: non-oxidized limonene, non-oxidized linalool, benzyl cinnamate, benzyl alcohol, farnesol, benzyl benzoate, α-isomethylionone, anis alcohol.
A statistically significant difference (p<0.05) between subjects with a positive patch test reaction to the individual fragrance marker compared with subjects with no fragrance positive reaction, univariate analysis accounting for the matching.

QoL in relation to number of positive patch test reactions to a fragrance ingredient/marker is illustrated in Figure 16. QoL and regarding severity of the patch test reaction in Figure 17.

Figure 16. Quality of life in relation to number of patch test reactions to fragrance markers/ingredients

Error bars: 95% CI

Bar chart with 95% confidence intervals (CI) illustrating quality of life measured with the Fragrance QoL index for men (green) and women (red) in relation to the number of patch test reactions they have. The higher the score the more impaired the QoL. Number of allergies are determined by the number of positive patch test reactions (+,++,+++) they have to any of the fragrance ingredients/markers tested. However, if a subject has a reaction to one of the mixes and to one of its constituents, it is counted as one allergy. If a person has reacted to one of the mixes and two different individual constituents, it is counted as two allergies etc. Correlation analyses: $P_{women} = 0.01$; $P_{men} = 0.33$.

Figure 17. QoL in relation to severity of the patch test reaction to a fragrance marker/ingredient
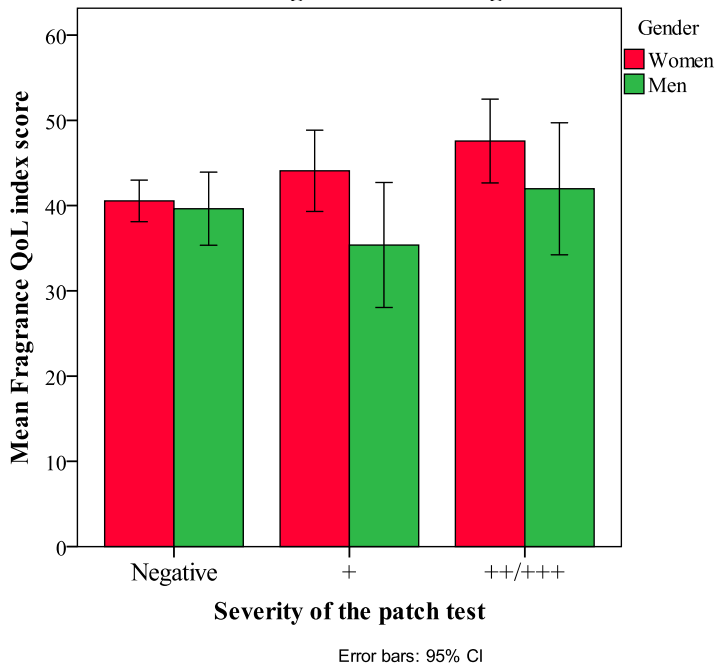
Error bars: 95% CI

Bar chart with 95% confidence intervals (CI) illustrating the difference in QoL in relation to intensity of a patch test reaction to a fragrance marker/ingredient in women (red) and men (green).
Correlation analyses: $P_{women} = 0.008$; $P_{men} = 0.877$.

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6. Discussion

6.1. General discussion

This section includes comments and considerations on the methodological aspects of the studies and a general discussion on the results of the studies.

6.2. Methodological considerations

6.2.1. Patch testing procedure

The patch test procedure is the gold standard for diagnosing contact allergy. It is not 100% specific or 100% sensitive, thus some subjects are false positive and some false negative. Many factors are standardized to ensure an accurate procedure in the testing and in the reading of the patch test reaction[72,73]. Thus, most factors are minimized but not completely eliminated as bias of the patch test method such as intra- and inter-observer variability in evaluation of the patch test reaction [74], preparation of the patch test material [72], intra-individual variation [75], and environmental factors (seasonal changes[76], sunlight exposure[77], and medication[78]). It is not possible to patch test with all fragrance ingredients because more than 2500 different fragrance ingredients exist[15] and there would not be sufficient space on the back for the testing. The fragrance ingredients included in the baseline series comprise the fragrance ingredients that often cause contact allergy and those most frequently used. However, it is possible that a subject patch tested with the screening markers of the baseline series has a fragrance allergy to another fragrance ingredient not represented in the baseline series.

In all the studies a patch test recording of "not tested, sensitized" (NT:S) was classified as not tested. This was done as it was uncertain how NT:S was interpreted at each centre. This bias is minor, as less than 1.2% of subjects included in all 4 studies had NT:S registered. Most of these were NT:S to FM I.

6.2.2. Database

The National Contact Dermatitis database contains uniform information on all consecutively patch tested subjects, which is considered one of the strengths of the study. The data are based on: 1) a large population representing the entire Danish patch tested eczema population (manuscript I & II), 2) uniform information, and 3) easy access to data. The uniform information consists not only of the patch test results, date, exposures or clinical relevance, but also demographic characteristics e.g. the MOAHLFA index. This is an internationally applied characterization of eczema patients, which makes it easier to compare groups also across international borders [79]. Weaknesses of the database are those of general character for all database studies.

6.2.3. Selection bias

All study populations were selected via the National Contact Dermatitis database, this should minimize selection bias. In study Part 2, the questionnaire studies were carried out as postal surveys. Performing the cheaper form of an electronic survey was considered; however, the empirical evidence on its effectiveness is still inconclusive [80]. In order to increase the response rates to ensure a representative group, we carefully customized and planned the questionnaire to the characteristics of an eczema population. To further increase the response rate in the case-control

study, a reminder postcard and a second questionnaire were posted. Furthermore, all posted questionnaires included a stamped, addressed return envelope. A representative number of subjects participated as the response rates of the different studies were: 55.7% responded with detailed narratives of the influence fragrance allergy had on their lives; 65.7% responded in the case-control survey; and 72.5% in the retest study. These response rates were considered satisfactory.

In the study population in the development of the Fragrance QoL index (manuscript III) we found a significant gender difference in the response rates. This might have influenced the development of the items comprising the Fragrance QoL index. As a consequence the QoL issues that affect women might be more represented in the Fragrance QoL index than those affecting the men and this could partly explain why we did not find the same significant differences in QoL among men as we found for women. However, many other factors may also play a part in why we found gender differences.

In the case-control study population (manuscript IV) more cases than controls answered the questionnaire. However, this difference was not of statistical significance. A significant gender difference was observed, and individuals with higher age showed higher response rates. Therefore many of the analyses of QoL were stratified by gender. However, we did not stratify by age as this was one of the matching criteria and thus the case group and control group were alike.

A selection bias in who chooses to respond to a postal survey is widely accepted and has been shown in previous studies among eczema patients[81]. Typically, individuals greatly affected by their condition are more inclined to participate, along with subjects of higher age and women [82].

6.2.4. Confounding

One of the strengths of the case-control study is that the control group closely resembles the case group. They all participated in the exact same questionnaire survey. The control group was chosen with similar demographic characteristics: 1) all had been patch tested and thus constituted a homogeneous eczema group, 2) they had been consecutively patch tested at the same hospital, thus came from the same geographic area, 3) they were matched on age, 4) on gender, and 5) and on patch test year. Overall, no differences between the case and control groups were found on these parameters, as would be expected. The matching and the selection of patch tested subjects from the same hospital were done to minimize the effects of confounding factors that could influence the results. However, we did not control for all confounding factors, e.g., occupational eczema. The questionnaire survey was conducted in the same period for the case group and the control group and it is unlikely that seasonal changes, which influence clinical manifestation of eczema [83,84], would have confounding effects.

6.2.5. Choice of reference group

The choice of a reference group in the case-control questionnaire survey is considered as an important aspect of this study. Both groups were similar in many demographic characteristics; they were also similar in prevalence's of eczema. However, the groups still homogeneous in certain aspects, e.g., among the young men there was an overrepresentation of occupational dermatitis in the reference/control group. Occupational eczema has a large impact on the QoL [54,66]. Thus, an analysis among young men may give rise to a misinterpretation of the effect fragrance allergy has on QoL. We tried to control for the factors that showed a significant difference in disease burden in a logistic regression model, taking all the significant factors into account, and we did not find any significant difference on the QoL measurements.

6.2.6. Recall bias

Recall of events/disease severity declines with time [85,86] . This might lead to an overestimation or underestimation [87]. This bias would most likely be the same for the case group as for the control groups as we attempted to minimize this bias by matching for when they had been patch tested, age and gender. Furthermore, in the Fragrance QoL index all items were to be answered regarding how the respondents felt at the time of answering which should diminish recall bias[88]. Nevertheless, recall bias cannot be entirely eliminated, but the extent of this bias is probably minimal.

6.2.7. Information bias

We strove to minimize information bias in the construction and validation of the each item in the questionnaires. This included a retest of the items comprising the Fragrance QoL index. However, we did not perform a retest of all items included in the case-control questionnaire to assess reliability of all the items, which can be considered a weakness of the study.

6.2.8. Data management and statistics

Great lengths were taken to ensure the validity of the data entering and handling[88]. All variables of the questionnaires were checked for missing data and outlying values by frequency tables, and cross tabulations to check for internal consistency. If inconsistencies occurred, they were checked in the original questionnaire. All statistical methods used in this thesis were discussed with a bio-statistician to ensure the most correct approach was applied.

6.3. Result-oriented discussion study Part 1

The next section is a general discussion of the results from manuscript I and manuscript II.

6.3.1 The FM II as a diagnostic screening marker of fragrance allergy

The prevalence of allergy to FM II was relatively high (4.5%) and it ranked second among the fragrance screening markers after the FM I. Most of the reactions were of clinical relevance. The FM II proved to be an important screening marker of fragrance allergy as it contributed to identifying 202 subjects (15.6%) with a fragrance allergy, who would otherwise have gone undetected. This is also in agreement with other studies [89-92]. Thus, the FM II is now permanently implemented as part of the standard screening markers of allergy in the European Baseline Series. Screening with a mixture of different fragrance ingredients as opposed to screening with the individual fragrance ingredient has some advantages. First and foremost this mimics how persons are exposed to the fragrance ingredients in real life. We know a cocktail effect develops, which is the synergistic response after exposure of a blend of fragrance ingredients[23,93]. Thus, a subject with a fragrance allergy who is tested with the individual fragrance ingredients may not elicit a positive patch test reaction, but when tested with the mixture, a positive reaction is detected because of the synergistic effect. The disadvantage of testing only with the mixes is that it remains unknown which fragrance ingredient(s) causes the allergy. The European Union Cosmetic Directives states that 26 fragrance ingredients, which also happen to be the most frequent causes of allergy, shall be listed on the ingredient label of cosmetic products[94]. This labelling makes it possible to avoid specific fragrance ingredients by reading the ingredient label, providing that the fragrance ingredient(s) are included among the 26 fragrance ingredients. Of these 26 fragrance ingredients, 8 comprise the FM I and 6 comprise the FM II. Thus, if only screening with the mixes, a subject with an allergy to one of the mixes would still not know which individual fragrance ingredient(s) to avoid. Furthermore, for diagnostic purposes it is easier to establish clinical relevance of a positive patch test reaction to

one of the 26 fragrance ingredients than to the mix. Consequently, additionally patch testing with the individual fragrance ingredients would be of key importance both for an individual and on a public health level[95]. On the individual level, it would be easier for the clinician to establish a correct diagnosis and the fragrance-allergic individual would be able to avoid the fragrance ingredient and thereby avoid manifestation of disease. On the public health level, it is important to monitor and ensure that epidemics of allergy to specific fragrance ingredients do not occur[96]. However, it would be of even greater help if not only the 26 fragrance ingredients were labeled, but all fragrance ingredients used in the product. Many other fragrances ingredients, essential oils and extract are not to be labelled although they are known fragrance allergens[97,98]. Currently, the 26 fragrance ingredients are considered the most frequent causes of fragrance allergy, but we know that allergy to fragrance ingredients change over time [27,28,99] as "new" scents are being introduced by the perfume industry[27]. Thus, if all fragrance ingredients, essential oils and extracts were labelled, it would also be easier to identify "new" upcoming problematic fragrance ingredients and to provide better circumstances for proper diagnosis.

6.3.2 Exposures to fragrance ingredients

Cosmetic products are the main source of exposure regarding fragrance allergy. However, many other sources exist in our daily living: washing detergents, cleansing products, children's toys and topical medicine[12,95,100]. As expected, many different cosmetic product groups were also listed as the cause of fragrance allergy. Deodorants were found to be the leading cause of fragrance allergy, closely followed by lotion, fine fragrances and shampoo. It was not surprising that deodorants were on the top of the list as previous studies have also highlighted these products as problematic in relation to fragrance allergy[101]. Increased attention is needed by the perfume industry regarding the use of fragrance ingredients in deodorants as the skin area on which deodorants are applied is especially sensitive [102,103]. We found that allergy to HICC and FM II in men was associated with fragrance allergy caused by deodorants, which could indicate that these fragrances should be used in lower doses or not at all in deodorants. The risk assessment of fragrance ingredients is based on expose to only the individual ingredient and not when used in combination, which is problematic as synergistic responses occur, when exposed to several different fragrances [23,93]. A cosmetic product may contain hundreds of different fragrance ingredients[16,95]. Many people are exposed to these ingredients via many different cosmetic products, household products [104-106] and some industrial products. Thus, exposure to fragrance ingredients in combination should also be taken into account in risk assessment of these ingredients. Some fragrance ingredients are particularly popular[107] and as consumers are exposed to many different products, the cumulative exposure concentration could be very high. This should also be taken into account when making a risk assessment of a fragrance ingredient and using fragrance ingredients in cosmetic products.

6.4. Result-orientated discussion study Part 2

This section includes a general discussion of the results described in manuscript III, which includes the validation of the Fragrance QoL index. Also a general discussion of the results from the matched case-control study, manuscript IV, is described.

6.4.1. Quality of life and fragrance allergy − The QoL instrument

A new well-validated disease-specific QoL instrument for subjects with a fragrance allergy now exists in Danish and has been translated to English. In the development of the Fragrance QoL index, we aimed to achieve as high a degree of reliability and validity as possible. This was accomplished

by using the narratives of subjects with a fragrance allergy as a basis to develop all 13 items of the Fragrance QoL index. In the validation analyses convergent validity to other QoL instruments (DLQI), convergent validity to self-estimated disease severity, reliability, ability to distinguish changes in disease severity, factor analyses, and Rasch analyses, all proved that it fulfilled the standard validation criteria of a good QoL instrument. Strong correlations between the Fragrance QoL index and SF36v2 were not present and the SF36 did not seem able to sufficiently discriminate many factors, e.g., disease severity. This illustrates the disadvantages of using a generic instrument which for some conditions is simply too generic, and is not sensitive enough to discriminate between specific diseases or within a single disease[108]. Nevertheless, some studies have found the SF36 sufficient to assess QoL in relation to eczema, but their study population differs from ours. As our study population was not selected to participate because of clinical characteristics, as for many of the other studies[109] (e.g. hand eczema[110], occupational dermatitis[111] or atopic dermatitis). Furthermore, our population was selected after contact to the hospital and it is hoped that most had received a proper diagnosis, and treatment. Consequently, our study population may not be as "ill" which could explain why we do not see the same impact on QoL measured with the SF36 as other studies have found[111,112]. This is in line with a follow-up study of patients with occupational dermatitis, which likewise found that SF36 did not correlate well with disease severity[111].

6.4.2. Quality of life and fragrance allergy

A clear gender difference was observed regarding QoL and having a fragrance allergy. Women were significantly impaired in their QoL compared with their control group, a difference, which was not found among the men. One reason for this difference could be that more men had occupational dermatitis, which we know affects the QoL[46,48]. For contact dermatitis, it is well known that gender differences exist; foremost it is well documented that more women than men are affected[113,114], which most likely is due to their different exposure patterns[113]. We also know that women and men have different obligations in their daily lives, both at work at home[48], which can affect their disease and affect their QoL, which our study also indicates. Thus, the gender differences observed in relation to QoL could partly be explained by the fact that women in general are more exposed to fragrance ingredients than are men, and thereby women have to implement more changes in their daily behaviour to avoid fragrance ingredients. Moreover, it is a substantial sacrifice for women to forego fragrances in their daily lives. In the test-retest study of changes in disease severity, it seemed that individuals with a fragrance allergy did not improve as much in QoL, when there was improvement in eczema compared with their controls. Although this observation was not of statistical significance, it indicates that underlying factors other than eczema play a role in their QoL.

A significant association between severity of the fragrance allergy and the QoL was observed among women. The stronger the patch test reaction, the greater the QoL impairment. This could be explained by the more severe the patch test reaction, the lower the elicitation threshold in the individual and the easier it would be to provoke an allergic response[115,116], thus the greater QoL impairment. The number of fragrance allergies a woman has was also shown to have a significant association with increased impairment of QoL. Again this could be explained by the more allergies a person has, the more exposures that person has to avoid in his or hers daily life which affects QoL. Additionally, having multiple allergies might cause a lower elicitation threshold of the contact dermatitis because of synergistic effects[23,93], which influence QoL.

Women of the youngest age group, most recently diagnosed with a fragrance allergy had a significantly higher impact on their QoL compared with those diagnosed earlier. An interpretation could be that they have learned to live with their condition. This perception is in line with other studies which have found that receiving a proper diagnosis of the contact allergy provides knowledge that enables avoidance of the allergen and improvement in QoL[49,52].

QoL in relation to allergy to specific fragrance ingredients/markers showed that some particular fragrance ingredients were associated with impairment of QoL among women. HICC, FM I, FM II, MP and HICC were all statistically significantly associated with an increased impairment of QoL compared with other eczema patients. Most notably was HICC, which showed the greatest QoL impairment in compared with the other screening markers of fragrance allergy. Most likely this is because of its widespread use in relatively high concentrations[117] and thus easy elicitation of the contact allergy. This is also a fragrance ingredient noted by the Scientific Committee on Consumer Safety (SCCS), which has recently published an opinion [118] where they recommend that HICC should not be used in cosmetic products. It is also noticeable that the oxidized limonene and oxidized linalool show a very high impairment of QoL; however, not of statistical significance. These two fragrances are also widely used by the perfume industry[12,119,120]. For many years patch testing with these two fragrance ingredients was done with the pure ingredient; however, recent studies have shown that this is not a sufficient method of investigation. As some fragrance ingredients can oxidize after air exposure and become very potent allergens[121-125]. Therefore screening for allergy to fragrance ingredients that can auto- oxidize should be performed with oxidized forms and not the pure forms[126]. Otherwise allergy to these fragrance ingredients will be underestimated[127-129] as our study also suggests. In general, it can be said, that it is a difficult and a complex matter to perform the screening for fragrance allergy[130] and to ensure 100% sensitivity of the test. Knowing, that, the fragrance ingredients people are exposed to, are far too many in numbers to be able to test with them all [131], that the concentration and vehicle used for patch testing has to be optimal[132,133] and that some fragrance ingredients can change after air exposure to become even more potent allergens[122-125,132,134]. Furthermore, that synergistic responses may occur, when exposed to mixtures of fragrance ingredients[23,93].

Identification of the factors that influence the QoL in fragrance allergic persons is important for a relevant counselling of the patients when they are diagnosed with a fragrance allergy. It is known that skin care education for other eczema populations helps prevent eczema[70,135]. Tailoring the guidance by including knowledge of which factor play major parts in their QoL would most likely be very helpful in the prevention of allergic contact dermatitis to fragrance ingredients. However, the preventive treatment should not only be on patient's shoulders. The preventive measures must also include legislators, manufacturers and retailers so that the products sold are safe to use[136]. The products should not contain allergenic fragrance ingredients in concentrations that may elicit an allergic reaction in more than a minority of individuals. Dose response studies can help determine the elicitation potentials and set the bar for maximum concentrations allowed[115,137]. However, there still needs to be an evaluation of cocktail effect and multiple exposure sources for the same allergen. Prevention could also target improving the ingredient labelling of cosmetic products. The easier it is to read the label, the easier it is for the clinician to give the correct diagnosis, easier to conduct cosmetovigilance, easier for the patient to avoid the allergen, which all in all lead to less disease and a better QoL.

7. Conclusion

This PhD thesis contributes to the study and characterisation of individuals with a fragrance allergy. We find that fragrance allergy is a common, relevant condition that affects the QoL of individuals in Denmark. Around 10% of all individuals patch tested in Denmark have a fragrance allergy. Most of the positive patch test reactions to fragrance ingredients/markers are of clinical relevance. Women are overrepresented and the mean age of diagnosis is typically around 44 years of age. The QoL impairment was significantly increased in women with a fragrance allergy.

Conclusions of the individual manuscripts:

Manuscript I:
Fragrance mix II contributed significantly as a screening marker of fragrance allergy. Positive patch test reactions were observed in 553 patients (4.5%) and of these, 72.2% were judged to be clinically relevant. FM II ranked second in detecting fragrance allergy, after FM I. If FM II had not been included as a screening marker in the baseline series 15.6% (n=202) of individuals with fragrance allergy would not have been identified by the other fragrance screening markers (FM I, MP or HICC).

Manuscript II:
Deodorants were the leading exposure in causing fragrance allergy, especially among men. Seemingly, deodorants have an unhealthy composition of the fragrance ingredients that constitute the FM II. Cosmetic products were the main cause of allergic contact dermatitis. Deodorants accounted for 25%, scented lotion 24.4%, fine fragrances 16.0% and shampoo 13.0%. A gender difference was apparent as deodorants were significantly more likely to be listed as the cause of fragrance allergy in men (OR 2.2) than in women. Correlation was observed between deodorants listed as the cause of allergy and allergy detected by fragrance mix II (FM II) and hydroxyisohexyl 3-cyclohexene carboxaldehyde.

Manuscript III:
The especially developed Fragrance QoL index was found to be a technically valid, potentially useful, instrument for assessment of QoL among subjects with fragrance allergy. It had significant good correlations with the DLQI (r_S=0.70), self-estimated disease severity (r_S=51), and it showed good reliability, reproducibility (ICC=0.92), and ability to distinguish changes in disease severity. The factor analysis and Rasch test indicated that there were no latent factors and calculations of a total score for the Fragrance QoL index was agreeable.

Manuscript IV:
Fragrance allergic individuals are just as affected in their quality of life as are other eczema patients. However, women, and in particular young women recently diagnosed, seem to be even more impaired in their QoL compared with other eczema patients. Women were in general more affected in their QoL compared with their control group (p=0.014), which was not found among men. Several factors played a significant role on impairment of QoL in women: number of fragrance allergies, severity of the patch test reaction and allergy to specific fragrance ingredients/markers. The fragrance ingredient which was associated with the most impairment was HICC, which is also one of the most frequent causes of fragrance allergy.

Contact allergy to fragrances is a common condition. Prevention of this important health problem needs joint efforts of scientists, authorities and industry.

8. Future research

This thesis demonstrates the importance of continual re-evaluation of the screening methods used in the detection of fragrance allergy. The fragrance ingredients people are exposed to change over time as new scents continually are introduced by the perfume industry. The screening methods should reflect the fragrance ingredients people are exposed to in their daily lives.

These studies confirm that when determining QoL in a population with a specific disease it is of importance, that the instrument used is aimed at that particular disease. General instruments will not be able to distinguish the differences in QoL for subjects with a fragrance allergy. A future study will be the validation of the English version of the Fragrance QoL index in an English speaking eczema population. Furthermore, future studies will include translation of the Fragrance QoL index to Spanish, French and German and likewise a validation in the respective countries.

It is known that a cocktail effect exists for exposure to a mixture of fragrance ingredients. However, more knowledge of the pathophysiological mechanisms involved is needed. Furthermore, to determine if some combinations of fragrance ingredients potentially are more harmful than others.

More studies on how the guidelines for instruction of individuals with a fragrance allergy should be conducted. As the preventive treatment mainly relays, at the moment, on how the patient is able to avoid the allergen(s). Thus, it is of importance that they be instructed sufficiently taking the issues that matter for their QoL into account.

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10. Manuscripts

CONTACT DERMATITIS

Fragrance mix II in the baseline series contributes significantly to detection of fragrance allergy

Maria V Heisterberg[1], Klaus E Andersen[2], Christian Avnstorp[3], Berit Kristensen[4], Ove Kristensen[4], Knud Kaaber[5], Grete Laurberg[6], Torkil Menné[1], Niels Henrik Nielsen[7], Mette Sommerlund[8], Jens Thormann[9], Niels K Veien[6], Susanne Vissing[10] and Jeanne D Johansen[1]

[1]National Allergy Research Centre, Department of Dermato-allergology, Gentofte Hospital, University of Copenhagen, 2900 Hellerup, Denmark, [2]Department of Dermatology, Odense University Hospital, Odense, Denmark, [3]Dermatology Clinic, Roskildevej 264, Rødovre, Denmark, [4]Dermatology Clinic, Bredgade 50, Kalundborg, Denmark, [5]Dermatology Clinic, Bredgade 30, Herning, Denmark, [6]Dermatology Clinic, Vesterbro 99, Ålborg, Denmark, [7]Dermatology Clinic, Bindeledet 15, Bagsværd, Denmark, [8]Department of Dermatology, Århus University Hospital, Århus, Denmark, [9]Dermatology Clinic, Skovgade 23C, Vejle, Denmark, and [10]Dermatology Clinic, Gl. Hovedgade 14, Hørsholm, Denmark

Background: Fragrance mix II (FM II) is a relatively new screening marker for fragrance contact allergy. It was introduced in the patch test baseline series in Denmark in 2005 and contains six different fragrance chemicals commonly present in cosmetic products and which are known allergens.

Aim: To investigate the diagnostic contribution of including FM II in the baseline series by comparing it with other screening markers of fragrance allergy: fragrance mix I (FM I), *Myroxylon pereirae* and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC).

Method: Retrospective study of 12 302 patients consecutively patch tested with FM II by members of the Danish Contact Dermatitis Group 2005–2008.

Results: FM II gave a positive patch test in 553 patients (4.5%), and in 72.2% of these patients the reaction was judged to be clinically relevant. FM II ranked second in detecting fragrance allergy, after FM I. If FM II had not been included as a screening marker in the baseline series, 15.6% ($n = 202$) of individuals with fragrance allergy would not have been identified by the other fragrance screening markers (FM I, *M. pereirae* or HICC).

Conclusion: FM II contributes substantially to detecting fragrance allergy. It ranked second among the fragrance screening markers tested in the baseline series and detects individuals with an allergy who otherwise would not have been identified.

Key words: allergic contact dermatitis; clinical relevance of a patch test; cosmetics; FM II; fragrance; fragrance mix II. © John Wiley & Sons A/S, 2010.

Fragrance contact allergy is caused mainly by exposure to cosmetic products, but exposures to household and industrial products may contribute (1). The most common fragrance allergens are included in the European baseline series (2). However, because the fragrance ingredients consumers are exposed to through cosmetic products are continually changing (3), a new screening marker of fragrance allergy, the fragrance mix II (FM II), was introduced (4). FM II consists of six
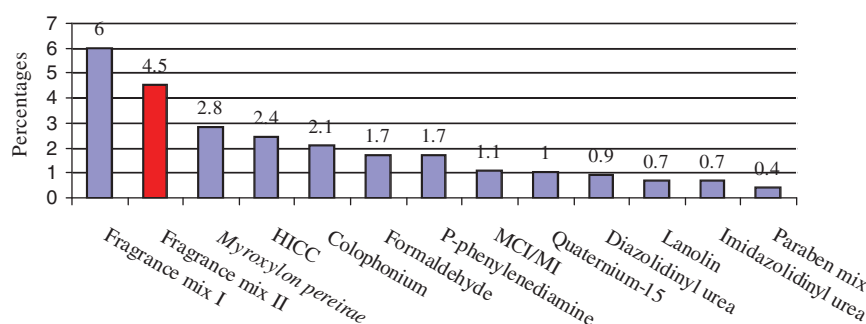
Fig. 1. Prevalences of positive patch test reactions to screening markers associated with cosmetic allergy among 12 302 subjects. HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone.

different fragrance compounds [hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), citral, farnesol, coumarin, citronellol, and α-hexyl cinnamal], all known to have sensitization properties and which are used in cosmetics (5). In Denmark FM II was introduced in the European baseline series in 2005 together with HICC with the aim of detecting more fragrance allergic individuals.

The objective of this study was to evaluate the contribution of FM II as a screening marker of fragrance allergy in a large population in comparison with other markers in the baseline series.

Material

Data were retrieved from a clinical database where the Danish Contact Dermatitis Group (DCDG) contributes with information. At the time of study DCDG comprised three dermatology departments (University Hospitals in Gentofte, Odense and Århus), and seven dermatology practices (Rødovre, Aalborg, Herning, Vejle, Bagsværd, Hørsholm, and Kalundborg). We included subjects patch tested with FM II in the baseline series. Testing spanned 2005–2008 as the FM II was introduced in the patch test baseline series in 2005. We included 12 302 subjects: 8063 females and 4239 males, mean age 44.8 years ($\pm$18.2; range 1–97 years). Table 1 shows the group's demographic characteristics.

The patch test results of subjects tested with FM II were compared with patch test results of other fragrance screening markers and also to other markers associated with cosmetic ingredient allergy (Fig. 1). The fragrance screening markers were fragrance mix I (FM I) 8% petrolatum (pet.), HICC 5% pet., *Myroxylon pereirae* 25% pet., and colophonium 20% pet. Colophonium is considered a fragrance marker as it is used in cosmetics and may cross-react with *M. pereirae* and FM I (6). However, allergy to colophonium from adhesives (colophonium or its derivatives) used in footwear (7), paper, diapers, and sanitary wear is also common (8). This may make colophonium a less specific marker of

Table 1. MOAHLFA index of consecutively patch-tested eczema patients and patients with a positive patch test reactions to fragrance mix II (FM II)

Index	Tested subjects		FM II positive	
	Numbers	%	Numbers	%
M	4239	34.5	136*	24.6
O	1468	11.9	71	12.8
A	2109	17.1	103	18.6
H	4619	37.5	220	39.8
L	625	5.1	24	4.3
F	2287	18.6	129*	23.3
AA	7389	60.1	329	59.5
Total	12 302	100	553	100

MOAHLFA index is an initialization: M: male; O: occupational causation of dermatitis; A: atopy; H: hand dermatitis; L: leg dermatitis; F: face affected by dermatitis; and AA: Age $\geq$ 40 years. *χ^2-test, $P < 0.05$.

fragrance allergy; consequently, comparisons of fragrance allergy in this study were done both with and without colophonium.

The other cosmetic-related screening markers were formaldehyde 1% aq., quaternium-15 1% pet., diazolidinyl urea 2% pet., imidazolidinyl urea 2% pet., *p*-phenylenediamine 1% pet., methylchloroisothiazolinone (and) methylisothiazolinone (MCI/MI) 0.01% aq., paraben mix 16% pet., and lanolin 30% pet.

FM II 14% consists of six different fragrances: 2.5% HICC, 1% citral, 2.5% farnesol, 2.5% coumarin, 0.5% citronellol, and 5% α-hexyl cinnamal in pet.

Clinical relevance was evaluated and registered according to standardized guidelines set by DCDG. In this study, clinical relevance covers current and/or past relevance based on (i) medical history, (ii) results of patch and/or use tests, (iii) ingredient labelling, or (iv) chemical analysis.

Methods

DCDG members used three different suppliers of the compounds tested: (i) TRUE Test® (MEKOS

Laboratories AS, Denmark), (ii) Trolab® (Hermal, Reinbeck, Germany), and (iii) Chemotechnique® (Chemotechnique Diagnostics, Vellinge, Sweden). The concordance rate between test material from different suppliers is generally high (9, 10), and in this study all materials were viewed as equal. No comparison of allergy rates was conducted between the different suppliers in this study.

The patch tests were done according to international guidelines (11) using Finn Chambers® (8 mm; Epitest Ltd Oy, Tuusula, Finland) applied on the back with Scanpor® tape (Norgesplaster A/S, Alpharma, As, Norway) and occluded for two days. Readings were done on D3 or D4 according to the recommendation of the International Contact Dermatitis Research group (12). Positive patch test reactions of +, ++, and +++ were defined as a contact allergy. An irritant reaction, a doubtful reaction (+?), or a negative reading was interpreted as a negative response.

Statistics

Data administration and statistical analysis were done in SPSS® version 15. Demographic characteristics of subjects tested with FM II were registered according to the MOAHLFA index (Table 1). FM II was compared with 12 other screening markers associated with cosmetics shown as crude percentages of positive patch test reactions and clinically relevant reactions. The prevalence of cumulative fragrance allergy frequency was determined and expresses the number of patients with a positive patch test to one or more fragrances among all the subjects tested. Likewise, a prevalence of 'preservative allergy' was determined. Concomitant reactions were determined between FM II and other fragrance markers and also their association, which are expressed by odds ratios (ORs) with 95% confidence intervals (CIs). The OR expresses the odds of having a positive patch test to FM II and a positive patch test to the investigated marker. χ^2 tests were performed. P values below 0.05 were considered significant.

Results

A positive reaction to FM II among 12 302 consecutively patch-tested individuals was observed in 4.5% ($n = 553$). The sex distribution was uneven: 417 females and 136 males, which is a significant over-representation of females ($P < 0.001$), OR = 1.6 (CI 1.4–2.0) expressing the OR of females having a positive patch test compared to males. There was a significant association between FM II allergy and facial dermatitis (Table 1). A positive patch test reaction to one or more of the fragrance markers (FM I, FM II, *M. pereirae* and HICC) was detected in 1298 (10.6%) of the participants. If colophonium was included as a fragrance screening marker, 1484 (12.1%) subjects had a positive patch test reaction. Allergy to fragrances had a higher prevalence than did allergy to other potential cosmetic ingredients: preservatives 4.0% ($n = 491$), p-phenylenediamine 1.7% ($n = 196$), or lanolin 0.7% ($n = 90$) (Table 2).

Clinical relevance of a positive patch test to FM I, FM II, and HICC were all above 70%, whereas *M. pereirae* and colophonium had approximately 50% of the reactions judged to be clinically relevant. None of the other screening markers had the same high prevalences of clinical relevance as FM I, FM II, and HICC (Table 3).

Concomitant reactions between FM II and each of the other fragrance markers are illustrated for each marker in Fig. 2a–d. There was a significant association between a positive reaction to each of the fragrance markers and FM II allergic subjects. In particular, HICC and FM II had a strong association with 243 (40.4%) concomitant reactions and an OR of 187.2. HICC alone identified 292 patients with fragrance allergy. If HICC had not been included as a screening marker, 49 subjects (16.8%) would not have been detected by only testing with FM II (Fig. 2c). The FM I identified 737 subjects with fragrance allergy, which is 56.3% of all patients (1298) with a positive patch test to one or more of the fragrance allergy markers (FM I, FM II, HICC, and *M. pereirae*). When comparing FM II allergic patients with the cumulative number of subjects with an allergy to FM I, *M. pereirae*, and

Table 2. Prevalences of allergy to 'fragrances', 'preservatives', hair dye, and other markers related to cosmetics

	Screening markers related to cosmetics	Number of positive patch tests (%)	
Fragrance	Fragrance mix I, fragrance mix II, *Myroxylon pereirae,* and hydroxyisohexyl 3-cyclohexene carboxaldehyde	1298	(10.6)
	Fragrance mix I, fragrance mix II, *Myroxylon pereirae*, hydroxyisohexyl 3-cyclohexene carboxaldehyde, and colophonium	1484	(12.1)
Preservative	Formaldehyde, quaternium-15, diazolidinyl urea, imidazolidinyl urea, methylchloroisothiazolinone/methylisothiazolinone, paraben mix	491	(4.0)
Hair dye	*p*-phenylenediamine	209	(1.7)
Other marker	Lanolin	90	(0.7)

Numbers patch-tested: 12 302.

Table 3. Prevalence of clinically relevant patch test reactions

Screening markers	Numbers positive	Numbers relevant in regard of eczema (%)	
A: Fragrance mix I	737	523	(71.0)
A: Fragrance mix II	553	399	(72.2)
A: *Myroxylon pereirae*	347	175	(50.4)
A: Hydroxyisohexyl 3-cyclohexene carboxaldehyde	292	224	(76.7)
B: Colophonium	262	124	(47.3)
C: *p*-phenylenediamine	209	108	(51.7)
D: Formaldehyde	204	105	(51.5)
D: MCI/MI	134	66	(49.3)
D: Quaternium-15	126	62	(62.2)
D: Diazolidinyl urea	111	47	(42.3)
E: Lanolin	90	44	(48.9)
D: Imidazolidinyl urea	78	27	(34.6)
D: Paraben mix	49	25	(51.0)
Cosmetic allergy	2034	1286	(63.2)

Markers of: A: fragrance allergy; B: Colophonium associated with fragrances; C: Hair colouring agent; D: Preservatives; and E: Lanolin. MCI/MI = methylchloroisothiazolinone/methylisothiazolinone.
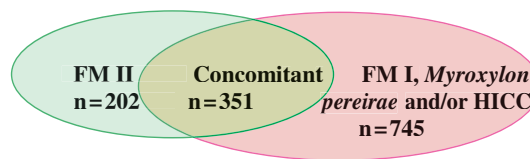

Fig. 3. Illustration of concomitant reactions to fragrance mix II (FM II) and subjects allergic to fragrance mix I (FM I), *Myroxylon pereirae*, and/or hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC). Subjects with fragrance allergy identified with FM II, FM I, *Myroxylon pereirae* and HICC (*n* = 1298). FM II additionally identified 202 (15.6%) allergic subjects.

identify additionally 338 (26.0%) fragrance allergic individuals as opposed to only testing with FM I and *M. pereirae*. When including colophonium as a fragrance screening marker, 322 fragrance allergic subjects would still be missed, if not testing with FM II and HICC, which is only 16 more subjects being identified when including colophonium (Table 4).

HICC, concomitant reactions were observed in 351 individuals. As FM II alone identified 553 allergic subjects, 15.6% of the FM II allergic subjects (*n* = 202) would not be detected by the other standard fragrance screening markers FM I, *M. pereirae,* and HICC (Fig. 3). A similar comparison was conducted between the group of subjects with an allergy to FM II and/or HICC and the group of subjects allergic to one or more of the other fragrance markers FM I, *M. pereirae,* and/or colophonium (Table 4). The comparison shows that FM II and HICC would

Discussion

Fragrance contact allergy is common among eczema patients in Denmark. In this study, 10.6% (1298) of patch-tested eczema patients (*n* = 12302) were fragrance allergic. This makes fragrance allergy the second most common cause of contact allergy after nickel, in accordance with other studies (13–15).

FM II identified 553 (4.5%) patients with a fragrance allergy and thus ranked second in the detection of fragrance allergy and in detecting allergy to cosmetic ingredients. There were significantly

Fig. 2. (a) Illustration of concomitant reactions to fragrance mix II (FM II) and fragrance mix I (FM I). Subjects with fragrance allergy identified by FM II and FM I = 1073. A significant statistical association was observed between the two groups, χ^2, $P < 0.0000001$. OR = 14.0 (CI 11.5–16.9). (b) Illustration of concomitant reactions to fragrance mix II (FM II) and *Myroxylon pereirae*. Subjects with fragrance allergy identified by FM II and *Myroxylon pereirae* = 836. A significant statistical association between the two groups was observed, χ^2, $P < 0.0000001$. OR = 5.3 (CI 4.0–7.1). (c) Illustration of concomitant reactions to fragrance mix II (FM II) and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC). Subjects with fragrance allergy identified by FM II and HICC = 602. A significant statistical association was observed 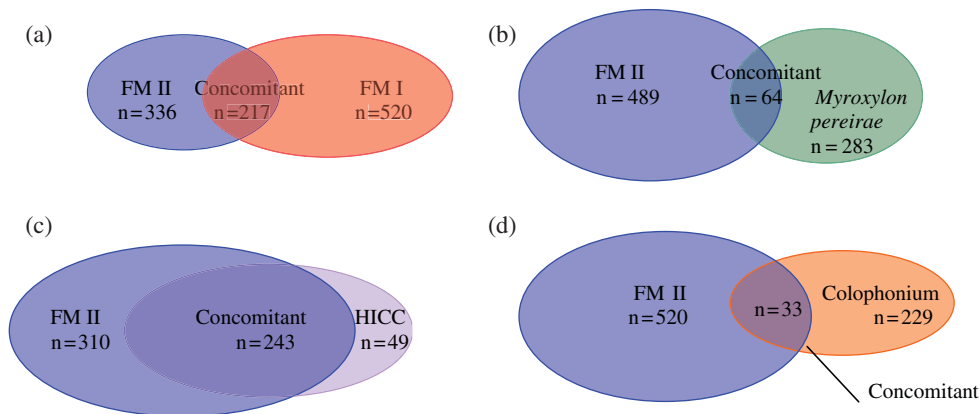between the two groups, χ^2, $P < 0.000001$. OR = 187.2 (CI 135.3–259.6). (d) Illustration of concomitant reactions to fragrance mix II (FM II) and colophonium. Subjects with fragrance allergy identified by FM II and colophonium = 782. A significant statistical association was observed among the two groups, χ^2, $P < 0.0000001$. OR = 3.2 (CI 2.2–4.7).

Table 4. Comparison of patch test reactions to fragrance mix II (FM II) and/or hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) with other markers of fragrance allergy: fragrance mix I (FMI) and *Myroxylon pereirae* (Group 1) and FM I, *Myroxylon pereirae* and colophonium (Group 2)

		Other fragrance screening markers (FM I, *Myroxylon pereirae*, and/or colophonium)				
		Positive		Negative		
		Group 1	Group 2	Group 1	Group 2	Total
FM II	Positive	264	280	338	322	602
and/or	Negative	696	882	11 004	10 818	11 700
HICC	Total	960	1162	11 342	11 140	12 302

A significant positive association was found between positive reactions to FM II and/or HICC and to one or more positive reactions to the other screening markers (χ^2 test, $P < 0.001$).

more females than males with allergy to FM II, OR = 1.6. This sex difference has been reported in other studies on fragrance allergy (13, 15–17), only one recent publication (18) reports the opposite, where FM II was significantly more frequent among males. The discrepancy compared to our study may be because of different age distribution among males and females or because of exposure differences.

Facial dermatitis was significantly more common among patients with a positive patch test to FM II and is viewed as a classical manifestation of fragrance allergy (6, 19, 20).

Most of the positive patch test reactions (>70%) to FM II were of clinical relevance as were the reactions to HICC and FM I (Table 3). These relatively high frequencies of clinical relevance imply that the tests have a high diagnostic value. *M. pereirae*, however, differed from the other three markers of fragrance allergy in having a much lower frequency of clinical relevance at 50.4%. This could imply that *M. pereirae* is not as specific a marker of fragrance allergy as the other fragrance markers, or it could reflect the difficulty in establishing *M. pereirae* exposure sources. The use of *M. pereirae* in cosmetics as a fragrance is banned in the EU but extracts of *M. pereirae* are allowed up to a maximum of 0.4% concentration and is not required to be labelled as a fragrance ingredient. *M. pereirae* may also be used in other products than cosmetics where labelling is not required. Likewise, colophonium had a clinical relevance of less than 50% because of the same reasons as for *M. pereirae*.

A decrease in prevalence of positive patch test reactions to FM I have been reported in recent studies (15, 17, 21); nevertheless, it is the screening marker of fragrance allergy with the highest prevalence of positive reactions, 6.0% in our study. If, however, FM I had been used as the sole screening marker in our study, only 56.8% of the patients

with fragrance allergy would have been identified. This is a lower figure than described by Larsen in 1998 (22), who estimated that FM I would identify 67% of all fragrance allergic patients. The declining prevalence of allergy measured with FM I is not an indication of a decline in fragrance allergy in general, but rather a likely result of a change in fragrances' composition and exposure (3).

HICC is one of the six fragrance compounds constituting the FM II and is also tested as a single compound with twice the concentration (HICC 5%). Concomitant reactions were anticipated between HICC and FM II and occurred in 40.4% of subjects positive to HICC and/or FM II. If HICC had not been tested as a single compound but only as a constituent of FM II, 16.8% of the HICC allergic patients would have been missed, confirming HICC as an important individual screening marker for fragrance allergy (17, 23–25). HICC and FM II have a complementary effect as some are detected by the individual fragrance and even more by FM II. We did not investigate the complementary effect of testing with the other individual fragrances constituting FM II, but a similar effect would be anticipated, as observed when testing with FM I and its individual fragrances. A previous study, where 1701 subjects were tested with FM II and its individual fragrances (24), reports of 50 subjects with a positive patch test reaction to FM II (14%), and of these 48% would have been detected by the individual fragrances. Further studies with larger study populations are needed on this subject to draw any conclusion.

The relatively high prevalences of allergy to HICC reported in this study (2.4%) and also in other studies (17, 24, 26, 27) might be the result of its frequent use in cosmetic products (28) and the use of high concentrations compared to the safe use dose (29–31). A study from the UK showed that almost 30% of 300 cosmetic and household products contained HICC (32), and in a Danish study, 72% of 25 cosmetic products contained HICC (28). According to the EU Cosmetic Directive HICC is one of the 26 fragrances to be declared specifically on the ingredient label of cosmetic products, when present at doses of 0.001% in leave-on products and 0.01% in rinse-off products (33). The European Commission's Scientific Committee on Consumer Products (SCCP) recommended 200 ppm as maximum amount of HICC in cosmetic products (34) and International Fragrance Association (IFRA) has recently further limited their recommended HICC concentrations (35). Despite these attempts to diminish the high exposure to HICC, no decrease in allergy has yet been observed (23).

It is not known if other fragrance ingredients with a similar scent and allergenic potential are used to

substitute HICC or other of the FM II ingredients, as observed for isoeugenol in FM I (36). Another issue of relevance for prevention is auto-oxidation of FM II ingredients and formation of more allergenic oxidation products. Geranial, which is a component of citral, one of the ingredients in FM II, can oxidize to epoxygeranial, which increases the sensitization potential (37). Other candidates for investigation could be farnesol and citronellol, but no studies are available yet.

In our study an association between positive patch tests to FM II and each of the other fragrance markers (FM I, *M. pereirae*, HICC, and colophonium) were all significant. In a previous study, positive patch tests to FM I and HICC were independently also associated with a reaction to FM II, but no association was found between FM II and colophonium or *M. pereirae* (24). This divergence may be because of a smaller sample size ($n = 1701$) compared with our study.

In conclusion, FM II contributed significantly to the identification of fragrance allergic patients, and 26.0% of fragrance allergic patients would have been missed if FM II and HICC had not been included as markers of fragrance allergy in the baseline series. If only FM II had been excluded, 15.6% would not have been identified with a fragrance allergy. In a high proportion of cases with a positive patch test to FM II and/or HICC, the reactions were clinically relevant. This further emphasizes their importance as screening markers for diagnosing fragrance allergy.

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Address:
Maria Vølund Heisterberg
National Allergy Research Centre
Department of Dermato-allergology
Gentofte Hospital
University of Copenhagen
2900 Hellerup
Denmark
Tel: 0045 3977 7310
Fax: 0045 3997 7118
e-mail: mavohe01@geh.regionh.dk

10.2. Manuscript II

Deodorants are the leading cause of allergic contact dermatitis to fragrance ingredients*

Maria V. Heisterberg[1], Torkil Menné[1], Klaus E. Andersen[2], Christian Avnstorp[3], Berit Kristensen[4], Ove Kristensen[4], Knud Kaaber[5], Grete Laurberg[6], Niels Henrik Nielsen[7], Mette Sommerlund[8], Jens Thormann[9], Niels K. Veien[6], Susanne Vissing[10] and Jeanne D. Johansen[1]

[1] *Department of Dermato-allergology, National Allergy Research Centre, Gentofte Hospital, University of Copenhagen, 2900 Hellerup, Denmark,* [2] *Department of Dermatology, Odense University Hospital, 5000 Odense, Denmark,* [3] *Dermatology Clinic, 2610 Rødovre, Denmark,* [4] *Dermatology Clinic, 4400 Kalundborg, Denmark,* [5] *Dermatology Clinic, 7400 Herning, Denmark,* [6] *Dermatology Clinic, 9000 Ålborg, Denmark,* [7] *Dermatology Clinic, 2880 Bagsværd, Denmark,* [8] *Department of Dermatology, 8000 Århus University Hospital, Århus, Denmark,* [9] *Dermatology Clinic, 7100 Vejle, Denmark, and* [10] *Dermatology Clinic, 2970 Hørsholm, Denmark*

Summary

Background. Fragrances frequently cause contact allergy, and cosmetic products are the main causes of fragrance contact allergy. As the various products have distinctive forms of application and composition of ingredients, some product groups are potentially more likely to play a part in allergic reactions than others.
Aim. To determine which cosmetic product groups cause fragrance allergy among Danish eczema patients.
Method. This was a retrospective study based on data collected by members of the Danish Contact Dermatitis Group. Participants ($N = 17\,716$) were consecutively patch tested with fragrance markers from the European baseline series (2005–2009).
Results. Of the participants, 10.1% had fragrance allergy, of which 42.1% was caused by a cosmetic product: deodorants accounted for 25%, and scented lotions 24.4%. A sex difference was apparent, as deodorants were significantly more likely to be listed as the cause of fragrance allergy in men (odds ratio 2.2) than in women. Correlation was observed between deodorants listed as the cause of allergy and allergy detected with fragrance mix II (FM II) and hydroxyisohexyl 3-cyclohexene carboxaldehyde.
Conclusion. Deodorants were the leading causes of fragrance allergy, especially among men. Seemingly, deodorants have an 'unhealthy' composition of the fragrance chemicals present in FM II.

Key words: allergic contact dermatitis; clinically relevant patch tests; cosmetics; deodorants; fragrance.

Cosmetic products cover wide range of different consumer products, and almost everyone has daily contact with a

Correspondence: Maria Vølund Heisterberg, Department of Dermato-allergology, National Allergy Research Centre, Gentofte Hospital, University of Copenhagen, 2900 Hellerup, Denmark. Tel: +45 3977 7310; Fax: +45 3997 7118. E-mail: mavohe01@geh.regionh.dk

cosmetic product. The EU Directive gives the following definition: 'A cosmetic product is any substance or preparation intended to be placed in contact with the various external parts of the human body or with the teeth and the mucous membranes of the oral cavity, with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, and/or correcting body odours, and/or protecting them or keeping them in good condition' (1).

Several aspects contribute to a cosmetic product's ability to cause fragrance allergy (2). Foremost, a product must contain sensitizing fragrance ingredients.

Sensitization can occur after a single significant exposure or after multiple exposures (2, 3), and once sensitization has occurred, a lower dose can cause an elicitation response (4). In our study, we use the term fragrance allergy synonymously with allergic contact dermatitis.

A wide range of fragrance ingredients exists, approximately 2500 different substances (5); many are known to be sensitizers in humans and are used in cosmetic products (6–8).

The individual fragrance ingredients are used in various combinations, and some cosmetic products contain hundreds of individual fragrance ingredients (9). Other principal factors contributing to a product's ability to cause allergy are related to its composition and intended use conditions. For example, the following may all play a role in a cosmetic product's ability to elicit fragrance allergic contact dermatitis: the nature of fragrance ingredients, as some may have synergistic effects (10); the concentration and potency of the allergenic fragrance ingredients; the application site; the frequency of application; the duration of exposure; and the user's skin barrier function (2, 11–13).

The purpose of this study was to determine the distribution of cosmetic product groups listed as the cause of fragrance allergic contact dermatitis among Danish eczema patients. Furthermore, our aim was to investigate sex differences and to evaluate whether there was an association between the cosmetic product listed as having caused a fragrance allergy and the different fragrance markers detecting an allergy.

Materials

Data were retrieved from a clinical database containing patch test results, patient characteristics, and exposure sources. All patients were examined by members of the Danish Contact Dermatitis Group (DCDG). During the study period (January 2005 to June 2009) the DCDG comprised three dermatology departments (university hospitals in Gentofte, Odense, and Århus) and seven dermatology clinics (Rødovre, Aalborg, Herning, Vejle, Bagsværd, Hørsholm, and Kalundborg). All patients had been patch tested with fragrance markers included in the baseline series: fragrance mix I (FM I), fragrance mix II (FM II), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) 5%, and *Myroxylon pereirae*/balsam of Peru 25% in petrolatum. FM I contains eight individual fragrance compounds: 1% cinnamal, 1% cinnamyl alcohol, 1% geraniol, 1% isoeugenol, 1% eugenol, 1% hydroxycitronellal, 1% *Evernia prunastri* (oak moss absolute), 1% α-amyl cinnamal and an emulsifier 5% sorbitan sesquioleate. FM II is composed of six different fragrances: 2.5% HICC, 1% citral, 2.5% farnesol, 2.5% coumarin, 0.5% citronellol and 5% α-hexyl cinnamal in pet.

A total of 17 716 subjects were consecutively patch tested: 11 610 women and 6106 men. The mean age was 44 years (standard deviation 18.3). Table 1 shows the study participants' demographic characteristics.

Relevant exposure sources causing a positive patch test reaction are registered in the database. The exposure sources are categorized as either 'leave-on' or 'rinse-off' products (Table 2) and further into specific cosmetic product groups (Table 3). If a cosmetic product could not be specified because it was unknown or did not fit any of the predetermined categories, it was registered as 'unspecified leave-on' or 'unspecified rinse-off'. Patients could have more than one specific cosmetic product recorded.

Methods

The patients included had been patch tested with at least one of the fragrance markers from the European baseline series (FM I, FM II, *M. pereirae* and HICC). The

Table 1. MOAHLFA index of consecutively patch tested eczema patients and patients with a fragrance allergy caused by a cosmetic product

Index	Tested subjects		Cosmetic fragrance allergy	
	No.	%	No.	%
M	6106	34.5	190*	25.2
O	2067	11.7	97	12.9
A	3115	17.6	137	18.2
H	6625	37.4	272	36.1
L	815	4.6	23*	3.1
F	3370	19.0	248*	32.9
AA	10465	59.1	488*	64.8
Total	17716	100	753	100

MOAHLFA index: M, male; O, occupational cause of dermatitis; A, atopy; H, hand dermatitis; L, leg dermatitis; F, facial dermatitis; and AA ≥ 40 years.
*χ^2-test, $p < 0.05$.

Table 2. Leave-on or rinse-off cosmetic products listed as the exposure causing fragrance allergy

	Leave-on		Rinse-off		All
	No.	%	No.	%	No.
Women	556	75.9	176	24.1	732
Men	162	69.2	72	30.8	234
Total	718	74.3	248	25.7	966

Table 3. The cosmetic product groups listed as having caused fragrance allergic contact dermatitis

Cosmetic product categories	Men and women	Women		Men	
	n	n	%	n	%
Unspecified stay-on products	286	224	29.8	62	26.1
Deodorant	146	91	12.4	55	23.5
Scented lotion	142	123	16.8	19	8.1
Unspecified rinse-off products	104	77	10.5	27	11.5
Fine fragrances	93	85	11.6	8	3.4
Shampoo	76	57	7.8	19	8.1
Liquid soap	63	41	5.6	22	9.4
Aftershave	16	2	0.3	14	6.0
Lipstick	11	9	1.2	2	0.9
Sun lotion	6	5	0.7	1	0.4
Hairstyling product	6	5	0.7	1	0.4
Shaving foam	5	1	0.1	4	1.7
Mascara	4	4	0.5	0	0.0
Hair dye	4	4	0.5	0	0.0
Eyeshadow	2	2	0.3	0	0.0
Makeup cream	2	2	0.3	0	0.0
Sum of cosmetic product within each category listed as the cause of fragrance allergic contact dermatitis	966	732		234	

patch tests were performed according to international guidelines (14) with Finn Chambers® (8 mm; Epitest Ltd Oy, Tuusula, Finland) applied on the back with Scanpor tape® (Norgesplaster A/S, Alpharma, As, Norway) and kept in place for 2 days. Readings were performed on day 2, 3 or 4, and on day 7, according to the recommendations of the International Contact Dermatitis Research Group (15).

Data administration and statistical analysis were perfomed using SPSS version 15 and OPENEpi (www.openepi.com). Percentages of the cosmetic product groups listed as causing a positive patch test reaction to a fragrance marker were calculated. χ^2-tests for characteristic differences were performed, and $p < 0.05$ was considered to be significant.

Results

Fragrance contact allergy to one or more of the fragrance markers was found in 1790 (10.1%) of the participants. Cosmetic products were the cause of fragrance allergic contact dermatitis in 753, comprising 42.1% of those with fragrance allergy, or 4.3% of the subjects consecutively examined for contact allergy. Some patients had more than one cosmetic product listed as causing their allergy; 966 product groups were listed. The majority of cosmetic products listed were 'leave-on' products (74.3%) rather than 'rinse-off' products (25.7%).

In general, many different cosmetic product categories were listed as causing fragrance allergic contact dermatitis (Table 3); 576 products had been listed as belonging to specific product categories. The commonest sources of allergic contact dermatitis were deodorants (25.3%), scented lotions (24.4%), fine fragrances (16.0%), shampoos (13.0%), liquid soaps (10.8%), aftershaves (2.7%), lipsticks (1.9%) and the remaining categories had frequencies of 1% or less (Fig. 1).

A sex difference was apparent in the distribution of cosmetic products listed as causing fragrance allergic contact dermatitis (Fig. 2). Deodorants, in particular, played a large role in men, accounting for 37.9% of the 145 products listed as causing fragrance allergic contact dermatitis among men, which was highly significant ($p < 0.001$). Scented lotions and fine fragrances played the largest role in women, accounting for 28.5% and 19.7%, respectively, of the products listed (n = 436) and the sex difference was highly significant ($p < 0.001$). No sex difference was observed in the reporting of shampoo as the cause of fragrance allergic contact dermatitis.

Figure 3 shows the role of the four most common products listed as having caused a positive patch test reaction to the different screening markers of the baseline series. There was a significant correlation between products listed as having caused allergy and the different markers (χ^2-test, $p < 0.001$). FM II and HICC were overrepresented in deodorants. Scented lotion
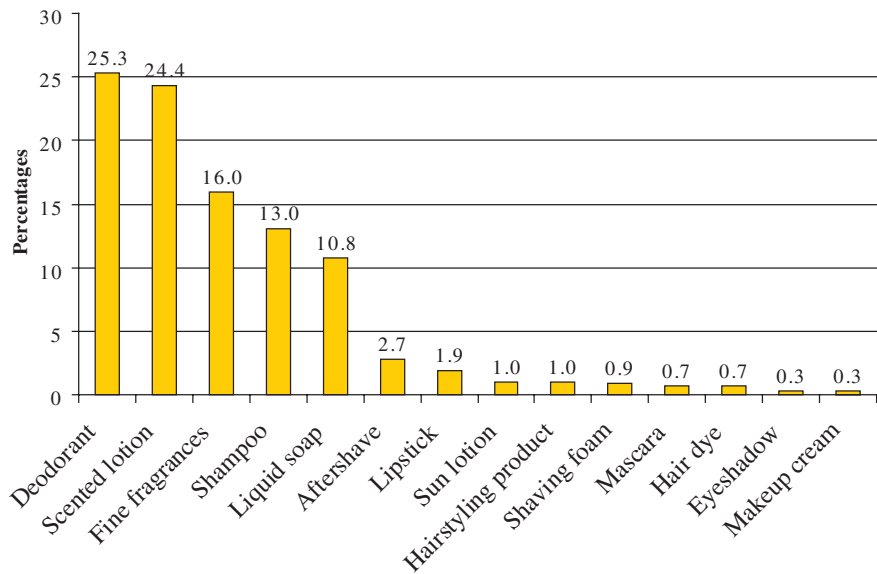
Fig. 1. Prevalence of specific cosmetic product groups listed as having caused fragrance allergy. The total number of specific products listed was 576.

and shampoo were more likely to be associated with fragrance allergic contact dermatitis detected by FM I and *M. pereirae*.

Among all the deodorants listed (n = 213) as having caused fragrance allergic contact dermatitis, an FM II allergy (34.3%) was more likely than an FM I (28.2%), HICC (24.9%) or *M. pereirae* (12.7%) allergy (Table 4).

Discussion

Adverse skin reactions caused by cosmetics are an increasing problem in the population of Denmark (16). The most frequent causes of cosmetic allergy have been shown to be fragrances (7, 11, 17, 18). Many different cosmetic product groups can cause allergic contact dermatitis; according to our study, it appears
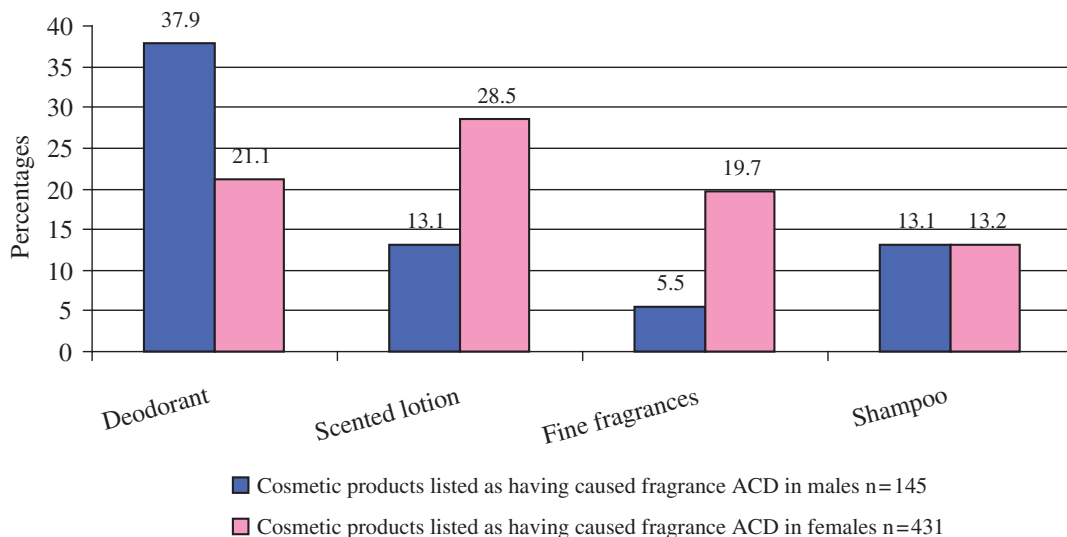
Fig. 2. Sex distribution of the four most frequent cosmetic products listed as having caused fragrance allergic contact dermatitis (ACD). A statistical sex difference in deodorants listed as the cause of fragrance allergy was observed ($p < 0.001$). The odds ratio for a deodorant listed as the cause of fragrance allergy in men versus women was 2.3 [confidence interval (CI) 1.5−3.5]. Likewise, a statistical sex difference was seen for scented lotion and fine fragrances as the cause of fragrance allergic contact dermatitis ($p < 0.001$). They were more frequent among women: the odds ratio for a cream with a scent was 2.6 (CI 1.6−4.5), and the odds ratio for a fine fragrance was 4.2 (CI 2.0−9.4). No sex difference was observed for shampoo listed as the cause of fragrance allergic contact dermatitis.

Fig. 3. The prevalence of each of the four most frequent cosmetic products responsible for fragrance allergy detected by different fragrance markers of the baseline series.

that the use of deodorants is especially associated with an increased risk of fragrance allergic contact dermatitis. We found deodorants listed as the leading causes of fragrance allergic contact dermatitis among eczema patients. Likewise, a study of the general population in Denmark reported deodorants as the leading causes of allergic and irritant contact dermatitis (16).
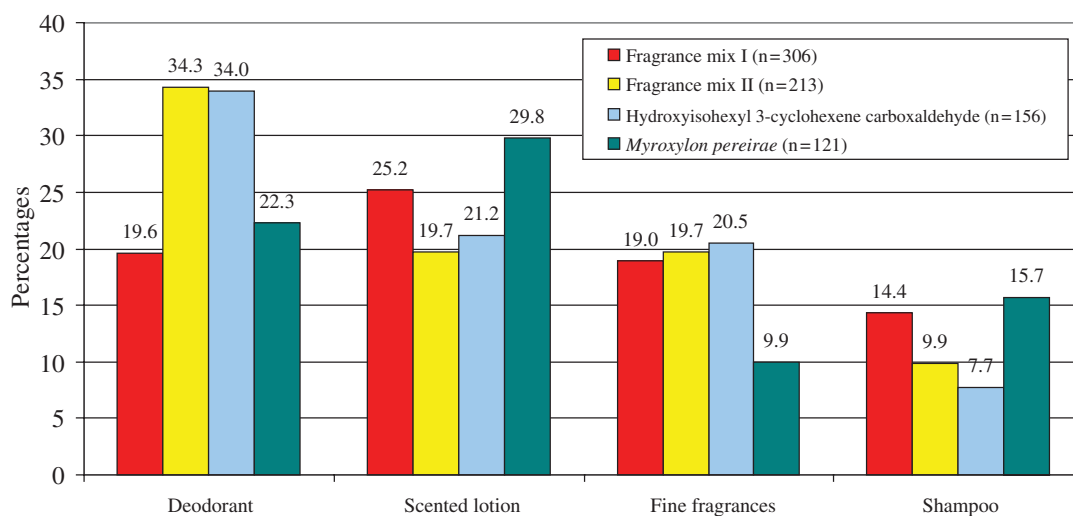
Deodorants are also related to first-time symptoms of fragrance allergy. A study of 925 eczema patients and a control group of 806 persons, randomly selected

from the population, reported a statistically significant correlation between development of a rash resulting from a scented deodorant as a first-time symptom (odds ratio: 2.3–2.9) and a later diagnosis of fragrance allergy (19). In a German study (20), eczema patients were patch tested with their own deodorants; 501 deodorants were tested, and 6.2% caused allergic reactions.

The sex difference in the use of cosmetic products is obvious, and a difference was expected with regard to

Table 4. The distribution of cosmetic product groups according to the fragrance screening markers that had a positive and clinically relevant patch test reaction (positive +, ++, +++)

	Fragrance screening markers of the baseline series							
		Fragrance mix I		Fragrance mix II		Hydroxyisohexyl 3-cyclohexene carboxaldehyde		*Myroxylon pereirae*	
Product	n	n	%	n	%	n	%	n	%
Deodorant	213	60	28.2	73	34.3	53	24.9	27	12.7
Scented lotion	188	77	41.0	42	22.3	33	17.6	36	19.1
Fine fragrances	144	58	40.3	42	29.2	32	22.2	12	8.3
Shampoo	96	44	45.8	21	21.9	12	12.5	19	19.8
Liquid soap	84	37	44.0	17	20.2	16	19.0	14	16.7
Aftershave	23	9	39.1	6	26.1	3	13.0	5	21.7
Lipstick	12	4	33.3	5	41.7	0	0.0	3	25.0
Sun lotion	10	4	40.0	2	20.0	3	30.0	1	10.0
Hairstyling product	6	3	50.0	2	33.3	1	16.7	0	0.0
Shaving foam	6	4	66.7	1	16.7	0	0.0	1	16.7
Mascara	4	2	50.0	0	0.0	1	25.0	1	25.0
Hair dye	4	3	75.0	0	0.0	0	0.0	1	25.0
Eyeshadow	5	2	40.0	1	20.0	1	20.0	1	20.0
Makeup cream	3	1	33.3	1	33.3	1	33.3	0	0.0

A cosmetic product could be listed as the cause of allergic contact dermatitis resulting from more than one fragrance marker.

which products were reported as having caused fragrance allergic contact dermatitis. Deodorants were significantly more likely to be listed as the cause of fragrance allergic contact dermatitis in men than in women (odds ratio 2.3), whereas women were significantly more likely to report a scented lotion or a fine fragrance as the cause of fragrance allergic contact dermatitis.

Many factors may explain why deodorants in particular are associated with a high risk of developing a fragrance allergic contact dermatitis. The environment in the axillae is moist and occluded, and this, in combination with the presence of hair follicles, can increase the penetration of certain allergens (21, 22). Shaving also increases penetration, and thus the risk of contact allergy (23). In a case study, 14 fragrance-allergic patients were asked to use one of their own deodorants in both the axillae and on the upper arm for 1 week. Twenty deodorants were tested; 12 of these (60%) caused eczema in the axillae, whereas only four (20%) caused eczema on the upper arm. The deodorants that caused eczema contained 1.3–8.6-fold higher concentrations of allergenic fragrance substances than those products that did not cause eczema (24). To provoke an allergic reaction, a lower concentration of a fragrance allergen is needed in the axillae than in other parts of the body. This could be explained by less fragrance evaporating than on non-occluded sites and the concentration of the fragrance substance remaining high for a longer time (25). As a lower concentration threshold is need in the axillae to provoke an elicitation response to an allergen (22), it could be argued that a lower concentration of allergen can cause sensitization when administered in the axillae; the study on first-time symptoms previously mentioned could be an indication of this (19). Another reason why deodorants are responsible for allergic contact dermatitis caused by fragrances is that they may contain irritants that help to deliver a stronger danger signal (26), facilitating the sensitization response (27) to an allergen and the elicitation response (28, 29).

The differences in formulation of deodorants (aerosol sprays, roll-ons, and sticks) also seem to play a role in the bioavailability of allergenic fragrance substances. One small study investigated a deodorant spray and a deostick with the same concentrations of allergenic fragrance substances tested in the antecubital of 7 fragrance allergic patients. Five of these subjects reacted to the deodorant spray, whereas only 1 reacted to the deostick (30). The effects of using different deodorant formulations have not yet been systematically investigated.

In our study, we identified a correlation between a deodorant being listed as the cause of allergy and a relevant, positive patch test reaction to HICC or FM II. This could be explained by frequent exposure to the single-compound fragrances of FM II used in deodorants (19, 31). A UK study on the labelling of cosmetic and household products revealed that deodorants had a mean of 7.8 (3–13) different fragrance ingredients in each deodorant, and almost 30% of all products investigated (n = 300) contained HICC (32). Likewise, a study from Denmark reported that 50% of deodorants on the market contained HICC (31). It will be interesting to see whether HICC allergy will decrease after the recent reduction in the maximal concentration recommended by the International Fragrance Association (33).

This study confirms that deodorants play a major role in allergic contact dermatitis caused by fragrances. Deodorants seem to have an unfortunate composition of FM II fragrance ingredients, leading to allergic contact dermatitis. As deodorants are used in sensitive areas of the body, it could be argued that these sensitizing fragrance ingredients should either be avoided or used in lower concentrations in deodorants than in other types of product.

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10.3. Manuscript III

Fragrance allergy and quality of life -
Development and validation of a disease specific quality of life instrument

Maria V Heisterberg, Torkil Menné and Jeanne D Johansen.
National Allergy Research Centre, Department of Dermato-allergology,
Copenhagen University Hospital Gentofte, University of Copenhagen, 2900 Hellerup, Denmark

Abstract

Background: Fragrance allergy is a lifelong condition which may give rise to permanent or recurrent contact dermatitis and may affect quality of life (QoL). The effect on QoL has not yet been investigated and no disease specific QoL instrument for fragrance allergy exists.

Objective: To develop and validate a disease specific instrument to investigate QoL among fragrance allergic subjects.

Method: A QoL instrument (Fragrance QoL index) was developed based on narratives from 68 subjects with a fragrance allergy and consisted of 13 items. It was tested in a mail survey among 1650 participants patch tested at Gentofte University Hospital (2000-2010). The survey included other QoL instruments (DLQI and SF36v2) and questions on eczema severity. The response rate was 66%.

Results: The fragrance QoL index showed a significant and strong correlation to the DLQI (r_S=0.70), and disease severity. But only weak correlation to SF36 (MCS: r_S=-0.22 and PCS: r_S=-0.31). Furthermore, a good reliability and responsiveness to changes in disease severity was seen.

Conclusion: The fragrance QoL index is a good instrument to investigate QoL among subjects with fragrance allergy. It had good correlations with the DLQI; self estimated disease severity and showed good reliability, reproducibility and ability to distinguish changes in disease severity.

Introduction

Allergic contact dermatitis to fragrance ingredients is common among eczema patients. Approximately 16% of eczema patients investigated for contact dermatitis have an allergy to a fragrance ingredient (1-3). Once diagnosed with an allergy it is a lifelong condition. No cure exists and thus, the primary treatment is allergen avoidance and symptomatic treatment of dermatitis flare ups. However, allergen avoidance can be difficult as fragrance ingredients are present in many different consumer products. A behavioural change to avoid the allergen will inevitably cause changes in the daily activities (4), which may affect QoL. Little is known of this amongst subjects with allergic contact dermatitis to fragrance ingredients. Previous studies on QoL and dermatitis found an impaired QoL in these subjects (5-11). A few studies have also investigated QoL in contact dermatitis patients and found an increased impairment in QoL (9;11-13). However, no studies have investigated fragrance allergy and QoL.

Health related QoL can be assessed by using a questionnaire and several different validated versions exist (12): 1) generic questionnaires, which can be used in healthy as well as in people with a disease; 2) speciality specific questionnaires, which are used in subjects with a disease within a certain medical field and 3) disease-specific questionnaires, which are aimed at people with a specific disease. Often they are used in combination to give the best estimate of the impairment of QoL (14). Many different disease-specific QoL instruments have been developed for skin diseases as the specialty specific (Dermatology Quality of Life Index) and/or generic questionnaires were not considered to perform sufficiently in assessing the QoL (15-18). The aim of this study was to develop and validate a disease specific QoL instrument for subjects with allergic contact dermatitis to fragrance ingredients.

Method

The study comprised two phases: Phase 1: Development of a disease-specific QoL instrument for subjects with fragrance allergy. Phase 2: Validation of the QoL instrument.

Participants
All participants had been patch tested with at least the Baseline series at Gentofte University Hospital between the 1st of January 2000 and the 31st of December 2010. Participants were aged 18 to 70 years old and registered in the National Contact Dermatitis Database administered by the National Allergy Research Centre. The participants in phase 1 of the study were all subjects with a positive patch test to at least one fragrance marker (n=122) and were randomly selected from all subjects who had a positive patch test in the database using Microsoft Sql Server 2008. Participants in phase 2 of the study consisted of a case-control group. The case group (n= 550) had at least one positive patch test reaction to a fragrance marker/ingredient (the fragrance positive group). They were randomly selected from the National Contact Dermatitis Database of all subjects with a positive patch test to a fragrance marker. The control group were selected from the same database and consisted of subjects who had *no* fragrance positive patch test reaction (the non-fragrance positive group n=1100) and they were matched to the fragrance positive subjects on age (± 1 year), gender and patch test year (± 1 year). The response rate was 65.7% (n=1084) and no statistically significant difference was seen in the response rate of the fragrance positive (68.9%) and the non-fragrance positive (64.1%) was seen (X^2 test p= 0.054). Furthermore, when stratified by patch test

year, no differences were seen between the response rates of fragrance positive and non-fragrance positive subjects over time.

Phase 1: Development of the Fragrance QoL index
A postal survey was sent to 122 subjects with a fragrance allergy. The survey consisted of a postal letter and a stamped, addressed reply envelope. The letter said: "*You have been to the Dermato-allergology Department, Gentofte Hospital and been diagnosed with perfume allergy. We are trying to find out how perfume allergy affects quality of life and would be grateful if you could help us with this. There is no obligation to do so. Please describe how your allergy affects your everyday life, include all the influences it may have on your work life, social life, personal relationships and leisure activities, or any in other aspects your allergy affect your life. Your response will be treated confidentially.*"
A content analysis of the respondents' narratives about their life with fragrance allergy was performed and formed the basis for "the fragrance QoL index". A schematic illustration of the development and validation of the fragrance QoL index is shown in Figure 1. It consists of 13 items which are answered on a visual analogue scale from 0-10. The time frame covered by the items was set to "currently", as in this present moment. It was translated to English (Figure 2) according to standardized methods of translation (19). A summarized score for the average fragrance QoL index can be calculated and the higher the score the more impaired the QoL. The VAS scores from each item were summarized for those subjects where 11 or more items are answered. If two or more items were left unanswered the response was excluded. In item 6, there was the possibility of answering not relevant which was counted as 0. In item 12 the score was reversed (10=0, 9=1,……, 0=10). The Danish version of the fragrance QoL index was translated into English according to standardized methods (19), see Figure 2.

Phase 2: The postal survey
A postal survey package was sent to the 1650 participants. It included a questionnaire and a stamped, addressed return envelope. A reminder postcard was sent to increase the response rate and furthermore the questionnaire was sent a second time together with a stamped, addressed return envelope. The questionnaire consisted of three QoL instruments: the newly developed fragrance QoL index, a specialty specific QoL instrument and a generic QoL instrument. Furthermore, it included general questions on eczema and exposure factors for fragrance allergy.

Self estimated disease severity
Disease severity was assessed on a visual analogue scale (VAS) in two different questions. One question was addressed at "their eczema at the present time" and the other at "when the eczema was worst". The questions were phrased: "How do you assess the degree of your eczema on a scale from 0 to 10, where 0 corresponds to no eczema and 10 correspond to very severe eczema? *Eczema is a condition of the skin with redness, swelling and itching, possibly with watery blisters and peeling of the skin. The condition will typically be recurrent, but may also have been present as a single episode.*"

Assessment of Quality of life
A dermatology specific QoL questionnaire, the Dermatology Quality of Life index (DLQI) was used. It consists of 10 questions in relation to well-being within the last week. A summarized score can be calculated with a maximum of 30 and minimum of 0 (20). The higher the score, the more impaired the QoL. The DLQI has been widely used (21;22) and translated to Danish (23). Permission to use the DLQI was granted by Dr. Mohammad Khurshid Azam Basra, Department of

Dermatology, Cardiff University School of Medicine, Heath Park, Cardiff, CF14 4XN, United Kingdom.

A generic health QoL questionnaire, the SF36v2 was used. It consists of 36 questions all related to the general health within the last 4 weeks. It is widely used in the field of dermatology including eczema patients (5-8). It has been translated into more than 50 languages including Danish (24-29). It yields 8 dimensions of functional health and well-being scores and also psychometrically-based physical (PCS) and mental (MCS) health summary scores, all are scored between 0-100. The lower the score the more impaired the QoL. The scores were calculated in Health Outcome Scoring Software 4.5, which was licensed through www.qualitymetric.com, as was permission to use the SF36v2 questionnaire.

Re-test

A re-test was conducted to test for reliability of the questions in the fragrance QoL index and to evaluate it's responsiveness to change. It was sent approximately 3-6 months after the questionnaire survey to 193 subjects (fragrance positive N= 71 and non-fragrance positive n=121) together with a return envelope. The participants of the re-test were randomly selected using an inbuilt SPSS function among initial respondents to the postal survey. The re-test was consisted of a postal questionnaire and a stamped, addressed return envelope. The re-test questionnaire comprised the Fragrance QoL index and a question on disease severity. Changes in disease severity was calculated ($VAS_{test} - VAS_{re\ test}$) and if the change was > 1 point it was considered a change in disease severity. A re-test is often conducted in close time relation to the test (30). However, in our study, the time span was 3 - 6 months and as eczema is a disease that fluctuates in severity over time, the test for reliability was illustrated not only with the crude intraclass correlations (ICC), but also with ICC adjusted for change in disease severity (31). The re-test was sent once and the response rate was 72.5% (n=140).

Database information

The National Contact Dermatitis Database is managed by the National Allergy Research Centre at the Department of Dermato-Allergology at Gentofte University Hospital. It contains information on patch test date, reactions, relevance of patch test (past or present) and demographic characteristics. All patch tests were done according to international guidelines (32) using Finn Chambers® applied on the back with Scanpore tape® (Vitalfo Scandinavia, AB, Allerød, Denmark) for a period of 2 days. Readings were done on day 2, 3 or 4 and 7 according to the recommendation of the International Contact Dermatitis Research Group (33). During the 10 year study period (2000-2010) there have been developments in the diagnostics of fragrance allergy. At Gentofte University Hospital the Fragrance mix II and hydroxyisohexyl 3-cyclohexene carboxaldehyde were introduced in the Baseline series in 2005 (1). From 2007, all patients who were investigated for contact allergy were additionally patch tested with our perfume series, which consist of the 26 fragrances which must be declared on cosmetic products according to the EU Cosmetic Directive (34). Furthermore, from January 2010 the oxidized forms of both limonene and linalool were also included as screening markers of fragrance allergy (supplied by Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden). However, by matching the controls for patch test year the possible bias due to change in patch test materials over time will be minimized.

Statistics and data management

Data entry was done manually in SPSS Data Entry Builder (SPSS® Inc., Chicago, IL, USA). Statistical analysis of correlations, crude percentages, mean and standard deviation were performed

using the SPSS for windows version 19 (SPSS® Inc., Chicago, IL, USA). Calculations on the SF36v2 dimensions were done in Health Outcome Scoring Software 4.5 and the Rasch analysis was performed with Winstep Software (www.winstep.com). All tests for statistically significant differences between fragrance positive and non-fragrance positive groups were done accounting for matching in univariate analysis or logistic regression analysis, and $p < 0.05$ was considered significant. The binary logistic regression analyses were checked by Hosmer-Lemeshow Goodness of fit test. Interpretation of the Cronbach's alpha: excellent when $\alpha \geq 0.9$; good: $0.8 \leq \alpha < 0.9$; acceptable: $0.7 \leq \alpha < 0.8$; questionable: $0.6 \leq \alpha < 0.7$; Poor: $0.5 \leq \alpha < 0.6$; and unacceptable when $\alpha < 0.5$. The Rasch analysis was done to give a range of details for assessing whether summarizing of the item scores into the fragrance QoL index score was justified. Factor analysis was performed to identify groups of inter-related variables, to see how they were related to each other. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy tested whether the partial correlations among variables were small. A KMO value above 0.05 is satisfactory for factor analysis to proceed. The Bartlett's test of sphericity was used to test the null hypothesis, if the variables in the population correlation matrix were uncorrelated.

Results

Demographics and clinical characteristics of the participants
A disease specific instrument to investigate the QoL among subjects with a fragrance allergy (the fragrance QoL index) was developed, Figure 1 and 2.
The point prevalence of eczema was 52.1 % and the 1-year prevalence of having eczema was 76.7 % with no overall differences between fragrance positive and non-fragrance positive subjects (Table 1). However, women with a fragrance allergy had a significant higher 1-year prevalence of eczema (p=0.042) compared with their control group. Duration of eczema was more frequently reported to be present *(almost) all the time* by women with fragrance allergy (p=0.041) compared with women with no-fragrance allergy (Table1). No difference was seen in the self-estimated "eczema when worst" between the fragrance positive group and their controls but a significant difference (p=0.048) on self- estimated severity of eczema "at the present moment" was observed between men with fragrance allergy and their controls.

The tests for validation of the fragrance QoL index
Convergent validity of the fragrance QoL index was assessed by the correlations with SF36 and the DLQI (Spearman correlations and scatter plot). All Spearman correlations were of statistical significance (Table 2) for both the fragrance positive and the non-fragrance positive subjects. Notably, a strong positive correlation (Spearman; r_S=0.70) was seen between the DLQI and the Fragrance QoL index, illustrated in Figure 3. However, correlation between the fragrance QoL index and the SF36 showed only moderate to weak inverse correlations both for the mental component summary score (MCS; r_S = -0.22) and the physical component summary score (PCS; r_S = -0.31).
The fragrance index showed a strong statistically significant correlation to self-estimated disease severity (Figure 4), as did the DLQI. However, this was not seen for the SF36, which showed weak correlations to disease severity (Figure 4.C and D).
Reliability
Internal consistency of the fragrance QoL index score was tested with Cronbach's alpha coefficient, (α =0.9), showing excellent consistency. The response rate of the re-test was 72.5% (n=140) and

there was no significant difference between the response rate of the fragrance positive and the non-fragrance positive.

The reproducibility of the fragrance QoL showed good intra-item correlations (ICC) in the test-retest study (Table 3). To avoid interference from improvement or worsening of disease an analysis of only "stable" subjects was conducted, shown in Table 3 as ICC $_{DU}$, which comprised 71 subjects. A change in eczema severity measured on the visual analogue scale (VAS$_{test}$-VAS$_{retest}$) of > 1 point was considered a change in disease severity (30). For most of the items (n=7) the ICC$_{DU}$ values showed good reliability (>0.8) and adequate reliability (>0.72) for four of the items. However, two items showed values of ICC$_{DU}$ of less than 0.70.The test-retest reliability is also illustrated in a Bland and Altman plot (Figure 5), which is a graphical method to compare two measurements (35). It shows the difference of the mean of the fragrance QoL index score in the test and retest compared to the difference of the mean of the self estimated disease severity measured on the VAS in both the test and re-test. Horizontal lines are drawn at the mean difference and at the limits of agreement, which are defined as the mean difference ±1.96 times the standard deviation of the differences. The plot illustrates a good agreement between the test and retest.

Responsiveness to change
One criterion for validation of the Fragrance QoL is that it should be able to discriminate between subjects with different disease severity. This is illustrated visually in a scatter plot (Figure 6) and tested with Pearson's test, which was highly statistically significant with r =0.76.
Rasch analysis
Rasch analyses were performed on the VAS data from the subjects with and without fragrance allergy as well as separately for women and men. The Rasch model that describes the probability of the scores as a function of the subject's QoL and the question's relevance in that respect showed a good fit to the data. The model explained 50 – 60% of the variation, and the unexplained (random) variation was distributed over a series of decreasing independent components. All items were positively correlated, and Cronbach's α was about 0.9. It showed that the items display a one dimensional measure of quality of life without indications of heterogeneity or disturbance of outliers.

Factor analysis
A factor analysis was also made although the VAS data could only approximately be assumed to follow a Gaussian distribution. Nevertheless, it showed that the items described a major principal component of the variation, while the secondary orthogonal components decreased gradually. Orthogonal varimax rotation analyses were performed separately for fragrance positive and non-fragrance positive; and for women and men; and for the total number of respondents. All Barlett's test of spericity were significant. Thus, the hypothesis that the intercorrelation matrix involving these 13 variables is an identity matrix is rejected. All analyses showed high KMO values (>0.9). Scree plots indicated that the first factor described 40−50% of the variation, while the following three factors accounted for 5−10% of the variation.

Overall, the results of these validation analyses indicate that the fragrance QoL index is technically valid and potentially useful for assessment of QoL for subjects with a fragrance allergy.

Discussion

This paper describes the development and validation of a novel instrument to investigate QoL among fragrance allergic subjects. The fragrance QoL index consists of 13 questions answered on a visual analogue scale. The validation was preformed in a series of analyses, which all showed it to be a technically valid and potentially useful instrument for assessment of fragrance QoL. When evaluated among 1084 subjects it showed a good correlation with the DLQI, which is often viewed as the golden standard when measuring QoL within the field of dermatology. The fragrance QoL index also showed a significant positive correlation to self-estimated eczema severity (spearman correlation r_S ==0.51) as did the DLQI indicating that they are good instruments for assessing QoL in relation to severity of disease. However, the SF36v2 did not show convergent validity to the Fragrance QoL index nor to the DLQI or self estimated disease severity. The spearman correlations were of statistically significant but the coefficients were low indicating weak correlations. This was surprising as other studies have found good correlation between SF36 and the DLQI and disease severity (8;36;37). However, there is a debate on how well the DLQI and the SF36 performs in determining QoL in subjects with skin diseases (38-40) and the variable results seen in the many different studies using the SF36 may be due to the diversity of study populations investigated (41). Our study population differ form most other study populations as they were not included because of specific clinical characteristics which is done for many of the other studies(40;42;43). Furthermore, our population was selected after treatment and it is hoped that most had received a proper diagnosis, and treatment(44). Consequently, our study population may not be as "ill" which could explain why we do not see the same impact on QoL measured with the SF36 as other studies have found(40;45). We specifically chose a relatively long study period of 10 years to be able to investigate the influence of time on the QoL, which will be reported in a separate publication. The results from this study imply that the SF36v2 is not sufficiently sensitive to reflect the QoL impairments for subjects with fragrance allergic contact dermatitis. The matching of the control group for age, gender and patch test year may have overcome some biases as the similarity among the case group and the control was quite good regarding the response rate, gender, age and prevalence of eczema. However, it is fairly difficult to find an ideal control group for investigation of fragrance allergy, e.g., healthy controls would not score at all on the DLQI, which we wanted to compare our newly developed questionnaire to, but they may distinguish better on the SF36 compared to fragrance allergic subjects.

The fragrance QoL index also showed good reliability, Cronbach's alpha was 0.9, indicating excellent internal consistency. The test-retest reliability assessed with the intraclass coefficient was 0.92, which reflects a good reproducibility by the subjects for the fragrance QoL index score. Responsiveness to change with disease severity was good as indicated in the scatter plot. Overall, all the validation analysis of the fragrance QoL index indicates that all the items are valid and it is justifiable to combine them into the fragrance QoL index score.

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Correspondence to:
Maria Vølund Heisterberg, MD
National Allergy Research Centre, Department of Dermato-allergology, Copenhagen University Hospital Gentofte, University of Copenhagen, 2900 Hellerup, Denmark.
e-mail:maria.voelund.heisterberg@regionh.dk
Tel: 0045 3977 7305
Fax: 0045 3997 7118

Table 1. Clinical and demographic characteristics of fragrance positive and non-fragrance positive in the questionnaire study N=1084

Respondents

	Fragrance positive						Non-fragrance positive						Total	
	Women		Men		Women and men		Women		Men		Women and men		Women and men	
	n (mean)	% (SD)	n (mean)	% (SD)	n (mean)	% (SD)	n (mean)	% (SD)	n (mean)	% (SD)	n (mean)	% (SD)	n (mean)	% (SD)
Responders	290	76.5%	89	23.5%	379	35.0%	551	78.2%	154	21.8%	705	65.0%	1084	-
Age	(45.8)	(12.6)	(48.9)	(11.9)	(46.5)	(12.5)	(46.6)	(12.3)	(49.7)	(11.0)	(47.3)	(12.1)	(47.1)	(12.2)
Prevalence of Eczema														
1 year prevalence	215[a]	81.7%	54	68.4%	269	78.7%	357	75.3%	99	76.3%	456	75.5%	725	76.7%
Point prevalence	160	56.7%	42	47.7%	202	54.6%	271	50.0%	81	53.3%	352	50.7%	554	52.1%
Duration of eczema														
Once, less than 2 weeks	9[b]	3.5%	4	5.2%	13	3.9%	18	3.9%	2	1.5%	20	3.3%	33	3.5%
Once, longer than 2 weeks	11[b]	4.2%	3	3.9%	14	4.2%	40	8.6%	12	9.1%	52	8.7%	66	7.1%
Many times	135[b]	51.9%	42	54.5%	177	52.5%	249	53.4%	65	49.2%	314	52.5%	491	52.5%
(almost) all the time	105[b]	40.4%	28	36.4%	133	39.5%	159	34.1%	53	46.2%	212	35.5%	345	36.9%
Severity of eczema VAS														
Eczema presently	(3.6)	(2.8)	(3.1)[c]	(2.5)	(3.5)	(2.7)	(3.6)	(3.0)	(3.9)	(3.0)	(3.7)	(3.0)	(3.6)	(2.9)
Eczema when worst	(7.8)	(2.4)	(7.2)	(2.7)	(87.6)	(2.5)	(7.7)	(2.5)	(7.6)	(2.3)	(7.6)	(2.5)	(7.6)	(2.5)
Other skin diseases														
Atopic dermatitis*	62	21.4%	16	18.0%	78	20.6%	98	17.8%	22	14.3%	120	17.0%	198	18.3%
Other skin disease	118	40.7%	24	27.0%	142	37.2%	214	38.5%	38	24.7%	250	35.5%	395	36.1%
Other allergies ** (other than fragrance)	183[d]	63.1%	44[e]	49.4%	227[f]	59.9%	222	40.3%	54	35.1%	276	39.1%	503	46.4%

n: Number of respondents

mean: Mean of age, and severity of eczema measured on the visual analogue scale for eczema currently and when worst.

SD: Standard deviation

%: Crude percentages of all respondents who answered the item. If respondents had not answered the item they were counted as missing in the analysis. Statistical tests were performed accounting for the matching for each of the items

(univariate analyses and logistic regression analyses)

* Atopic dermatitis as defined by Hanifin et al. One major, at least 3 minor criteria.

** Positive patch test reaction to a marker of the baseline series other than a fragrance screening marker (Fragrance mix I, fragrance mix II, hydroxyisohexyl 3-cyclohexene carboxaldehyde and Myroxylon pereirae.

[a] Fragrance positive women compared with control women in a logistic regression analyses accounting for the matching, p= 0.042

[b] Fragrance positive women had a significantly different duration of eczema compared with control women in a logistic regression analyses accounting for the matching, p= 0.041

[c] Fragrance positive men score significantly lower on the visual analogue scale on self estimated eczema severity (3.1) currently compared with non-fragrance positive men (3.9); Univariate analysis accounting for the matching, p=0.048.

[d] Fragrance positive women compared with control women in a logistic regression analyses accounting for the matching, p= 0.001

[e] Fragrance positive men compared with control men in a logistic regression analyses accounting for the matching, P=0.028

[f] Fragrance positive women and men compared with controls in a logistic regression analyses accounting for the matching, P= 0.001.

Table 2. Spearman correlation between the fragrance QoL index score and SF36v2 and DLQI

| | The Fragrance index | | | | | | DLQI | |
| | Fragrance positive | | Non-fragrance positive | | All respondents | | All respondents | |
	r_S	P value	r_S	P value	r_S	P value	r_S	P value
DLQI	0.70	<0.001	0.74	<0.001	0.72	<0.001	-	-
SF36 MCS	-0.22	<0.001	-0.30	<0.001	-0.28	<0.001	-0.19	<0.001
SF36 PCS	-0.31	<0.001	-0.31	<0.001	-0.31	<0.001	-0.23	<0.001

DLQI: The dermatology quality of life index.
SF36 MCS: Mental component summary score of the SF36 questionnaire.
SF36 PCS: Physical component summary score of the SF36 questionnaire.
r_S: Spearman correlation coefficient.
Fragrance positive: a positive patch test to at least one fragrance marker.
Non-fragrance positive: no positive patch test to a fragrance marker.

Table 3. Correlation between the fragrance index in the test and re-test N=140

| Item | Test | | Re-test | | Intraclass coefficient | |
	mean	(SD)	mean	(SD)	ICC	ICC $_{DU}$
1	3.88	(3.02)	3.66	(3.12)	0.77	0.86
2	4.60	(3.78)	5.37	(3.74)	0.78	0.80
3	4.93	(3.49)	4.29	(3.04)	0.77	0.77
4	5.47	(3.20)	4.74	(3.14)	0.72	0.66
5	4.03	(3.40)	3.71	(3.13)	0.72	0.72
6	3.49	(4.06)	3.73	(3.98)	0.81	0.89
7	0.54	(1.36)	0.76	(2.08)	0.68	0.72
8	2.63	(2.74)	2.59	(2.95)	0.83	0.91
9	2.74	(3.02)	2.58	(3.38)	0.72	0.79
10	2.26	(3.02)	2.64	(3.22)	0.76	0.99
11	2.49	(3.46)	3.16	(3.90)	0.82	0.89
12	6.02	(3.57)	6.55	(3.45)	0.75	0.82
13	1.54	(2.60)	1.46	(2.44)	0.63	0.61
Disease severity	3.47	(2.88)	3.17	(2.82)	0.56	-
Fragrance QoL index score	41.82	(25.62)	43.07	(26.31)	0.86	0.92

Legend: All ICC values were highly significant, p<0.001.
ICC: Intraclass coefficient.
ICC$_{DU}$: Only including respondents with no change in disease severity (n=71), a change of > 1 point on the visual analogue scale (VAS$_{test}$-VAS$_{retest}$) was considered a change in disease severity.
Mean: mean of the VAS.
SD: Standard deviation

Figure 1. Schematic illustration of the development of the fragrance QoL index

122 individuals with a fragrance allergy were randomly selected to participate in a postal survey. All had been patch tested at Gentofte University Hospital (2000-2010) and all had a positive reaction to at least one fragrance marker. A questionnaire was sent twice with a stamped, addressed return envelope to increase the response rate.

→ 4 recipients did not wish to participate and 50 did not respond

↓

68 recipients responded to the questionnaire with detailed narratives of how their fragrance allergy affected their quality of life (59 women and 9 men).

↓

Their narratives were categorized and formed the basis for 13 items comprising the fragrance QoL index and were discussed in a panel of experts on contact allergy to ensure relevance.

↓

1. Pilot study: a semi-structured telephone interview or person-to-person interview testing each item for clarity, relevance and comprehension in subjects with a fragrance allergy (n=3) and without a fragrance allergy (n=7). Furthermore, a panel discussion was held among experts in the field of contact allergy.

↓

Revision of the questionnaire according to comments from the interviews and discussion.

↓

2. Pilot study: a semi-structured telephone interview or person-to-person interview testing each item for clarity, relevance and comprehension among those with a fragrance allergy (N=4) and individuals who were not fragrance allergic (N=6).

↓

Minor revisions to the questionnaire according to comments from the interviews and discussion

↓

The fragrance QoL index was tested in a postal questionnaire survey together with SF36v2, DLQI and disease severity questions among 550 persons with fragrance allergy and 1100 without a fragrance allergy; the response rate was 66%.

↓

A retest of the fragrance QoL index was done in 193 persons; the response rate was 72.5%.

↓

Data were manually entered into a database and checked for typing errors. Statistical analyses were made in SPSS and quality metric health outcome scoring software. Rasch test was done in Winstep Software.

→ The fragrance QoL index was translated into English according to standardized methods.

Figure 2. The fragrance QoL index

Your own evaluation of your rash

The following statements concern how you currently feel about your rash. Please put a cross on the line that best agree with how you feel. The numbers range from 0 to10, where 0 is completely disagree, 5 is partly agree and 10 is strongly agree.

1. Your rash has had a negative effect on your quality of life.

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

2. You must take special measures in your daily life to avoid situations that could provoke your rash.

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

3. You are often bothered by fissures and/or cracked skin.

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

4. You are often bothered by itchy skin.

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

5. You are often bothered by pain or smarting.

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

6. Your rash has caused you to take special measures in order to do your work/studies, *e.g. use gloves, be exempt from certain duties, change job/studies, stop working, or other similar measures to avoid provoking your rash.*

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree — ☐ Not applicable

7. You restrict physical contact with your family/friends in order to avoid provoking your rash.

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

8. Your rash often makes you irritable or stressed.

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

9. You are often worried about being exposed to things that can provoke your rash.

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

10. You feel less attractive because of your skin disease.

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

11. You miss being able to smell nice.

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

12. You know what provokes your rash. *This means that you have a clear idea of what provokes/exacerbates your rash.*

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

13. You feel people should show more consideration towards your illness.

Completely disagree — 0 1 2 3 4 5 (Partly) 6 7 8 9 10 — Strongly agree

Figure 3. Scatter plot of the correlation between the Fragrance QoL index and the DLQI
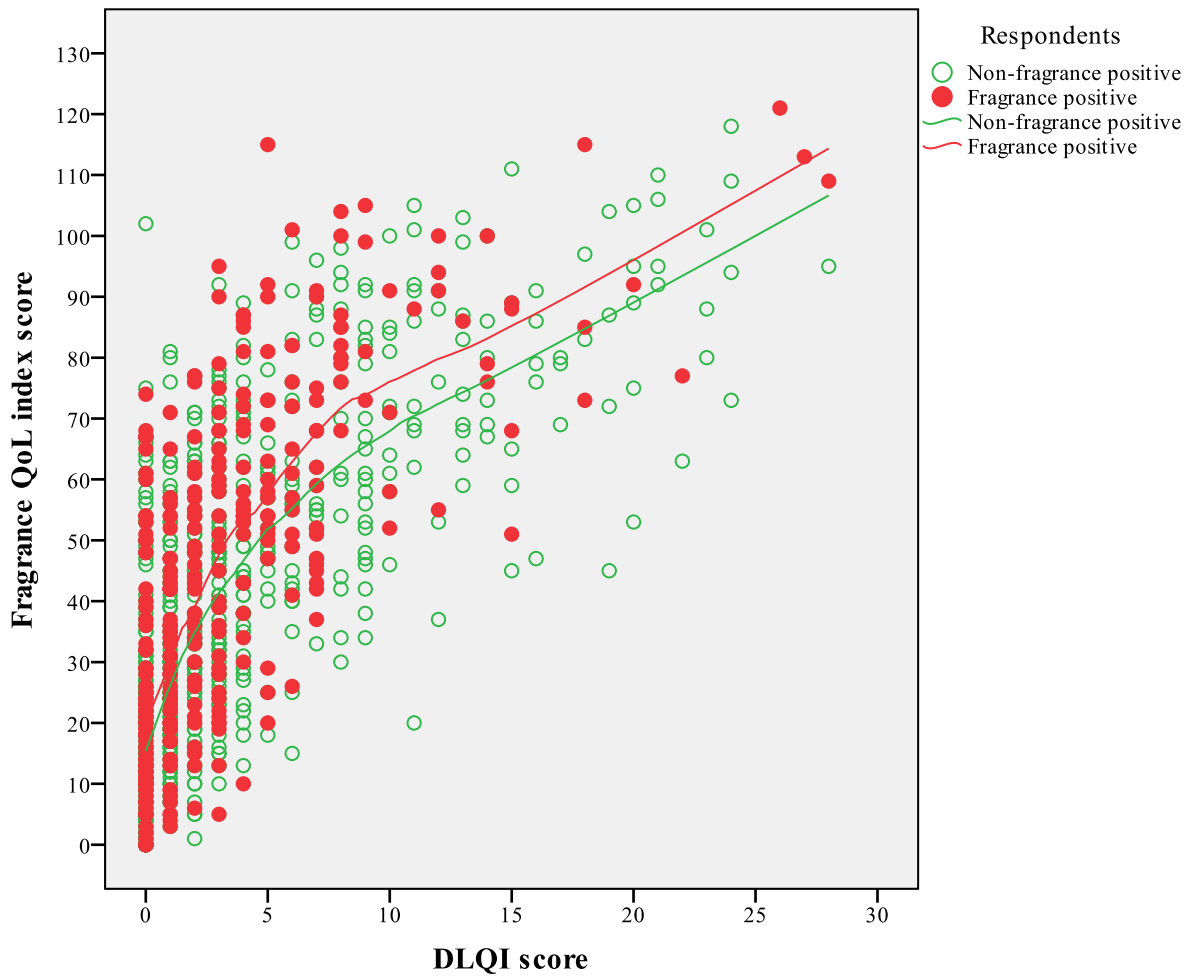
Fragrance positive: at least one positive patch test reaction to a fragrance screening marker/ingredient
Non-fragrance positive: no positive patch test reaction to any fragrance screening marker/ingredient.
Each line represents an estimate (kernel) of the relation between the means for the fragrance positive (red) and the non-fragrance positive (green). A significant nonlinear correlation is observed between the DLQI and the fragrance QoL index scores (Spearman correlation, p<0.001).

Figure 4. Correlation between quality of life (QoL) and self estimated disease severity

A. QoL measured with the fragrance QoL index

B. QoL measured with the DLQI

C. QoL measured on the SF36-MCS

D. QoL measured on the SF36-PSC

■ Fragrance positive respondents; rS=0.51, p<0.001
■ Non-fragrance positive respondents; rS= 0.55, p<0.001

■ Fragrance positive respondents; rS=0.56, p<0.001
■ Non-fragrance positive respondents; rS=0.60, p<0.001

■ Fragrance positive respondents; rS= -0.14, p<0.015
■ Non-fragrance positive respondents; rS= -0.15, p<0.001

■ Fragrance positive respondents; rS= -0.17, p<0.002
■ Non-fragrance positive respondents; rS= -0.25, p<0.001

Legend: Self-estimated disease severity was measured on a visual analogue scale (VAS), where 0 is no eczema and 10 is very severe eczema. The quality of life was measured with (A) the fragrance QoL index , (B) DLQI , (C) SF36v2: mental component summary score (MCS) and (D) SF36v2: physical component summary score (PCS).
r_S: Spearman correlation coefficient.
Non-fragrance positive: patch test negative reaction to all fragrance screening markers/ingredients.
Fragrance positive: at least one positive patch test reaction to a fragrance screening marker/ingredient.

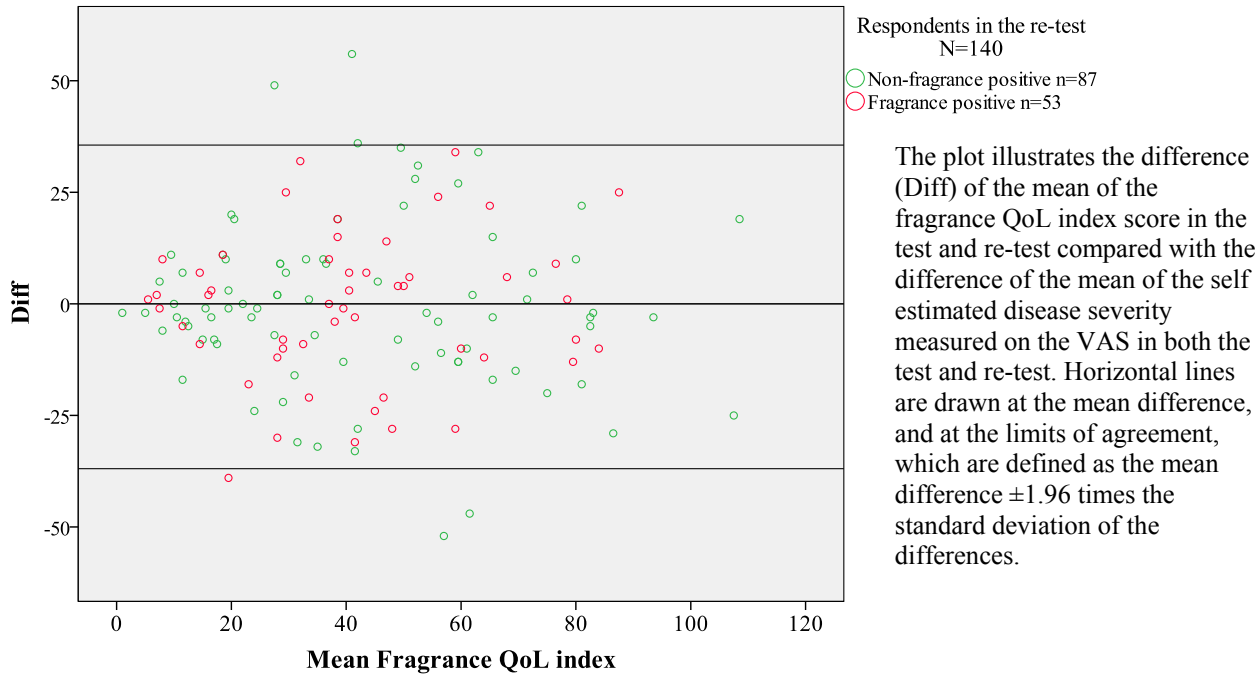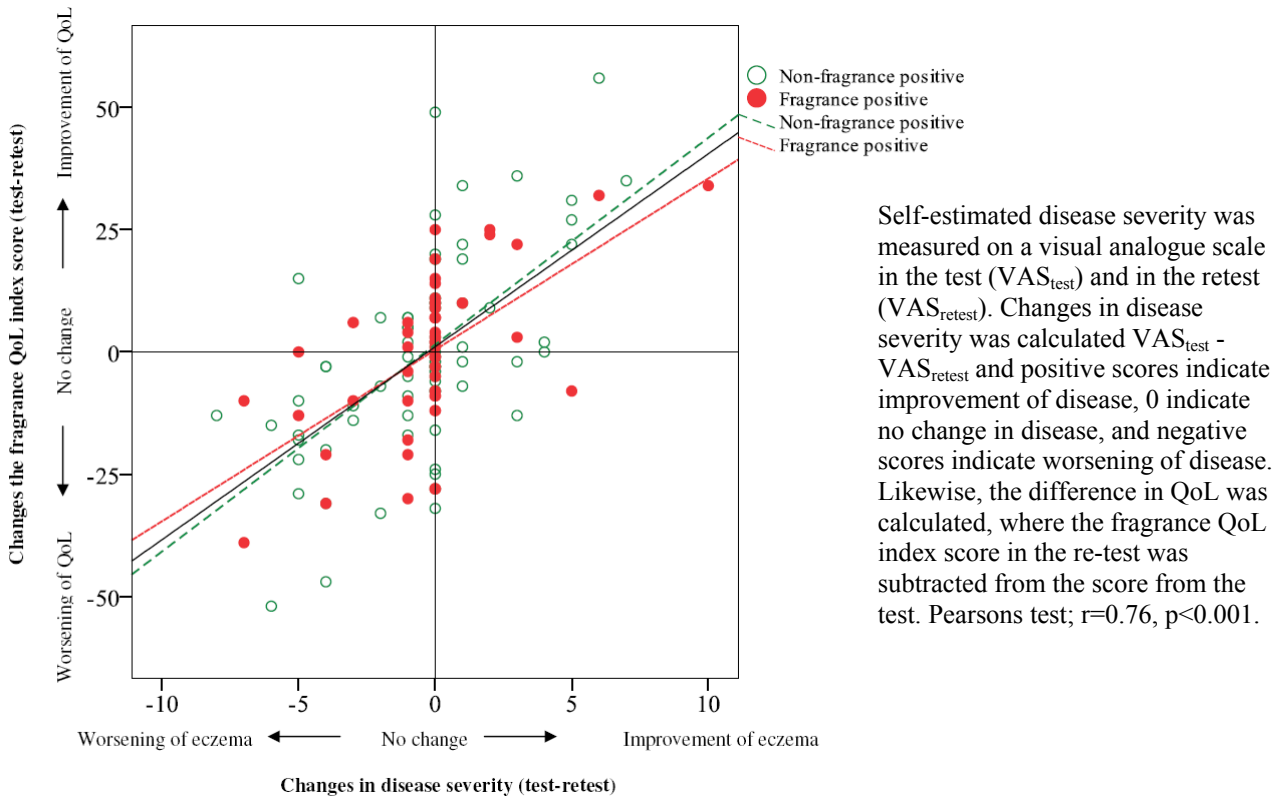## Figure 5. Bland & Altman plot illustration of the correlation of the test-retest

Respondents in the re-test
N=140
○ Non-fragrance positive n=87
○ Fragrance positive n=53

The plot illustrates the difference (Diff) of the mean of the fragrance QoL index score in the test and re-test compared with the difference of the mean of the self estimated disease severity measured on the VAS in both the test and re-test. Horizontal lines are drawn at the mean difference, and at the limits of agreement, which are defined as the mean difference ±1.96 times the standard deviation of the differences.

Figure 6. Scatter plot of the Fragrance QoL responsiveness to change with changes in disease severity (test-retest)

○ Non-fragrance positive
● Fragrance positive
Non-fragrance positive
Fragrance positive

Self-estimated disease severity was measured on a visual analogue scale in the test (VAS_{test}) and in the retest (VAS_{retest}). Changes in disease severity was calculated VAS_{test} - VAS_{retest} and positive scores indicate improvement of disease, 0 indicate no change in disease, and negative scores indicate worsening of disease. Likewise, the difference in QoL was calculated, where the fragrance QoL index score in the re-test was subtracted from the score from the test. Pearsons test; r=0.76, p<0.001.

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10.4. Manuscript IV

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**Fragrance allergy and quality of life –
a case control study**

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Maria V Heisterberg, Torkil Menné and Jeanne D Johansen.
National Allergy Research Centre, Department of Dermato-allergology,
Copenhagen University Hospital Gentofte, University of Copenhagen, 2900 Hellerup, Denmark

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Abstract

Background: Fragrance ingredients can cause contact allergy, which may affect quality of life (QoL). However, few studies have investigated this topic.

Objective: Investigation of QoL life among subjects with a fragrance allergy compared to other eczema patients.

Method: A case–control survey was sent to subjects with positive patch test reaction to a fragrance ingredient/marker (n=550) and to a control group (n=1100). It contained questions on eczema and the newly developed fragrance QoL index. Participants had been consecutively patch tested at Gentofte University Hospital (2000-2010). Response rate was 65.7%. Information on patch test data were retrieved from the National Contact Dermatitis Database.

Results: An increase in impairment of QoL was observed in women with fragrance allergy compared to the control group (p=0.042), which was not found among men. Several factors played a significant role on impairment of QoL in women: number of fragrance allergies, severity of the patch test reaction, age together with recent diagnosis and allergy to specific fragrance ingredients/markers.

Conclusion: Fragrance allergic subjects are just as affected in their quality of life as other eczema patients. However, women, and in particular recently diagnosed young women, seem to be more impaired in their QoL compared to other eczema patients.

Introduction

Allergy to a fragrance ingredient is a life long condition. Symptoms fluctuate and may be potentially avoidable if not exposed to the allergen. However, avoidance can be difficult because many different consumer products contain allergenic fragrances (1;2). The main exposures are cosmetic products comprising many different product categories: soaps, shampoos, cleansing wipes, deodorants, creams, sun protecting lotions, fine fragrances, aftershaves etc. Other exposures to fragrance ingredients could be in topical medicaments, toys, cleaning agents and detergents.
A change in behaviour in persons with a fragrance allergy when trying to avoid fragrances may be expected and also an increased awareness or even fear of exposure, which could influence their QoL. We know that QoL in subjects with eczema is impaired (3-5), not only due to the disease activity but other factors also play a role (6). However, little is known of the impairment of QoL in fragrance allergic subjects. The aim of this study was to assess if and how fragrance allergy affects QoL in fragrance allergic subjects compared to subjects without fragrance allergy using the newly developed and validated instrument, the fragrance QoL index. Furthermore, to investigate if certain fragrances ingredients affect QoL more than others and if the number of fragrance allergies and the severity of the patch test reaction affect QoL.

Method:

Study design
The study was designed as a case-control study. A questionnaire was sent by post to all participants and included a stamped, addressed, return envelope. The procedures involved in conducting the survey have been described previously.

Study population
All participants were age 18−70 years of age and had been consecutively patch tested at Gentofte University Hospital during 2000-2010. The case group (n=550) included individuals with at least one positive patch test to a fragrance ingredient/marker. The control group (n=1100) was non-fragrance positive individuals, which means they had no positive patch test reactions to fragrance markers/ingredients. The non-fragrance positive subjects were matched on age (± 1 year), gender and patch test year (± 1 year) to the fragrance positive subjects.

Quality of life measure:
The fragrance QoL index is a disease specific instrument to investigate QoL in fragrance allergic subjects. It consists of 13 items reflecting their subjective feelings at the time they fill in the questionnaire. The items were answered on a visual analogue scale from 0 to 10. A summarized fragrance QoL index score can be calculated with a maximum of 130 and minimum of 0. The fragrance QoL index has recently been validated in one study in a Danish eczema population (Manuscript III) and translated to English using standardized methods (7;8).

Database information
The National Contact Dermatitis Database is managed by the National Allergy Research Centre at the Department of Dermato-Allergology at Gentofte University Hospital. It contains information on patch test date, reactions and demographic characteristics. All patch tests were done according to international guidelines (9) using Finn Chambers® applied on the back with Scanpore tape® (Vitalfo Scandinavia, AB, Allerød, Denmark) for a period of 2 days. Readings were done on Day 2, 3 or 4 and 7 according to the recommendation of the International Contact Dermatitis Research Group (10). Patch test reactions were read and classified into different categories. In this paper we will investigate the positive reactions comprising: plus (+), plus (++), and plus (+++) reactions and the negative reactions, which refer to all the other reactions. During the 10 years study period (2000-2010) there have been developments in the diagnostics of fragrance allergy. At Gentofte

University Hospital the Fragrance mix II and hydroxyisohexyl 3-cyclohexene carboxaldehyde were introduced in the Baseline series in 2005 (11). From 2007, all patients investigated for allergy were additionally patch tested with our perfume series, which consist of the 26 fragrances which must be declared on cosmetic products according to the EU Cosmetic Directive (12). Furthermore, from January 2010 the oxidized forms of both limonene and linalool were also included as screening markers of fragrance allergy. However, by matching controls for the patch test year a possible bias due to the change in patch test materials over time will be minimized. Age groups were made on the basis of percentiles, to ensure a representative number of fragrance positive subjects in each group. The two fragrance mixes (fragrance mix I and fragrance mix II) consist of several different fragrance ingredients (13-15) and some of the participants had also been tested with these individual fragrance ingredients. Thus we counted the number of fragrance allergies in the following way: It was counted as one allergy when a subject had a positive patch test reaction to an individual fragrance ingredient or to a mix where they had not been tested with the individual fragrances, or had been negative to them. It was also counted as one allergy when a subject had a positive patch test reaction to one of the mixes and one of its constituents. It was counted as two allergies if a subject had a positive patch test reaction to a mix and two of its constituents etc.

Statistics and data management

Statistical analyses were performed using the Statistical Product and Service Solution package (SPSS® Inc., Chicago, IL, USA) for windows version 19. Standard methods were used for the descriptive statistics, crude percentages, mean and standard deviation. The analyses for differences between case group and control group were done accounting for the matching in: 1) a conditional logistic regression model, which is a model designed for analysing responses in a case-control setting where one or several controls are matched to one case; 2) univariate analyses performed accounting for the matching. Several logistic regression analyses and Mann Whitney tests were preformed stratified by case group and control group to test for differences and confounding factors within each group. The binary logistic regression analyses was checked by Hosmer-Lemeshow goodness of fit test. In all the statistical analyses, a p value of < 0.05 was considered significant.

Results

The demographics and disease burden of the study population

The questionnaire was sent to 1650 participants and 1084 (65.7%) responded. No difference was found in response rates between fragrance positive women and non-fragrance positive women (p=0.22), and likewise for the men (p=0.07). No age difference was found in the response rates between fragrance positive and non-fragrance positive control groups. There was no difference in the point prevalence of having eczema between women with a fragrance allergy and their controls (p=0.07), and likewise for the men (p=0.41) (Table 1). For both men and women having a fragrance allergy showed a statistically significant association with having other allergies (p_{women}= 0.001; p_{men}= 0.03). Rhinitis was statistically significantly more frequent in fragrance positive men compared to non-fragrance positive men (p=0.006). This was not observed among the women. Surprisingly, having been diagnosed with anxiety was significantly more frequent among the non-fragrance positive women than fragrance positive women (p=0.04) and a similar tendency was seen among the men; however, not of statistical significance (p=0.17). Men with fragrance allergy were significantly more frequently diagnosed with diabetes than non-fragrance positive men (p=0.037), which was not seen among the women. Multiple chemical sensitivity (MCS) was mainly observed among women (n=24), only one non-fragrance positive man had MCS according to Lacour's criteria for diagnosing MCS (16). Significantly more women with fragrance allergy had MCS than non-fragrance positive women (p=0.016).

The Quality of life and fragrance allergy stratified by gender
Quality of life measured with the Fragrance QoL index showed a statically significant difference between the fragrance positive case group and the non-fragrance positive control group (p=0.042). However, this difference was only among the women, where a significant QoL impairment was observed (p=0.014) and no significant difference was observed among the men (p=0.732). When performing a multi regression analysis separately for women and men including parameters showing statistically significant differences in disease burden/conditions (Table 1) we found that QoL impairment was still significantly associated with having a positive patch test reaction to a fragrance ingredient for women (p=0.042). For the men and their controls we again found no significant association to QoL and fragrance allergy (p=0.163).
The fragrance positive women showed a significant difference compared with the non-fragrance positive women in how much they worry about being exposed to things that can provoke their rash; they also felt that people should be more considerate of their illness, and they felt greater impairment of physical contact compared with their control group. Some gender similarities were observed as both men and women with a fragrance allergy felt that they more frequently had to take special measures to avoid situations that could provoke their rash; they all missed to smell nice and felt they had a better understanding of what provokes their rash compared to the control groups (Table 2).
Gender differences were quite apparent in many aspects regarding QoL and fragrance allergy. In overall, men were not as affected by fragrance allergy in their QoL. Thus, results from the men are shown in the tables and figures and but not mentioned in the text.

The Quality of life in women at different at age groups and stratified by patch test year
Impairment of QoL for women with fragrance allergy was not significantly correlated with age (Pearson's test; p=0.24). However, for the non fragrance positive women a significant inverse correlation was observed as they were less affected in their QoL with higher age (Pearson's test; p=0.02). Impairment of QoL for each of the age groups stratified by time of patch test showed a significant difference in the youngest age group. This difference is shown in figure 1. and illustrates the QoL impairment for different age groups over time. Thus, the more recently women in the youngest age group had been diagnosed with a fragrance allergy, the greater QoL impairment (trend test, p=0.03). This increased QoL impairment in the youngest age group most recently diagnosed with a fragrance allergy was also significant compared with the older age groups (QoL in the youngest age group of women diagnosed in 2009 and 2010 compared to the older age groups; Univariate analysis p=0.016).

Quality of life in women and allergy to specific fragrance markers
Allergies to some fragrance ingredients/markers affect QoL, more than others, among the women. In our study population the prevalence of having a positive patch test reaction to a fragrance marker was highest for fragrance mix I (n=174), followed by *Myroxylon pereirae* (n=94) and fragrance mix II (n=54). The top ten fragrance markers of allergy among those who responded to the questionnaire survey are shown in Table 3 together with their QoL impairment. A significant greater QoL impairment was detected among women with an allergy to fragrance mix I (FMI), fragrance mix II (FMII), and *Myroxylon pereirae* (MP). The strongest significance was seen for hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), which also showed the greatest QoL impairment of all the individual fragrance markers among the women.

Quality of life in women and multiple allergies
In women, a gradual significant increase in impairment of QoL in relation to number of positive patch test reactions to fragrance ingredients/markers (p=0.001) was seen for the women (Figure 2).

Quality of life in women and severity of the patch test reaction
The severity of patch test reactions vary, the stronger the reaction the higher the score (+,++,+++). A plus one reaction (+) was observed in 200 subjects, a plus two reaction (++) was observed in 171 subjects and a plus three reaction (+++) in 8 subjects. As only 8 subjects had a plus 3 reaction we combined these subjects with those who had a plus two reaction in order to give a better description of the population (Figure 3). Women displayed a significant linear increase in QoL impairment in relation to severity of the patch test reaction (correlation analysis; p=0.008).

Discussion

The results of this matched case-control study show that subjects with a fragrance allergy are affected in their QoL. Gender differences in QoL were apparent throughout the results as fragrance allergy in general seems to affect women more than men. Thus, women with a fragrance allergy show a significant greater QoL impairment compared to their control group. It is well known that gender differences exist in contact allergy and it is well documented that more women than men are affected (17;18); most likely due to their different exposure patterns (17). Women and men have different obligations in their daily lives both at work and at home (19) which can affect their disease and thus affect their QoL, which our study also indicates. The gender differences we observe in relation to QoL may be explained by the fact that women in general are more exposed to fragrance ingredients compared with men and therefore women have to make a greater effort and more changes in their behavior to avoid fragrance ingredients. In addition, it is a significant sacrifice for them to miss fragrances in their daily lives. We found that especially young women who had been examined for allergy recently show a significant greater QoL impairment. It could be argued that this tendency is seen because it is more difficult to live with fragrance allergy at the present day because of different exposure patterns than 10 years ago. However, then we would expect to see this impact on QoL in all the age groups recently diagnosed, which we do not. Thus, it is more likely an illustration of how young women in particular find it very difficult when initially diagnosed, but then learn to live with their fragrance allergy and manage it. Previous studies have documented that the impact of QoL in relation to being patch tested showed improvement in QoL (20), especially among subjects with a positive patch test reaction (5).
A factor that may influence why men with a fragrance allergy did not display the affect in QoL, as were seen among the women, could partly be because of confounders. For example, the prevalence of occupational eczema was higher among non-fragrance positive men (22.1%) compared with fragrance positive men (15.7%) and occupational eczema has been linked to increased QoL impairment (21;22). Although this difference was not of statistical significance it indicates that the study population is heterogeneous and other factors could exist and be attributed to the fact, that only minor differences were observed in QoL among fragrance positive men and their control group, compared to that observed for the women.
Allergy to some fragrance ingredients/markers clearly play a larger role than others on the impact on QoL in women. Allergy to HICC, in particular, is associated with a greater QoL impairment in women. This may be due to its wide spread use in consumer products (23;24), which makes the fragrance ingredient difficult to avoid. Furthermore, the allowed concentration of HICC in cosmetic products has been too high for many years (25-27) and thereby exposure to products containing HICC could lead to sensitization and elicitation of a contact allergy. Thus, industry must take steps to lower doses of fragrance ingredients in products to below elicitation doses otherwise subjects with a fragrance allergy will be at risk of getting a reaction, which also affects their QoL.
Severity of the patch test reaction was significantly associated with increased impairment of QoL and this could be interpreted such that the more severe the patch test reaction, the lower the

elicitation threshold in the individual and the more likely it would be to provoke an allergic response (25;28), leading to a greater QoL impairment. The number of fragrance allergies a subject has was also shown to have a significant association with QoL impairment. Again, this could be an indication that the more allergies a person has, the more exposures one has to avoid and maybe because of synergistic effects it is more likely to get an elicitation of the contact allergy (29;30), which influence QoL.

Overall, the control group seems to be a good match for subjects with a fragrance allergy. However, it is difficult to find an appropriate control group. We chose other eczema patients as we expect they would have a similar disease burden of eczema and thus the differences we observed be attributed to the fragrance allergy. We did find that some other diseases/conditions had significantly different prevalence among the fragrance positive and their controls in women and men, respectively (Table 1). However, when adjusting for these significant differences in a multiple logistic regression analysis no difference was observed; thus women still had a significant greater QoL impairment while men did not show any significant effect on QoL. To further eliminate bias the control group was chosen among subjects examined at the same hospital and thereby we could eliminate referral bias. The controls were matched on gender and age to minimize those as confounding effects. Furthermore, controls were matched for time of patch test to minimize any confounding effect of examination procedures in particular (31;32), but also differences in referral and exposure patterns.

In conclusion, QoL is affected by having a fragrance allergy. Women, and in particular young women, with recent diagnosis of fragrance allergy are worst affected. Number of fragrance allergies and severity of the allergy influence the impact on QoL. Furthermore, having an allergy to FMI, FMII, MP, and especially to HICC is associated with a significant greater QoL impairment.

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Correspondence to:
Maria Vølund Heisterberg, MD
National Allergy Research Centre, Department of Dermato-allergology, Copenhagen University Hospital Gentofte, University of Copenhagen, 2900 Hellerup, Denmark.
e-mail:maria.voelund.heisterberg@regionh.dk
Tel: 0045 3977 7305
Fax: 0045 3997 7118

Table 1. Demographic and disease burden of the study population N=1084

	Respondents									
	Women					Men				
	Fragrance positive		Non-fragrance positive			Fragrance positive		Non-fragrance positive		
	n	(%)	n	(%)	p	n	(%)	n	(%)	p
Participants	424	(33.3)	848	(66.7)	-	126	(33.3)	252	(66.7)	-
Responders	290	(68.4)	551	(65.0)	0.22	89	(70.6)	154	(61.1)	0.07
Eczema										
Point prevalence	160	(56.7)	271	(50.0)	0.07	42	(47.7)	81	(52.6)	0.41
Atopic dermatitis[i]	62	(21.4)	98	(17.8)	0.21	16	(18.0)	22	(14.3)	0.47
Occupational eczema	45	(15.5)	80	(14.5)	0.70	14	(15.7)	34	(22.1)	0.21
Other allergies[ii]	**183**	**(63.1)**	**222**	**(40.3)**	**0.001**	**44**	**(49.4)**	**54**	**(35.1)**	**0.03**
Other skin diseases	118	(40.7)	214	(38.5)	0.52	24	(27.0)	38	(24.7)	0.60
psoriasis	17	(5.9)	53	(9.6)	0.06	4	(4.5)	10	(6.5)	0.52
acne	26	(9.0)	41	(7.4)	0.44	7	(7.9)	8	(5.2)	0.41
urticaria	43	(14.8)	65	(11.8)	0.21	5	(5.6)	6	(3.9)	0.51
skin diseases not mentioned above	52	(17.9)	89	(16.2)	0.51	11	(12.4)	19	(12.3)	0.97
Other disease[iii]										
Rhinitis	94	(34.3)	159	(30.6)	0.28	**34**	**(41.5)**	**35**	**(24.1)**	**0.006**
Food allergy	70	(25.5)	104	(20.2)	0.08	12	(14.6)	22	(15.4)	0.89
Asthma	52	(19.0)	102	(19.6)	0.84	18	(21.7)	22	(15.1)	0.22
Depression	52	(19.0)	102	(19.5)	0.87	10	(12.0)	23	(16.2)	0.40
Diabetes	17	(6.2)	32	(6.1)	0.94	**14**	**(16.7)**	**11**	**(7.6)**	**0.037**
Anxiety	**16**	**(5.9)**	**53**	**(10.2)**	**0.04**	2	(2.4)	9	(6.3)	0.17
Heart disease	11	(4.0)	35	(6.7)	0.13	8	(9.6)	14	(9.7)	0.99
COLD[iv]	8	(3.0)	25	(4.8)	0.22	1	(1.2)	8	(5.6)	0.14
Stroke	4	(1.5)	18	(3.4)	0.11	5	(6.0)	10	(7.0)	0.79
Heart attack	2	(0.7)	9	(1.7)	0.27	6	(7.1)	7	(4.9)	0.49
MCS[v]	**14**	**(4.8)**	**10**	**(1.8)**	**0.016**	0	-	1	(0.6)	-

N: Numbers

%: Crude percentages. If respondents had not answered the item they were counted as missing in the analysis.

Logistic regression accounting for the matching; P- values <0.05 considered statistically significant are highlighted red. Fishers exact test was performed with small samples, but no statistical significant differences were found.

[i]. Atopic dermatitis, UK criteria (34)

[ii] : A positive patch test to a marker of the baseline series other than a fragrance marker/ingredient.

[iii]. The diagnosis was established by the question "Has your doctor ever told you that you have/have had ……"?.

[iv]. COLD: Chronic Obstructive Lung Disease

[v]:MCS: Multiple Chemical Sensitivity syndrome; La Cour's criteria (16)

Table 2. Quality of life measured with the fragrance QoL index

| QoL instruments / Fragrance QoL index | Respondents — Women and men | | | | | Respondents — Women | | | | | Respondents — Men | | | | | Fragrance positive women vs. fragrance positive men Mann-Whitney test |
| | Fragrance positive N= 379 | | Non-fragrance positive N=705 | | Univariate analysis accounting for the matching | Fragrance positive N=290 | | Non-fragrance positive N=551 | | Univariate analysis accounting for the matching | Fragrance positive N=89 | | Non-fragrance positive N=154 | | Univariate analysis accounting for the matching | |
	Mean	SD	Mean	SD	p	Mean	SD	Mean	SD	p	Mean	SD	Mean	SD	p	p
Item 1. Eczema and QoL	4.1	3.2	3.9	3.2	0.156	4.3	3.3	3.8	3.3	0.059	3.7	2.9	4.0	3.0	0.518	0.165
Item 2. Avoid situations	5.2	3.6	4.0	3.7	<0.001	5.3	3.6	4.2	3.8	<0.001	4.5	3.4	3.3	3.4	0.010	0.075
Item 3. Fissures and cracks	4.7	3.5	4.7	3.7	0.862	4.8	3.5	4.6	3.7	0.446	4.4	3.6	5.0	3.6	0.284	0.388
Item 4. Itching	5.6	3.4	5.3	3.6	0.218	5.6	3.4	5.2	3.6	0.128	5.4	3.3	5.5	3.3	0.761	0.590
Item 5. Pain and smarting	4.1	3.4	4.1	3.6	0.774	4.2	3.4	4.0	3.7	0.444	3.8	3.2	4.2	3.5	0.391	0.333
Item 6. Work and school	3.4	4.0	3.2	4.0	0.371	3.6	4.1	3.2	4.0	0.175	2.7	3.9	3.1	3.9	0.514	0.228
Item 7. Impaired physical contact	0.9	2.1	0.5	1.5	0.001	1.0	2.3	0.5	1.5	<0.001	0.5	1.2	0.4	1.4	0.755	0.639
Item 8. Irritation and stress	2.8	3.0	2.7	3.1	0.627	2.8	3.1	2.7	3.2	0.468	2.5	2.8	2.6	3.0	0.743	0.568
Item 9. Worried	3.3	3.2	2.5	3.1	<0.001	3.5	3.4	2.5	3.1	<0.001	2.6	2.6	2.2	2.7	0.353	0.093
Item 10. Less attractive	2.6	3.2	2.6	3.3	0.861	2.8	3.4	2.7	3.4	0.636	1.8	2.5	2.0	3.0	0.614	0.082
Item 11. Miss to smell nice	3.7	3.6	1.9	3.1	<0.001	4.1	3.7	2.0	3.1	<0.001	2.6	3.1	1.4	2.7	0.004	0.002
Item 12. Understand triggers	7.2	3.1	5.3	3.8	<0.001	7.3	3.0	5.5	3.8	<0.001	6.6	3.3	4.4	3.7	<0.001	0.102
Item 13. Consideration from people	2.0	2.9	1.4	2.4	<0.001	2.2	3.0	1.5	2.5	0.001	1.4	2.3	0.9	1.8	0.105	0.150
Fragrance QoL index score	44.0	27.3	40.4	27.0	0.042	45.7	28.2	40.5	27.5	0.014	38.4	23.6	39.6	25.0	0.732	0.05

N: number of respondents

Mean: The items were answered on a visual analogue scale (0-10). The higher the score the more affected the respondents are.

SD: Standard deviation

P values with statistical significant difference (p<0.05) are highlighted red.

Fragrance positive: at least one positive patch test reaction to a screening marker/ingredient of fragrance allergy.

Non-fragrance positive: no positive patch test reaction to a screening marker/ingredient of fragrance allergy.

Table 3. The prevalence of positive patch test reactions to the top 10 fragrance markers/ingredients and QoL in subjects tested with these fragrance ingredients/markers

| | Positive patch test reactions | | | | Fragrance QoL index score | | | | | |
| Patch test markers of fragrance allergy | Women | | Men | | Women | | | Men | | |
	Numbers positive/tested	%	Numbers positive/tested	%	Fragrance positive Mean (SD)	Non fragrance positive Mean (SD)	p-value	Fragrance positive Mean (SD)	Non fragrance positive Mean (SD)	p-value
Fragrance mix I	174/825	21.1	51/239	21.3	46.0 (28.0)	41.4 (27.7)	0.03	39.2 (23.2)	39.1 (25.0)	0.89
Myroxylon pereirae	94/838	11.2	22/241	9.1	48.2 (28.0)	41.7 (27.8)	0.02	30.8 (20.7)	40.1 (24.8)	0.13
Fragrance mix II	54/524	10.3	17/160	10.6	50.8 (27.3)	43.0 (28.2)	0.04	36.9 (26.3)	42.1 (25.4)	0.49
Oxidized linalool	7/68	10.3	1/23	4.3	53.6 (32.7)	49.5 (25.8)	0.39	54.0 (-)	43.0 (30.1)	0.81
Oxidized limonene	6/67	9.0	3/22	13.6	53.5 (41.4)	49.3 (25.0)	0.38	47.3 (22.8)	42.9 (31.1)	0.99
Evernia furfuracea	25/281	8.9	10/90	11.1	48.6 (22.3)	45.9 (28.7)	0.44	39.1 (23.9)	45.8 (25.9)	0.53
Hydroxycitronellol	49/669	7.3	7/193	3.6	34.8 (26.5)	43.6 (27.8)	0.18	29.5 (20.6)	40.5 (24.9)	0.27
Evernia prunastri	48/760	6.3	20/219	9.3	48.4 (24.8)	42.4 (27.9)	0.07	37.4 (18.1)	39.6 (24.9)	0.64
HICC	32/671	4.8	14/194	7.2	56.6 (26.3)	42.2 (27.6)	0.002	29.5 (23.6)	41.2 (24.9)	0.13
Isoeugenol	25/670	3.0	6/193	3.1	45.1 (30.0)	42.9 (27.7)	0.41	47.4 (30.7)	39.9 (24.6)	0.61

QoL: quality of life.
HICC: hydroxyisohexyl 3-cyclohexene carboxaldehyde.
Mean: mean of the Fragrance QoL index score; high score indicate greater QoL impairment.
SD: Standard deviation
The screening for fragrance allergy comprises of 31 markers/ingredients: The 10 listed above and methyl 2-octynoate, cinnamal, citral, eugenol, cinnamyl alcohol, α-hexyl cinnamal, butylphenyl methylpropional, amyl cinnamal, geraniol, benzyl salicylate, coumarin, amyl cinnamyl alcohol, majantol, citronellol, and the following did not have any positive reactions: non-oxidized limonene, non-oxidized linalool, benzyl cinnamate, benzyl alcohol, farnesol, benzyl benzoate, α-isomethylionone, anis alcohol.
A statistically significant difference (p<0.05) between subjects with a positive patch test reaction to the individual fragrance marker compared with subjects with no fragrance positive reaction, univariate analysis accounting for the matching.

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Figure 1. Quality of life in women with fragrance allergy in different age groups stratified by patch test year
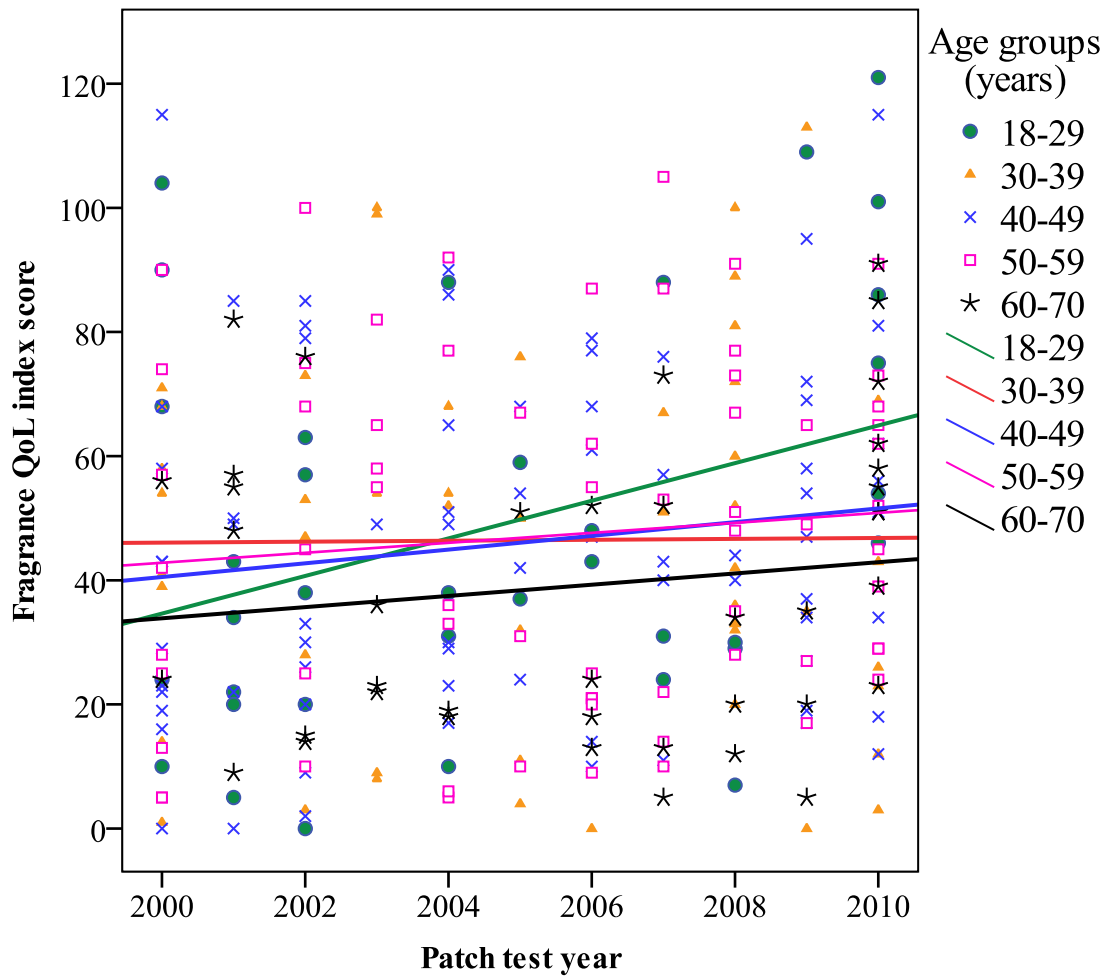

Scatter plot with trend lines illustrating QoL in women in different age groups stratified by patch test year. Each line represents the linear association between the QoL during different patch test years for each age group: the green line (age group 18-29; trend test, p=0.03); orange line (age group 30-39; p= 0.95); blue line (age group 40-49; p= 0.20); pink line (age group 50-59; p=0.39) and black line (age group 60-70; p=0.32).

Figure 2. Quality of life in relation to number of patch test reactions to fragrance ingredients/markers

Error bars: 95% CI
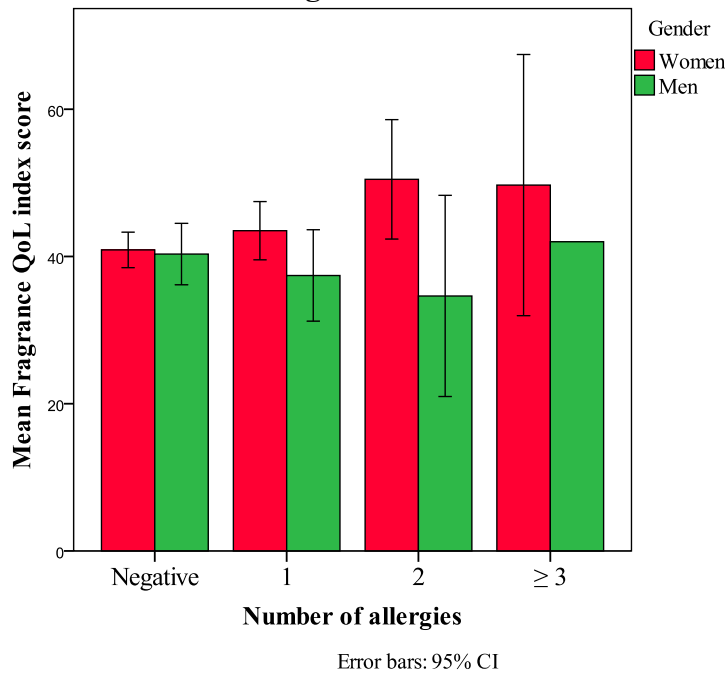
Bar chart with 95% confidence intervals illustrating quality of life measured with the fragrance QoL index for men (green) and women (red) in relation to the number of patch test reactions they have. The higher the score the more impaired the QoL. Number of allergies are determined by the number of positive patch test reactions (+,++,+++) they have to any of the fragrance ingredients/markers tested. However, if a subject has a reaction to one of the mixes and to one of its constituents, it is counted as one allergy. If a person has reacted to one of the mixes and two different individual constituents, it is counted as two allergies etc. Correlation analyses: $P_{women} = 0.01$; $P_{men} = 0.33$.

Figure 3. Quality of life in relation to severity of the patch test reaction to a fragrance ingredient/marker (negative, +,++/+++) in women and men
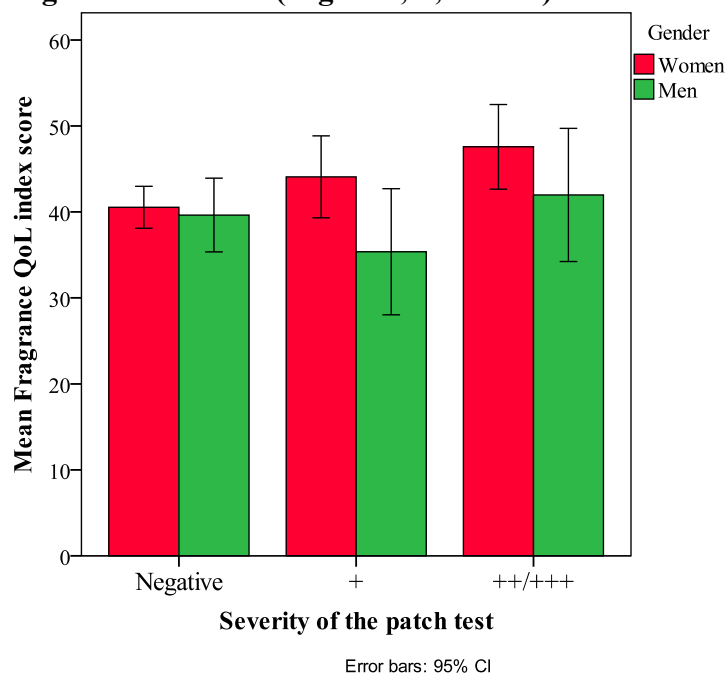
Error bars: 95% CI

Bar chart with 95% confidence intervals (CI) illustrating the difference in QoL in relation to intensity of a patch test reaction to a fragrance marker/ingredient in women (red) and men (green).
Correlation analyses: $P_{women} = 0.008$; $P_{men} = 0.877$.

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