UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCES

Allergy to natural ingredients and polyethylene glycols in cosmetic and pharmaceutical products

PhD Thesis

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This thesis has been submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen, Denmark, on September 1st 2021

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ISBN nr. 978-87-93624-97-9

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This thesis is based on four original manuscripts. The manuscripts are referred to by the following Roman numerals throughout the thesis:

PART I:

I. Bruusgaard-Mouritsen MA, Johansen JD, Zachariae C, Kirkeby CS, Garvey LH. Natural ingredients in cosmetic products - A suggestion for a screening series for skin allergy. *Contact Dermatitis.* 2020 Oct;83(4):251-270.

II. Bruusgaard-Mouritsen MA, Johansen JD, Garvey LH. Facial contact dermatitis caused by cosmetic-relevant allergens. Accepted for publication in *Contact Dermatitis* (August 31st 2021).

PART II:

III. Bruusgaard-Mouritsen MA, Johansen JD, Garvey LH. Clinical manifestations and impact on daily life of allergy to polyethylene glycol (PEG) in ten patients. *Clin Exp Allergy*. 2021 Mar;51(3):463-470.

IV. Bruusgaard-Mouritsen MA, Jensen BM, Poulsen LK, Johansen JD, Garvey LH. Optimizing investigation of suspected allergy to polyethylene glycols. *J Allergy Clin Immunol.* 2021 May 27:S0091-6749(21)00825-3.

Preface

This thesis is based on scientific work carried out at the National Allergy Research Centre, the Dermatology Department, the Allergy Clinic and the Laboratory of Medical Allergology at the Department of Dermatology and Allergy, Copenhagen University Hospital – Herlev and Gentofte between 2017 and 2021. The projects received financial funding from the Ministry of Environment, the Aage Bangs Foundation and Harboe Fonden. All are gratefully acknowledged.

First, I would like to express my sincere gratitude to my principal supervisor Professor Jeanne Duus Johansen. Thank you for your excellent scientific guidance, for sharing your outstanding knowledge on dermatology and for always keeping the door to your office open. It has been an honour to work under your great leadership.

I am also deeply grateful to Lene Heise Garvey for introducing me to the allergologic field. Thank you for sharing your immense clinical knowledge on allergology and for your superb supervision. Your passion for allergic patients is a true inspiration.

To Claus Zachariae, thank you for introducing me to the clinical aspects of dermatology.

I would also like to say a special thank you to Lars K. Poulsen. Thank you for your invaluable scientific knowledge on laboratory techniques and immunological aspects of allergy; and thank you Bettina Margrethe Jensen for all your kind help in the laboratory, including analysing and interpreting results. Working with you in the laboratory was filled with laughter and positivity.

I also wish to thank all my colleagues at the National Allergy Research Centre and the Laboratory of Medical Allergology for creating an inspiring and supportive work and research environment.

A special thanks to the nurses, secretaries and doctors at the Dermatology Department for helping with patient recruitment and testing patients; and to the nurses, secretaries and doctors at the Allergy Clinic for helping with practical issues in the clinic. Also, a big thank you to all the patients and participants in the studies conducted in this thesis — without you, these studies would not have been possible.

I especially wish to thank my parents and my parents-in-law. Thank you for your continuous support, encouragement and logistic help whenever needed.

Finally, a heartfelt thanks to my ever-supportive husband, Mads, and our daughters, Emma and Isabella, for your unconditional love and for always believing in me.

Maria Anna Bruusgaard-Mouritsen

Copenhagen, September 2021

Abbreviations

App	Application
CAS	Chemical Abstract Service
CI	Confidence Interval
COVID	Corona virus disease
EAN	European Article Number
FDA	American Food and drug Administration
FMI	Fragrance Mix I
FMII	Fragrance Mix II
HR test	Basophile histamine release test
IgE	Immunoglobulin E
INCI	International Nomenclature of Cosmetic Ingredients
mm	Millimeter
MCI	Methylchloroisothiazolinone
MI	Methylisothiazolinone
MOAHLFA index	**M**ale, **O**ccupational dermatitis, **A**topic dermatitis, **H**and dermatitis, **L**eg dermatitis, **F**ace dermatitis, **A**ge > 40 years).
mRNA	Messenger RiboNucleic Acid
MW	Molecular weight
OR	Odds ratio
PEG	Polyethylene glycol
Pet	Petrolatum
PMA	Phorbol 12-myristate 13-acetate
SPT	Skin prick test
w/v	Weight per volume

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Summary in English

Natural ingredients and the excipients polyethylene glycols (PEGs) are commonly used in cosmetic and pharmaceutical products. Some ingredients in cosmetic and pharmaceutical products are well-known allergens. Others are rarely reported as allergens and may be overlooked. Knowledge about rare allergens´ allergenic potential, including investigation procedure and diagnosis is sparse.

In PART 1 of this thesis, the objectives were to identify the most common natural ingredients in cosmetic products used in Denmark, propose a screening test series with natural ingredients relevant for immediate-type and delayed-type allergy (manuscript I) and evaluate this screening test series in patients with facial dermatitis (manuscript II). In addition, to characterize patients with allergic facial dermatitis and cosmetic-induced allergic facial dermatitis and evaluate patch test reactions to 27 selected cosmetic-relevant allergens (manuscript II).

In manuscript I, the presence of natural ingredients in 10,067 cosmetic products on the Danish market were investigated by use of Kemiluppen, a non-profit application helping consumers avoid problematic substances in cosmetic products. A total of 121 different natural ingredients were found in ≥ 30 cosmetic products. A screening test series with 21 cosmetic-relevant natural allergens was developed based on natural ingredients commonly listed in Kemiluppen and additionally described in the literature as known allergens.

In manuscript II, a prospective skin test study was conducted with the screening test series with natural ingredients developed in manuscript I which was tested on patients with facial dermatitis. In total, 66 patients were included. The most common patch test positive cosmetic-relevant natural ingredients were linalool hydroperoxides, propolis and limonene hydroperoxides. Potato and peanut were the most common prick test positive cosmetic-relevant natural ingredients, however, without any relation to the use of cosmetic products. The 66 patients filled in a questionnaire about their facial dermatitis and use of natural ingredients in cosmetic products. Facial dermatitis affected nearly all patients´ quality of life and caused limitations to their daily life. A total of 43 patients (65.2%) preferred cosmetic products branded as “natural” for healthier (65.2%), less allergenic (50%) and/or environmental (34.8%) reasons.

We also performed a retrospective study investigating the prevalence, risk factors and relevance of cosmetic-relevant allergens of facial dermatitis patients among 8740 patients aged ≥ 18 years patch tested at the Dermatology Department at Gentofte Hospital from 2010 to 2019. A total of 26.2% were diagnosed with facial dermatitis. Risk factors for facial dermatitis were female gender and atopic dermatitis. Of these, 30.6% had cosmetic-induced facial dermatitis. Risk factors for cosmetic-induced facial dermatitis were female gender and age > 40 years. Atopic dermatitis was associated with a lower risk of developing cosmetic-induced facial dermatitis. The most common cosmetic-relevant allergens were fragrances and preservatives.

In PART 2 of this thesis, the objectives were to characterize patients with PEG allergy (manuscript III), evaluate skin prick test (SPT) and *in vitro* reactivity over time to different MW PEGs, and assess cross-sensitization patterns in PEG allergy (manuscript IV).

In manuscript III, clinical manifestations of immediate-type allergy and impact on daily life among 10 PEG-allergic patients diagnosed at the Allergy Clinic at Gentofte Hospital between 2010 and 2019 were reported. Detailed clinical history was obtained from patient files supported by a retrospective questionnaire. Pharmaceutical products and cosmetic products were primary causes of PEG allergy. Anaphylaxis was primarily caused by analgesic tablets, antibiotic tablets and depot-steroids. Eight patients had experienced at least one adrenaline-requiring anaphylactic reaction prior to the diagnosis. Seven patients had repeated reactions before diagnosis (median 3, range 2–6). Median time from first reaction to diagnosis was almost two years (median 20 months, range 2-120 months). Impact on daily life improved after diagnosis with a median likert score of 7 before diagnosis compared to 4 after diagnosis. After diagnosis, accidental re-exposure was reported in 4/10 patients despite great efforts to avoid it, however, none reported severe life-threatening reactions after diagnosis.

In manuscript IV, the 10 PEG-allergic patients from manuscript III and 16 non-PEG-allergic healthy volunteers were skin prick tested once or twice 26 months apart with lower MW PEGs (PEG 300, 3000, 6000) followed by high MW PEG (PEG 20,000) in stepwise, increasing concentrations and polysorbate 80 and poloxamers. Patients previously testing positive to PEG 3000 and/or 6000 on SPT also tested positive to PEG 20,000. Patients with a longer interval since diagnosis tended to test negative to low MW PEGs and positive only to high MW PEG. During SPT, three patients developed systemic urticaria despite careful titration. Eight patients were cross-sensitized to poloxamer 407 and three to polysorbate 80. All controls tested negative. An optimized

investigation algorithm for patients with suspected PEG allergy was developed. The algorithm was based on a titrated stepwise SPT with PEGs of increasing MW thereby minimizing the risk of inducing anaphylaxis during investigation. *In vitro* Basophil Histamine Release test (HR test) showed limited usefulness. *In vitro* HR test with passive sensitization was not useful.

In conclusion, it was shown that natural ingredients are widely used in cosmetic products. Only few of the selected natural ingredients in this study seem to have an allergenic potential great enough to qualify regular testing in standard investigation series. Facial dermatitis is common and frequently caused by cosmetics. Fragrances and preservatives are still the most common causes of facial dermatitis. Facial dermatitis affects the patients´ quality of life. Further preventive actions and optimization of investigation procedures should be implemented.

Allergy to PEG is rare, difficult to diagnose and affects patients´ daily life due to the widespread use of PEG in cosmetics and pharmaceutical products. Skin test reactivity to PEG can decrease over months to years. Titrated SPT with high MW PEG 20,000 in increasing concentrations can be diagnostic, when lower MW PEGs test negative. An optimized investigation algorithm based on skin prick testing is recommended when PEG allergy is suspected until an effective *in vitro* diagnostic test has been developed. Cross-sensitization between PEGs and poloxamer 407 and polysorbate 80 is common, but the clinical implications remain unknown.

Dansk resumé

Naturlige ingredienser og fyldstoffer som polyethylen glycol (PEGs) bruges hyppigt i kosmetiske produkter og lægemidler. Nogle ingredienser i kosmetik og lægemidler er kendte allergener. Andre angives sjældent som allergener og kan derfor overses. Viden om sjældne allergeners allergifremkaldende potentiale, herunder udredningsprocedure og diagnose er sparsom.

I DEL 1 af denne afhandling var målene at identificere de mest almindelige naturlige ingredienser i kosmetiske produkter, der anvendes i Danmark, og foreslå en screeningstestserie med naturlige ingredienser relevant for straksallergi og kontaktallergi (manuskript I) og evaluere denne screeningstestserie hos patienter med ansigtseksem (manuskript II). Desuden at karakterisere patienter med allergisk ansigtseksem og kosmetik-induceret allergisk ansigtseksem og evaluere lappetestreaktioner på 27 udvalgte kosmetiske allergener (manuskript II).

I manuskript I blev hyppigheden af naturlige ingredienser i 10.067 kosmetiske produkter på det danske marked undersøgt ved brug af Kemiluppen, en non-profit applikation, der hjælper forbrugere med at undgå problematiske stoffer i kosmetiske produkter. Der blev i alt fundet 121 forskellige naturlige ingredienser i ≥ 30 kosmetiske produkter. En screeningstestserie med 21 kosmetik-relevante naturlige allergener blev udviklet ud fra de naturlige ingredienser, der hyppigst indgik i Kemiluppen og yderligere var beskrevet i litteraturen som kendte allergener.

I manuskript II udførte vi en prospektiv hudtestundersøgelse med screeningstestserien med naturlige ingredienser, der var udviklet i manuskript I, som blev testet på patienter med ansigtseksem. I alt blev 66 patienter inkluderet. De kosmetik-relevante naturlige ingredienser, der hyppigst forårsagede en positiv lappetest, var linalool hydroperoxider, propolis og limonene hydroperoxider. Kartoffel og peanut var de hyppigste priktest-positive kosmetik-relevante naturlige ingredienser, dog uden nogen relation til brugen af kosmetiske produkter. De 66 patienter udfyldte et spørgeskema omhandlende deres ansigtseksem samt brug af kosmetiske produkter med naturlige ingredienser. Ansigtseksem påvirkede næsten alle patienters livskvalitet og forårsagede begrænsninger i deres daglige liv. I alt foretrak 43 patienter (65,2%) kosmetiske produkter som var mærket "naturlige" af sundere (65,2%), mindre allergifremkaldende (50%) og/eller af miljømæssige (34,8%) årsager.

Vi gennemførte også en retrospektiv undersøgelse, der undersøgte forekomst, risikofaktorer og relevans af kosmetik-relevante allergener hos ansigtseksempatienter blandt 8740 patienter ≥ 18 år som var lappetestet på hudafdelingen på Gentofte Hospital fra 2010 til 2019. I alt var 26,2% diagnosticeret med ansigtseksem. Risikofaktorer for ansigtseksem var kvindeligt køn og atopisk eksem. Af disse havde 30,6% kosmetik-induceret ansigtseksem. Risikofaktorer for kosmetik-induceret ansigtseksem var kvindeligt køn og alder > 40 år. Atopisk eksem var forbundet med en lavere risiko for at udvikle kosmetik-induceret ansigtseksem. De mest almindelige kosmetik-relevante allergener var parfumestoffer og konserveringsmidler.

I DEL 2 af denne afhandling var målene at karakterisere patienter med PEG-allergi (manuskript III), evaluere hudpriktest (SPT) og *in vitro*-reaktivitet over tid for PEGs med forskellig molekylvægt (MW), og vurdere kryds-sensibiliseringsmønstre ved PEG-allergi (manuskript IV).

I manuskript III blev kliniske manifestationer ved straksallergi og påvirkning af dagligdagen vurderet blandt 10 PEG-allergiske patienter diagnosticeret på Allergiklinikken på Gentofte Hospital mellem 2010 og 2019. Detaljeret klinisk sygehistorie blev indhentet fra patientjournaler samt et retrospektivt spørgeskema. Lægemidler og kosmetiske produkter var primære årsager til PEG-allergi. Anafylaksi var primært forårsaget af smertestillende tabletter, antibiotika og depotsteroider. Otte patienter havde oplevet mindst en adrenalin-krævende anafylaktisk reaktion inden diagnosen. Syv patienter havde flere reaktioner før diagnosen (median 3, interval 2-6). Mediantiden fra første reaktion til diagnose var næsten to år (median 20 måneder, interval 2-120 måneder). Påvirkning af dagligdagen blev forbedret efter diagnosen med en median likert score på 7 før diagnosen sammenlignet med 4 efter diagnosen. Efter diagnosen blev der rapporteret utilsigtet re-eksponering hos 4/10 patienter på trods af store bestræbelser på at undgå det; der var dog ingen som havde haft livstruende reaktioner efter diagnosen var stillet.

I manuskript IV blev de 10 PEG-allergiske patienter fra manuskript III og 16 ikke-PEG-allergiske kontrolpersoner priktestet én eller to gange med 26 måneders mellemrum med lavere MW PEGs (PEG 300, 3000, 6000) efterfulgt af høj MW PEG (PEG 20.000) i trinvis stigende koncentrationer samt polysorbate 80 og poloxamer 407. Patienter, der tidligere testede positiv på PEG 3000 og/eller 6000 på SPT, testede også positiv på PEG 20.000. Patienter med et længere interval siden diagnosen havde tendens til at teste negativt på lav MW PEGs og positivt på høj MW PEG. I forbindelse med SPT udviklede tre patienter systemisk nældefeber trods omhyggelig titrering. Otte patienter var krydssensibiliserede over for poloxamer 407 og tre over for polysorbate 80. Alle

kontrolpersoner testede negative. En optimeret udredningsalgoritme til patienter, hvor der er mistanke om allergi over for PEGs, blev udviklet. Algoritmen var baseret på en titreret trinvis SPT med PEGs med stigende MW, hvilket minimerede risikoen for at inducere anafylaksi under undersøgelsen. *In vitro* Basophil Histamine Release test (HR test) viste begrænset anvendelighed. *In vitro* HR test med passiv sensibilisering var ikke brugbar.

Vi konkluderede, at naturlige ingredienser er meget udbredt i kosmetiske produkter. Kun få af de udvalgte naturlige ingredienser i dette studie synes at have et allergisk potentiale stort nok til at kvalificere regelmæssig testning i standardtestserier i forbindelse med udredning. Ansigtseksem er hyppig og ofte forårsaget af kosmetik. Parfumestoffer og konserveringsmidler er stadig de hyppigste årsager til ansigtseksem. Ansigtseksem påvirker patienternes livskvalitet. Yderligere forebyggende foranstaltninger og optimering af undersøgelsesprocedurer bør implementeres.
Allergi over for PEG er sjælden, vanskelig at diagnosticere og påvirker patienternes dagligdag på grund af den udbredte brug af PEG i kosmetiske produkter og lægemidler. Hudtestreaktivitet over for PEG kan mindskes over måneder til år. Titreret SPT med stigende koncentrationer af høj MW PEG 20.000 kan være diagnostisk, når lavere MW PEGs er negative. En optimeret udredningsalgoritme baseret på hudpriktest anbefales, når der er mistanke om PEG allergi, indtil en effektiv *in vitro* test til diagnostik er blevet udviklet. Krydssensibilisering mellem PEGs og poloxamer 407 og polysorbate 80 er almindelig, men de kliniske konsekvenser er endnu ukendte.

1. Introduction

Cosmetic and pharmaceutical products contain numerous ingredients, some of which cause either immediate-type or delayed-type allergic reactions. The most common allergenic ingredients in these products have already been identified, but new allergens continuously emerge. Some allergens are rare but may still have significant impact on patients´ daily lives.

Thousands of ingredients are available for composing cosmetic products, many of which seem to have an allergenic potential judged from exhaustive reviews.[1–5] However, no complete overview exists. At the same time, there is no general strategy for assessing and limiting existing skin allergens in cosmetic products.[6] This means that there is a risk of skin allergy for consumers, when using cosmetics. Some ingredients have been identified as relatively frequent causes of allergy in cosmetic products.[1,7] Others are reported occasionally or even very rarely. A lack of awareness about new allergens means that it may be easy to overlook an allergic cause of skin reactions such as facial dermatitis, which is a common disease entity affecting around 14% of patients being patch tested in Europe.[8]

Cosmetic products branded as "natural" have increased in popularity.[7,9] Although some natural ingredients are known as potential sensitizers, such as most fragrances and compositae, other natural ingredients, not thought to be allergenic, may have the potential to cause allergic reactions. To date, a thorough investigation of natural ingredients in cosmetic products on the European market and their allergenic potential has not been conducted. In this thesis, we set out to investigate the most frequent causes of allergy to cosmetic products with a focus on natural ingredients and potentially overlooked allergens of both immediate and delayed-type allergy.

Like cosmetics, pharmaceutical products often contain numerous excipients, many of which are not familiar to consumers, patients, or healthcare personnel. The regulation is different for pharmaceutical products than for cosmetics, and there is great variation in rules for declaration of excipients in pharmaceutical products; polyethylene glycols (PEGs) is an example of this. They are excipients widely used in cosmetic and pharmaceutical products.[10] Allergy to PEG is rare and despite the potential to cause severe anaphylactic reactions, only little is known about PEGs as sensitizers.[10] At the present time, there is limited published information about PEGs as allergens

including clinical manifestations in PEG-allergic patients, diagnostic tests and identification of high-risk patients.

Over-all objective

The over-all objectives of this thesis were to investigate the causes of allergy to cosmetic products with a focus on natural ingredients as potentially overlooked allergens; and to investigate the impact and optimized diagnosis of rare allergens in immediate-type allergic reactions to pharmaceuticals, with PEG as an example. This was done in four studies (manuscript I, II, III, IV). Manuscript I and II concern the first part and manuscript III and IV the second part of the objective.

2. Background

PART 1 Allergy to cosmetic products with a focus on natural ingredients

Cosmetic products

Cosmetic products are widely used worldwide and the range of cosmetic products is expanding rapidly due to a large consumer population.[7,11–13] The European Cosmetic Regulation "Regulation (EC) N° 1223/2009" defines a cosmetic product as "any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) 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".[14]
Cosmetics are topical products used to enhance the appearance of the body and many cosmetic products are designed for use on the face and hair. Common cosmetic products include skin lotions/cleaners, deodorants, shampoos, hair styling products, perfumes and make-up products e.g. lipstick, mascara, eye shadow, foundation and rouge. Women use more cosmetics than men and more women than men are diagnosed with facial dermatitis. However, there is also an increasing consumption of cosmetics by men as well as adolescents and children.[11–13]

The chemical composition of cosmetic products is increasingly complex and ingredients have become more diverse. It is estimated that approximately 10% of the general population experience delayed-type or immediate-type allergic reactions to cosmetics and this number is expected to increase.[1,15,16]

Natural ingredients

The terms "natural cosmetics" and "natural ingredients" are vague as there is no legislative definition of what natural covers. The European Cosmetic Regulation defines natural ingredients in cosmetics according to the origin of the ingredients and the production method.[14] Nevertheless, the term "natural ingredients" can be interpreted in several ways as the natural ingredients may be extracted from nature; originally found in nature even though they are made synthetically; or extracted from nature and subsequently chemically modified in a laboratory.

The market for natural cosmetic products is estimated to be approximately 5% of the total Danish cosmetic market.[9] An increase is expected in the coming years driven by a growing consumer focus on the usage of sustainable raw materials.
In particular, the product groups creams and lotions, shampoos and cleansing products make up a significantly amount of the total market for natural cosmetic products.[9]

The popularity of natural cosmetic products among consumers is based on the assumption that they are healthier, safe without 'unnecessary chemistry' and have environmental benefits.[9,17] However, natural ingredients are not necessarily less harmful or more healthy than synthetic, non-natural products as the natural cosmetic products may contain extracts from plants or flowers which may be allergenic. Thus, allergy cannot be prevented by choosing natural cosmetic products. Cosmetic products can contain up to 100% natural ingredients, exclusive water, but natural cosmetic products are generally formulated in the same way as conventional cosmetic products. Therefore, the natural cosmetic products may consist of the same ingredients, with the exception that, for example, a natural version of an ingredient is used instead of a non-natural ingredient. Unnecessary chemistry, such as potentially allergenic preservatives may be avoided, as some plant substances can have a preservative effect, but overall, natural cosmetic products do not preclude the use of preservatives or perfumes compared to conventional cosmetic products. Although cultivation of selected plants in cosmetic products may have a smaller environmental impact, natural cosmetics are not themselves protective of the environment, as manufacturing, packaging, use, and disposal after use are not prerequisites for a product to be labelled as “natural”.[9]
Natural cosmetic products have been classified as more expensive than conventional products in an investigation performed by The Danish Ministry of Environment.[9] This is based on more expensive ingredients, an increased production price to avoid compromising the quality of the product, and the expense of labeling schemes which are required for documentation of natural ingredients in the cosmetic products. Overall, there is no evidence that natural cosmetics are better, e.g. less allergenic, healthier or better for the environment than other cosmetics.

The EU Cosmetic Regulation “Regulation (EC) N° 1223/2009” regulates cosmetic ingredients and cosmetic products on the European market for consumer safety.[14] All cosmetic products must comply with the Regulation. This also applies to natural cosmetic products meaning they must be safe to use.[14] Natural ingredients, including plant-derived ingredients (botanicals) in cosmetic products must be stated on the product label by International Nomenclature of Cosmetic Ingredients [INCI] names.[18] INCI names are standardised, internationally recognized names

designed to help identifying the chemical nature of each ingredient in a cosmetic product. INCI names for plant-derived ingredients are based on the botanical source in Latin followed by the common name in parentheses and possibly the plant part from which the ingredient has been produced, and type of preparation. Thus, an INCI name does not indicate a particular chemical composition, standard or purity.[18] The botanical ingredient is also assigned a CAS Registry Number which is "a globally accepted identifier of a chemical substance" that is supposed to "designate only one substance".[19] But in plant-derived ingredients, the CAS number refers to mixtures of unspecified composition which only identifies the plant source but not the chemical composition or unique chemical substances. This means that all plant-derived extracts coming from a particular plant species will be assigned the same CAS Registry number irrespective of derivation e.g. from seeds, leaves or roots, and which method has been used. There can also be numerous INCI names for ingredients derived from one plant describing the same material but provided with different CAS numbers. This adds confusion to the understanding of the nomenclature of ingredients in cosmetic products.

Allergic reactions to (natural) ingredients in cosmetics, symptoms and diagnosis

Contact allergy, also named delayed-type allergic reactions or type IV allergy, and less frequently immediate-type allergy to ingredients in cosmetics have been reported in the literature.[1–5,7,20,21]

Contact allergy and allergic contact dermatitis

Contact allergy is mediated by a T-cell mechanism causing inflammation, named allergic contact dermatitis at the skin site of exposure. The pathophysiological mechanism of contact dermatitis can be divided into two phases; a sensitization phase, where naïve T-cells are primed to recognize the allergen in question, proliferate and differentiate to effector T-cells. Most of the T-cells activated during the primary response will die when the allergen is removed, but a minority will develop into memory T-cells, which means that the individual has become sensitized.[22,23] The second phase of the reaction is elicitation, where re-exposure to the specific allergen is responsible for the recruitment and activation of the sensitized T-cells, resulting in inflammation i.e. the clinical manifestations of allergic contact dermatitis with erythema, oedema, infiltration, and possibly vesicles.[22,23]

Allergic contact dermatitis due to cosmetics is often suspected in patients with facial dermatitis who present for patch testing.[15] Common causes of facial allergic contact dermatitis are fragrances and preservatives but many other cosmetic ingredients are contact allergens and have been shown

to be common causes of facial dermatitis.[24–26] Investigations of facial allergic contact dermatitis may be complex due to excessive numbers of potential allergenic ingredients in cosmetics and consequently, the allergy may be overlooked. This also relates to new allergens, such as natural ingredients, emerging in cosmetic products.

Patch testing is the gold standard when diagnosing allergic contact dermatitis.[27] The diagnosis of allergic contact dermatitis is established when a patch test is positive and clinical relevance is found during exposure assessment. The exposure assessment is often conducted by reviewing the ingredient lists of products used by the patients. This assessment can also be performed by a chemical analysis of a product to establish the presence of an allergen.[27] Patch testing with the products used by the patient will in case of a positive or sometimes a doubtful response add evidence to the relevance assessment or even detect otherwise over-looked allergens.[17] Patch testing is further described in the Method section.

Contact dermatitis can also be caused by exposure to irritants, irritant contact dermatitis. The mechanism is a non-specific immune activation caused by damage to the skin barrier, but the exact pathways are still poorly understood.[23] Therefore, no test exists for diagnosing irritant contact dermatitis and it is not possible clinically to distinguish allergic and irritant contact dermatitis. The diagnosis of irritant contact dermatitis depends on a negative (or not relevant positive) patch test and sufficient exposure to irritants at the site of dermatitis. Many cosmetic products contain irritants and irritant contact dermatitis may be an overlooked condition caused by cosmetic products.

Immediate-type allergy and contact urticaria

Immediate-type allergy caused by ingredients in cosmetics is less commonly reported.[1]

Cosmetics are applied topically, and the most frequent symptom of immediate-type allergy to cosmetics is contact urticaria, a transient localized wheal and flare reaction appearing within 60 minutes upon exposure of an allergen. It is an IgE-mediated reaction resulting in histamine release from cross-linked IgE-receptors on the mast cell surface. This means that the skin reaction may spread and become more generalized, or extracutaneous manifestations may occur, in rare cases even anaphylaxis, referred to as Contact Urticaria Syndrome (CUS).[1] The IgE-mechanism will be further elaborated in the subsequent section on polyethylene glycols.

Diagnosis is made from a clinically relevant history and a positive skin prick test, prick-prick test, use test or provocation with the patient's own products and relevant ingredients. In some special cases, specific IgE may be available for detection such as for chlorhexidine.[28]

Natural cosmetics, such as emollients and moisturizers with plant protein derivatives, e.g. wheat and oat known with the potential to cause immediate-type allergy when ingested, are increasing in popularity as ingredients in cosmetics.[1,29,30] Protein sensitization can occur through various routes; gastrointestinal, respiratory and percutaneous, especially via an impaired skin barrier.[31–33] It is yet to be understood if and how these pathways of sensitization interact. But severe reactions from cosmetic applications with natural ingredients have been described, especially when the allergens have undergone modification such as hydrolysis. Wheat is probably the most well-known example. In Japan, more than 2000 cases of hydrolysed wheat-induced allergic reactions have been described following the use of a former popular facial soap with hydrolysed wheat gluten.[34,35] Severe allergic reactions have also been described following application of milk-containing topical products in milk-allergic patients.[31]

Contact urticaria, like contact dermatitis, comes both in an immunological and a non-immunological form.[1] Non-immunological urticaria is localized contact urticaria without previous sensitization to a specific allergen. The mechanism is not well understood and symptoms would often be milder and more confined than for immunological contact urticaria.[1]

To complicate matters further, contact dermatitis may also be caused by contact with protein-containing material, called protein contact dermatitis.[36] The symptoms are typically chronic dermatitis with acute flares upon contact with the protein-containing material. The condition is diagnosed by a positive prick-prick test to the suspected natural materials.[27] This is a condition most often described in occupations handling food and affecting the hands and arms.[37,38]

Prevalence of allergy to natural ingredients

To date, there are only very few systematic investigations and no exhaustive studies on the prevalence of allergy to natural ingredients due to the comprehensive use and unclear definition of what natural ingredients cover. Thus, it is impossible to give an overall estimate of the prevalence. Nor has there been any studies on how commonly natural ingredients are used in cosmetic products. In a recently conducted systematic review by Alinaghi et al., the most common natural ingredients causing allergic contact dermatitis in the general population were found to be fragrance mix I (3.5%), *myroxylon pereirae* (1.8%) and colophonium (1.3%).[39] Immediate-type reactions, i.e. immunological contact urticaria have also been described and in 2016, a review of cases due to plant-derived and animal-derived cosmetic ingredients was published and later an

encyclopaedia appeared.[1,3–5] It seems that apart from a few allergens, such as modified wheat, most evidence relies on the reporting of a few cases, which are not always well-described.

Diagnosing allergy to natural ingredients in cosmetics

The patch testing regimen for contact allergy to natural ingredients varies. In most centers, patients are tested with the European baseline series containing a few selected natural ingredients, including colophonium, ingredients in fragrance Mix I and II, lanolin alcohol, *myroxylon pereirae*, and sesquiterpene lactone mix. In 2020, propolis was added to the European baseline series following increasing focus on this ingredient´s allergenic potential.[40] The concentrations used are based on the recommendations from the European Society of Contact Dermatitis. In many cases, special series are added to the test panel, however, none which focus on natural ingredients. It is recommended to add patch tests with the patient's own cosmetic products.[27] In cases where a specific product is suspected, a repeated open application test can be performed, which mimics the normal repeated use of a cosmetic product.[27]

Skin prick testing with the patient's own products, standardized commercial skin prick test preparations and prick-prick test with fresh food are used during investigation of immediate-type allergy to natural ingredients in cosmetic products.

Diagnosing allergic contact dermatitis and immediate-type allergy to natural ingredients can be challenging. New natural allergens continuously emerge without guidelines or recommendations on test methods. It may be difficult and sometimes impossible to test patients with the exact same formulation and concentration as in the cosmetic product, since the INCI name or CAS number do not specify the chemical composition of the ingredient.

PART 2 Immediate-type allergy to polyethylene glycols

Polyethylene glycols

Polyethylene glycols (PEGs) were discovered for the first time in 1859 by two different chemists.[41] Since then, PEGs have been increasingly used as excipients in pharmaceutical, cosmetic and household products, but only rarely in food products. PEGs have various functions, e.g. as active ingredients in laxatives, optimizing the properties of a product or acting as a carrier molecule in chemo-therapeutics and other pegylated drugs.[10]

PEGs are generally considered safe and biologically inert and exposure to PEGs is nearly impossible to avoid due to the extensive use in everyday products.[10] Most recently, PEGs have

gained attention as an excipient in the mRNA-based COVID-19 vaccines from BioNTech/Pfizer and Moderna. During the first days of vaccination in the UK, two cases of anaphylaxis directed the suspicion against PEGs.[42]

PEGs are hydrophilic polymers of varying molecular weight (MW) and chain length. They are synthesized by polymerization of ethylene oxide, and water (figure 1). Molecular weights range between 200 and 50,000 g/mol.[10,43] PEGs can be divided into low MW PEGs which are viscous, clear liquids and high MW PEGs which are waxy, white solids.[10,44]

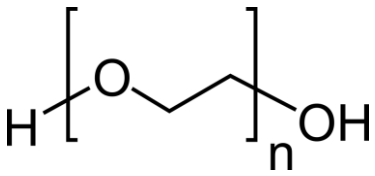

Figure 1. Chemical structure of polyethylene glycol. n = number of ethyleneoxide units.

PEGs have numerous synonyms, e.g. Macrogol, Kleanprep, Alkox, Carbovax, Polygol, Oxyethylene polymer, Polyoxyethylene diol and Polyoxyethylene ether (POE) among others.[10] The PEG nomenclature is inconsistent and varies between products and countries. In cosmetic products and food, PEGs are described by the average number of ethylene oxide units, for example, PEG 100, and in food products also as E1521.[10,45] In drugs and other pharmaceutical products, PEGs are commonly described by the synonym macrogol and the total molecular weight of the number of ethylene oxide units. The molecular weight of ethylene oxide is 44 g/mol and macrogol 4400 g/mol is calculated as 100 × 44 = 4400. Thus, PEG 100 and macrogol 4400 is the same compound but named differently depending on the product. In addition to this, PEGs can be added to products as mixtures of different oligomer sizes with various molecular weights. This means that PEG 4400 often constitutes a mixture of PEG molecules with both high and low MW but with an average molecular weight of 4400 g/mol.[10,46] PEG-allergic patients are at particular risk of re-exposure due to the widespread use, insufficient or misleading product labelling and lack of a standardized nomenclature.[10,47]

Structurally related derivatives

There are several structurally PEG-related derivatives that may have the potential to cause cross-sensitization in immediate-type allergy. PEG derivatives with a similar chemical structure are PEG ethers, PEG fatty acid esters, PEG amine ethers, PEG castor oils, PEG soy sterols, PEG-propylene glycol polymers (poloxamers) and PEG sorbitans (polysorbates). Although only limited knowledge of cross-sensitization exists, at least the two latter are relevant to PEG immediate-type allergy.[10,43,44,48,49] Cross-sensitization patterns have only been rarely investigated and the clinical relevance needs further clarification.[10,49,50]

Poloxamers are excipients generally recognized as safe and with low toxicity. They were introduced by Wyandotte Chemical Corporation during the late 1940s as the first block copolymers for industrial purposes.[51] Today, poloxamers are known with the synonyms Pluronic, Kolliphor and Synperonic and cover a large range of solids, pastes and liquids.[50,52] Poloxamers are commonly used in cosmetic and pharmaceutical products as surfactants, stabilizers, and solubilizers.[50]

Poloxamers are synthetic, non-ionic, amphiphilic copolymers arranged in a triblock structure (figure 2). The triblock structure is formed by a hydrophobic central chain of polypropylene glycol surrounded by two hydrophilic chains of PEG on each side.[50] There exist more than 50 poloxamers. Poloxamer 188 and poloxamer 407 are the most prevalent due to their great solubility in water.[53] All poloxamers have a similar chemical structure but differ in their molecular weight due to the variable number of polypropylene glycol and PEG units.[53] Therefore, each type of poloxamer has a different hydrophilic-lipophilic balance. Molecular weights varies from 1,100 to 14,000 g/mol.[52] The generic term “poloxamer” is commonly followed by a numerical value of three digits: the first two digits, multiplied × 100, indicates the molecular weight of the hydrophobic core of propylene glycol, and the last digit, multiplied × 10 gives the percentage of the hydrophilic PEG content. As an example, poloxamer 407 has a propylene glycol molecular mass of 4000 g/mol (56 propylene glycol units) and a 70% PEG content (101 PEG units).[54,55]

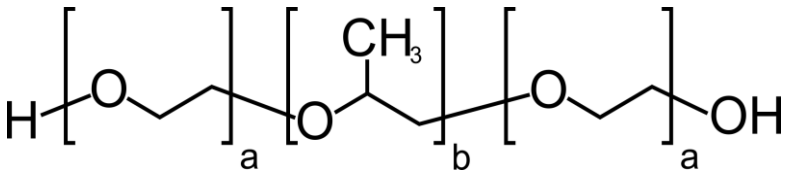

Figure 2. Chemical structure of poloxamer. a = PEG. b = polypropylene glycol.

Polysorbates are known with numerous synonyms, the most common being Tween®, Kolliphor, Scattics, Alkest, Canarcel, and E433 (food additive).[10]

Polysorbates are synthetic, non-ionic, amphiphilic surfactants derived from pegylated sorbitan esterified with a lipophilic group of fatty acids; e.g. lauric acid or oleic acid.[56]

The generic term "polysorbate" is followed by a number, e.g. 20, 40, 60 or 80. The number represent the total number of PEG units within the polymer chain and the lipophilic group associated with the pegylated sorbitan portion. As such, e.g. Polysorbate 80 has 80 PEG units linked to the lipophilic group of fatty acids, oleic acid (figure 3). Hence, in polysorbate 80, the PEG MW is 80 × 44 = 3520 g/mol. Polysorbate 20 has 20 PEG units linked to the lipophilic group of fatty acids, lauric acid. In polysorbate 20, the PEG MW is 20 × 44 = 880 g/mol. Polysorbates are oily liquids commonly used as emulsifiers, solubilizers and stabilizers in cosmetic and pharmaceutical products as well as food agents.[56] Polysorbate 80 has been used in vaccines and biologic pharmaceutical drugs for years. Most recently, attention has been drawn to polysorbate 80 as an excipient in the non-mRNA based COVID-19 vaccines Vaxzevria from AstraZeneca, Janssen vaccine from Johnson & Johnsen and NVX-CoV2373 vaccine from Novavax.[57–59] Polysorbate 20 is used as an excipient in the SARS-CoV-2 Sanofi vaccine with polysorbate 80 as an adjuvant.[59]

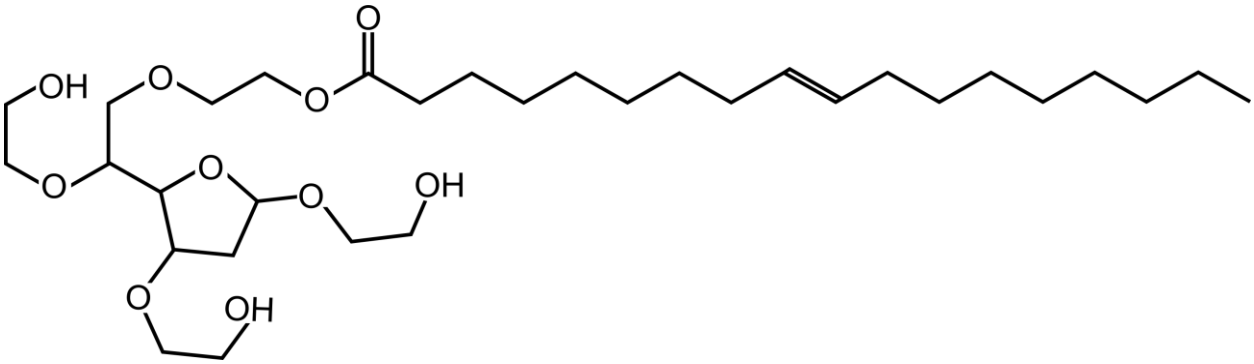

Figure 3. Chemical structure of polysorbate 80.

Allergic reactions to polyethylene glycols in pharmaceutical products, symptoms and diagnosis

Immediate-type allergy to polyethylene glycols

Immediate-type allergy is divided into two phases: a sensitization phase and an elicitation phase. In the sensitization phase, allergen exposure leads to the production and secretion of specific IgE-antibodies. These bind to high affinity IgE-receptors on mast cells and basophil granulocytes.

In the elicitation phase, allergen re-exposure results in the allergen binding of IgE-antibodies on the mast cell surface and cross-linking of two IgE-receptors. Within seconds the mast cell and the basophile granulocyte degranulate and release histamine and other mediators such as leukotrienes, tryptase, prostaglandins and heparin.

Immediate-type PEG allergy presents with symptoms from urticaria to severe allergic reactions including anaphylactic shock. Immediate-type PEG allergy was first described in 1977 in a 50-year old male patient who developed pruritus, erythema and exanthem following application of two PEG-containing antifungal agents.[60] Today, the most common products causing severe immediate-type allergic reactions are pharmaceutical products such as bowel preparations, depot steroid injections and tablets. However, immediate-type reactions to cosmetic products containing PEGs have also been described.[49,61–65]

Immediate-type allergy to PEG is rare but the true prevalence is unknown. A review by Wenande et al. found 37 case reports of PEG allergy between 1977 and 2016.[10] Since 1989, the American Food and Drug Administration (FDA) has registered 133 reports of anaphylaxis caused by PEGs. Four cases of PEG-induced anaphylaxis caused by laxatives are reported every year in the USA.[66] Several case reports and small case series have been published in the past decades from all over the world, especially since January 2021 due to the increased focus on PEG allergy following the worldwide implementation of the PEG-containing mRNA-based COVID-19 vaccines.[42,59,67,68] The first patient with PEG allergy in Denmark was diagnosed in 2010 and since then, 18 patients have been diagnosed at the Allergy Clinic at Gentofte Hospital; eight of them within the previous two years. As a result of continued extensive use of PEG, improved investigation procedures and increased awareness of PEG allergy, the prevalence is expected to rise.[10,62,66]

PEG-allergic patients often present with repeated, severe allergic reactions or anaphylaxis to structurally different drugs and other products, but PEG is rarely suspected as the culprit. Lack of awareness of PEG allergy among healthcare professionals as well as lack of standardized investigation guidelines compromise a correct diagnosis.

The diagnosis of PEG allergy is based on a convincing clinical history of an allergic reaction to one or more PEG-containing drugs combined with tests traditionally used in drug allergy investigation i.e. skin prick test, intradermal test or oral provocation, supplemented by *in vitro* tests.

At the Allergy Clinic at Gentofte Hospital, patients are investigated with a skin prick test series containing a panel of different MW PEGs. This test series has been continuously developed since 2010. Skin prick testing is not without risk for the patient as systemic reactions can occur following skin prick test.[10,69] Other tests, such as intradermal test and graded oral provocation with PEG-containing products are used in other allergy centers, however, these tests are associated with a high risk of inducing anaphylaxis.[10,43,66,69–74] Currently, the specificity and sensitivity for all these test modalities are unknown.

It has been suggested, that PEG allergy is primarily caused by an IgE-mediated mechanism.[62,75]

For some drug allergens, *in vitro* test reactivity can decline or be lost over time following lack of exposure. This has previously been shown for IgE to ethylene oxide, chlorhexidine and penicillin.[76–79] In PEG-allergic patients, it is unknown whether *in vivo* or *in vitro* reactivity decrease over time or if allergenic reactivity remains dormant until reactivated by re-exposure. Nor is it known whether PEG allergy can disappear permanently.

Contact allergy to polyethylene glycols

Contact allergy to PEG has been reported in the literature.[80–86] In all reported cases, contact allergy was caused by low MW PEGs and involved an impaired skin barrier. In a recent study of 836 patients, a high prevalence of 4.2% positive patch test reactions to PEG 400 (100%) was seen related to topical use of nitrofurazone preparations containing PEGs for skin infections etc.[87] In the Dermatology Department at Gentofte Hospital, all patients with facial dermatitis suspected of contact allergy have been patch tested with a special facial series containing rare allergens in cosmetics. PEG 400 (100%) by Allergeaze® and the related polysorbate 80 (5.0% in pet) delivered by Chemotechnique® have been part of this series. From 2015 to 2020, between 635 and 665 women were tested in total with each allergen, and no positive reactions were found (unpublished data). Thus, in our clinic PEG 400 is a rare contact allergen. The focus in this thesis will therefore be on immediate-type allergy to PEGs.

3. Objectives

PART 1 Allergy to cosmetic products with a focus on natural ingredients

Manuscript I Natural ingredients in cosmetic products – a suggestion for a screening series for skin allergy

- To identify the most common natural ingredients in cosmetic products used in Denmark.
- To investigate the allergenic potential of the most commonly used natural ingredients in cosmetic products based on published literature.
- To propose a screening test series with natural ingredients in cosmetics relevant for immediate-type and delayed-type allergy.

Manuscript II Facial dermatitis caused by cosmetic-relevant allergens

- To evaluate the screening test series with natural ingredients developed in manuscript I.
- To characterize patients with cosmetic-induced allergic facial dermatitis.
- To establish an overview of contact allergy to selected common cosmetic-relevant allergens.

PART 2 Immediate-type allergy to polyethylene glycols

Manuscript III Clinical manifestations and impact on daily life of allergy to polyethylene glycol (PEG) in ten patients

- To characterize clinical features of patients with PEG allergy.
- To investigate time to diagnosis and impact of a PEG allergy diagnosis on the daily life of patients diagnosed with allergy to PEG.

Manuscript IV Optimizing investigation of suspected allergy to polyethylene glycols

- To evaluate skin prick test reactivity over time to different MW PEGs.
- To evaluate *in vitro* reactivity over time to different MW PEGs by using histamine release test and histamine release test with passive sensitization.
- To assess cross-sensitization patterns in PEG allergy.

4. Methods

Test methods relevant for manuscript I-IV

The following test methods were applied in this thesis:
In PART 1, patch testing and skin prick testing were performed.
In PART 2, skin prick testing and basophil histamine release test with and without passive sensitization were performed.

Testing for contact allergy

Patch test

Patch testing is performed on the upper back using contact allergens suspended in petrolatum or aqua in aluminum 8-millimeter Finn® Chambers attached with Scanpore tape for 48 hours. Patch test readings on exposure site are done on day 2, day 3 or 4, and day 7, and based on palpation of the skin reaction and visual scoring. Reactions are classified according to the European guidelines as an allergic reaction graded into +1 (weak positive reactions: erythema, infiltration and possibly papules), +2 (strong positive reaction: erythema, infiltration, papules and vesicles) or +3 (extreme positive reaction: intense erythema, infiltration and coalescing vesicles), a negative reaction, an irritant reaction, or a doubtful reaction. Both irritant and doubtful reactions were interpreted as negative reactions in this thesis.[27]

Testing for immediate-type allergy

Skin tests and *in vitro* tests are used when investigating immediate-type allergy. Skin testing is recommended a minimum of four to six weeks after the allergic reaction to a potential allergen to avoid false-negative results.[88]
In skin tests, mast cells in the skin are exposed to the suspected allergen and if the test is positive, a wheal and flare response will appear on the skin caused by an IgE-dependent activation of mast cells.

Skin prick test

In skin prick test (SPT), a small volume with a high concentration of an allergen is pricked into the epidermis using a lancet. The test is performed on the forearm with the allergen extract, sometimes in duplicate. The reaction is read after 15 minutes. A saline solution is used as a

negative control and histamine 10 mg/ml is used as a positive control. A positive reaction is defined as a wheal diameter ≥ 3 mm.[89]
When testing fresh food, a prick-prick technique is utilized, by first pricking the fresh food with the lancet and then pricking the skin.

At the Allergy Clinic at Gentofte Hospital, a SPT series containing excipients of varying molecular weight including PEG 300, PEG 3000, PEG 6000, and the related polymers polysorbate 80 and poloxamer 407 has been used since 2010 when investigating PEG allergy. An extended series including PEG 20,000 has been used since 2014.

Basophil histamine release test (HR test)
The *in vitro* basophil histamine release test (HR test) detects IgE-mediated reactions.[90] This test method is safe for the patient as there is no risk of inducing a systemic reaction, but due to the short-lived basophil granulocytes, the HR test has to be performed within 24 hours of the blood sample being drawn.
In this study, the test was performed on the day of blood sampling. Initially, blood was centrifuged, following replacement of plasma with 1,4- piperazinediethanesulfonic acid (PIPES) buffer. Glass fiber-coated microtiter plates were added 50 µL diluted blood and 50 µL stimulant (polyclonal goat anti-human IgE, phorbol 12-myristate 13-acetate (PMA) and ionomycin, or PEG 300, PEG 3000, PEG 6000, PEG 20,000, poloxamer 407 or polysorbate 80 in six concentrations. The plates were incubated for 60 minutes at 37°C. Released histamine was determined by making o-phthaldialdehyde-histamine fluorescent complexes quantified on a Histareader.[91]

Basophil histamine release test with passive sensitization
If a patient has non-releasing basophiles or if the blood sample is more than 24 hours old, histamine release can be measured after passive sensitization.
In this test, patients´ sera were incubated with fresh buffy coat blood (from the blood bank) and added 10 pg/ml recombinant human IL-3 and stored overnight at 8°C. The buffy coat blood was washed with PIPES buffer followed by ice-cold stripping buffer, thereby removing IgE from donor basophils. The IgE-stripped cells were incubated with serum for 1 hour at 37°C. The cell suspension (25 µL) and stimulants (25 µL) were added to glass fiber-coated microtiter plates. Released histamine was quantified as described above.[91]

Part 1 Allergy to cosmetic products with a focus on natural ingredients (manuscripts I and II)

Market survey and literature study (manuscript I)

A market survey was conducted between June and September 2017 to obtain an overview of the most common natural ingredients in cosmetic products in Denmark. To check as many products as possible, the non-profit application "app" Kemiluppen (in English translated to "The Chemicals Magnifying Glass") was used. It was developed in 2015 by The Danish Consumer Council THINK Chemicals, an initiative under the Danish Consumer Council, which is a non-governmental organization that helps consumers avoid problematic chemical substances when shopping for consumer products, including cosmetics.[92] The app can be downloaded free of charge to iPhone™ and Android™ users.

When a consumer scans the European Article Number (EAN) barcode of a cosmetic product with a smartphone camera, the consumer automatically uploads product pictures and ingredients in the application. If the product is uploaded for the first time, the product is investigated by THINK Chemicals, and product name, category and International Nomenclature of Cosmetic Ingredients (INCI) labeling is manually entered in an anonymized database. If this has already been done, the consumer is instantly informed of potential problematic substances in the product.[93] The cosmetic ingredients are evaluated by THINK Chemicals according to different lists of problematic chemicals and sent to the manufacturer to verify that the entered details are correct.[94]

In 2017, Kemiluppen contained 10,067 various cosmetic products with a total of 208,341 labeled ingredients covering 4432 different ingredients. In this study, all cosmetic products were reviewed for plant- and animal-derived ingredients by reading the ingredient list of all cosmetic products included in the application.

Following the market survey, a literature search on selected natural ingredients was conducted to examine how frequently the naturally derived ingredients were described in the literature and related to allergic reactions in cosmetics or other topically administered products. The literature search was conducted using the Medline/PubMed database. The words "contact allergy" OR "urticaria" was used in combination with (by using AND) a specific ingredient. Articles published until June 2019 describing immediate-type or delayed-type allergic reactions to the specific natural ingredient were included in the search. In addition, bibliographical references from identified reports were reviewed and standard textbooks on natural cosmetic ingredients were consulted.[3–5]

Development of a screening test series

A screening test series containing natural ingredients was developed using two combined selection criteria:

1. Natural ingredients included in ≥ 30 cosmetic products in the application "Kemiluppen".
2. Natural ingredients described as the causative allergen in ≥ 20 articles or in cosmetic or topical pharmaceutical products in > 3 patient cases.

The screening test series consisted of a patch test series and a skin prick test series.

Database study (manuscript II)

The National Database of Contact Allergy

In 2001, The Ministry of Environment founded The National Allergy Research Centre which established the surveillance database "The National Database of Contact Allergy". The aim of this database was to monitor the prevalence of contact dermatitis. The database contains patch test results and demographic and clinical data for dermatitis patients patch tested by Dermatology Departments at Danish University Hospitals and dermatologists in private practices in Denmark who are members of Danish Contact Dermatitis Group. Data are registered from the internationally recognized MOAHLFA (**M**ale, **O**ccupational dermatitis, **A**topic dermatitis, **H**and dermatitis, **L**eg dermatitis, **F**ace dermatitis, **A**ge > 40 years) variables and relevance of positive patch test reactions.

Patient data were extracted from The National Database of Contact Allergy. All patients diagnosed with facial dermatitis from 2010 to 2019 at the Dermatology Department at Gentofte Hospital were included. Data included age and patient information from the MOAHLFA index. If a patient was diagnosed with facial dermatitis, patch test results from 27 selected cosmetic-relevant allergens from the department´s specific extended series were additionally extracted. These 27 cosmetic-relevant allergens were selected by the participating researchers. The patch test procedure was standardized according to the guidelines from the European Society of Contact Dermatitis.[27]

Skin test study (manuscript II)

Patients included were investigated with the screening test series with cosmetic-relevant natural allergens developed by our research group in manuscript I. In addition, the patients received a questionnaire about facial dermatitis and natural ingredients (appendix I).

The study population included consecutive patients ≥ 18 years of age investigated for facial dermatitis at the Dermatology Department at Gentofte Hospital, during a 12-month period from June 2020 to May 2021. Exclusion criteria were age < 18 years and standard exclusion criteria for patch testing and skin prick testing (antihistamine tablet within the last three days, current systemic immunosuppressive treatment, topical treatment with corticosteroids on test area within the last seven days, active dermatitis in patch test area or known pregnancy or breast feeding).

Part 2 Immediate-type allergy to polyethylene glycols (manuscripts III and IV)

Questionnaire study (manuscript III)

All patients aged ≥ 18 years and diagnosed with PEG allergy at the Allergy Clinic at Gentofte Hospital from September 2010 to August 2019 were invited to participate (n=12). The diagnosis was made by an anamnesis of one or more allergic reactions to products containing PEGs in combination with a positive SPT to one or more MW PEGs. One patient declined participation, and one patient had died, making 10 patients eligible for inclusion in the study. The first patient was enrolled in 2017, but patients were consecutively enrolled until August 2019. Patients were diagnosed three weeks to eight years prior to inclusion in the study.
Invitation to participate in the study included a letter with information about the study, a consent form, and a questionnaire.

The included patients received a questionnaire about PEG allergy (appendix II). The questionnaire comprised 11 questions about PEG exposure, suspected causes, self-reported allergy symptoms, and the impact on the patients´ daily life scored retrospectively on a likert scale from 0 to 10 before and after diagnosis, where 0 corresponded to no impact on daily life and 10 corresponded to severe impact on daily life. The patients were also asked what they perceived to be the most important information from the healthcare professionals when diagnosed with PEG allergy.
In addition, a detailed clinical history was obtained from patient records and allergy investigations that had been performed at the time of diagnosis.

Skin prick test and *in vitro* studies (manuscript IV)

This study included the same 10 PEG-allergic patients who participated in the questionnaire study. Of these, eight patients were diagnosed until 2017 and consented to participate twice with a second visit 26 months later in 2019. One patient later declined the second visit. Two patients were

included after 2017 and only participated once. Sixteen non-PEG-allergic healthy volunteers, matched for age and gender, served as controls.
The study included SPT results, blood samples and histamine release test results from the initial allergy work-up as well as prospective SPT results, blood samples and *in vitro* test results from the current study.

Skin prick testing
Patients and controls were tested with a skin prick test series developed at the Allergy Clinic at Gentofte Hospital. PEGs and derivatives were prepared at the Laboratory of Medical Allergology, Copenhagen University Hospital Gentofte. The test series included: Lower MW PEGs: PEG 300 (100%), PEG 3000 (50% w/v), PEG 6000 (50% w/v), polysorbate 80 (20% w/v) and poloxamer 407 (10% w/v).These were tested stepwise with 20 minutes observation between each step. If only local reactions occurred, SPT was performed stepwise with PEG 20,000 in increasing concentrations of 0.01%, 0.1%, 1%, 10% and 20% (w/v) until a positive response was reached. If SPT was negative, duplicates were performed. The controls were tested with all components in duplicate. Saline solution was used as a negative control and histamine 10 mg/ml was used as a positive control. A positive reaction was defined as a wheal ≥ 3 mm.

Blood sampling and in vitro testing
Blood samples were drawn once for controls and patients participating once in the study, and twice 26 months apart for patients who participated twice. Blood samples were drawn prior to SPT. Blood samples were analyzed with histamine release test with and without passive sensitization.

Histamine release test (HR test) was performed on the day of blood sampling at the Laboratory of Medical Allergology at Gentofte Hospital. PEG 300, PEG 3000, PEG 6000, PEG 20,000, poloxamer 407, polysorbate 80 (Sigma-Aldrich), anti-IgE (KPL, Gaithersburg, MD, USA) and phorbol 12-myristate 13-acetate (PMA) + ionomycin (both from Sigma-Aldrich) were used for the HR test. HR test was only performed at the first study visit. Histamine release test with passive sensitization was performed in the final inclusion period on all available blood samples, from both study visits, from included patients and controls with the same substances as used in the HR test.

Ethical considerations

The market survey conducted in manuscript I did not include patients why there was no need for ethical approval of this study. The regional Human Ethics Committee approved the study protocol of the database study and the skin test study in manuscript II (Project ID H-19088990), and the questionnaire study in manuscript III and clinical study in manuscript IV (Project H-17021145). Patients were included after giving oral and written informed consent.

The Danish Data Protection Agency approved storage of data for manuscript II (international reference: HGH-2017-046) and manuscript III and IV (international reference: HGH-2017-078).

Permission to collect data from the National Database of Contact Allergy were given by the Danish Clinical Quality Program – National Clinical Registries. In May 2021, data were extracted from the database.

All participants (patients and controls) in manuscript IV were financially compensated according to the number of completed visits (maximum 2 visits) with receiving 500 DKK (approximately 70 EUR) per visit as well as travel expenses. All data are presented anonymized.

5. Results and discussion of main findings

PART 1 Allergy to cosmetic products with a focus on natural ingredients (manuscripts I and II)

Identification of common potentially allergenic natural ingredients in cosmetics, development and evaluation of a screening test series (manuscript I and II)

Development of a screening test series (manuscript I)

In total, 10,067 cosmetic products on the Danish market were investigated. We identified 121 different natural ingredients that were included in at least 30 cosmetic products. This indicates that natural ingredients are commonly used in cosmetic products. The natural ingredients comprised 117 plant-derived ingredients and 4 animal-derived ingredients.

Not all the 121 natural ingredients were reported in the literature to cause allergic skin reactions from cosmetics. We selected the 21 ingredients described as most allergenic in the literature for further investigation and categorized them into three groups depending on whether they were reported to cause immediate-type (milk, peach, peanut and white potato), delayed-type (beeswax, cinnamon, compositae plants, eucalyptus, lanolin, lavender, lemon, lemongrass, liquorice, mint, orange, propolis, rose, tea tree, ylang-ylang) or immediate-type and delayed-type (oat, soybean and wheat) allergic reactions.

Based on the information gathered from the database search and the literature study, a cosmetic screening series for potentially allergenic natural ingredients in cosmetic products was composed. The screening test series consisted of a patch test series with the following allergens: Beeswax, cinnamal, eucalyptus oil, lanolin, lavender oil, lemon oil, lemongrass oil, liquorice, mint oil, oat, orange oil, propolis, rose oil, sesquiterpene lactone mix, tea tree oil, wheat, and ylang-ylang oil, and a skin prick test series with: Beeswax, milk, oat, peach, peanut, potato, propolis, soy, wheat, and the inhalational allergens birch, grass, and mugwort, to circumvent potential cross-sensitization from there.

Only few natural ingredients were described as causing immediate-type allergic reactions when used in cosmetic products despite their ability to induce anaphylactic reactions when ingested, which is known for wheat, milk and peanut.[34,95–98] A possible reason for this is that proteins from

the natural ingredients either are too small to contain at least two IgE-binding epitopes, or they are too large to penetrate the layers of the skin to interact with mast cells to elicit an allergic rection. It was recently shown that hydrolyzed wheat proteins with polypeptide lengths < 30 amino acids or < 3500 Da are safe for use in cosmetics as they cannot trigger an immediate-type allergic reaction.[98] To elicit an allergic reaction, the hydrolyzed wheat proteins must have ≥ two IgE-binding epitopes of at least 15 amino acid residues each. Hydrolyzed wheat can penetrate intact skin, but the hydrolyzed wheat protein used in a formerly popular Japanese soap, which caused sensitization to hydrolyzed wheat, had an average molecular weight of approximately 50,000 Da which cannot penetrate the skin. However, as the soap was designed for facial use, sensitization may have occurred through the rhino-conjunctival and/or oral mucosa by help from surfactants.[98] The pathogenesis of sensitization through the skin from food proteins is yet to be fully elucidated.

Evaluation of screening series (manuscript II)

A total of 66 consecutive patients with facial dermatitis (62 females, 4 males) and a mean age of 47.9 ± 17.0 years were in addition to their standard investigation tested with the screening test series of 21 natural ingredients developed in manuscript I.

A positive patch test to at least one allergen from the screening test series was found in nine patients (13.6%). Only five allergens elicited a positive patch test reaction and the most common allergens were hydroperoxides of linalool (6.1%), propolis (4.5%) and hydroperoxides of limonene (3%) (manuscript II, table 3). While hydroperoxides of linalool and limonene are known plant-derived sensitizers and has been recommended for screening in national series, they have not yet been accepted for the European baseline series. Clinically relevant exposures to hydroperoxides of linalool and limonene were investigated and found in four out of nine patients in everyday products. This was primarily cosmetic products such as soap, cream and massage oil, but also detergents.

More attention has recently been drawn to propolis and other natural ingredients in cosmetic products.[17,99–102] Propolis is an animal-derived ingredient included in this screening test series.[103] Prior to this study, propolis was implemented in the European baseline series in 2020 for standard investigation following an increased focus on propolis in cosmetic products as a potential sensitizer.[102] In a recent study from Germany, an upward trend of contact allergy to propolis was seen with a prevalence of 3.94% during the period 2015-2018.[104,105] We saw a similar frequency in our small study (manuscript II). However, no clinically relevant exposure to propolis was found in this study.

A positive skin prick test to at least one allergen from the screening test series was found in 12 patients (18%) divided among five allergens, with the two most common being potato (10.6%) and peanut (4.5%). All these patients had tolerated eating potato and peanut and had inhalational allergy to birch and grass, thus, the positive skin prick test represented cross-sensitization without relevance for cosmetic products. We did not find any relevant exposure from the patients´ own products.

Surprisingly, only few of the tested allergens in the screening test series elicited a positive response. Reasons for this may be that their allergenic potential in cosmetic products is too small to elicit an allergic reaction and allergy from natural cosmetics is only a minor problem. It may also be a consequence of different chemical compositions in the allergens used for testing and the allergens from the same plant used in a cosmetic product with another chemical composition. In addition, the preparation and processing of the natural ingredients, e.g. hydrolysis or heat treatment, may cause the ingredients to become more or less allergenic. This also means that in clinical practice, the patients are not tested with the exact modified natural ingredient which they are exposed to following topical application. Instead, they are tested with commercially available standard preparations or the ingredients "as is" which not necessarily have the same content as in the cosmetic product. An example of this is wheat; cosmetic products contain hydrolyzed wheat, but patients in this study were tested with regular, non-modified wheat in the clinic, as modified wheat is not available for testing. The test concentrations used in this study were based on recommended standard test concentrations, yet, these may underestimate the true number of allergic reactions. In addition, our sample size was small, and the study could be considered a pilot study.

Facial dermatitis, quality of life and views on natural ingredients (manuscript II)

All 66 patients received a questionnaire regarding symptoms of facial dermatitis, effect on quality of life and the patients´ view on natural ingredients. The response rate was 100%.

Almost half of the patients (40.9%) had current facial symptoms which had lasted months to years. This shows that facial dermatitis is a chronic problem in many patients. Several of the patients (43.9%) were further affected by their dermatitis in other areas of the body, as also neck (24.4%), arms (24.2%), trunk/shoulders (18.2%), legs (18.2%), hands (16.7%), or feet (6.1%) were involved. Half of the patients suspected cosmetic products as the cause of their facial dermatitis. The wide-ranging impact of facial dermatitis was highlighted by the fact that nearly all patients

(94%) experienced affected quality of life. In addition, 62.1% experienced limitations to their everyday life.

Studies on facial dermatitis and questionnaire studies regarding quality of life in patients with facial dermatitis have primarily been performed on patients who also had hand dermatitis or atopic dermatitis.[106–108] Nonetheless, their findings are in line with this study showing that facial dermatitis is associated with low quality of life in atopic dermatitis patients primarily due to social limitation and itching. The patients in this study additionally mentioned sleep problems, visual impairment due to swelling, pain and concerns about what the symptoms were and about the future as limitations. Also work-related limitations and the time-consuming perspective of doctor appointments for investigation were reported. These findings highlight the need for continuous efforts to improve investigation and quickly identify current causes and exposures followed by correct and effective treatment.

Cosmetic products containing natural ingredients are popular due to a consumer demand for cosmetic products with natural health effects and without unnecessary chemicals. In this study, a total of 43 patients (65.2%) preferred cosmetic products branded as "natural" due to reasons they were healthier (65.2%), less allergenic (50%) and/or to be better for the environment (34.8%). Of these, 77% label checked the cosmetic products for natural ingredients on a regular basis when shopping for cosmetic products. This confirms the findings in an Italian questionnaire study where 48% of the patients used natural topical products.[21] Although a total of 60.6% of the patients in this study were aware of allergy to natural ingredients, only 6.1% of the patients expected they might be allergic to natural ingredients in cosmetic products. As only few patients suspected natural ingredients as the cause of their dermatitis, it is important for healthcare personnel to test patients with their own products during investigation procedures.[17] This was surprisingly only done in a few of the patients in our study despite of being a part of the standards of the department, maybe demonstrating the difficulties in investigating these complex patients.

Facial dermatitis and common causes of contact allergy to cosmetic products (manuscript II)

We investigated characteristics of facial dermatitis and cosmetic-induced facial dermatitis in adult patients patch tested between 2010 and 2019 at the Dermatology Department at Gentofte Hospital. Overall, 8740 consecutive adult patients aged 18-99 years had been tested during 2010-2019. A total of 2292 patients (26.2%) were diagnosed with facial dermatitis. This is similar to other studies were the prevalence of facial dermatitis has been reported to be 15.4-27.4%.[109–111]

There was no significant age difference between the patients with facial dermatitis and patients without facial dermatitis (48.4 ± 16.7 years vs. 48.3 ± 16.9 years, $P < 0.78$). Analyses based on a multivariate logistic regression model showed that female gender (OR 2.0, 95% CI: 1.8–2.3) and atopic dermatitis (OR 2.5, 95% CI: 2.3–2.8) were both associated with a significantly higher risk of facial dermatitis. In this study, more than 80% of the patients with facial dermatitis were women. Thus, our data support the findings of other studies that facial dermatitis is more common in women than men.[109,112–114]

A total of 701 patients (30.6%) were diagnosed with facial dermatitis caused by cosmetics. In other studies, the prevalence of cosmetic-induced dermatitis was found to be between 9.8%-47.3%.[115–119] In total, 637 patients were diagnosed with facial allergic contact dermatitis, 56 patients with facial irritant contact dermatitis and 8 patients had both diagnoses. Analyses based on a multivariate logistic regression model showed that female gender (OR 2.1, 95% CI: 1.6–2.7) and age above 40 years (OR 1.3, 95% CI: 1.0–1.6) were associated with a significantly higher risk of cosmetic-induced facial dermatitis compared to not having cosmetic-induced facial dermatitis. In this study, almost 90% of the patients with cosmetic-induced facial dermatitis were women. The high number is believed to be caused by the fact that women more often use cosmetic products along with women more often seeking medical help. Interestingly, atopic dermatitis was associated with a significantly lower risk (OR 0.6, 95% CI: 0.5–0.8) of cosmetic-induced facial dermatitis. In contrast, other studies have found that both female gender and atopic dermatitis were associated with a significant increased risk of adverse reactions to cosmetic products.[112,113,117,119] We believe the significantly lower risk of cosmetic-induced facial dermatitis in atopic dermatitis patients in our study is explained by an underestimation of a diagnosis of irritant contact dermatitis to cosmetics. Atopic dermatitis skin is more susceptible to irritants, which may be caused by some cosmetic products, however, skin symptoms may be assumed to be a symptom of atopic dermatitis, thereby disregarding or overlooking the diagnosis of irritant contact dermatitis.

Common allergens (manuscript II)

Among 701 patients with cosmetic-induced facial dermatitis, the most frequent positive patch test results to 27 allergens commonly used in cosmetic products were caused by fragrance allergens (fragrance mix I (FMI), fragrance mix II (FMII), *myroxylon pereirae*, hydroperoxides of linalool and limonene) in 324 patients (46.2%). A positive patch test to fragrance allergens was associated with a significantly higher risk of cosmetic-induced facial dermatitis compared to non-cosmetic-induced facial dermatitis (FMI: OR 9.2, 95% CI: 6.9–12.2, FMII: OR 7.5, 95% CI: 5.0–11.3,

myroxylon pereirae: OR 5.1, 95% CI: 3.3–8.0, hydroperoxides of linalool: OR 4.7, 95% CI: 3.0–7.4, and hydroperoxides of limonene: OR 5.2, 95% CI: 3.0–9.1). Fragrances are known to be among the most common allergens in cosmetic products and the prevalence of contact allergic reactions to fragrances has increased during recent years.[26,120,121] This study demonstrate that fragrances in cosmetic products are still the most prominent cause of contact dermatitis due to a lack of prevention and regulation.

In 119 patients (17%), at least one positive patch test reaction to preservatives was found. A positive patch test to methylisothiazolinone (MI) (OR 2.4, 95% CI: 1.7–3.4), methylchloroisothiazolinone/methylisothiazolinone (MCIMI) (OR 2.1, 95% CI: 1.5–3.1), formaldehyde (OR 4.6, 95% CI: 2.7–7.6) or imidazolidinyl urea (OR 5.7, 95% CI: 1.1–29.6) was associated with a significantly higher risk of cosmetic-induced facial dermatitis. MI was introduced in European cosmetic products in 2005 and has since then given rise to a growing epidemic of facial allergic dermatitis and hand dermatitis in most of the industrialized world. In Denmark, the prevalence of contact dermatitis to MI increased significantly from 1.5% in 2005 to 5.7% in 2013.[122–127] From 2001-2009, facial dermatitis affected 20-25% of the patients with MI allergy but in 2013 this number had increased to 41%.[128] In 2017, a regulation was introduced with the purpose of decreasing the prevalence of contact dermatitis to MI. The regulation reduced the allowed MI concentration in rinse-off products and completely banned the use of MI in leave-on cosmetics. This has had some effect and in 2019 the overall prevalence of contact dermatitis to MI had decreased to 3.3%.[123]

Following FMI, FMII, MI, and MCI/MI, colophonium was the fifth most common patch test positive allergen (4.8%) and associated with a significantly higher risk of cosmetic-induced facial dermatitis compared with non-cosmetic-induced facial dermatitis (8.7% vs. 2.9%, OR 3.1, 95% CI: 2.1–4.6). Colophonium is a well-known sensitizer and recently found to be among the eight most common allergens causing contact dermatitis in the general population along with FMI and FMII.[39] A current clinical relevance was found in all patients allergic to FMI I and FMII, but only in approximately 50% of the patients positive to colophonium.

In the market survey with Kemiluppen described in manuscript I, colophonium was included in less than 30 products out of more than 10,000 investigated cosmetic products and thus, a very rare ingredient in cosmetic products (unpublished observation). A positive patch test to colophonium may therefore represent cross-sensitization as other facial exposures to colophonium-containing products are unlikely. In 61 colophonium-positive patients in this study, 33 patients (54.1%) were

also patch test positive to at least one fragrance-related allergen with FMI (22/33) and FMII (13/33) as the most common. Although colophonium is not an accepted fragrance allergen, a statistically significant association between colophonium and FMI has been found and it is possible that colophonium could indicate a perfume allergy.[129,130] The results show the importance of natural ingredients as common causes of contact allergy.

Part 2 Immediate-type allergy to polyethylene glycols (manuscripts III and IV)

Patient characteristics and response to questionnaire (manuscript III)

All ten included PEG-allergic patients responded to the questionnaire.

There were six males and four females with a median age of 35 years (range 18-64 years). Three patients were known with allergic rhinoconjunctivitis; none had a history of reactions to PEG-free drugs, venoms or vaccinations, and none of the patients had a vaccination containing PEG, polysorbate 20 or polysorbate 80 since diagnosis. One patient has had an anaphylactic reaction to an electrolyte tablet soluble in water (a sports drink) containing an unknown concentration of PEG. PEGs are also added to other diet supplements such as vitamin pills and serves as an anti-foaming agent in food and drinks labelled as E1521, but no other patients had a history of reactions to food or food supplements.

All reactions reported by the 10 patients had been immediate-type allergic reactions with symptom onset within 10 minutes of exposure and symptoms ranged from urticaria and itching to anaphylaxis with circulatory or respiratory compromise. Eight patients (80%) had at least one episode of adrenalin-requiring anaphylaxis. This is in line with a review by Wenande et al., where 76% of 37 cases had experienced reactions fulfilling the criteria for anaphylaxis following exposure to PEG.[10]

The main products causing anaphylaxis were oral medication (analgesic tablets, antibiotic tablets, antacids, and laxatives) and depot-steroid injections. Especially laxatives and depot-steroid injections are described as known exposures in the literature.[10,66,131–133] Exposure assessment is a very important part of PEG allergy investigation and severe allergic reactions to structurally different product should raise suspicion towards PEG allergy. Detailed clinical manifestations, culprits and test results from 10 patients with PEG allergy can be seen in manuscript III, table 2.

The rarity of this allergy was underlined by none of the patients being aware of PEG allergy before the diagnosis. One patient had suspected an excipient to be the culprit. Despite several contacts

with the medical system, none of the healthcare professionals who had been treating the patients for anaphylaxis suspected PEG as the culprit, except for one paramedic. The lack of awareness of the allergy also led to misdiagnoses in several patients, including urticaria, idiopathic anaphylaxis, or allergy to the active ingredient in drugs, e.g. penicillin prior to the diagnosis. Cases with misdiagnosed patients have been reported in the literature.[47,104] Time from first reaction to diagnosis was almost two years (median 20 months, range 2-120 months) and seven patients reported repeated reactions (median 3, range 2-6) until the diagnosis was established. Other case reports have also described repeated reactions in PEG allergic patients.[43,61,63,69,74,133,134]

Despite great efforts to avoid PEG after diagnosis was established, almost half of the patients reported accidental re-exposure from everyday products, such as cosmetics, e.g. soap and shampoo and less often pharmaceutical products, e.g. tablets and a steroid cream. Symptoms were immediate but mild, and none of the reactions required adrenaline treatment or hospitalization. The number of re-exposures highlights how difficult it is to avoid PEG and how important it is to inform the patient about the many exposure routes. Many cosmetic products contain only very low molecular weight PEG, probably with less potential of causing severe allergic reactions, thus, the number of true re-exposures may be underestimated. Three of the patients in this study only had one allergic reaction and seemed less reactive in SPT. It is possible that these patients can tolerate limited PEG exposure over time. Other patients in this study have had repeated severe reactions with years in between and these patients seem to retain their reactivity to PEG. It is currently unknown if there are patients who permanently loose sensitization to PEG. Due to the risk of developing severe reactions upon inadvertent re-exposure, patients at the Allergy Clinic at Gentofte Hospital are told that PEG allergy covers all MW PEGs and is for life.

We found that allergy to PEG has severe impact on the patients´ daily life. Median likert score before diagnosis was 7 (range 0-10) compared to 4 (range 0-8) after diagnosis. Although a correct diagnosis improved daily life of the patients, seven patients reported experiencing limitations after diagnosis. Especially right after the diagnosis was made, patients reported stress, anxiety and the overwhelming fear of new anaphylactic reactions from the widespread use of PEG in everyday products, including cosmetics, hygiene products and over-the-counter medication such as analgesics, antibiotics, antacids and laxatives.

We have developed a list of clinical scenarios where PEG allergy should be suspected to help healthcare professionals unfamiliar with the clinical presentation of PEG allergy (manuscript IV, table II):

- Repeated, severe allergic reactions/anaphylaxis to ≥ 2 structurally different drugs/products e.g. tablets, depot injections, antacids, PEG-based laxatives.
- Severe allergic reactions to only some formulations, or doses, of same generic drug.
- Severe allergic reactions to drugs, where allergy to the active ingredient has been excluded on testing (e.g. antibiotics, analgesics).
- Severe allergic reactions to drugs containing PEG or PEG derivatives (polysorbate 80, poloxamers).
- Severe allergic reactions to vaccines containing PEG (mRNA vaccines) or PEG derivatives (polysorbate 80, poloxamers).
- Severe allergic reactions to PEGylated drugs, where allergy to the active drug is excluded.
- Severe unexplained allergic reactions in connection with surgery or invasive procedures.

Patients were asked what they perceived as the most valuable information when diagnosed with PEG allergy. They reported that information about the widespread use of PEG, which products are "high-risk" products, as well as learning how to check if a product contains PEGs, e.g. by reading the package insert and/or the manufacturers product information were most valuable. In addition, receiving an allergy warning card, follow-up appointments and continued access to advice from a doctor at the Allergy Clinic were listed as very important. Most patients have had several contacts to the Allergy Clinic, after the diagnosis was made, asking advice about prescribed treatments. Additionally, other healthcare professionals have contacted the Allergy Clinic seeking advice on how to avoid PEG exposure in connection to e.g. medical treatments and surgical procedures.
Due to the rarity of this allergy, it cannot be expected that other healthcare professionals are aware of the allergy. Patients therefore have a massive responsibility to avoid accidental re-exposure and unfortunately also to educate healthcare professionals who are unaware of PEG allergy. Thus, PEG allergy requires patient empowerment for patients to take responsibility for their own allergy and being one step ahead all the time to avoid inadvertent re-exposure. This includes informing hospitals, general practitioners, specialists, dentists, hairdressers and tattoo artists of their allergy before every upcoming appointment so consultations and treatments can be planned without PEG exposure.

Some patients also requested a list of "safe" PEG-free drugs or a PEG-containing product database, however, these are not possible to compile as the PEG content may vary in different formulations of the same drug, in different doses of the same formulation and in new parallel imports of drugs. Therefore, it is important always to check each individual drug for PEG and preferably with help from the local pharmacist to ensure that drugs and over-the-counter products are PEG-free. Patients must identify their own safe drugs and other products and always bring their own medication, e.g. pain killers and antihistamine in case of hospitalization or accidental re-exposure when not at home.

Skin prick test and *in vitro* studies (manuscript IV)

Skin prick testing

Ten patients were skin prick tested at time of diagnosis and nine patients had a positive SPT to PEG 3000 and/or PEG 6000. One patient initially tested negative to PEG on SPT four weeks after the initial reaction but tested positive to the PEG derivatives poloxamer 407 and polysorbate 80. As SPT is recommended four to six weeks following exposure, it is possible, that PEG would have tested positive if tested later.[88] The patient was included in this study three and a half years later and tested positive to PEGs of varying MW on SPT at the first study visit.

In seven patients, primarily patients with the longest time interval since diagnosis, reactivity decreased over time with loss of reactivity to a lower MW PEG (PEG 3000 and/or PEG 6000). The fastest decline was 26 months as seen between the two study periods. These seven patients continued to test positive to PEG 20,000 in varying concentrations indicating that SPT with PEG 20,000 in increasing concentrations can be used to increase diagnostic sensitivity of SPT. This is especially important in patients with a long delay between their clinical reaction and allergy work-up as patients who have been exposed a longer time ago may be negative on SPT to low MW PEGs. A PEG allergy diagnosis could mistakenly be missed in these patients if they are not tested with high MW PEG. In two patients, reactivity increased over time and it remained stable in one. The control group with 16 participants tested negative to SPT in all concentrations in duplicate suggesting a high specificity to this test, although this has not been calculated.

An important finding of this study is that allergenicity increases with increasing MW suggesting there is no upper threshold for positivity.[10,69] This means that if SPT is positive for lower MW PEG, higher MW PEG will also test positive. Thus, in a patient who is SPT positive to PEG 3000,

further testing with PEG 6000 or higher MW PEGs is not necessary and will only increase the risk of developing a systemic reaction.

It is debated whether there is a lower threshold for reactivity. This is particularly relevant when advising patients to either completely avoid PEG-containing products, or to continue to use products containing lower MW PEGs than SPT was positive to.[66,69] At the Allergy Clinic at Gentofte Hospital, PEG-allergic patients are warned against PEG of all MWs even if lower MW PEGs test negative. This is supported by results in our study, where two patients at the second study visit showed increased reactivity to PEGs with positive SPT to lower MW compared with an earlier visit. This increased reactivity could be explained by unknown accidental re-exposure, and it is possible that minor asymptomatic exposures, e.g. from cosmetics, creams, soaps or tablet coatings containing low MW PEG can maintain or even increase allergenic reactivity. Also the lack of standardized labelling, admixture with other MW PEGs and lack of information about the concentration of PEG in products makes it difficult to identify the risk of a reaction on exposure[10,47] PEG concentrations are likely to be lower in tablet coatings than the tablet core and we are aware that a few patients in this cohort can tolerate continuous use of tablets with PEG in the tablet coating. It could be that these patients are desensitizing themselves by this continuous small amount of exposure on a regular basis.

Despite the small amount of allergen exposure during SPT, this procedure can induce systemic reactions and anaphylaxis in patients with a history of severe allergic reactions. Therefore, SPT should only be performed with stepwise increasing concentrations.[10,69] Three patients developed systemic urticaria to lower MW PEG (PEG 3000) during SPT despite careful stepwise SPT and were not tested with PEG 20,000. One of these patients had previously tested positive to PEG 20,000 0.01% at the time of diagnosis. All three patients were treated successfully with an oral antihistamine tablet not containing PEG.

Cross-sensitization to PEG-derivatives

Between diagnosis and last study visit, eight patients tested positive to poloxamer 407 while three tested positive to polysorbate 80. Only limited literature exists regarding sensitization patterns. The clinical relevance of cross-sensitization is currently unknown.[10,66] Only one patient from our clinic diagnosed with PEG allergy has had a clinical reaction to polysorbate 80 and another patient had a severe clinical reaction (cardiac arrest) to a poloxamer 407-containing device.[43,66]

The clinical implications of cross-sensitization between PEG and PEG-derivatives have become of utmost importance within the last year. The mRNA-based COVID-19 vaccines from BioNTech/Pfizer and Moderna contain PEG 2000, and most of the other available non-mRNA-based vaccines contain varying amounts of polysorbate 80.[57,58,135,136] Therefore, identifying a safe COVID-19 vaccine for PEG-allergic patients is important.

In vitro testing

Currently, SPT is considered the safest method for patients when diagnosing PEG allergy, although systemic reactions to SPT are reported in the literature and also seen in three patients in this study.[10,69] HR testing carries no risk of allergen exposure for the patient and has shown promising results for allergy to e.g. chlorhexidine, peanut and pollen.[28,91,137] Therefore, the potential of the HR test with and without passive sensitization as a diagnostic tool in diagnosing PEG allergy were investigated.

HR test was positive in two patients in this study, but the HR results were only consistent with the SPT results in one of the patients, who was diagnosed with PEG allergy just one month prior to inclusion in this study. Four patients had inconclusive tests. This was most likely due to non-releasing basophils. Non-releasing basophils is a known limitation of this test where basophils are unresponsive and thus not releasing histamine following IgE-dependent stimuli. This is found in 10-20% of the general population.[138] In this study, an interesting observation was, that three of the patients with unresponsive basophils had systemic urticarial reactions during SPT. Four patients had a negative HR test.

Previously, two out of four patients who had a HR test performed at the time of diagnosis tested positive at that time and results for one patient was published at the time.[63] These two patients both tested negative in the HR test at first study visit, but both remained positive on skin prick test to high MW PEG. This suggests that HR test may be useful during investigation if used shortly after exposure and clinical reactions, but only has limited use when there have been longer intervals between exposure and HR testing. Recently, another Danish group showed limited use of HR testing in the diagnosis of PEG allergy.[74]

Histamine release test with passive sensitization was negative in all patients and cannot be recommended as a diagnostic tool in PEG allergy. HR test and HR test with passive sensitization were negative in all 16 controls.

A reliable *in vitro* test for suspected PEG allergy which would eliminate the risk of inducing systemic reactions remains to be developed. An anti-PEG IgE assay has recently shown promising results in a small cohort of patients but there are no commercial IgE-detecting assays available yet.[139]

Investigation algorithm for patients suspected of allergy to PEG

Optimization of safe diagnostic tests is paramount to PEG-allergic patients and healthcare personnel but there is currently not an internationally accepted, standardized provocation model for diagnosing PEG allergy. At the Allergy Clinic at Gentofte Hospital, skin prick testing is considered the safest diagnostic method for diagnosing allergy to PEG.

Based on our results and clinical experience, we have suggested an investigation algorithm for patients suspected of allergy to PEG. The algorithm is based on a titrated stepwise approach of skin prick testing with PEG 300, PEG 2000, PEG 3000, PEG 6000, and PEG 20,000 in stepwise, increasing concentrations as well as poloxamer 407 and polysorbate 80. This investigation algorithm is currently highly relevant for patients with suspected PEG allergy who should be investigated prior to a COVID-19 vaccine. PEG 2000 has been included in the new algorithm as the mRNA-based COVID-19 vaccines contain PEG 2000. SPT with PEG 20,000 should only be performed if SPT with lower MW PEGs is negative but the clinical suspicion of PEG allergy is strong. This minimizes the need for less safe test modalities (intradermal and provocation testing) which have been repeatedly reported to cause severe systemic reactions.[10,43,66,69–73] Intradermal and provocation testing are only recommended in rare cases if clinical suspicion is strong and the full algorithm has shown negative results.

After our studies ended, another six patients have been diagnosed with PEG allergy at the Allergy Clinic. All six patients have been investigated and diagnosed using this algorithm. None of the patients developed systemic reactions during investigation. Two of these six patients only had a positive reaction to PEG 20,000. If these two patients only had been tested with low MW PEGs, they would not have been diagnosed with PEG allergy with a high risk of experiencing inadvertent re-exposure or alternatively, they could have been exposed to a graded challenge with a risk of inducing a severe systemic reaction.

6. Considerations on methodology

In the following section, considerations on methods not covered or only briefly described in manuscripts I-IV are elaborated.

Part 1 Allergy to cosmetic products with a focus on natural ingredients (manuscripts I and II)

Market survey and literature study (manuscript I)

Natural ingredients

There is no specific legislative or clear official definition that address natural ingredients in cosmetic products. In this study, we defined natural ingredients as plant- or animal-derived. The arbitrary definition was inspired by the European Cosmetic Regulations´ definition of natural ingredients in cosmetics referring to the origin of the natural ingredients. However, other interpreted definitions could have led to other investigated ingredients.

The application "Kemiluppen"

The application (app) "Kemiluppen" contains more than 10,000 cosmetic products scanned by Danish consumers since 2015. As the app "Kemiluppen" is 100% anonymous, it is not possible to obtain personal data for the consumers using the app. Thus, it is unknown whether there is an overrepresentation of a specific gender, a certain age group or consumers with a preference within cosmetic products.

This app helps consumers to avoid chemical substances such as fragrance contact allergens, and consumers using the app may be more focused on health, allergy and avoidance of certain ingredients or products compared with consumers not using this app with a risk of selection bias.

The app can only be accessed using an electronic device, why users may be younger and belong to a more resourceful group of the Danish population compared with the general consumer. This could possibly exclude consumers with less resources and the older generation.

Even though the app does not contain all cosmetic products available on the Danish market, the use of this app provides access to many more cosmetic products than it would have been possible to investigate if a regular market study had been carried out and cosmetic products in beauty stores, supermarkets, hair dressing salons etc. had been physically examined.[140–142] The strength of this app is that it is continuously updated with the newest products scanned by consumers while outdated products are removed. This is thoroughly managed by the non-profit organization of

THINK Chemicals without economic interests in consumers that could otherwise risk affecting consumers in certain directions.[93]

Literature search

A thorough literature search was performed to identify the most commonly described natural ingredients according to our definition (ingredients described as the causative allergen in cosmetic or topical pharmaceutical products in > 3 patient cases or in ≥ 20 articles from other (or unknown) exposure sources). The increased focus on natural ingredients and their allergenic potential in cosmetic products is still relatively new, and not all patient cases or studies are described in the literature. Therefore, by our literature search criteria, we may have excluded natural ingredients with potential to cause allergic symptoms that are not well described in the literature.

Database study (manuscript II)

Study population

Patient data were extracted from the National Database of Contact Allergy and included consecutive patients ≥ 18 years of age, patch tested at the Dermatology Department, Gentofte Hospital from January 2010 to December 2019. Patients could have been referred to the department for other reasons than facial dermatitis and did not solely consist of patients with facial dermatitis. Patients patch tested more than once were either included at first registration of facial dermatitis or at first patch test if without a diagnosis of facial dermatitis to avoid incorrect time of inclusion. Patch test reactions from patients already sensitized to a relevant cosmetic-relevant allergen before 2010 were not included.

Study design

The database study presented descriptive data from a database why statistical associations between risk factors and outcomes only could be interpreted as associations without causality.

Diagnostic considerations

Patch test readings have been assessed through European acknowledged scoring criteria.[27] Patch test preparations and readings were performed by trained specialist nurses throughout the period to minimize the influence of inter-individual variation affecting the patch test procedure and interpretation of patch test reactions.[143,144] These considerations also apply to the skin test study.

The selection of specific cosmetic-relevant patch test allergens

This selection was based on the most common ingredients in cosmetic products included in the European baseline series and the supplemental series selected by the participating researchers. The exclusion of other allergens not specifically interpreted as cosmetic-relevant, or the exclusion of allergens from other test series, yet potentially relevant, may have affected the true results of the etiology of facial dermatitis in this study. We also relied on standard test concentrations, which may not always be optimal.

Skin test study (manuscript II)

Study population

Participants for the study were recruited among patients investigated for facial dermatitis. It is a possibility that the patients who agreed to participate in the study were more severely affected by their facial dermatitis than patients who did not wish to participate. Patients treated with immunosuppressive treatment were excluded as it can influence the reproducibility of a positive patch test reaction.[145]

Due to lock-down under the COVID-19 epidemic, fewer patients were included in testing with the screening test series with natural ingredients. It is possible that if more patients had been tested, perhaps some of the rarer allergens could have turned out as important.

Test materials, preparation and patch test readings.

All test preparations used in the skin test study were based on already established concentrations available as standardized commercial patch test preparations or standardized commercial skin prick test preparations. Although some preparations were not used in standardized test series, all test preparations had been used in the department prior to the study. Testing several concentrations, or even for some less described allergens increasing the test concentration, might have given more positive reactions and provided additional information.

In this study, an irritant reaction or a doubtful reaction was interpreted as negative reactions.[27] However, a doubtful reaction may both signify a true contact allergy or an irritant reaction. The weak reaction may be due to insufficient test concentrations, caused by cross-reacting substances from the true allergen, or technical issues. It is important to be critical and if a suspicion exists, further tests such as re-patch test or use tests should be performed.

Questionnaire study

The questionnaire was a non-validated questionnaire constructed by the participating researchers based on existing knowledge and assumptions on causality. The questionnaire contained specific questions about the patient´s facial dermatitis, including possible exposures and associated limitations of daily life, as well as questions concerning the patient´s view on natural ingredients. Eight colleagues without facial dermatitis were pretested to reduce the risk of bias due to misinterpretation of the questions. To elude response bias, the questionnaire was completed by the patients while waiting for results of the skin prick test performed on the first day of inclusion. Selection bias was avoided as the response rate was 100%, however, the answers may represent patients with more severe facial dermatitis as patients with less severe facial dermatitis in Denmark more often are referred to dermatologists in private practice and less commonly referred to a Dermatology Department, as mentioned above. Thus, the answers are presumably related to a high representation of a subgroup of patients with severe facial dermatitis.[146]

Part 2 Immediate-type allergy to polyethylene glycols (manuscripts III and IV)

Questionnaire study (manuscript III)

Study population

All patients diagnosed with PEG allergy were asked to complete a questionnaire regarding PEG allergy. No control group was included as answering the questionnaire would not have made sense for patients without PEG allergy.

Questionnaire

The purpose of this questionnaire study was to establish a detailed knowledge of PEG allergy, including the patients´ perspective on living with the allergy. No other questionnaire studies have been performed in PEG-allergic patients.

The questionnaire was a non-validated questionnaire containing specific questions about PEG and PEG allergy as well as questions that made it possible to elaborate on an answer.

The questions were constructed by the participating researchers based on current knowledge supplemented with questions from the patient´s point of view.

There was no risk of selection bias as all patients included in the study were invited to participate. The response rate was 100% and thus representative of the population of interest.

As this was a retrospective cohort study there was a risk of recall bias. The patients were diagnosed a few weeks to several years before answering the questionnaire and recall may have decreased.

However, most of the PEG allergic patients still remember their often severe and repeated allergic reactions. Bias due to misinterpretation of the questions were tried avoided by pre-testing seven colleagues without PEG allergy.

Skin prick test and *in vitro* studies (manuscript IV)

This is the first study to investigate skin test reactivity over time, cross-sensitization patterns in PEG allergy, and *in vitro* studies in a cohort of ten PEG allergic patients. As PEG allergy is rare, the size of this cohort is a strength of the study.

Study population

Two groups were included in this study: Participants with PEG allergy and a control group without PEG allergy. Participants were gender and age matched. The study population was small, and analyses contained too few participants to reach statistical power. However, the size of the PEG allergic group was sufficient to draw preliminary conclusions from both the SPT study and *in vitro* test studies.

Test materials and preparation

Since 2010, patients with suspected PEG allergy at the Allergy Clinic at Gentofte Hospital have been investigated with a continuously developed skin prick test series consisting of the following agents: PEG 300, PEG 3000, PEG 6000, poloxamer 407 and polysorbate 80. In 2014, PEG 20,000 was added to the series. Most recently, PEG 2000 was additionally added to the SPT series following the use as an excipient in the mRNA-based COVID-19 vaccines. This SPT series has proven valuable when diagnosing patients. To help healthcare professionals worldwide with limited experience of investigation procedures in suspected PEG allergy, a detailed procedure for preparing solutions for skin prick testing for PEG 300, PEG 3000, PEG 6000, PEG 20,000 in various concentrations, poloxamer 407 and polysorbate 80 according to the Allergy Clinic at Gentofte Hospital has been provided (manuscript IV, Table E1). The preparation of test materials has been in use for more than ten years but is not internationally recognized as no gold standard test exist. The compounds used are classified as laboratory chemicals and may be subject to local legislation. No studies have been done on sterility or stability. At our centre, solutions are used for six months when stored at room temperature.

Skin prick testing

Skin prick testing with PEGs and derivatives have been employed for more than a decade at our department. Unfortunately, due to the low number of patients with PEG allergy, it is not possible to calculate sensitivity and specificity for this test. However, we do believe the specificity of our PEG SPT series is high as 16 healthy controls in this study had negative SPT results in all MW and concentrations. Further, 539 non-PEG-allergic patients have been investigated at the Allergy Clinic at Gentofte Hospital as part of routine allergy work-up between 2012 and 2019 and all tested negative. No patients have yet tested positive to the SPT series without a history of relevant symptoms following exposure to PEG-containing products.

Currently, there is no internationally accepted gold standard for diagnosing PEG allergy. An ideal diagnostic test should be able to produce a positive response at the lowest PEG concentration in order not to cause anaphylaxis in the PEG-allergic patient. Recommendations for investigating patients with suspected PEG allergy is based on experiences from few patients which makes it difficult to assess specificity and sensitivity of a test.[10] Skin prick testing, intradermal testing and oral provocation models are currently available, but especially the latter two have been described causing severe allergic reactions including anaphylaxis in PEG-allergic patients why we consider SPT the safest method.

Histamine release test

Histamine release test (HR test) is a valuable tool in investigation and diagnosis of various immediate-type allergies.[76–79] The advantage of this method is that it is safe for the patient because it does not expose the patient to a given allergen. As our group in the past were able, albeit only in a single patient, to formally demonstrate the IgE-dependency of the PEG-induced histamine release, we wanted to investigate this further in all patients diagnosed with PEG allergy until 2019.

In this study, two patients tested positive in the HR test. The two patient assays and the (+) control (anti-IgE) can be seen in manuscript IV, figure E1. In a HR assay, a dose-response curve would be bell-shaped (U-shaped) if a large concentration window was used (figure 4). This means that with increasing concentrations of a stimulant, the histamine release will increase until maximum release and will then decline with further increase of stimulant.

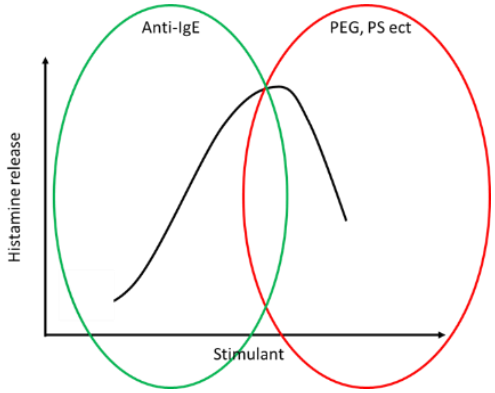

Figure 4. A positive HR assay will form a bell-shaped dose-response curve in a large concentration window. The (+) control (anti-IgE) illustrates the expected outcome for the dose-response curve.

In this study, PEG 300, PEG 3000, PEG 6000, PEG 20,000, poloxamer 407, polysorbate 80, anti-IgE and phorbol 12-myristate 13-acetate (PMA) + ionomycin were titrated in a 10-fold dilution range from 10 mg/ml to 0.1 µg/ml and tested in six concentrations. For some of the PEG agents (e.g. ethylene glycol) an optimal concentration was found in the middle of the tested concentrations. For PEG 3000, PEG 6000, PEG 20,000 and poloxamer 407, the optimal concentrations for the two patients testing positive were found at the most diluted ones, with a positive response at the lowest concentrations, i.e. at the right part of the bell-shaped dose-response curve normally seen for histamine release. This means that the most optimal scenario would have been to use a larger titration range with higher dilutions. Unfortunately, this was not possible in our experimental design as only the last two tested patients were positive in the HR test. At this time, it was too late to change the dilution range in the HR test.

The finding of decreased histamine release at increasing concentrations could suggest that the concentrations used were potentially in the toxic range. While a toxic response to high doses cannot fully be excluded, normally such a response would be accompanied by unspecific histamine release, none of which was observed. Even though we have not been able to include lower doses, we expect, that histamine release is a result of IgE-mediated mast cell degranulation and not due to a toxic response.

7. Conclusion and perspectives for future research

PART 1 Allergy to cosmetic products with a focus on natural ingredients

We have shown that natural ingredients are widely used in Danish cosmetic products (manuscript I). The definition of "natural" used in this study was arbitrary and an increased focus on developing a standardized, internationally accepted definition of "natural" will be useful in the future. Several natural ingredients (in this study defined as plant- or animal-derived) have been described with an allergic potential when examining the literature. Future research on emerging natural allergens in cosmetic products is relevant to determine their allergenic potential.

In future studies, the risk of topical sensitization to food proteins and development of immediate-type allergy or exercise-induced symptoms could be investigated. It has been shown, that in rare cases, patients with allergy to ingested wheat may experience wheat-dependent exercise-induced anaphylaxis (WDEIA) characterized by an allergic reaction following ingestion of wheat and physical exercise. It is not known whether this condition also applies for patients using wheat or other food protein-containing topical products followed by physical exercise. The interaction or independence between topical products and oral allergy to food proteins also needs further elucidation.

The skin test series with natural ingredients only showed few positive test results when tested as described in manuscript II. This could be caused by the limited number of patients participating in the study due to COVID-19, so larger studies are needed. It could be interesting to agree on a series with natural ingredients, which could be tested in a European multi-center study. Increased focus and improved regulation on plant-derived ingredients in cosmetic products are necessary to improve nomenclature and investigation with the correct chemical composition of plant-derived ingredients in cosmetic products.

We found that facial dermatitis is common among patch tested patients and a significant proportion of patch tested facial dermatitis patients are allergic to cosmetic ingredients. Fragrances and preservatives are still the most prominent causes of contact dermatitis from cosmetic products, indicating continued exposure among consumers to these contact allergens in too high concentrations. Although regulations have been improved for consumer protection, these are not sufficient. We found that nearly all facial dermatitis patients reported limitations to their daily life and/or affected quality of life with socio-economic consequences due to sick leave, impaired work-performances and healthcare appointments for investigation and diagnosis in many patients. Thus, the consequences of facial dermatitis before and after diagnosis are wide ranging. More

investigations on risk assessment and prevention in order to continuously improve regulations are needed.

PART 2 Immediate-type allergy to polyethylene glycols

We have shown that clinical manifestations in patients with confirmed PEG allergy are often dramatic and affects the patients´ daily life (manuscript III). Repeated severe allergic reactions and delayed diagnosis caused stress and anxiety among the patients. We found that the diagnosis of PEG allergy improved the patients´ daily life compared with before diagnosis. This was mainly due to information at time of diagnosis on how to avoid PEG and how to manage the allergy in everyday life. Several of the patients asked for the possibility to exchange experiences with other PEG-allergic patients. There are currently only a few patient-initiated online forums in English where patients can get in touch with others with the same diagnosis. Development of focus groups in order to help patients should be investigated and implemented. A Facebook group is currently being created by a newly diagnosed patient from the Allergy Clinic. In addition, continued increased awareness about PEG allergy, clinical presentation and common culprits, and improved investigation, diagnosis and information about this allergy in society and among healthcare professionals needs further elaboration. This will help healthcare personnel prevent patients from getting substandard care due to fear of anaphylaxis on introducing new treatments and unnecessary exposure. With increased awareness of PEG allergy, the frequency of PEG-allergic patients is expected to increase.

Insufficient product labelling and non-standardized nomenclature in cosmetic, pharmaceutical and food products are challenging to patients and healthcare workers with limited knowledge about PEG allergy. Future studies should address this problem to prevent re-exposure and help patients navigate in everyday products.

All included patients had been diagnosed with PEG allergy from a clinically relevant exposure history in combination with a positive skin prick test to PEG. We have suggested an optimized investigation algorithm for patients suspected of PEG allergy based on titrated stepwise skin prick testing with PEGs of increasing MW (manuscript IV). This will minimize the risk of inducing anaphylaxis in sensitized patients and prevent a false negative SPT in patients referred with a long delay since their initial reaction. Even SPT may induce systemic reactions in some patients why a diagnostic *in vitro* test would help patients being diagnosed without being exposed to the allergen. *In vitro* studies with histamine release test with and without passive sensitization testing unfortunately showed limited use in the investigation of PEG allergy. Although the HR data were

preliminary, two of the patients did react in the HR test shortly after their clinical reactions. This should encourage further work in the field of developing sensitive biological assays and future studies should aim at developing an optimized diagnostic method for PEG allergy without allergen exposure. As an IgE-mediated mechanism seems to be involved in immediate-type PEG allergy, these studies could include investigations of the immunologic mechanisms behind PEG allergy and possibly developing an IgE assay for PEGs and structurally related polymers including determining a lower threshold for reactivity, preferably based on molecular weight and amount of PEG. The latter would be helpful to patients who potentially are allowed exposure to small amounts of PEG as total avoidance of PEG causes considerable stress to patients due to the large number of products they need to avoid. A commercial risk-free standard method for diagnosing PEG allergy would also help improve generalizability across healthcare systems and countries and uncover the true prevalence of PEG allergy.

It was found that SPT reactivity varies over time and between patients. Overall, patients could be classified into two reactivity groups; a group with increasing reactivity and a group with decreasing reactivity. Further investigations are needed to determine reasons for increasing or decreasing reactivity and whether this allergy can disappear in some patients. Until this has been done, patients are informed that PEG allergy is lifelong at our centre.

The route of sensitization to PEG is unknown. It is unclear whether sensitization to PEG is elicited by PEG or a PEG-derivative. Overall, very limited information is available on cross-reactivity patterns with poloxamers and polysorbates. Cross-sensitization between PEGs and the derivatives poloxamer 407 and polysorbate 80 was common in this study, but the clinical implications remain unknown and needs to be elucidated. Various pharmaceutical products are pegylated including vaccines and chemotherapeutics. The mRNA-based COVID-19 vaccines from BioNTech/Pfizer and Moderna with PEG 2000 are both pegylated vaccines, while polysorbate 80 has been used in various vaccines for many years, and most recently also in the non-mRNA-based COVID-19 vaccines. Cross-sensitization has been highly relevant during the worldwide COVID-19 epidemic. Pegylated drugs, including vaccines which are likely to be based on the mRNA-technology in the future, and their clinical relevance in PEG-allergic patients, needs to be investigated as it is of utmost importance not to limit these patients from optimal treatment now and in the future.

8. References

1. Verhulst L, Goossens A. Cosmetic components causing contact urticaria: a review and update. *Contact Dermatitis*. 2016;75(6):333-344.
2. Dooms-Goossens A. Cosmetics as causes of allergic contact dermatitis. *Cutis*. 1993;52(5):316-320.
3. de Groot AC. *Monographs in Contact Allergy, Volume 1. Non-Fragrance Allergens in Cosmetics (Part 2).* Boca Raton, FL: CRC Press Taylor and Francis. 2018. 728 p
4. de Groot AC. *Monographs in Contact Allergy, Volume 1: Non-Fragrance Allergens in Cosmetics (Part 1).* Boca Raton, FL: CRC Press Taylor and Francis. 2016. 744 p
5. de Groot AC. *Monographs in Contact Allergy, Volume 2. Fragrances and Essential Oils.* Boca Raton, FL: CRC Press Taylor and Francis. 2019. 946 p
6. The SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation 10th revision. https://ec.europa.eu/health/sites/default/files/scientific_committees/consumer_safety/docs/sccs_o_224.pdf. Accessed August 23, 2021.
7. Goossens A. Contact-Allergic Reactions to Cosmetics. *J Allergy*. 2011;2011:1-6.
8. Giménez-Arnau AM, Deza G, Bauer A, et al. Contact allergy to preservatives: ESSCA* results with the baseline series, 2009–2012. *J Eur Acad Dermatology Venereol*. 2017;31(4):664-671.
9. Miljø og fødevareministeriet. Kortlægning Af Økologiske Og Naturlige Kosmetiske Produkter, nr. 153; 2016. https://www2.mst.dk/Udgiv/publikationer/2016/12/978-87-93529-52-6.pdf. Accessed September 1, 2017.
10. Wenande E, Garvey LH. Immediate-type hypersensitivity to polyethylene glycols: a review. *Clin Exp Allergy*. 2016;46(7):907-922.
11. Ficheux A-S, Gomez-Berrada M-P, Roudot A-C, Ferret P-J. Consumption and exposure to finished cosmetic products: A systematic review. *Food Chem Toxicol*. 2019;124(Feb):280-299.
12. Hall B, Tozer S, Safford B, et al. European consumer exposure to cosmetic products, a framework for conducting population exposure assessments. *Food Chem Toxicol*. 207AD;45(11):2097-2108.
13. Hall B, Steiling W, Safford B, et al. European consumer exposure to cosmetic products, a framework for conducting population exposure assessments Part 2. *Food Chem Toxicol*. 2011;49(2):408-422.
14. Regulation (EC) No 1223/2009 of the European Parliament and of the council of 30 November 2009 on cosmetic products. https://eur-lex.europa.eu/eli/reg/2009/1223/oj. Accessed July 30, 2021.
15. Zirwas MJ. Contact Dermatitis to Cosmetics. *Clin Rev Allergy Immunol*. 2019;56(1):119-128.
16. Zukiewicz-Sobczak WA, Adamczuk P, Wróblewska P, et al. Allergy to selected cosmetic ingredients. *Postep Dermatol Alergol*. 2013;30(5):307-310.
17. Gilissen L, Huygens S, Goossens A. Allergic contact dermatitis caused by topical herbal remedies: importance of patch testing with the patients' own products. *Contact Dermatitis*. 2018;78(3):177-184.
18. International Cosmetic Ingredient Nomenclature Committee (INC) - Personal Care Products Council. https://www.personalcarecouncil.org/resources/inci/background-information/international-cosmetic-ingredient-nomenclature-committee-inc/. Accessed July 30, 2021.
19. CAS REGISTRY | CAS. https://www.cas.org/cas-data/cas-registry. Accessed July 30, 2021.
20. Mertens S, Gilissen L, Goossens A. Allergic contact dermatitis caused by cocamide diethanolamine. *Contact Dermatitis*. 2016;75(1):20-24.
21. Corazza M, Borghi A, Gallo R, et al. Topical botanically derived products: use, skin reactions, and usefulness of patch tests. A multicentre Italian study. *Contact Dermatitis*. 2014;70(2):90-97.
22. Martin SF, Rustemeyer T, Thyssen JP. Recent advances in understanding and managing contact dermatitis. *F1000Research*. 2018;20(7):1-10.
23. Martin SF, Bonefeld CM. Chapter 6 - Mechanisms of Irritant and Allergic Contact Dermatitis. In: Contact Dermatitis (Sixth Edition). (Johansen JD, Mahler V, Lepoittevin JP, Frosch PJ, eds):2021;

95-120.
24. Rolls S, Owen E, Bertram CG, et al. What is in? What is out? Updating the British Society for Cutaneous Allergy facial series. *Br J Dermatol*. 2021;184(1):151-155.
25. Horton E, Uter W, Geier J, et al. Developing a cosmetic series: Results from the ESSCA network, 2009-2018. *Contact Dermatitis*. 2021;84(2):82-94.
26. Park ME, Zippin JH. Allergic Contact Dermatitis to Cosmetics. *Dermatol Clin*. 2014;32(1):1-11.
27. Johansen JD, Aalto-Korte K, Agner T, et al. European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice. *Contact Dermatitis*. 2015;73(4):195-221.
28. Garvey LH, Krøigaard M, Poulsen LK, et al. IgE-mediated allergy to chlorhexidine. *J Allergy Clin Immunol*. 2007;120(2):409-415.
29. Pootongkam S, Nedorost S. Oat and wheat as contact allergens in personal care products. *Dermatitis*. 2013;24(6):291-295.
30. Vansina S, Debilde D, Morren M-A, Goossens A. Sensitizing oat extracts in cosmetic creams: is there an alternative? *Contact Dermatitis*. 2010;63(3):169-171.
31. Porcaro F, Caminiti L, Crisafulli G, Guglielmo F, Pajno GB. Anaphylaxis to cutaneous exposure to bovine colostrum based cream. *Asian Pacific J Allergy Immunol*. 2019;37(1):9-11.
32. Jarmoc LM, Primack WA. Anaphylaxis to Cutaneous Exposure to Milk Protein in a Diaper Rash Ointment. *Clin Pediatr (Phila)*. 1987;26(3):154-155.
33. Laurière M, Pecquet C, Bouchez-Mahiout I, et al. Hydrolysed wheat proteins present in cosmetics can induce immediate hypersensitivities. *Contact Dermatitis*. 2006;54(5):283-289.
34. Yagami A, Aihara M, Ikezawa Z, et al. Outbreak of immediate-type hydrolyzed wheat protein allergy due to a facial soap in Japan. *J Allergy Clin Immunol*. 2017;140(3):879-881.
35. Nakamura M, Yagami A, Hara K, Sano-Nagai A, Kobayashi T, Matsunaga K. Evaluation of the cross-reactivity of antigens in Glupearl 19S and other hydrolysed wheat proteins in cosmetics. *Contact Dermatitis*. 2016;74(6):346-352.
36. Goossens A, Amaro C, Mahler V. Chapter 16 - Protein Contact Dermatitis. In: Contact Dermatitis (Sixth Edition). (Johansen JD, Mahler V, Lepoittevin JP, Frosch PJ, eds):2021; 355-364.
37. Hjorth N, Roed-Petersen J. Occupational protein contact dermatitis in food handlers. *Contact Dermatitis*. 1976;2(1):28-42.
38. Amaro C, Goossens A. Immunological occupational contact urticaria and contact dermatitis from proteins: a review. *Contact Dermatitis*. 2008;58(2):67-75.
39. Alinaghi F, Bennike NH, Egeberg A, Thyssen JP, Johansen JD. Prevalence of contact allergy in the general population: A systematic review and meta-analysis. *Contact Dermatitis*. 2019;80(2):77-85.
40. Wilkinson M, Gallo R, Goossens A, et al. A proposal to create an extension to the European baseline series. *Contact Dermatitis*. 2018;78(2):101-108.
41. Bailey Jr. F, Koleske J. Chapter 3 - Early History of the Poly(alkylene oxide)s. In: Alkylene Oxides and Their Polymers. 1990:27-34.
42. Garvey LH, Nasser S. Anaphylaxis to the first COVID-19 vaccine: is polyethylene glycol (PEG) the culprit? *Br J Anaesth*. 2021;126(3):e106-e108.
43. Wenande E, Kroigaard M, Mosbech H, Garvey LH. Polyethylene glycols (PEG) and related structures: overlooked allergens in the perioperative setting. *A&A case reports*. 2015;4(5):61-64.
44. Calogiuri G, Foti C, Bilo MB, Garvey LH. Macrogols: A misleading cause of drug hypersensitivity diagnosis. *Clin Immunol Endocr Metab Drugs*. 2017;4(1):9-13.
45. Younes M, Aggett P, Aguilar F, et al. Refined exposure assessment of polyethylene glycol (E 1521) from its use as a food additive. *EFSA journal Eur Food Saf Auth*. 2018;16(6):e05293.
46. Jang H-J, Shin CY, Kim K-B. Safety Evaluation of Polyethylene Glycol (PEG) Compounds for Cosmetic Use. *Toxicol Res*. 2015;31(2):105-136.
47. Povoski SP, Davis PD, Colcher D, Martin Jr EW. Single molecular weight discrete PEG compounds: emerging roles in molecular diagnostics, imaging and therapeutics. *Expert Rev Mol Diagn*. 2013;13(4):315-319.
48. Yamasuji Y, Higashi Y, Sakanoue M, et al. A case of anaphylaxis caused by polyethylene glycol analogues. *Contact Dermatitis*. 2013;69(3):183-185.
49. Badiu I, Guida G, Heffler E, Rolla G. Multiple drug allergy due to hypersensitivity to

polyethylene glycols of various molecular weights. *J Investig Allergol Clin Immunol*. 2015;25(5):368-369.
50. Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. *Pharm Res*. 2006;23(12):2709-2728.
51. Russo E, Villa C. Poloxamer hydrogels for biomedical applications. *Pharmaceutics*. 2019;11(12):671.
52. Ruel-Gariépy E, Leroux J-C. In situ-forming hydrogels-review of temperature-sensitive systems. *Eur J Pharm Biopharm*. 2004;58(2):409-426.
53. Urbán-Morlán Z, Castro-Ríos R, Chávez-Montes A, et al. Determination of poloxamer 188 and poloxamer 407 using high-performance thin-layer chromatography in pharmaceutical formulations. *J Pharm Biomed Anal*. 2008;46(4):799-803.
54. Singh-Joy S, McLain VC. Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105 benzoate, and poloxamer 182 dibenzoate as use. *Int J Toxicol*. 2008;27(SUPPL. 2):93-128. https://journals.sagepub.com/doi/10.1080/10915810802244595?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed.
55. Patel HR, Patel RP, Patel MM. Poloxamers: A pharmaceutical excipients with therapeutic behaviors. *Int J PharmTech Res*. 2009;1(2):299-303.
56. Jones MT, Mahler H-C, Yadav S, et al. Considerations for the Use of Polysorbates in Biopharmaceuticals. *Pharm Res*. 2018;35(8):148.
57. ANNEX I SUMMARY OF PRODUCT CHARACTERISTICS. https://www.ema.europa.eu/en/documents/product-information/covid-19-vaccine-janssen-epar-product-information_en.pdf. Accessed July 30, 2021.
58. ANNEX I SUMMARY OF PRODUCT CHARACTERISTICS. https://www.ema.europa.eu/en/documents/product-information/vaxzevria-previously-covid-19-vaccine-astrazeneca-epar-product-information_en.pdf. Accessed July 30, 2021.
59. Banerji A, Wolfson AR, Wickner PG, et al. COVID-19 Vaccination in Patients with Reported Allergic Reactions: Updated Evidence and Suggested Approach. *J Allergy Clin Immunol Pract*. 2021;9(6):2135-2138.
60. Fisher AA. Contact urticaria due to polyethylene glycol. Cutis. 1977 Apr;19(4):409-12.
61. Cerdá VJ, Pacheco RR, Witek JD, De La Calle FMM, De La Sen Fernández ML. Immediate hypersensitivity to polyethylene glycols in unrelated products: When standardization in the nomenclature of the components of drugs, cosmetics, and food becomes necessary. *Allergy, Asthma Clin Immunol*. 2019;15(1).
62. Wenande EC, Skov PS, Mosbech H, Poulsen LK, Garvey LH. Inhibition of polyethylene glycol-induced histamine release by monomeric ethylene and diethylene glycol: a case of probable polyethylene glycol allergy. *J Allergy Clin Immunol*. 2013;131(5):1425-1427.
63. Shah S, Prematta T, Adkinson NF, Ishmael FT. Hypersensitivity to polyethylene glycols. *J Clin Pharmacol*. 2013;53(3):352-355.
64. Co-Minh HB, Demoly P, Guillot B, Raison-Peyron N. Anaphylactic shock after oral intake and contact urticaria due to polyethylene glycols. *Allergy*. 2007;62(1):92-93.
65. Hyry H, Vuorio A, Varjonen E, Skyttä J, Mäkinen-Kiljunen S. Two cases of anaphylaxis to macrogol 6000 after ingestion of drug tablets. *Allergy*. 2006;61(8):1021-1021.
66. Stone CA, Liu Y, Relling M V, et al. Immediate hypersensitivity to polyethylene glycols and polysorbates: more common than we have recognized. *J allergy Clin Immunol Pract*. 2019;7(5):1533-1540.e8.
67. Krantz MS, Liu Y, Phillips EJ, Stone CA. COVID-19 vaccine anaphylaxis: PEG or not? *Allergy Eur J Allergy Clin Immunol*. 2021;76(6):1934-1937.
68. Cox F, Khalib K, Conlon N. PEG That Reaction: A Case Series of Allergy to Polyethylene Glycol. *J Clin Pharmacol*. 2021;61(6):832-835.
69. Sellaturay P, Nasser S, Ewan P. Polyethylene Glycol–Induced Systemic Allergic Reactions (Anaphylaxis). *J Allergy Clin Immunol Pract*. 2021;9(2):670-675.
70. Borderé A, Stockman A, Boone B, et al. A case of anaphylaxis caused by macrogol 3350 after injection of a corticosteroid. *Contact Dermatitis*. 2012;67(6):376-378.

71. Oyarzabal NA, Longo Areso N, Bernedo Belar N, Popolizio IG, Arregui AV, Balza de Vallejo OV. Anaphylactic shock due to allergy to macrogol 4000 contained in SonoVue®. *Case Reports Clin Med*. 2017;06(06):143-147.
72. Antón Gironés M, Roan Roan J, de la Hoz B, Sánchez Cano M. Immediate allergic reactions by polyethylene glycol 4000: two cases. *Allergol Immunopathol (Madr)*. 36(2):110-112.
73. Dewachter P, Mouton-Faivre C. Anaphylaxis to macrogol 4000 after a parenteral corticoid injection. *Allergy*. 2005;60(5):705-706.
74. Brandt N, Garvey LH, Bindslev-Jensen U, Kjaer HF, Bindslev-Jensen C, Mortz CG. Three cases of anaphylaxis following injection of a depot corticosteroid with evidence of IgE sensitization to macrogols rather than the active steroid. *Clin Transl Allergy*. 2017;7:2.
75. Bommarito L, Mietta S, Nebiolo F, Geuna M, Rolla G. Macrogol hypersensitivity in multiple drug allergy. *Ann Allergy Asthma Immunol*. 2011;107(6):542-543.
76. Opstrup MS, Poulsen LK, Malling HJ, Jensen BM, Garvey LH. Dynamics of plasma levels of specific IgE in chlorhexidine allergic patients with and without accidental re-exposure. *Clin Exp Allergy*. 2016;46(8):1090-1098.
77. Opstrup MS, Malling HJ, Krøigaard M, et al. Standardized testing with chlorhexidine in perioperative allergy-a large single-centre evaluation. *Allergy Eur J Allergy Clin Immunol*. 2014;69(10):1390-1396.
78. Opstrup MS, Mosbech H, Garvey LH. Allergic sensitization to ethylene oxide in patients with suspected allergic reactions during surgery and anesthesia. *J Investig Allergol Clin Immunol*. 2010;20:269-270.
79. Hjortlund J, Mortz CG, Stage TB, Skov PS, Dahl R, Bindslev-Jensen C. Positive serum specific IgE has a short half-life in patients with penicillin allergy and reversal does not always indicate tolerance. *Clin Transl Allergy*. 2014;29(4):34.
80. Fisher AA. Immediate and delayed allergic contact reactions to polyethylene glycol. *Contact Dermatitis*. 1978;4(3):135-138.
81. Prieto A, Baeza ML, Herrero T, et al. Contact dermatitis to Furacin®. *Contact Dermatitis*. 2006;54(2):126-126.
82. Moreno Escobosa MC, Moya Quesada MC, Cruz Granados S, Amat López J. Contact dermatitis to antibiotic ointments. *J Investig Allergol Clin Immunol*. 2009;19(6):510-511.
83. Guijarro S, Sánchez-Pérez J, García-Díez A. Allergic contact dermatitis to polyethylene glycol and nitrofurazone. *Am J Contact Dermat*. 1999;10(4):226-227.
84. Hannuksela M, Pirilä V, Salo O. Skin reactions to propylene glycol. *Contact Dermatitis*. 1975;1(2):112-116.
85. Maibach H. Polyethyleneglycol: allergic contact dermatitis potential. *Contact Dermatitis*. 1975;1(4):247-247.
86. Bajaj A, Gupta S, Chatterjee A, Singh K. Contact sensitivity to polyethylene glycols. *Contact Dermatitis*. 1990;22(5):291-292.
87. Özkaya E, Kılıç S. Polyethylene glycol as marker for nitrofurazone allergy: 20 years of experience from Turkey. *Contact Dermatitis*. 2018;78(3):211-215.
88. Kroigaard M, Garvey LH, Gillberg L, et al. Scandinavian Clinical Practice Guidelines on the diagnosis, management and follow-up of anaphylaxis during anaesthesia*. *Acta Anaesthesiol Scand*. 2007;51(6):655-670.
89. Heinzerling L, Mari A, Bergmann K-C, et al. The skin prick test-European standards. *Clin Transl Allergy*. 2013;3(1):3.
90. Skov P, Mosbech H, Norn S, Weeke B. Sensitive glass microfibre-based histamine analysis for allergy testing in washed blood cells. Results compared with conventional leukocyte histamine release assay. *Allergy*. 1985;40(3):213-218.
91. Larsen LF, Juel-Berg N, Hansen KS, et al. A comparative study on basophil activation test, histamine release assay, and passive sensitization histamine release assay in the diagnosis of peanut allergy. *Allergy Eur J Allergy Clin Immunol*. 2018;73(1):137-144.
92. Danish Consumer Council THINK Chemicals | Forbrugerrådet Tænk Kemi. https://kemi.taenk.dk/english. Accessed August 25, 2021.
93. Bennike NH, Oturai NB, Müller S, et al. Fragrance contact allergens in 5588 cosmetic products identified through a novel smartphone application. *J Eur Acad Dermatology Venereol*.

2018;32(1):79-85.
94. The Danish Consumer Council THINK Chemicals' test methods | Forbrugerrådet Tænk Kemi. https://kemi.taenk.dk/bliv-groennere/danish-consumer-council-think-chemicals-test-methods. Accessed April 15, 2019.
95. Husain Z, Schwartz RA. Peanut allergy: an increasingly common life-threatening disorder. *J Am Acad Dermatol*. 2012;66(1):136-143.
96. Mousan G, Kamat D. Cow's milk protein allergy. *Clin Pediatr (Phila)*. 2016;55(11):1054-1063.
97. Flom JD, Sicherer SH. Epidemiology of cow's milk allergy. *Nutrients*. 2019;11(5):1051.
98. Burnett C, Bergfeld WF, Belsito D V., et al. Safety assessment of hydrolyzed wheat protein and hydrolyzed wheat gluten as used in cosmetics. *Int J Toxicol*. 2018;37(1_suppl):55S-66S.
99. de Groot AC. Linalool Hydroperoxides. *Dermatitis*. 2019;30(4):243-246.
100. de Groot AC. Limonene Hydroperoxides. *Dermat contact, atopic, Occup drug*. 2019;30(6):331-335.
101. Rajpara S, Wilkinson MS, King CM, et al. The importance of propolis in patch testing–a multicentre survey. *Contact Dermatitis*. 2009;61(5):287-290.
102. Wilkinson M, Gonçalo M, Aerts O, et al. The European baseline series and recommended additions: 2019. *Contact Dermatitis*. 2019;80(1):1-4.
103. Nyman GSA, Tang M, Inerot A, Osmancevic A, Malmberg P, Hagvall L. Contact allergy to beeswax and propolis among patients with cheilitis or facial dermatitis. *Contact Dermatitis*. 2019;81(2):110-116.
104. Uter W, Gefeller O, Mahler V, Geier J. Trends and current spectrum of contact allergy in Central Europe: results of the Information Network of Departments of Dermatology (IVDK) 2007–2018*. *Br J Dermatol*. 2020;183(5):857-865.
105. Schubert S, Geier J, Dickel H, Buhl T, Ruëff F, Löffler H. Contact sensitization to propolis in the Information Network of Departments of Dermatology (IVDK) 2013-2019 and market survey of propolis commerce in Germany. *Contact Dermatitis*. 2021;Aug 22:doi:10.1111/cod.13960.
106. Holm JG, Agner T, Clausen ML, Thomsen SF. Quality of life and disease severity in patients with atopic dermatitis. *J Eur Acad Dermatology Venereol*. 2016;30(10):1760-1767.
107. Boonchai W, Charoenpipatsin N, Winayanuwattikun W, Phaitoonwattanakij S, Sukakul T. Assessment of the quality of life (QoL) of patients with dermatitis and the impact of patch testing on QoL: A study of 519 patients diagnosed with dermatitis. *Contact Dermatitis*. 2020;83(3):182-188.
108. Blome C, Radtke MA, Eissing L, Augustin M. Quality of Life in Patients with Atopic Dermatitis: Disease Burden, Measurement, and Treatment Benefit. *Am J Clin Dermatol*. 2016;17(2):163-169.
109. Kasemsarn P, Iamphonrat T, Boonchai W. Risk factors and common contact allergens in facial allergic contact dermatitis patients. *Int J Dermatol*. 2016;55(4):417-424.
110. Li LF, Liu G, Wang J. Prevalence and 1-year outcome of facial allergic contact dermatitis in patients patch tested in a university hospital. *Contact Dermatitis*. 2007;57(3):187-190.
111. Katz AS, Sherertz EF. Facial dermatitis: patch test results and final diagnoses. *Am J Contact Dermat*. 1999;10(3):153-156.
112. Garg T, Agarwal S, Chander R, Singh A, Yadav P. Patch testing in patients with suspected cosmetic dermatitis: A retrospective study. *J Cosmet Dermatol*. 2018;17(1):95-100.
113. Warshaw EM, Buchholz HJ, Belsito D V., et al. Allergic patch test reactions associated with cosmetics: Retrospective analysis of cross-sectional data from the North American Contact Dermatitis Group, 2001-2004. *J Am Acad Dermatol*. 2009;60(1):23-38.
114. Alani JI, Davis MDP, Yiannias JA. Allergy to cosmetics: a literature review. *Dermatits*. 2013;24(6):283-290.
115. Davies RF, Johnston GA. New and emerging cosmetic allergens. *Clin Dermatol*. 2011;29(3):311-315.
116. Biebl KA, Warshaw EM. Allergic Contact Dermatitis to Cosmetics. *Dermatol Clin*. 2006;24(2):215-232.
117. Hafner M de FS, Rodrigues AC, Lazzarini R. Allergic contact dermatitis to cosmetics: retrospective analysis of a population subjected to patch tests between 2004 and 2017. *An Bras Dermatol*. 2020;95(6):696-701.
118. Travassos AR, Claes L, Boey L, Drieghe J, Goossens A. Non-fragrance allergens in specific

cosmetic products. *Contact Dermatitis*. 2011;65(5):276-285.
119. Lindberg A, Tammela M, Boström Å, et al. Are adverse skin reactions to cosmetics underestimated in the clinical assessment of contact dermatitis? A prospective study among 1075 patients attending Swedish patch test clinics. *Acta Derm Venereol*. 2004;84(4):291-295.
120. Bennike NH, Zachariae C, Johansen JD. Trends in contact allergy to fragrance mix I in consecutive Danish patients with eczema from 1986 to 2015: a cross-sectional study. *Br J Dermatol*. 2017;176(4):1035-1041.
121. Bennike NH, Zachariae C, Johansen JD. Non-mix fragrances are top sensitizers in consecutive dermatitis patients - a cross-sectional study of the 26 EU-labelled fragrance allergens. *Contact Dermatitis*. 2017;77(5):270-279.
122. The Scientic Committee on Cosmetic Products and Non-Food Products Intended for Consumers. Opinion Concerning Methylisothiazolinone (Colipa No. P94). https://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out_201.pdf Accessed August 24, 2021.
123. Havmose M, Thyssen JP, Zachariae C, Menné T, Johansen JD. The epidemic of contact allergy to methylisothiazolinone—An analysis of Danish consecutive patients patch tested between 2005 and 2019. *Contact Dermatitis*. 2021;84(4):254-262.
124. Lundov MD, Thyssen JP, Zachariae C, Johansen JD. Prevalence and cause of methylisothiazolinone contact allergy. *Contact Dermatitis*. 2010;63(3):164-167.
125. Lundov MD, Opstrup MS, Johansen JD. Methylisothiazolinone contact allergy - a growing epidemic. *Contact Dermatitis*. 2013;69(5):271-275.
126. Johnston GA. The rise in prevalence of contact allergy to methylisothiazolinone in the British Isles. *Contact Dermatitis*. 2014;70(4):238-240.
127. Urwin R, Wilkinson M. Methylchloroisothiazolinone and methylisothiazolinone contact allergy: a new 'epidemic.' *Contact Dermatitis*. 2013;68(4):253-255.
128. Schwensen JF, White IR, Thyssen JP, Menné T, Johansen JD. Failures in risk assessment and risk management for cosmetic preservatives in Europe and the impact on public health. *Contact Dermatitis*. 2015;73(3):133-141.
129. Belloni Fortina A, Cooper SM, Spiewak R, Fontana E, Schnuch A, Uter W. Patch test results in children and adolescents across Europe. Analysis of the ESSCA Network 2002-2010. *Pediatr Allergy Immunol*. 2015;26(5):446-455.
130. Paulsen E, Andersen KE. Colophonium and Compositae mix as markers of fragrance allergy: Cross-reactivity between fragrance terpenes, colophonium and Compositae plant extracts. *Contact Dermatitis*. 2005;53(5):285-291.
131. Gökay SS, Çelik T, Sari MY, Ekinci F, Yildizdaş RD, Yilmaz HL. Urticaria as a rare side effect of polyethylene glycol-3350 in a child: Case report. *Acta Clin Croat*. 2018;57(1):187-189.
132. Lee SH, Hwang SH, Park JS, Park HS, Shin YS. Anaphylaxis to polyethylene glycol (Colyte®) in a patient with diverticulitis. *J Korean Med Sci*. 2016;31(10):1662-1663.
133. Wylon K, Dölle S, Worm M. Polyethylene glycol as a cause of anaphylaxis. *Allergy, Asthma Clin Immunol*. 2016;12(1).
134. Jakubovic BD, Saperia C, Sussman GL. Anaphylaxis following a transvaginal ultrasound. *Allergy Asthma Clin Immunol*. 2016;12(1):3.
135. ANNEX I SUMMARY OF PRODUCT CHARACTERISTICS. https://www.ema.europa.eu/en/documents/product-information/comirnaty-epar-product-information_en.pdf. Accessed July 30, 2021.
136. ANNEX I SUMMARY OF PRODUCT CHARACTERISTICS. https://ec.europa.eu/health/documents/community-register/2021/20210713152599/anx_152599_en.pdf. Accessed July 30, 2021.
137. RefLab. Standard/Non-standard Materials. https://reflab.dk/allergy-testing/histamine-release-test/standard-materials/. Accessed June 26, 2020.
138. Nguyen KL, Gillis S, MacGlashan DW. A comparative study of releasing and nonreleasing human basophils: Nonreleasing basophils lack an early component of the signal transduction pathway that follows IgE cross-linking. *J Allergy Clin Immunol*. 1990;85(6):1020-1029.
139. Zhou Z-H, Stone CA, Jakubovic B, et al. Anti-PEG IgE In Anaphylaxis Associated with Polyethylene Glycol. *J Allergy Clin Immunol Pract*. 2021;9(4):1731-1733.e3.

140. Opstrup M, Johansen J, Bossi R, Lundov M, Garvey L. Chlorhexidine in cosmetic products - a market survey. *Contact Dermatitis*. 2015;72(1):55-58.
141. Yazar K, Boman A, Lidén C. p-Phenylenediamine and other hair dye sensitizers in Spain. *Contact Dermatitis*. 2012;66(1):27-32.
142. Yazar K, Boman A, Lidén C. Potent skin sensitizers in oxidative hair dye products on the Swedish market. *Contact Dermatitis*. 2009;61(5):269-275.
143. Bruze M, Isaksson M, Edman B, Björkner B, Fregert S, Möller H. A study on expert reading of patch test reactions: inter-individual accordance. *Contact Dermatitis*. 1995;32(6):331-337.
144. Svedman C, Isaksson M, Björk J, Mowitz M, Bruze M. "Calibration" of our patch test reading technique is necessary. *Contact Dermatitis*. 2012;66(4):180-187.
145. Wee J, White J, McFadden J, White I. Patch testing in patients treated with systemic immunosuppression and cytokine inhibitors. *Contact Dermatitis*. 2010;62(3):165-169.
146. Fincham J. Response rates and responsiveness for surveys, standards, and the Journal. *Am J Pharm Educ*. 2008;72(2):43.

9. Manuscripts

Manuscript I. Bruusgaard-Mouritsen MA, Johansen JD, Zachariae C, Kirkeby CS, Garvey LH. Natural ingredients in cosmetic products - A suggestion for a screening series for skin allergy. *Contact Dermatitis.* 2020 Oct;83(4):251-270.

Received: 25 February 2020 | Revised: 31 March 2020 | Accepted: 2 April 2020

DOI: 10.1111/cod.13550

ORIGINAL ARTICLE

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Natural ingredients in cosmetic products—A suggestion for a screening series for skin allergy

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Funding information
The Danish Environmental Protection Agency, Grant/Award Number: A grant to The National Allergy Research Centre

Abstract

Background: Naturally derived cosmetic product ingredients of both plant and animal origin are being included increasingly in product formulations in order to cater to consumer preferences. They may be an overlooked cause of reactions to cosmetic products in some patients with dermatitis.

Objectives: To identify naturally derived cosmetic product ingredients with allergenic potential (type I and type IV) and propose a cosmetic screening test series.

Methods: The study was conducted in two steps. The first step was a market survey using a nonprofit application helping consumers avoid problematic substances in cosmetic products. The application contained 10 067 cosmetic products that were label checked for naturally derived cosmetic product ingredients. The second step was a literature search to examine how frequently the naturally derived ingredients were described and related to allergic reactions in cosmetics or other topically administered products.

Results: We identified 121 different naturally derived cosmetic product ingredients that were included in at least 30 cosmetic products. In total, 22 ingredients were selected for a screening test series.

Conclusions: We propose a supplemental patch test and a prick test screening series with naturally derived cosmetic product ingredients for patients with skin reactions to cosmetic products, aiming to identify a cause in more patients than is currently possible.

KEYWORDS

cosmetics, food proteins, natural ingredients, patch test, skin prick test

1 | INTRODUCTION

Cosmetic products labeled as natural are increasing in popularity.[1,2] This is due to a general belief among consumers that these products are safe and have health and environmental benefits.[2,3] However, there is no clear official or legislative definition of what natural covers.[2] According to European cosmetic regulations, natural ingredients in cosmetics refer to the origin of the ingredients in the products and production method.[2] In this study, we define natural ingredients as plant- and animal-derived ingredients. Cosmetic products with and without natural ingredients are known to cause skin reactions, but these are underreported, as people tend to just stop using the cosmetic product suspected of causing a skin reaction without seeking medical advice. In addition, due to an often-delayed onset of symptoms most patients do not to suspect the cosmetic product to be the culprit.[3,4]

Type IV allergy from cosmetics is well known and many allergens have been identified.[1] Type I allergy and protein contact dermatitis caused by food proteins are less commonly diagnosed in relation to cosmetics and may be difficult to identify due to the many different natural ingredients used.[5] New allergens are continuously described, but no recommendations exist regarding test methods or how to select natural ingredients for testing. To date, only very few systematic investigations of the allergenicity of the burgeoning number of new cosmetic product ingredients being included in cosmetic product formulations have been performed. We hypothesize that some patients with dermatitis or other skin reactions, where an allergen is not identified, might be reacting to naturally derived cosmetic product ingredients. Our aim is to establish a screening test series with the natural ingredients commonly used in cosmetic products relevant for both type I and type IV allergies. This can potentially identify new causative allergens among patients with type I and type IV allergies suspected to be caused by cosmetic products.

2 | METHODS

The study was conducted in two steps. The first step was a market survey using the application (app) "Kemiluppen" developed in 2015 by The Danish Consumer Council THINK Chemicals, an initiative under the Danish Consumer Council, which is a nongovernmental organization helping consumers avoid problematic chemical substances when shopping for consumer products.[6] The second step was a literature search to examine how frequently these potential allergens were described and related to allergic reactions in cosmetics or other topically administered products.

2.1 | The application "Kemiluppen"

A market survey was conducted between June and September 2017 using "Kemiluppen" (in English translated to "The Chemicals Magnifying Glass"), a free and nonprofit smartphone app providing consumers an easy overview of allergenic, carcinogenic, and other problematic ingredients or suspected endocrine-disrupting chemicals in cosmetic products. The app functions by the consumer scanning the European Article Numbering (EAN) barcode of a cosmetic product with their smartphone camera, and the consumer uploads pictures of the product and ingredients in the app. The product is further investigated by THINK Chemicals, which manually enters the product name, category and International Nomenclature of Cosmetic Ingredients (INCI) labeling in an anonymized database. The database contains no information on the individual consumer who is scanning the product. THINK Chemicals evaluates the ingredients according to different lists of problematic chemicals, and the ingredient list is also sent to the manufacturer to verify that the entered details are correct.[7] If this has already been done, consumers are instantly informed of possible problematic substances in the product.[8]

In June 2017, Kemiluppen included 10 067 products containing a total of 208 341 labeled ingredients covering 4432 different ingredients. On average, each cosmetic product contained 21 ingredients. The ingredients of all cosmetic products included in the app were reviewed for plant- and animal-derived ingredients by the first author of this article.

2.2 | Literature review

A literature search was conducted in the Medline/PubMed database combining the words "contact allergy" OR "urticaria" in association with (by using AND) the individual ingredients listed in Table 1. Inclusion criteria were articles published until June 2019 describing type I or type IV allergic reactions to the respective natural ingredients. Relevant bibliographical references from identified reports were also reviewed. In addition, standard textbooks on cosmetic ingredients, including natural ingredients, were consulted, in particular Monographs in Contact Allergy Volumes 1 and 2 by Anton C. de Groot.[9-11]

2.3 | Development of a screening test series

To establish a screening test series with relevant natural ingredients, we used two selection criteria:

1. Natural ingredients in Kemiluppen that were included in at least 30 cosmetic products

and

2. Ingredients described as the causative allergen:

in cosmetic or topical pharmaceutical products in more than three patient cases.

or

in ≥20 articles from other (or unknown) exposure sources.

A total of 21 ingredients fulfilled the selection criteria and were examined further.

SAS Enterprise Guide software, version 7.1 (SAS Institute, Cary, North Carolina) was used for data management.

3 | RESULTS

In total, we identified 121 different natural ingredients that were included in at least 30 cosmetic products. The 117 plant-derived and 4 animal-derived ingredients that were included in at least 30 cosmetic products are listed in Table 1. Of these, 18 plant-derived ingredients and 3 animal-derived ingredients fulfilled the criteria to be investigated further.

3.1 | Review of 21 selected ingredients from the Kemiluppen database

In total, 21 ingredients were selected for further investigation and categorized into three groups depending on whether they were reported

TABLE 1 The 121 naturally derived cosmetic product ingredients included in at least 30 cosmetic products in Kemiluppen

Ingredients derived from [common name]:	Botanical or animal source	Quantity in cosmetic products
Cinnamon/cinnamal	***Cinnamomum zeylanicum, Cinnamomum cassia***	**1627**
Aloe	*Aloe barbadensis*	1612
Shea nut	*Butyrospermum parkii nut*	1310
Bee products:	**In total:**	**865**
Beeswax/cera alba/cera flava	**Bee**	**798**
Propolis	**Bee**	**25**
Honey	Bee	25
Royal jelly	Bee	17
Sunflower*	***Helianthus annuus***	858
Jojoba	*Simmondsia chinensis*	714
Almond	*Prunus amygdalus*	701
Wheat	***Triticum vulgare***	**602**
Olive	*Olea europaea*	599
Algae, seaweed	Various species	555
Guar bean	*Guar hydroxypropyltrimonium*	500
Lanolin	**Sheep**	**475**
Soybean	***Glycine soja***	**441**
Coconut	*Cocos nucifera*	390
Avocado	*Persea gratissima*	371
Orange	***Citrus aurantium***	**370**
Argan tree	*Argania spinosa*	361
Chamomile*	***Chamomilla recutita, Chamaemelum nobile***	350
Apricot	*Prunus armeniaca*	349
Rosemary	*Rosmarinus officinalis*	338
Rose	**Various rose species**	**329**
Castor oil plant	*Ricinus communis*	307
Brazilian tropical palm tree	*Copernicia cerifera*	277
Corn	*Zea mays*	271
Macadamia	*Macadamia integrifolia*	270
Camellia	*Camellia oleifera*	266
Rape	*Brassica napus*	264
Lavender	***Lavandula augustifolia***	**230**
Mint	***Mentha piperita, Mentha spicata, Mentha aquatica***	**215**
Rice	*Oryza sativa*	212
Candelilla	*Euphorbia cerifera*	210
Pot marigold*	***Calendula officinalis***	205
Cocoa	*Theobroma cacao*	200
Grape wine	*Vitis vinifera*	194
Lemon	***Citrus limon***	**192**
Witch-hazel	*Hamamelis virginiana*	166
Sesame	*Sesamum indicum*	165
Milk	**Cow/horse/donkey**	**150**
Liquorice	***Glycyrrhiza glabra, Glycyrrhiza inflata***	**146**
Oat	***Avena sativa***	**143**

(Continues)

TABLE 1 (Continued)

Ingredients derived from [common name]:	Botanical or animal source	Quantity in cosmetic products
Pomegranate	*Punica granatum*	142
Silk	Insects	141
Pomelo	*Citrus grandis*	137
Cucumber	*Cucumis sativus*	132
Pelargonium	*Pelargonium graveolens*	125
Eucalyptus	***Eucalyptus globulus***	**124**
Safflower	*Carthamus tinctorius*	124
Field mustard	*Brassica campestris*	106
Salvia	*Salvia officinalis*	100
Tapioca	*Manihot esculenta*	95
Açaí palm	*Euterpe oleracea*	94
Apple	*Pyrus malus*	92
Flax	*Linum usitatissimum*	92
Acacia	*Acacia senegal*	90
Evening primrose	*Oenothera biennis*	90
Wild carrot	*Daucus carota*	87
Magnolia	*Magnolia officinalis*	82
Bergamot orange	*Citrus aurantium bergamia*	79
Lemongrass	***Cymbopogon flexuosus, Cymbopogon citratus***	**79**
Irish moss	*Chondrus crispus*	74
Common bamboo	*Bambusa vulgaris*	73
Coneflower*	***Echinacea purpurea, Echinacea angustifolia***	70
Australian tea tree	***Melaleuca alternifolia***	**68**
Mango	*Mangifera indica*	68
Orbignya	*Orbignya oleifera*	67
Moringa	*Moringa oliefera*	65
Ylang-ylang	***Cananga odorata***	**65**
Brazil nut	*Bertholletia excelsa*	64
Sugar	*Saccharum officinarum*	64
Ginger	*Zingiber officinale*	63
Patchouli	*Pogostemon cablin*	63
Levant cotton	*Gossypium herbaceum*	61
Linden	*Tilia vulgaris, Tilia cordata*	60
Blueberry	*Vaccinium angustifolium, Vaccinium corymbosum, Vaccinium myrtillus*	58
Jasmine	*Jasminum officinale*	57
Barley	*Hordeum vulgare*	55
Elder	*Sambucus nigra*	55
Lemon balm	*Melissa officinalis*	53
Raspberry	*Rubus idaeus*	51
Light Red Meranti	*Shorea stenoptera*	50
African oil palm	*Elaeis guineensis*	49
Candlenut	*Aleurites moluccana*	49
Arnica*	***Arnica montana***	48
Ginseng	*Panax ginseng*	47

TABLE 1 (Continued)

Ingredients derived from [common name]:	Botanical or animal source	Quantity in cosmetic products
Potato	***Solanum tuberosum***	**47**
Willow	*Salix nigra*	47
Birch	*Betula alba*	45
Burdock*	***Arctium lappa***	45
Gotu Kola	*Centella asiatica*	44
Maidenhair tree	*Ginkgo biloba*	44
Juniper	*Juniperus communis*	44
Peanut	***Arachis hypogaea***	**44**
Grapefruit	*Citrus paradise*	43
Mandarin	*Citrus nobilis*	43
Horsetail	*Equisetum arvense*	42
Passionflower	*Passiflora*	42
Lime	*Citrus aurantifolia*	40
Vanilla	*Vanilla planifolia, Vanilla tahitensis*	39
Coriander	*Coriandrum sativum*	38
Fennel	*Foeniculum vulgare*	38
Peach	***Prunus persica***	**38**
Blackcurrant	*Ribes nigrum*	37
Thyme	*Thymus vulgaris*	37
Gardenia	*Gardenia florida*	36
Yarrow*	***Achillea millefolium***	36
Cranberry	*Gardenia florida*	35
Hibiscus	*Hibiscus rosa sinensis*	35
Hop	*Humulus lupulus*	35
Larch tree	*Galactoarabinan*	35
Boxthorn	*Lycium barbarum*	34
Buckthorn	*Hippophae rhamnoides*	34
Borage	*Borago officinalis*	33
Iris	*Iris florentina*	33
May Chang	*Litsea cubeba*	32
Meadowfoam	*Limnanthes alba*	32
Melon	*Carica papaya*	32
Myrrh	*Commiphora myrrha*	32
Common nettle	*Urtica dioica*	32
Murumuru	*Astrocaryum murumuru*	31
Strawberry	*Fragaria ananassa*	31
Sandalwood	*Santalum album*	30

Note: The 18 plant-derived and 4 animal-derived ingredients that fulfilled the criteria to be investigated further are written in bold. The seven Compositae plants are also shown with an (*) asterisk.

to cause type I, type I and IV, or type IV allergic reactions. The ingredients causing type IV reactions were further categorized into two subgroups depending on whether they are well-known allergens, already routinely tested in many centers, or rarely reported allergens. The ingredients are listed alphabetically in each category, primarily by their vernacular name followed by their botanical name.

3.2 | Ingredients causing type I allergy

3.2.1 | Milk

Allergy to cow´s milk is the most common food allergy in children, affecting approximately 2% of all children.[12] Allergy onset is often in

TABLE 2 Type I allergic reactions to cow´s milk in cosmetic products reported in the literature

Year	Age	Sex	Exposure	Clinical symptoms	Relevant test results	Type of reaction	Reference
2019	16	M	Cutaneous application of a cream containing bovine colostrum to a surgical wound 6 mo after operation.	Urticaria, itching, and wheezing occurred within few minutes. Symptoms responded to systemic epinephrine, hydrocortisone, and chlorpheniramine maleate.	Skin prick test strongly positive to cow´s milk protein.	I	15
1996	35	F	Make-up remover containing casein.	Generalized skin pruritus, dizziness, tachycardia, and profuse sweating immediately after the application to the face.	Skin prick tests positive to cow´s milk and casein. α-Lactalbumin and β-lactoglobulin were negative. Specific IgE to cow's milk and casein were 8.90 and 3.70 kU/L, respectively. Specific IgE to the other milk proteins was negative.	I	17
1987	12 months	M	Casein containing ointment to an inflamed diaper area.	Two episodes of anaphylaxis following cutaneous application of ointment.	Significantly elevated specific IgE antibodies to milk and milk proteins.	I	18
1980	11 months	M	Application of ointment to the area of the napkin or diaper dermatitis.	Flushing, generalized urticaria, and angioedema involving face and lip and mild respiratory distress.	Skin prick test positive to whole cow´s milk, cow´s milk albumin, cow´s milk casein, and cow´s milk whey.	I	19

Abbreviations: F, Female; M, Male.

the first year of life.[13,14] The prevalence decreases to <1% in children ≥6 years of age, but can persist into adolescence and adulthood.[13] The mechanism may be immunoglobulin E (IgE) mediated, non-IgE mediated, or mixed IgE- and non-IgE mediated.[14] IgE-mediated type I allergic reactions account for 60% of reactions. Non–IgE-mediated reactions are mostly type IV allergic, delayed skin reactions.[13,14] In IgE-mediated reactions, symptoms may be from the skin, gastrointestinal, and/or respiratory system and can present as life-threatening anaphylaxis.[15] There are more than 25 different proteins in cow´s milk. Four caseins and the whey proteins—α-lactalbumin, β-lactoglobulin, bovine serum albumin, and lactoferrin—have been identified as allergens.[16] Exposure to milk proteins occurs by drinking cow´s milk or formula-based cow's milk, by being passed through breast milk or via the skin in children who are highly sensitive to milk.[15] Treatment is complete avoidance of milk-containing products, and if the child is breastfed, exclusion of dairy products from the mother's diet.[13] The milk-derived ingredients in the milk-containing cosmetic products registered in Kemiluppen are listed in various forms as milk, whey protein, milk lipids, non-fat dry milk extract, casein, and colostrum. Casein and whey are added to cosmetics for claimed antioxidant, moisturizing, and calming effects, although evidence is extremely limited. Four cases of type I anaphylactic reactions to cow´s milk–containing cosmetics, creams, and ointments following cutaneous application have been described in the literature, all in patients with known cow's milk allergy (see Table 2).[15,17-19]

Horse and donkey milk may also induce sensitization. A few cases describing type I sensitization induced by cosmetic products containing mare´s milk have been reported, as well as anaphylaxis to ingested mare´s milk.[20-24] Cross-sensitization between cow´s milk and mare´s milk is rare.[24,25] One case report has described type I and IV allergic reactions to donkey´s milk in a patient previously known with atopic dermatitis, who had been treated with donkey's milk–containing topical products.[26]

3.2.2 | Peach (*Prunus persica*)

Peaches belong to the Rosaceae family.[27] Peaches have two different sensitization routes. Allergy to peach in Northern and Central Europe is associated primarily with the oral allergy syndrome caused by sensitization to birch pollen and labile proteins known as profilins, like Bet v 1 and Bet v 2, due to cross-reactivity between pollen profilin and the peach profilin Pru p 4.[28,29] Profilins cross-react with homologous proteins in fruits from the Rosaceae family, such as peach, apple, and apricot.[30] Peach allergy is the most common food allergy among adults in Southern Europe.[31] In addition to the oral allergy syndrome, it can cause contact urticaria, anaphylaxis, and severe, potentially life-threatening reactions.[32] In Southern Europe, peach allergy is mediated primarily by Pru p 3, a lipid transfer protein (LTP).[28] LTPs are widespread plant food pan-allergens that are stable (due to being heat-resistant and pepsin-resistant) and are highly conserved proteins of around 10 kD.[33,34] Especially the peach surface fuzz has large amounts of LTP, but also the peel and cutin layers of peaches are rich in proteins and have a higher allergenicity than the pulp with its high carbohydrate content.[35,36] Because of the geographic distribution of LTP, sensitization probably occurs through the skin or airways. Rosaceae allergic patients who are hypersensitive to LTP frequently show both skin and in vitro cross-sensitization and cross-reactivity to many botanically unrelated fruits and vegetables.[37] Peach is added to cosmetics for anti-aging, antioxidant properties as well as skin-

recovering properties after UVB skin exposure.[38-40] To our knowledge, no allergic reactions to peach in cosmetics have been reported in the literature yet, but there are a number of reports of patients experiencing type I allergy while preparing LTP-containing fruit and vegetables, including peaches.[29,41]

3.2.3 | Peanut (*Arachis hypogaea*)

Peanut is a grain legume belonging to the Fabaceae family. It is one of the most allergenic foods and the most common cause of food-mediated type I allergy and anaphylaxis. Exposure is through ingestion, skin contact, and inhalation.[42] The mechanism is IgE mediated and symptoms range from mild urticaria to severe anaphylaxis, even with lethal outcome.[42,43] Allergy to peanuts often begins in childhood and affects 1% of children younger than 5-years-old. Only approximately 20% outgrow the allergy, which often makes peanut allergy a lifelong condition.[42] The estimated prevalence of peanut allergy in developed countries is between 0.6% and 1.0%.[42] Thirteen peanut proteins, Ara h 1 to Ara h 13, have been identified as allergens.[44] Sensitivity to the various peanut antigens differs among geographic locations.[44] Peanut allergic patients can have cross-reactivity to tree nut, soy, and other legumes such as beans, peas, lentils, and lupinus.[45] Peanuts can be processed into peanut oil and the derivatives hydrogenated peanut oil, peanut acid, or peanut glycerides. These are commonly used in foods such as salad dressings and margarine; in pharmaceutical products as vehicles for the active ingredient; in topical and other preparations; and in cosmetic products such as soap, skin cleansers, skin care products, and moisturizers.[46] There are, to our knowledge, no reports of allergic reactions to peanut-containing cosmetic products in peanut allergic patients. One case report described type I allergy from peanut butter used on the skin of a 31-year-old woman.[47] It has, however, been proposed that sensitization to peanuts may occur in children using peanut oil–containing products on a damaged or inflamed skin barrier.[48]

3.2.4 | White potato (*Solanum tuberosum*)

White potatoes belong to the Solanaceae family.[49] Potatoes are widely consumed in most of Europe and are frequently used when introducing solid food to infants.[50] Raw and cooked potatoes can cause type I allergic reactions. The symptoms are usually caused by contact or ingestion, or rarely by inhalation.[51-54] Peeling is the primary cause of potato contact allergy and is usually occupational among food handlers and caterers.[55] Localized reactions to raw potato have been reported in patients who do not react to cooked potatoes, due to heat denaturing of the allergenic proteins.[49] Allergic reactions caused by ingestion of raw potatoes are typically associated with oral allergy syndrome caused by allergens having IgE cross-reactivity with predominantly grass and tree pollen.[50] Type I allergic reactions to cooked potatoes have been reported rarely.[50] The common allergen of potatoes is patatin, a large glycoprotein that has been identified as an IgE-binding protein.[56] Patatin is a cross-reactive allergen in latex-associated potato allergy.[57] There are no reports of cases of allergic reactions to potato in cosmetics or other topical remedies.

3.3 | Ingredients described causing type I and IV allergies

3.3.1 | Oat (*Avena sativa*)

Oat is an edible grain belonging to the Poaceae family. Wild oat is the most common of the oat species and has high nutritional value due to its high protein content.[58] Oat is used as an ingredient in food where it may cause type I allergy.[5] Oat is further used in cosmetic products in various forms including oat kernels, oat meals, oat bran, oat protein, oat flour, oat starch, and oat peptide.[59] Moisturizing creams containing oat protein improve skin barrier function because of its anti-inflammatory, anti-oxidant, and anti-pruritic properties.[60] They are known as colloidal oatmeal products consisting of grinded oat boiled into an extract containing protein, lipids, vitamins, polysaccharides, saponins, flavonoids, and polyphenols.[59] Oat-containing moisturizers and bath therapies have been used widely used as topical treatments of various skin conditions, for example, atopic dermatitis, although the risk of being sensitized to oat through the skin increases when the skin barrier is impaired, as in atopic dermatitis.[60,61] This is seen in the cases below, where all patients but one were treated with oat-containing moisturizers or soap for atopic dermatitis and developed type I and/or IV allergy to oats in the cosmetic products (see Table 3).[62-64] One patient developed oral allergy syndrome after sensitization through the skin.[60] Also occupational allergic contact dermatitis is described in the literature.[65-67]

3.3.2 | Soybean (*Glycine max*)

Soybean is a legume belonging to the Fabaceae family.[45] Soy is among the eight most common allergenic foods, with exposure through skin contact, ingestion, and inhalation causing type I allergic reactions.[45] Allergic reactions include skin, gastrointestinal, respiratory, and anaphylactic reactions.[68] Sixteen IgE-reactive soy proteins, including Gly m 1 to Gly m 8 as the most allergenic, have been identified as allergens.[45,69] Food allergy to soy proteins has been described mainly in young children with atopic dermatitis, but may present later; in a study of 30 patients, the first allergic reaction to soy occurred at a mean age of 19 ± 10 years (range, 3-44 years).[70,71] The prevalence of soybean allergy is unknown.[68] Soy bean is a birch pollen–related allergenic food, and recent studies have shown an increased risk of soy allergy among Central European patients who are sensitized to birch pollen due to IgE cross-reactivity between the major birch pollen allergen Bet v 1 and the homologous soy protein, Gly m 4.[68,70,71] Soy allergic patients may also have cross-reactivity to peanuts, tree nuts, and other legumes such as beans, peas, lentils, and lupinus.[45] Soy derivatives are used as active ingredients in make-up and skin- and

TABLE 3 Type I and IV allergic reactions to oat in cosmetic products reported in the literature

Year	Age	Sex	Exposure	Clinical symptoms	Relevant test results	Type of reaction	Reference
2016	68	F	Cream.	Facial dermatitis.	Positive patch test to cream and *Avena sativa* oat extract 5% aq.	IV	64
2010	33	F	Cosmetic emollient cream moisturizer.	For 6 mo, facial rash immediately after application of creme. Later, itchy, swollen lips, and pruritic, erythematous papules and patchy lesions on the trunk after eating certain biscuits or bread containing oatmeal.	Positive prick tests to the cream and *Avena sativa* alcoholic extract. Elevated total IgE level (1328 kU/L) and slightly elevated specific IgE antibodies to oat (1.23 UA/mL). Patch test negative to emollient cream.	I	60
2007	0-15	8 NK	Emollient creams containing oat.	Five experienced atopic dermatitis flares, two experienced pruritus, one experienced widespread erythema.	Patch test positive to oat.	IV	137
2002	7	F	Oat cream applied to arms and trunk.	After 15 min, swollen lesions and contact urticaria where the cream had been applied.	Open patch test positive to oat. Oat-specific IgE was positive at 0.76 kU/L.	I	138
2000	3	F	Moisturizer.	Flare up after application on the right arm and the hands.	Patch test ++ positive to *Avena* in cream. Prick test positive to oat.	I and IV	62
1988	14 mo, 2, 14	1F, 2 M	Bathed with a product based on oatmeal extract.	Exacerbations of atopic eczema after baths.	Specific IgE antibodies to oat were positive. Patch test to oat was positive.	I and IV	63

Abbreviations: F, female; M, male; NK, not known.

hair-conditioning agents due to a high content of soy phytosterols.[72] Several allergic reactions, both type I and type IV, to soy and maleated soybean oil in cosmetic products, have been described in the literature (see Table 4). Maleated soybean oil is a reaction product of soybean oil and fumaric acid.[73] One atopic dermatitis patient with type I allergic symptoms related to soy-based ingredients in cosmetic products subsequently developed severe type I allergic symptoms including urticaria, dyspnea, and hypotension after eating soy products.[74]

3.3.3 | Wheat (*Triticum vulgare*)

Wheat is an edible grain belonging to the Poaceae family. It is the most common food grain worldwide due to its ability to grow in various climates.[75] Sensitization to wheat proteins can be oral, percutaneous, perimucosal, and/or rhinoconjunctival, and it may cause different disorders with different immunologic mechanisms.[75,76] Impaired skin barrier as in atopic dermatitis may increase the risk of sensitization via skin.[59] The IgE-mediated type I allergic reactions are most common with symptoms such as urticaria, abdominal pain, or systemic anaphylaxis. In addition, food-dependent exercise-induced anaphylaxis, occupational rhinitis, or asthma (known as Baker's asthma) are seen. Wheat gluten is a large group of proteins comprising approximately 85% of the proteins in wheat and consists of water-insoluble wheat proteins, including monomeric gliadins and highly polymeric glutenins.[77,78] These are implicated in IgE-mediated allergy to ingested wheat, and omega-5 gliadin is an important allergen in wheat-dependent exercise-induced anaphylaxis (WDEIA).[79] The remaining 15% of wheat proteins are water-soluble, nongluten proteins, including albumins and globulins, which also are implicated in wheat allergy and cross-react with grass pollen allergens.[75,80] Wheat protein and wheat gluten can be hydrolyzed enzymatically or chemically to its constituent amino acids to overcome insolubility, a process called deamidation.[81] Hydrolyzed wheat protein (HWP) and hydrolyzed wheat gluten (HWG) are added to skin care and hairdressing products for their emulsifying, stabilizing, moisturizing, and volumizing properties.[80,82,83] For almost two decades, severe allergic reactions to HWP and HWG in cosmetics have been described. There are numerous reports of type I allergic reactions and fewer reports of type IV allergic reactions (see Table 5).

3.4 | Ingredients well-known to cause type IV allergy

The cosmetics products registered in Kemiluppen contain various bee products: beeswax (*cera alba*), propolis (bee glue), honey, and royal jelly. This section focuses on propolis and beeswax, since these two bee products have been described primarily as causing contact allergic reactions in cosmetics.

TABLE 4 Type I and IV allergic reactions to soy in cosmetic products reported in the literature

Year	Age	Sex	Exposure	Clinical symptoms	Relevant test results	Type of reaction	Reference
2015	30	F	Cosmetic lotions containing soy-based ingredients.	Contact urticaria on fingers. Systemic urticaria, dyspnea and hypotension, after eating soy products.	Specific IgE 19.3 UA/mL. Skin prick test positive for soy extract (10 mg/mL), the cosmetic lotion and a commercially available soymilk.	I	74
2005	43	F	Face topicals containing soy.	Erythema of the nasal tip and on the cheeks.	Prior skin prick test positive to soy. Specific IgE antibodies were detected for soy (19.3 UA/mL).	I	139
2001	55	F	Facial cosmetic cream.	Erythema, swelling of the face.	Patch test positive to the cream, soybean extract and ceramide 3 together (2% petrolatum). Immediate slight erythema to soybean extract dilution 20% eth.	IV	140
2000	48	F	Maleated soybean oil in a facial moisturizer.	Face and neck dermatitis.	Patch test positive to cosmetic creams and maleated soybean oil. Repeated open application test with maleated soybean oil strongly positive.	IV	73
1996	44	F	Facial moisturizing cream.	Itching a few hours after application, dermatitis.	Patch test: + to myristyl lactate 0.5%, + to maleated soybean oil 1.5%.	IV	141
1995	45, 47, 62	3F	Skin repair cream.	Redness, itching and edema of the face and neck.	Patch test: ++ positive to skin repair cream and maleated soybean oil.	IV	142

Abbreviation: F, female.

3.4.1 | Propolis

Propolis, also known as bee glue, is produced by honeybees after collecting resinous material from plants, which they mix with saliva and beeswax to produce propolis. The bees use propolis to repair, strengthen, and narrow the entrance to their hives.[11] Due to geographic location and variability of plant species, the composition of propolis is highly variable. It has more than 300 constituents comprising primarily resin and beeswaxes and aromatic oils and pollen. Propolis has antibacterial, antifungal, and antiviral properties and has been used for thousands of years in pharmaceutical products, apitherapy, and folk medicine.[84] Furthermore, it is used as a dietary supplement and as an ingredient in cosmetic products. The most important allergens are esters of caffeic acid: "LB-1," phenethyl caffeate, benzyl caffeate, and geranyl caffeate.[11] Propolis may cross-react with other allergens and there is a well-known association between propolis and *Myroxylon pereirae* (balsam of Peru).[11] Many allergic reactions are caused by use of topical pharmaceutical and cosmetic products as well as occupational exposure in beekeepers.[11,98] Hausen et al have reported 114 original patients and de Groot has summarized the literature of 70 other reported patients in the literature of type IV allergy to propolis in cosmetic and pharmaceutical products.[11,85]

3.4.2 | Beeswax (*cera alba*)

Beeswax is produced and secreted from eight wax glands in the abdomen of the worker bees of the honey bee (*Apis mellifera*).[10] Beeswax is used by the bees to form cells of the honeycomb for honey storage and protection of the brood in the hive. It has more than 300 constituents, mainly esters of fatty acids and free long-chain alcohols, as well as residues of propolis and pollen.[10] Beeswax is used in cosmetic and pharmaceutical products, in food as a food additive (E901), for coating and glazing of candy and fresh fruit, for making beeswax candles, and for making vax and varnish for leather and wood.[10,86] A recent Swedish study published 17 cases of patients with type IV allergy to beeswax.[86] There are a further eight cases of type IV allergy to beeswax in cosmetics and five cases related to non-cosmetic products reported.[10] Some of these cases might be due to a reaction to propolis in propolis-contaminated beeswax. There are no reported cases of type I allergic reactions to beeswax.

3.4.3 | Cinnamon (*Cinnamomum zeylanicum*)

Cinnamon belongs to the Lauraceae family.[87] The essential cinnamon oil is extracted from the bark or leaves of the tree by distillation.[9] The main component of cinnamon bark oil is cinnamal, which gives cinnamon its scent and taste and has a strong sensitizing potential.[88,89] Cinnamal is used as a fragrance ingredient and often as a nature-identical chemical, and is one of the eight components in fragrance mix I (FMI).[88] The main component of cinnamon leaf oil is eugenol. Cinnamon is used as a spice and flavoring agent in food, sweets, gum and drinks, dentifrices, herbal-based products, and cosmetics.[90,91] Exposure can be through ingestion, skin contact, and inhalation. Allergic symptoms such as urticaria and dermatitis are seen.[90,92] More than 15 cases of type IV allergy to cinnamon oil in cosmetic or pharmaceutical products are described.[9] One of these patients developed a

TABLE 5 Type I and IV allergic reactions to wheat in cosmetic products reported in the literature

Year	Age	Sex	Exposure	Clinical symptoms	Relevant test results	Type of reaction	Reference
2018	34	M	Occupational anaphylaxis.	Type I allergic reactions with 1-y interval, 5 min after cleaning a tank containing an anti-wrinkle cream. First: Rhinitis, conjunctivitis and sneezing. Later: Contact urticaria, conjunctivitis and dyspnea.	Prick test positive for cosmetic product (10% aq.) and wheat extract. Negative for gluten extract.	I	143
2015	21-73	17 F, 1 M	Soap.	Urticaria, WDEIA.	Skin prick test positive to 0.01% Glupearl 19S solution.	I	131
2013	23	F	Sprayable hair conditioner and another hairspray containing HWP.	Rhinitis, conjunctivitis, dyspnea, angioedema of the eyelids, asthma-like symptoms at work (hairdresser). Contact urticaria, burning and tingling of the hands and soles when exercising.	Skin prick test and open application test positive to products containing HWP.	I	144
2013	22	F	Spray products containing HWP.	Urticaria, work-related sneezing, nasal itching, rhinitis. Two episodes of generalized urticaria and eyelid edema when exercising after having eaten wheat-containing food.	Skin prick test and open application test positive to products containing HWP.	I	144
2013	3	M	Moisturizing cream.	Urticaria on both arms and facial angioedema.	Prick test positive to moisturizing cream and HWP. Wheat prick test negative. Wheat flour-specific IgE positive at 0.61 kU/L. HWP-specific IgE positive at 2.96 kU/L.	I	145
2013	35-60	7 F	Soap.	Contact urticaria and WDEIA.	Prick test positive to wheat, bread, 0,1% soap solution supplemented with HWP-A in saline, and 1 mg/mL HWP-A in saline. Specific IgE for wheat and gluten were detected in all seven patients.	I	146
2012	18-46	3 F	Cosmetics, creams, eye-liners, shampoo.	Contact urticaria.	Skin prick test positive to HWP.	I	81
2012	23	M	Face cream.	Pruritic wheals on the face and neck, bilateral palpebral edema.	Patch test positive to the cream and to 1% HWP in water. Prick tests positive for hydrolyzed wheat extract, malt, cereal mix, oats.	I and IV	147
2011	49	F	Hydrolyzed wheat protein-supplemented soap.	Eyelid edema and dyspnoea eight times while working or walking. Facial wheals and nasal discharge after bathing.	Prick test positive to the soap (0.1% in saline). Wheat specific IgE 1.35 kU/L, gluten specific IgE 1.78 kU/L. Oral provocation tests induced eyelid edema, nasal discharge and dyspnea. Face wash with the soap induced facial wheals.	I	148
2010	28, 34	2 F	Cosmetics.	Recurrent contact urticaria, initially on hands, then more diffused immediately after applying cosmetics.	Skin prick test positive to the cosmetics and HWP.	I	83

TABLE 5 (Continued)

Year	Age	Sex	Exposure	Clinical symptoms	Relevant test results	Type of reaction	Reference
2010	18, 24	NK	Skin tensing cosmetics, facial cream.	Contact urticaria.	Prick test positive to skin tensing cosmetics containing HWP.	I	149
2009-2017	1 -93	2025 F 86 M	Soap or other products containing HWP.	Itching, eyelid edema, nasal discharge and/or wheals within several to 30 min after using soap or other products containing hydrolyzed wheat.	Skin prick test positive to Glupearl 19S solution.	I	150
2008	NK	7 F	Cosmetics, mainly facial cream.	Contact urticaria immediately after applying cosmetics (mainly facial cream), from different brands, containing HWP. Six had anaphylactic reactions or urticaria after eating preserved foods containing modified gluten. Neither had allergic reactions after eating bread.	Skin tests positive to the cosmetics containing HWP, and in case of food allergy, modified gluten. Skin tests negative to natural wheat flour, but specific IgE to wheat flour were positive in two cases. Specific IgE to gluten were positive in three patients.	I	151
2007	42	F	Moisturizing cosmetic cream.	Intense burning on face, neck and scalp several hours after applying a new moisturizing cosmetic cream and development of a florid, itchy rash over face and neck, which lasted several weeks, settling with the use of topical steroids.	Patch test negative to standard, cosmetic, hairdressing and facial series. Testing with the patient's own cosmetic cream showed a positive reaction. Patch test with the diluted constituents of the cosmetic cream identified an isolated allergy to HWP (50% aq.).	IV	152
2007	3	F	Emollient.	Scaly, erythematous lesions on the knees.	Patch test positive to the emollient and the individual components of the emollient for palmitoyl-HWP.	IV	153
2006	21-53	9 F	Moisturizers, shower gels, shampoos and conditioner.	Contact urticaria.	Positive skin prick tests with the suspected cosmetics and the identified HWP. Skin tests negative to wheat flour extract. Low-moderate levels of IgE specific of wheat flour or gluten.	I	78
2006	NK	3 NK	Shower gel, shampoo, mascara.	Generalized erythema, contact eczema, facial angioedema with generalized urticaria.	NK	NK	154
2002	46	F	Eye cream and body moisturizer.	Contact urticaria.	Skin prick test and open application tests positive to HWP.	I	77
2000	64	F	Moisturizing cosmetic cream.	Itchy, erythematous lesions on the eyelids, face and neck.	Patch test positive to cosmetic cream and to the HWP ingredient of the cream (10% aq.).	IV	82
2000	27	F	Cosmetic cream. Moisturizing body cream containing HWP.	Contact urticaria. Pruritic, erythematous, urticarial rash.	Skin prick test positive to HWP.	I	155

Abbreviations: F, female; M, male; NK, not known.

recurrence of dermatitis after eating cinnamon.[93] Other side effects have been described, and irritant contact dermatitis is seen when patients are patch tested with cinnamon oil 2% or higher.[9] Cinnamal is the second most frequently reported individual chemical causing allergy, with around 350 published cases.[89] There are also several cases of patients reacting to cinnamal in spices, foods, and flavorings, as well as occupational type IV allergy in bakers.[9]

3.4.4 | Compositae plants

Daisy flowers belongs to the Compositae family, which is the second largest family of plants in the world, comprising more than 20 000 species.[10,94] The whole plant, roots, leaves, stalks, flower heads, and extract of the leaves and flowers are commonly used in cosmetics, massage oils, essential oils, folk and traditional medicine, and tea and water/alcohol extracts due to supposed anti-inflammatory and other health effects.[10,94] The most commonly used Compositae plants in cosmetics and pharmaceutical products are *Helianthus annuus*, *Calendula officinalis*, *Arctium lappa*, *Arnica montana*, *Achillea millefolium*, *Chamomilla recutita* and *Chamaemelum nobile*, and *Echinacea purpurea* and *Echinacea angustifolia*.[95] Compositae plants are believed to be the most frequent cause of allergic contact dermatitis of all plants in Europe. Symptoms are often localized to hands and face but might spread to the rest of the body.[10,96] If the symptoms are not treated, contact dermatitis often progresses and becomes chronic. The primary sensitization pathway is via direct plant contact, but the plants cross-react with each other and the prevalence of polysensitization is high.[96,97] Severe systemic type IV allergic reactions can also be seen when ingested.[96,98] The most important allergens are sesquiterpene lactones and thiophenes/polyacetylenes, but not all allergens have yet been identified.[10,94] There are reports of hundreds of cases of type IV allergic reactions to Compositae plants in cosmetics, pharmaceuticals, and as occupational sensitization in masseurs, gardeners, greenhouse workers, florists, pharmacists, and drug sellers.[9,10,94,96,98] A few cases of possible type I allergy to chamomile in a cosmetic cream and enemas have been reported.[99,100] In pollen-food allergy syndrome, patients who are sensitized to mugwort pollen may develop type I allergy symptoms and even severe anaphylaxis upon ingesting the Compositae plant chamomile.[99-101]

3.4.5 | Eucalyptus (*Eucalyptus globulus* and *Eucalyptus citriodora*)

Eucalyptus belongs to the Myrtaceae family.[102] There are two main types of eucalyptus oils. *Eucalyptus globulus* oil is derived from the Tasmanian blue gum *E. globulus*, while *Eucalyptus citriodora* oil is derived from the citron-scent gum *E. citriodora*.[9] The composition of these two oils differs. The dominant ingredient in *E. globulus* oil is 1,8-cineole (eucalyptol) constituting more than 50% of the oil, whereas the main component in *E. citriodora* oil is citronellal. Both essential oils are produced by steam distillation of the leaves, buds, fruits, and bark from the tree and widely used as ingredients in perfume and cosmetics.[9,103] More than 17 patient cases of type IV allergy to eucalyptus oil primarily in topical pharmaceutical products but also in cosmetic products have been reported.[9]

3.4.6 | Lanolin

Lanolin is secreted from the sebaceous glands of sheep.[1] Lanolin consists primarily of wax esters, free fatty acids, and water, although the composition varies due to many factors, such as sheep breed, age, and habitat.[10,104,105] The derivative lanolin alcohol is a complex mixture of organic alcohols obtained by hydrolysis of lanolin. Lanolin and lanolin alcohol have great water-binding and emulsifying properties. They are easily absorbed into skin and hair and are commonly used in cosmetic and pharmaceutical products. Lanolin is also used in surgical dressings and adhesive bandages as well as in furniture and shoe polish, papers, print colors, wool clothing, fur, and leather.[10] There are numerous reports of contact allergy to lanolin and lanolin alcohol, with more than 73 cases of type IV allergy to lanolin and 222 cases of type IV allergy to lanolin alcohol in skin care products, herbal cosmetics, and topical pharmaceutical products.[10]

3.4.7 | Lavender (*Lavandula angustifolia*)

Lavender belongs to the Lamiaceae family and is often used as a garden plant, for flavoring in food, sweets, and drinks, and for odor in cosmetic and household products. The essential lavender oil is obtained from the flowers by steam-distillation and used in traditional herbal medicine and aromatherapy.[9] The essential oil contains the terpenes linalool, linalyl acetate, and caryophyllene, which are potentially allergenic. When lavender oil is exposed to air, the terpenes oxidize to strongly allergenic hydroperoxides.[9] Although fresh lavender oil might have limited allergenic potential, air-oxidized lavender oil can thus cause allergic contact dermatitis.[117] There are many reports of type IV allergy to lavender oil, primarily with occupational relevance among masseurs and in aromatherapy as well as in topical pharmaceutical products. There are more than 50 publications of type IV allergy to lavender.[9,106,107]

3.4.8 | Lemon (*Citrus limon*)

The lemon tree belongs to the Rutaceae family.[122] Lemon contains the allergen Cis I 3, an LTP causing type I allergic reactions, including allergic rhinoconjunctivitis, food allergy, and anaphylaxis after ingestion.[108,109] The essential lemon oil is obtained from cold-pressing the peel. The main constituent of the cold-pressed essential oil is the terpene D-limonene, a prehapten, which after air oxidation yields the hydroperoxides limonene-1-hydroperoxide and limonene-2-hydroperoxide. These are both strong contact allergens.[9] Lemon oil and limonene are used as ingredients in perfumery, aromatherapy, and other cosmetic products, and for many other purposes.[9] More than 10 patient cases of type IV allergy to lemon oil in cosmetic products have been reported.[9] Limonene is one of the most commonly used fragrance chemicals in cosmetics, with more than 100 reported cases of type IV allergy.[89]

3.4.9 | Lemongrass (*Cymbopogon* spp.)

Lemongrass belongs to the Poaceae family.[110] Lemongrass resembles the scent of lemon, and the essential oil is obtained by steam

distillation of the lemongrass leaves. There are two types of lemongrass oils: East Indian lemongrass oil derived from *Cymbopogon flexuosus* and West Indian lemongrass oil derived from *Cymbopogon citratus*. Patch testing is performed primarily with material from *Cymbopogon citratus*. Lemongrass leaves and the essential oil are among others used in a variety of cosmetic and pharmaceutical products.[111] The allergens in lemongrass oil are the two ingredients neral and geranial that constitute citral, the active component in lemongrass oil, which is used to measure the quality of the lemongrass oil.[9] Citral is a well-known fragrance allergen.[89] There are more than 25 reports of type IV allergy to lemongrass oil.[9,111]

3.4.10 | Mint (*Mentha piperita* and *Mentha spicata*)

Mint is grown all over the world and belongs to the Labiatae family, including the species spearmint (*Mentha spicata*) and the water mint (*Mentha aquatica*). Peppermint is a hybrid mint produced by crossing *Mentha aquatica* and *Mentha spicata*.[112] Various forms of the plant such as leaf, leaf water, leaf extracts, and oil are commonly used in cosmetics, personal hygiene products, and pharmaceutical products as preservatives due to their antioxidant and antimicrobial effects, in aromatherapy, and in food as spices due to their flavoring properties.[9] The major constituents of mint oils are menthol, menthone, and menthyl acetate. Exposure to mint is through the skin and by ingestion.[112] Most toothpaste contains spearmint, peppermint, or menthol, added to give it a pleasant scent and taste.[112] Both peppermint and spearmint oils are among the most common allergens in toothpaste, causing perioral dermatitis, stomatitis, cheilitis, gingivitis, and glossitis.[113] A large intake of mint-flavored sweets and chewing gum can cause similar symptoms.[112] Smoking of menthol cigarettes coupled with cutaneous exposure has been associated with urticaria.[114] More than 24 cases of type IV allergy to peppermint oil, 14 cases of type IV allergy to spearmint oil, and more than 20 cases of type IV allergy to menthol, all in relation to cosmetic and pharmaceutical products, have been reported in the literature.[9,89]

3.4.11 | Orange—bitter orange (*Citrus aurantium*) and sweet orange (*Citrus sinensis*)

The orange tree belongs to the Rutaceae family. There are two types of oranges, the popular sweet orange primarily cultivated in Brazil and the bitter orange from Paraguay, both native to China. The pulp from the oranges can be eaten fresh or processed into juice. The essential orange oil is obtained by cold-pressing the peel as a byproduct in the juice industry and is used in perfumes, cosmetics, and aromatherapy and for flavoring in food and drinks.[9] Orange oil consists of more than 90% of the prehapten D-limonene, which transforms to limonene hydroperoxides after light and air exposure and becomes strongly allergenic. There are more than 10 patient cases of type IV allergies to orange oil, primarily related to occupation allergy in aromatherapists, masseurs, and people working with perfumes.[9]

3.4.12 | Rose (*Rosa damascena*)

Roses belongs to the Rosaceae family. There are various roses in the cosmetic products in THINK Chemical's Kemiluppen database, but in this section, the focus is on the most commonly used rose in cosmetic products, *Rosa damascena*. The essential oil is obtained from rose flowers by hydro- or steam distillation. Rose oil is expensive, and it takes almost 4000 kg of rose flowers to produce 1 kg of rose oil. It is primarily used as fragrance in finer perfumes, skin products, aromatherapy, and as a fragrance in food and drinks.[115] Furthermore, rose oil might have physiological and psychological relaxation, analgesic, and anti-anxiety effects.[116] The main constituents of rose oil are citronellol and geraniol, which may be the main allergens. There are reports of more than 15 cases of type IV allergy to rose oil in cosmetic products and topical pharmaceutical preparations.[9]

3.4.13 | Australian tea tree (*Melaleuca alternifolia*)

The Australian tea tree belongs to the Myrtaceae family.[117] Tea tree oil is an essential oil hydro-distilled from the leaves of the tea tree.[9] It has a camphoraceous scent with a menthol-like cooling sensation.[117] Tea tree oil is used in cosmetic products, pharmaceuticals, aromatherapy, folk medicine, and household products because of its antimicrobial, antiseptic, and anti-inflammatory properties.[117,118] Tea tree oil is toxic when ingested in higher doses and it can cause skin irritation at higher concentrations. The essential oil contains antioxidants including terpenes that are potentially allergenic. When tea tree oil is exposed to air, light, or warmth, some of the terpenes autoxidize to *p*-cymene, which is representative for the oxidative degradation.[9] Autoxidation leads to the formation of peroxides and other strong sensitizers.[9] Thus, tea tree oil from freshly opened tea tree oil products may elicit no or weak reactions, which is why oxidized tea tree oil should be used for patch testing.[119] There are numerous reports of type IV allergy to tea tree oil with more than 195 patient cases.[9]

3.4.14 | Ylang-ylang (*Cananga odorata*)

The ylang-ylang tree belongs to the Annonaceae family.[120] The essential oil is produced by steam distillation of the flowers from the tree. The quality of the oil depends on the distillation time and is divided into four different grades of oil, with the finest oil being the ylang-ylang oil "extra super" and "extra," with a distillation time of only 30 minutes or less. The four qualities of the oil also differ in the composition of ingredients. The main ingredients in the finest oil are linalool and benzyl acetate. The content of these ingredients decreases in oils with longer distillation time, whereas the content of germacrene D increases with longer distillation time but is low in the finer oils. Ylang-ylang essential oil is primarily used as fragrance in finer perfumes, cosmetics and aromatherapy, and folk medicine and as flavor in food and drinks.[121] Earlier, dihydro-isoeugenol was the primary allergen in ylang-ylang oil and caused several cases of pigmented

contact dermatitis, especially in Japan. After elimination of dihydroisoeugenol, derivatives of geraniol and linalool are probably the main sensitizers.[122] There are more than 17 patient cases of type IV allergy to ylang-ylang oil.[9]

3.5 | Ingredients rarely reported to cause type IV allergy

3.5.1 | Liquorice (*Glycyrrhiza glabra*)

Liquorice is the root of plants belonging to the Fabaceae family, comprising more than 30 species, including *Glycyrrhiza glabra* and *Glycyrrhiza inflata*.[123] Liquorice is commonly used in pharmaceutical products, as a skin-whitening agent in cosmetics, and a skin conditioning agent in sunscreens.[123,124] Liquorice is available in water-soluble and oil-soluble versions. Glabridin is considered the main active agent, which inhibits the production of melatonin within the melanocyte via inhibition of tyrosinase activity and promotes depigmentation. This makes liquorice popular in skin-lightening cosmetics, especially in Asia.[125,126] In the literature, type IV allergic reactions are described in several patients using cosmetic products (see Table 6). The allergenic component is unknown.

4 | DISCUSSION

The aim of this study was to provide an overview of the use of naturally derived cosmetic product ingredients and to identify those most commonly used and those with a documented sensitizing capacity (type I and/or IV) in the literature. We investigated the ingredients of 10 067 cosmetic products based on ingredient labeling. We are not aware of any other similar study. We identified 121 different natural ingredients that were included in at least 30 cosmetic products. Of these, the 10 most commonly used were ingredients derived from cinnamon (cinnamal), aloe, Compositae plants, shea nut, bee products (cera alba and propolis), jojoba, almond, wheat, olive, and algae and seaweed, but only some of these were commonly reported to cause allergic skin reactions from cosmetics. Based on this new knowledge, a cosmetic screening series for potentially allergenic natural ingredients in cosmetic products is proposed.

4.1 | The most common ingredients reported causing type I allergy

Milk, peanuts, peaches, and potatoes are all food proteins known to cause type I allergy when ingested. Especially milk and peanuts are well-known potent allergens causing severe, potentially life-threatening type I allergic reactions. Patients who are highly sensitized to milk may also have severe allergic reactions following cutaneous exposure to milk protein–containing products on inflamed skin, which enhances the absorption of casein and whey leading to anaphylactic episodes. Four cases of type I allergic reactions to milk in cosmetics have been described in the literature, and these are by far the most serious systemic and anaphylactic reactions caused by cutaneous application of food proteins to the skin.[15,17-19]

The use of peanut oil in cosmetic products has frequently been debated due to an increase in the prevalence of peanut allergy and the widespread use in cosmetic products. Two potential problems are identified relating to peanut-containing cosmetic products: (a) the risk that patients can be sensitized to peanut through the skin and develop type I allergy and (b) the risk that patients with known peanut allergy will react to peanut-containing products when applied to the skin. The increase in the prevalence of peanut allergy may be caused by sensitization in the first 6 months of life through the use of cosmetic products containing peanut oil.[48] In a questionnaire study with

TABLE 6 Type IV allergic reactions to liquorice in cosmetic products reported in the literature

Year	Age	Sex	Exposure	Clinical symptoms	Relevant test results	Type of reaction	Reference
2017	60	M	Aftershave containing *Glycyrrhiza inflata*.	Facial dermatitis present for 6 mo.	Patch test positive to aftershave cream (++), *G. inflata* liquorice extract 1% in petrolatum and 1% in eth.	IV	123
2017	70	M	Aftershave containing *Glycyrrhiza inflata*.	Dermatitis of the face, neck, hands and legs.	Patch test positive for G. inflata liquorice extract 1% pet.	IV	123
2016	39	F	Two skin-lightening products.	Itchy, facial erythema.	Patch test positive to two skin-lightening products "as is" and liquorice flavonoid 2% pet.	IV	156
2015	76	F	Cosmetic cream to treat facial pigmented areas.	Facial erythema, mostly around the eyelids and cheek.	Patch test positive to oil-soluble liquorice extract 1% aq.	IV	125
2008	35	F	Facial cream.	Facial erythema and periorbital edema, 1 d after use.	Patch test positive to liquorice root extract 1% pet.	IV	124
1999	43	F	Facial cream, foundation and essence.	Itchy, reddish eruptions on the face of one-month duration.	Patch test positive to oil-soluble liquorice extracts at 0.5%, 1% and 5% pet.	IV	126

Abbreviations: F, female; M, male.

406 patients reporting symptoms on first contact with peanuts, only 19% had been knowingly exposed to peanut before the first documented reaction, implying a potential other route of sensitization.[127] Infants may be exposed to peanut proteins via nipple cream or other topical products for dermatitis, which often contain peanut oil.[128] In a study with 49 children with peanut allergy, more than 80% had been exposed to skin creams containing peanut oil on rashes in the first 6 months of life, which preceded the onset of symptoms of peanut allergy.[48] Topical exposure to peanut allergens may also occur through peanut butter caused by indirect exposure through the skin before peanuts are introduced into an infant's diet.[48] There is still uncertainty whether topical preparations containing peanut oil are safe to use in peanut-allergic patients. The refining process of peanut oil, which includes heat treatment, does not destroy allergenicity completely, which indicates that some allergens are heat stable even if they are present in only trace amounts in refined peanut oil.[129] As there is only insufficient data, there is no safe threshold at which nonallergic or peanut-allergic individuals can safely be exposed to peanut proteins through the skin, but due to latest research, the Scientific Committee on Consumer Safety suggests that refined peanut oil–containing preparations below a protein level of 0.5 ppm are safe for topical use among peanut-allergic patients.[128]

Although no cases have been reported on allergy to peach and potato in cosmetic products, there are several reports describing type I allergic reactions to both, while preparing or ingesting them. A potential sensitization through skin exposure, analogous to peanut, cannot be ruled out.

4.2 | The most common ingredients reported causing type I and type IV allergies

Wheat, oat, and soy have all been reported in the literature to cause both type I and type IV allergies as ingredients in cosmetic products. Wheat is the food protein most commonly causing sensitization through the skin. In Japan, more than 2000 cases of allergic reactions to hydrolyzed wheat gluten (HWG) in facial soaps and other cosmetic products containing 0.3% of HWG called Glupearl 19S have been described. Immediate allergic reactions with eyelid edema and contact urticaria during or after using the soap have been described in many patients, whereas in other patients, symptom onset was more than 2 years after starting to use the soap.[80] Most individuals with hydrolyzed wheat protein (HWP) allergy can eat wheat products, such as bread, pastries, and pasta.[130] However, some patients with contact allergic reactions to HWP and HWG, who are tolerant to food containing unmodified wheat protein, may experience severe allergic reactions when eating food containing deamidated gluten.[7] For example, more than half of the Japanese patients have experienced anaphylaxis after eating wheat-containing food and a number of patients have also experienced wheat-dependent exercise-induced anaphylaxis.[80,131]

New research has shown that HWP and HWG with a molecular weight < 3500 Da and polypeptide lengths ≤30 amino acids are safe for use in cosmetics.[80,130] HWP and HWG with polypeptide lengths ≤30 amino acids cannot trigger a type I allergic reaction as they must have at least two IgE-binding epitopes of at least 15 amino acid residues each to elicit an allergic reaction. By comparison, Glupearl 19S in the above-mentioned Japanese facial soap has an average molecular weight of about 50 000Da.[80] A protein of this size cannot penetrate intact skin. Instead, sensitization may have been achieved by skin exposure to surface-active chemicals (surfactants) present in soaps or detergents, in addition to direct contact to the immune system via rhinoconjunctival and/or oral mucosa. An impaired skin barrier also increases the risk of sensitization through the skin.

Oat has also been reported to cause both type I and IV allergy. Oat is commonly used in the treatment of atopic dermatitis. All reported patients with allergy to oat in cosmetics had atopic dermatitis, except one patient. In the patient cases listed above (Table 3), only one patient was described with subsequent oral allergy syndrome. The potential for sensitization to oat through cosmetics needs to be investigated, including symptoms after ingestion in combination with exercise.

Soy also has the potential to sensitize percutaneously and causes both type I and IV allergies, especially in patients with a reduced skin barrier function such as in atopic dermatitis. In one case described earlier in this article, a patient with atopic dermatitis had a type I allergic reaction to soy-based ingredients in cosmetic products, and subsequently developed anaphylaxis after eating soy products (Table 4).[74] Food allergy to soy proteins has been described mainly in young children with atopic dermatitis, potentially making these patients at higher risk of percutaneous sensitization.[71]

In general, the pathogenesis of percutaneous sensitization from food proteins and food allergy from percutaneous sensitization is yet to be fully elucidated.[74]

4.3 | The most common ingredients reported causing type IV allergy

Regarding well-known type IV allergens, the most common plant-derived sensitizers overall in the cosmetic products in Kemiluppen were cinnamon (cinnamal) and Compositae plants, whereas bee products and lanolin were the most common animal-derived ingredients. Compositae plants, cinnamon (cinnamal), lanolin, and the bee products propolis and cera alba are all well-known sensitizers and, except for cera alba, included in the European baseline series.[9-11]

Especially Compositae plants may be challenging to test. Standardization of patch testing is difficult, as various Compositae plants are of variable composition and the commercialized allergens available might be different from the ingredient included in a cosmetic or topical pharmaceutical product.[3] However, we rely on patch testing with the main sensitizers in Compositae plants complied in the sesquiterpene lactone mix in the baseline series possibly supplemented with a Compositae mix. Patients reacting to Compositae mix and not to sesquiterpene lactones often present with multiple positive reactions to fragrances and other compounds containing terpenes, such as

Myroxylon pereirae resin and colophonium, due to cross-reactivity.[132] Compositae plants may also induce type I allergic reactions when ingested or inhaled due to cross-reactivity to mugwort. A few cases of possible type I allergy to chamomile in cosmetic and pharmaceutical products have been reported.[99-101]

Cinnamal is a chemical substance that is found naturally, but most often produced by chemical synthesis. Cinnamal is an important fragrance allergen and one of the top 10 most frequent sensitizers in fragrances causing type IV allergic reactions.[122] Cinnamal is known to act as a direct histamine releaser, which is why symptoms may be confused with type I allergy.[133] Although there has been reported one case of a potential type I allergic reaction caused by cinnamal in a cosmetic product, the allergenic potential of both cinnamal and Compositae plants as type I allergens needs to be elucidated before they can be defined as type I allergens in cosmetic and pharmaceutical products.[103,134] For this reason Compositae plants and cinnamal are listed as type IV allergens.

Allergy to lanolin and lanolin alcohol is common and has been known for almost a century. A recent Danish study has shown an increase in the prevalence of lanolin contact allergy over more than a decade (2004-2015).[105] Although the specific allergens in lanolin are unknown, it has been suggested that the alcohol fraction of lanolin plays an important role, since reducing this part of the lanolin reduces the frequency of allergic reactions. Hence the derivative lanolin alcohol, and not lanolin "as is," is included in the European baseline series.[10] Propolis has been added recently to the European baseline series. The number of patients with type IV allergic reactions to propolis is expected to increase due to an extensive use of this ingredient and the increasing use of natural products.[135]

Eucalyptus, lavender, lemon, lemongrass, mint, orange, rose, tea tree, and ylang-ylang are known type IV sensitizers. They are all steam-distilled to essential oils, but the chemical composition of the individual essential oil varies depending on the plant, harvest, and distillation parameters.[5] Essential oils are often used as fragrances in perfumes and they are known perfume allergens. Reactions to essential oils include type I and type IV allergies, irritant contact dermatitis, and phototoxic reactions.[136] Most of the essential oils are included in either perfume test series or screening test series with essential oils. Liquorice is a less known sensitizer and only six cases of allergic reactions to liquorice in cosmetic products have been described in the literature.

4.4 | Strengths and limitations

More than 10 000 cosmetic products are included in the free and nonprofit smartphone application (app) that have been scanned anonymously by Danish consumers. Consumers using the app may be more focused on health, allergy, and avoidance of certain ingredients or products compared with the rest of the population, making them biased in their selection of cosmetic products. Due to the app being used on electronic devices, the consumers using the app might belong to a younger and more resourceful group of the Danish population compared to the general consumer. Although it is impossible to verify that the products were randomly selected, due to more than 10 000 cosmetic products included in the app, we believe that the Kemiluppen database is representative of the cosmetic products used by the Danish consumers.

Because there is no clear official or legislative definition of what "natural" covers, our definition of natural ingredients is arbitrary, although inspired by the European cosmetic regulation's definition of natural ingredients in cosmetics that refers to the origin of the ingredients in the products.[2] Other interpretations of "natural ingredients" may have led to other ingredients to be investigated in this study. The use of natural ingredients in cosmetic products is still relatively new, and not all allergic patients are identified and treated, or cases described in the literature. Therefore, by our literature selection criteria, we may have excluded natural ingredients, that, although not well described in the literature, may have the potential to cause allergic symptoms in patients. A selection of a screening test series with the 20 most common ingredients in the cosmetic products could have been another possible way to detect new allergens. However, by selecting the ingredients that were most commonly found in cosmetic products in addition to being described in the literature, we believe that the specific natural ingredients selected in this study were relevant for further investigation.

5 | CONCLUSION

To our knowledge, no other studies have reported the prevalence of allergic reactions to abundant natural ingredients in cosmetic products or developed a screening test series focusing exclusively on natural ingredients in cosmetic products. Based on the information gathered from the database search and literature study, we propose a screening series including patch testing and skin prick testing with the following ingredients:

- Patch test: Cera alba, cinnamal, eucalyptus oil, lanolin, lavender oil, lemon oil, lemongrass oil, liquorice, mint oil, oat, orange oil, propolis, rose oil, sesquiterpene lactone mix, tea tree oil, wheat, and ylang-ylang oil.
- Prick test: Cera alba, milk, oat, peach, peanut, potato, propolis, soy, wheat, and the cross-reacting inhalation allergens birch, grass, and mugwort, which cause potential cross-sensitization to certain foods.

In a future study, we will include these naturally derived cosmetic product ingredients in a supplemental screening test series on consecutive dermatitis patients. We believe that additional testing with these selected natural ingredients in patients with dermatitis may detect the cause of dermatitis in more patients than we are able to today. Standardization of patch-testing products with natural ingredients may be challenging, as the chemical composition of natural ingredients may vary considerably according to their origin, climate conditions, extraction procedures, preservation, and skin metabolism among other factors, thereby eliciting false-negative results. For this reason,

investigation should always include testing with the patients´ own products.

ACKNOWLEDGEMENTS

The authors would like to thank all employees at the Danish Consumer Council THINK Chemicals, an initiative under the Danish Consumer Council, for contributing data by the application "Kemiluppen."

AUTHOR CONTRIBUTIONS

Jeanne Duus Johansen: Conceptualization; funding acquisition; methodology; project administration; resources; supervision; writing-review and editing. **Claus Zachariae:** Conceptualization; methodology; writing-review and editing. **Christel Kirkeby:** Conceptualization; data curation; methodology; writing-review and editing. **Lene Garvey:** Conceptualization; methodology; project administration; supervision; writing-review and editing. **Maria Anna Bruusgaard-Mouritsen:** Conzeptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Writing-original draft; Writing-reviews & editing.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Goossens A. Contact-allergic reactions to cosmetics. *J Allergy (Cairo)*. 2011;2011:467071. https://doi.org/10.1155/2011/467071.
2. Miljø og fødevareministeriet. Kortlægning Af Økologiske Og Naturlige Kosmetiske Produkter, nr. 153; 2016. https://www2.mst.dk/Udgiv/publikationer/2016/12/978-87-93529-52-6.pdf. Accessed September 1, 2017.
3. Gilissen L, Huygens S, Goossens A. Allergic contact dermatitis caused by topical herbal remedies: importance of patch testing with the patients' own products. *Contact Dermatitis*. 2018;78(3):177-184.
4. Corazza M, Borghi A, Gallo R, et al. Topical botanically derived products: use, skin reactions, and usefulness of patch tests. A multicentre Italian study. *Contact Dermatitis*. 2014;70(2):90-97.
5. Verhulst L, Goossens A. Cosmetic components causing contact urticaria: a review and update. *Contact Dermatitis*. 2016;75(6):333-344.
6. Danish Consumer Council THINK Chemicals | Forbrugerrådet Tænk Kemi. https://kemi.taenk.dk/english. Accessed April 15, 2019.
7. The Danish Consumer Council THINK Chemicals' test methods | Forbrugerrådet Tænk Kemi. https://kemi.taenk.dk/bliv-groennere/danish-consumer-council-think-chemicals-test-methods. Accessed April 15, 2019.
8. Bennike NH, Oturai NB, Müller S, et al. Fragrance contact allergens in 5588 cosmetic products identified through a novel smartphone application. *J Eur Acad Dermatol Venereol*.2018;32(1):79-85.
9. de Groot AC. *Monographs in Contact Allergy, Volume 2. Fragrances and Essential Oils*. Boca Raton, FL: CRC Press Taylor and Francis; 2019:946.
10. de Groot AC. *Monographs in Contact Allergy, Volume 1: Non-Fragrance Allergens in Cosmetics (Part 1)*. Boca Raton, FL: CRC Press Taylor and Francis; 2016:744.
11. de Groot AC. *Monographs in Contact Allergy, Volume 1. Non-Fragrance Allergens in Cosmetics (Part 2)*. Boca Raton, FL: CRC Press Taylor and Francis; 2018:728.
12. Sicherer SH, Sampson HA. Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol*. 2014;133(2):291-307.
13. Mousan G, Kamat D. Cow's milk protein allergy. *Clin Pediatr (Phila)*. 2016;55(11):1054-1063.
14. Flom JD, Sicherer SH. Epidemiology of cow's milk allergy. *Nutrients*. 2019;11(5):1051.
15. Porcaro F, Caminiti L, Crisafulli G, Guglielmo F, Pajno GB. Anaphylaxis to cutaneous exposure to bovine colostrum based cream. *Asian Pac J Allergy Immunol*. 2019;37(1):9-11.
16. Wal J-M. Cow's milk allergens. *Allergy*. 1998;53(11):1013-1022.
17. Tabar AI, Alvarez MJ, Echechipía S, Acero S, Garcia BE, Olaguíbel JM. Anaphylaxis from cow's milk casein. *Allergy*. 1996;51(5):343-345.
18. Jarmoc LM, Primack WA. Anaphylaxis to cutaneous exposure to milk protein in a diaper rash ointment. *Clin Pediatr (Phila)*. 1987;26(3):154-155.
19. Lecks HI. Anaphylaxis from milk protein in diaper ointment. *JAMA*. 1980;244(14):1560.
20. Verhulst L, Kerre S, Goossens A. The unsuspected power of mare's milk. *Contact Dermatitis*. 2016;74(6):376-377.
21. Robles S, Torres MJ, Mayorga C, et al. Anaphylaxis to mare's milk. *Ann Allergy Asthma Immunol*. 2007;98(6):600-602.
22. Fanta C, Ebner C. Allergy to mare's milk. *Allergy*. 1998;53(5):539-540.
23. Doyen V, Leduc V, Corazza F, Mairesse M, Ledent C, Michel O. Protein contact dermatitis and food allergy to mare milk. *Ann Allergy Asthma Immunol*. 2013;110(5):390-391.
24. Gall H, Kalveram CM, Sick H, Sterry W. Allergy to the heat-labile proteins α-lactalbumin and β-lactoglobulin in mare's milk. *J Allergy Clin Immunol*. 1996;97(6):1304-1307.
25. Businco L, Giampietro PG, Lucenti P, et al. Allergenicity of mare's milk in children with cow's milk allergy. *J Allergy Clin Immunol*. 2000;105(5):1031-1034.
26. Peeters C, Herman A, Baeck M. Donkey's milk allergy. *Br J Dermatol*. 2017;177(6):1760-1761.
27. Asero R, Mistrello G, Roncarolo D, et al. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. *Int Arch Allergy Immunol*. 2000;122(1):20-32.
28. Gamboa PM, Cáceres O, Antepara I, et al. Two different profiles of peach allergy in the north of Spain. *Allergy*. 2007;62(4):408-414.
29. Asero R. Peach-induced contact urticaria is associated with lipid transfer protein sensitization. *Int Arch Allergy Immunol*. 2011;154(4):345-348.
30. González-Mancebo E, González-de-Olano D, Trujillo MJ, et al. Prevalence of sensitization to lipid transfer proteins and profilins in a population of 430 patients in the south of Madrid. *J Investig Allergol Clin Immunol*. 2011;21(4):278-282.
31. Fernández-Rivas M, González-Mancebo E, Rodríguez-Pérez R, et al. Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. *J Allergy Clin Immunol*. 2003;112(4):789-795.
32. Sánchez-López J, Gázquez V, Rubira N, et al. Food allergy in Catalonia: clinical manifestations and its association with airborne allergens. *Allergol Immunopathol (Madr)*. 2017;45(1):48-54.
33. Asero R, Pravettoni V. Anaphylaxis to plant-foods and pollen allergens in patients with lipid transfer protein syndrome. *Curr Opin Allergy Clin Immunol*. 2013;13(4):379-385.
34. Arena A. Anaphylaxis to apple: is fasting a risk factor for LTP-allergic patients?*Eur Ann Allergy Clin Immunol*. 2010;42(4):155-158.
35. Fernández-Rivas M, Cuevas M. Peels of Rosaceae fruits have a higher allergenicity than pulps. *Clin Exp Allergy*. 1999;29(9):1239-1247.

36. Asero R, Mistrello G, Amato S, Roncarolo D, Martinelli A, Zaccarini M. Peach fuzz contains large amounts of lipid transfer protein: is this the cause of the high prevalence of sensitization to LTP in Mediterranean countries?*Eur Ann Allergy Clin Immunol*. 2006;38 (4):118-121.
37. Asero R, Mistrello G, Roncarolo D, et al. Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. *Allergy*. 2002;57(10):900-906.
38. Yonezawa T, Momota R, Iwano H, et al. Unripe peach (*Prunus persica*) extract ameliorates damage from UV irradiation and improved collagen XVIII expression in 3D skin model. *J Cosmet Dermatol*. 2019;18(5):1507-1515.
39. Redondo D, Arias E, Oria R, Venturini ME. Thinned stone fruits are a source of polyphenols and antioxidant compounds. *J Sci Food Agric*. 2017;97(3):902-910.
40. Kim YH, Yang HE, Park BK, Heo MY, Jo BK, Kim HP. The extract of the flowers of *Prunus persica*, a new cosmetic ingredient, protects against solar ultraviolet-induced skin damage in vivo. *J Cosmet Sci*. 2002;53(1):27-34.
41. Cuesta-Herranz J, Lázaro M, De Las Heras M, et al. Peach allergy pattern: experience in 70 patients. *Allergy*. 1998;53(1):78-82.
42. Husain Z, Schwartz RA. Peanut allergy: an increasingly common life-threatening disorder. *J Am Acad Dermatol*. 2012;66(1):136-143.
43. Bush RK, Taylor SL, Nordlee JA. Peanut sensitivity. *Allergy Proc*. 1989;10(4):261-264.
44. Wu Z, Zhou N, Xiong F, et al. Allergen composition analysis and allergenicity assessment of Chinese peanut cultivars. *Food Chem*. 2016;196:459-465.
45. Cabanillas B, Jappe U, Novak N. Allergy to peanut, soybean, and other legumes: recent advances in allergen characterization, stability to processing and IgE cross-reactivity. *Mol Nutr Food Res*. 2018;62 (1):1700446.
46. Ring J, Möhrenschlager M. Allergy to peanut oil - clinically relevant? *J Eur Acad Dermatol Venereol*. 2007;21(4):452-455.
47. Mathias CG. Contact urticaria from peanut butter. *Contact Dermatitis*. 1983;9(1):66-68.
48. Lack G, Fox D, Northstone K, Golding J, Avon Longitudinal Study of Parents and Children Study Team. Factors associated with the development of peanut allergy in childhood. *N Engl J Med*. 2003;348 (11):977-985.
49. Tavidia S, Morton CA, Forsyth A. Latex, potato and tomato allergy in restaurateur. *Contact Dermatitis*. 2002;47(2):109-125.
50. de Swert LFA, Cadot P, Ceuppens JL. Diagnosis and natural course of allergy to cooked potatoes in children. *Allergy*. 2007;62(7): 750-757.
51. Castells MC, Pascual C, Esteban MM, Ojeda JA. Allergy to white potato. *J Allergy Clin Immunol*. 1986;78(6):1110-1114.
52. Obtułowicz A, Pirowska M, Wojas-Pelc A. Contact eczema of hands caused by contact with potato protein. *Ann Agric Environ Med*. 2016; 23(2):377-378.
53. de Lagrán ZM, de Frutos FJO, de Arribas MG, Vanaclocha-Sebastián F. Contact urticaria to raw potato. *Dermatol Online J*. 2009;15(5):14.
54. Jeannet-Peter N, Piletta-Zanin PA, Hauser C. Facial dermatitis, contact urticaria, rhinoconjunctivitis, and asthma induced by potato. *Am J Contact Dermat*. 1999;10(1):40-42.
55. Delgado J, Castillo R, Quiralte J, Blanco C, Carrillo T. Contact urticaria in a child from raw potato. *Contact Dermatitis*. 1996;35(3): 179-180.
56. Seppälä U, Alenius H, Turjanmaa K, Reunala T, Palosuo T, Kalkkinen N. Identification of patatin as a novel allergen for children with positive skin prick test responses to raw potato. *J Allergy Clin Immunol*. 1999;103(1 Pt 1):165-171.
57. Tavadia S, Morton CA, Forsyth A. Latex, potato and tomato allergy in a restaurateur. *Contact Dermatitis*. 2002;47(2):109-125.
58. Anderson OD. The spectrum of major seed storage genes and proteins in oats (*Avena sativa*). *PLoS One*. 2014;9(7):e83569.
59. Pootongkam S, Nedorost S. Oat and wheat as contact allergens in personal care products. *Dermatitis*. 2013;24(6):291-295.
60. Vansina S, Debilde D, Morren M-A, Goossens A. Sensitizing oat extracts in cosmetic creams: is there an alternative?*Contact Dermatitis*. 2010;63(3):169-171.
61. Pigatto P, Bigardi A, Caputo R, et al. An evaluation of the allergic contact dermatitis potential of colloidal grain suspensions. *Am J Contact Dermat*. 1997;8(4):207-209.
62. Pazzaglia M, Jorizzo M, Parente G, Tosti A. Allergic contact dermatitis due to avena extract. *Contact Dermatitis*. 2000;42(6):364.
63. Riboldi A, Pigatto PD, Altomare GF, Gibelli E. Contact allergic dermatitis from oatmeal. *Contact Dermatitis*. 1988;18(5):316-317.
64. Madsen JT, Andersen KE. 2-Amino-4-hydroxyethylaminoanisole sulfate - a coupler causing contact allergy from use in hair dyes. *Contact Dermatitis*. 2016;74(2):102-104.
65. Dempster JG. Contact dermatitis from bran and oats. *Contact Dermatitis*. 1981;7(2):122.
66. Pigatto PD, Polenghi MM, Altomare GF. Occupational dermatitis in bakers: a clue for atopic contact dermatitis. *Contact Dermatitis*. 1987;16(5):263-271.
67. Calzavara-Pinton PG, Tosoni C, Carlino A, Cattaneo R. Contact eczematous dermatitis caused by wheat and oats. *G Ital Dermatol Venereol*. 1989;124(6):289-291.
68. Holzhauser T, Wackermann O, Ballmer-Weber BK, et al. Soybean (*Glycine max*) allergy in Europe: Gly m 5 (β-conglycinin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. *J Allergy Clin Immunol*. 2009;123(2):452-458.
69. Cordle CT. Soy protein allergy: incidence and relative severity. *J Nutr*. 2004;134(5):1213S-1219S.
70. Ballmer-Weber BK, Holzhauser T, Scibilia J, et al. Clinical characteristics of soybean allergy in Europe: a double-blind, placebo-controlled food challenge study. *J Allergy Clin Immunol*. 2007;119(6): 1489-1496.
71. Mittag D, Vieths S, Vogel L, et al. Soybean allergy in patients allergic to birch pollen: clinical investigation and molecular characterization of allergens. *J Allergy Clin Immunol*. 2004;113(1):148-154.
72. Lin T-K, Zhong L, Santiago J, Lin T-K, Zhong L, Santiago JL. Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. *Int J Mol Sci*. 2017;19(1):70.
73. Le Coz CJ, Lefèbvre C. Contact dermatitis from maleated soybean oil: last gasps of an expiring cosmetic allergen. *Contact Dermatitis*. 2000;43(2):118-119.
74. Yagami A, Suzuki K, Nakamura M, et al. Case of anaphylactic reaction to soy following percutaneous sensitization by soy-based ingredients in cosmetic products. *J Dermatol*. 2015;42(9): 917-918.
75. Ricci G, Andreozzi L, Cipriani F, Giannetti A, Gallucci M, Caffarelli C. Wheat allergy in children: a comprehensive update. *Medicina (Kaunas)*. 2019;55(7):400
76. Chinuki Y, Morita E. Wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat protein in soap. *Allergol Int*. 2012; 61(4):529-537.
77. Pecquet C, Lauriere M, Huet S, Leynadier F. Is the application of cosmetics containing protein-derived products safe?*Contact Dermatitis*. 2002;46(2):123.
78. Laurière M, Pecquet C, Bouchez-Mahiout I, et al. Hydrolysed wheat proteins present in cosmetics can induce immediate hypersensitivities. *Contact Dermatitis*. 2006;54(5):283-289.
79. Palosuo K. Update on wheat hypersensitivity. *Curr Opin Allergy Clin Immunol*. 2003;3(3):205-209.
80. Burnett C, Bergfeld WF, Belsito DV, et al. Safety assessment of hydrolyzed wheat protein and hydrolyzed wheat gluten as used in cosmetics. *Int J Toxicol*. 2018;37(suppl 1):55S-66S.

81. Denery-Papini S, Bodinier M, Larré C, et al. Allergy to deamidated gluten in patients tolerant to wheat: specific epitopes linked to deamidation. *Allergy*. 2012;67(8):1023-1032.
82. Sanchez-Pérez J, Sanz T, García-Díez A. Allergic contact dermatitis from hydrolyzed wheat protein in cosmetic cream. *Contact Dermatitis*. 2000;42(6):360.
83. Olaiwan A, Pecquet C, Mathelier-Fusade P, Francès C. Contact urticaria induced by hydrolyzed wheat proteins in cosmetics. *Ann Dermatol Venereol*. 2010;137(4):281-284.
84. de Groot AC. Propolis: a review of properties, applications, chemical composition, contact allergy, and other adverse effects. *Dermatitis*. 2013;24(6):263-282.
85. Hausen BM, Wollenweber E, Senff H, Post B. Propolis allergy. (I). Origin, properties, usage and literature review. *Contact Dermatitis*. 1987;17(3):163-170.
86. Nyman GSA, Tang M, Inerot A, Osmancevic A, Malmberg P, Hagvall L. Contact allergy to beeswax and propolis among patients with cheilitis or facial dermatitis. *Contact Dermatitis*. 2019;81(2):110–116.
87. Mertens M, Gilissen L, Goossens A, Lambert J, Vermander E, Aerts O. Generalized systemic allergic dermatitis caused by *Cinnamomum zeylanicum* in a herbal tea. *Contact Dermatitis*. 2017;77(4):259-261.
88. Admani S, Hill H, Jacob SE. Cinnamon sugar scrub dermatitis: "natural" is not always best. *Pediatr Dermatol*. 2017;34(1):e42-e43.
89. Scientific Committee on Consumer Safety. SCCS/1459/11 Opinion on fragrance allergens in cosmetic products; 2011. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_073.pdf. Accessed October 29, 2019.
90. Isaac-Renton M, Li MK, Parsons LM. Cinnamon spice and everything not nice: many features of intraoral allergy to cinnamic aldehyde. *Dermatitis*. 2015;26(3):116-121.
91. Lauriola MM, De Bitonto A, Sena P. Allergic contact dermatitis due to cinnamon oil in galenic vaginal suppositories. *Acta Derm Venereol*. 2010;90(2):187-188.
92. Diba VC, Statham BN. Contact urticaria from cinnamal leading to anaphylaxis. *Contact Dermatitis*. 2003;48(2):119.
93. Leifer W. Contact dermatitis due to cinnamon; recurrence of dermatitis following oral administration of cinnamon oil. *AMA Arch Derm Syphilol*. 1951;64(1):52-55.
94. Paulsen E. Contact sensitization from Compositae-containing herbal remedies and cosmetics. *Contact Dermatitis*. 2002;47(4):189-198.
95. Paulsen E, Chistensen LP, Andersen KE. Cosmetics and herbal remedies with Compositae plant extracts - are they tolerated by Compositae-allergic patients? *Contact Dermatitis*. 2008;58(1):15-23.
96. Paulsen E, Andersen KE. Clinical patterns of Compositae dermatitis in Danish monosensitized patients. *Contact Dermatitis*. 2018;78(3):185-193.
97. Paulsen E, Andersen KE, Hausen BM. Sensitization and cross-reaction patterns in Danish Compositae-allergic patients. *Contact Dermatitis*. 2001;45(4):197-204.
98. Paulsen E. Systemic allergic dermatitis caused by sesquiterpene lactones. *Contact Dermatitis*. 2017;76(1):1-10.
99. Rudzki E, Rapiejko EZP, Rebandel P, Jaworski E. Oral allergy syndrome with contact urticaria from cosmetic creams. *Contact Dermatitis*. 1999;40(6):326-326.
100. Reider N, Sepp N, Fritsch P, Weinlich G, Jensen-Jarolim E. Anaphylaxis to camomile: clinical features and allergen cross-reactivity. *Clin Exp Allergy*. 2000;30(10):1436-1443.
101. Nakagawa M, Hanada M, Amano H. A case of anaphylactic reaction to chamomile tea in a patient with mugwort pollinosis. *Allergol Int*. 2019;68(3):396-398.
102. Gyldenløve M, Menné T, Thyssen JP. Eucalyptus contact allergy. *Contact Dermatitis*. 2014;71(5):303-304.
103. Zrira S, Bessiere JM, Menut C, Elamrani A, Benjilali B. Chemical composition of the essential oil of nine *Eucalyptus* species growing in Morocco. *Flavour Fragrance J*. 2004;19:172-175.
104. Miest RYN, Yiannias JA, Chang Y-HH, Singh N. Diagnosis and prevalence of lanolin allergy. *Dermatitis*. 2013;24(3):119-123.
105. Fransen M, Overgaard LEK, Johansen JD, Thyssen JP. Contact allergy to lanolin: temporal changes in prevalence and association with atopic dermatitis. *Contact Dermatitis*. 2018;78(1):70-75.
106. Bingham LJ, Tam MM, Palmer AM, Cahill JL, Nixon RL. Contact allergy and allergic contact dermatitis caused by lavender: a retrospective study from an Australian clinic. *Contact Dermatitis*. 2019;81(1):37-42.
107. Corazza M, Amendolagine G, Borghi A, Toni G, Lauriola MM. Aromatherapy and occupational allergic contact dermatitis: two further cases caused by lavender oil and other essential oils. *Contact Dermatitis*. 2019;81(5):378-408.
108. Thermo Scientific. Lemon. http://www.phadia.com/en/products/allergy-testing-products/immunocap-allergen-information/food-of-plant-origin/fruits/lemon/. Accessed May 21, 2019.
109. Ahrazem O, Ibáñez MD, López-Torrejón G, et al. Lipid transfer proteins and allergy to oranges. *Int Arch Allergy Immunol*. 2005;137(3):201-210.
110. Avoseh O, Oyedeji O, Rungqu P, Nkeh-Chungag B, Oyedeji A. Cymbopogon species; ethnopharmacology, phytochemistry and the pharmacological importance. *Molecules*. 2015;20(5):7438-7453.
111. de Groot A, Schmidt E. Essential oils, part V: peppermint oil, lavender oil, and lemongrass oil. *Dermatitis*. 2016;27(6):325-332.
112. Calapai G, Minciullo PL, Miroddi M, Chinou I, Gangemi S, Schmidt RJ. Contact dermatitis as an adverse reaction to some topically used European herbal medicinal products - Part 3: *Mentha × piperita - Solanum dulcamara*. *Contact Dermatitis*. 2016;74(3):131-144.
113. Sainio E-L, Kanerva L. Contact allergens in toothpastes and a review of their hypersensitivity. *Contact Dermatitis*. 1995;33(2):100-105.
114. Camarasa G, Alomar A. Menthol dermatitis from cigarettes. *Contact Dermatitis*. 1978;4(3):169-170.
115. de Groot AC, Schmidt E. *Essential Oils: Contact Allergy and Chemical Composition*. Boca Raton, FL: CRC Press Taylor Fr; 2016:1058.
116. Mohebitabar S, Shirazi M, Bioos S, Rahimi R, Malekshahi F, Nejatbakhsh F. Therapeutic efficacy of rose oil: a comprehensive review of clinical evidence. *Avicenna J Phytomed*. 2017;7(3):206.
117. Larson D, Jacob SE. Tea tree oil. *Dermatitis*. 2012;23(1):48-49.
118. de Groot AC, Schmidt E. Tea tree oil: contact allergy and chemical composition. *Contact Dermatitis*. 2016;75(3):129-143.
119. Rutherford T, Nixon R, Tam M, Tate B. Allergy to tea tree oil: retrospective review of 41 cases with positive patch tests over 4.5 years. *Australas J Dermatol*. 2007;48(2):83-87.
120. Tan LTH, Lee LH, Yin WF, et al. Traditional uses, phytochemistry, and bioactivities of *Cananga odorata* (Ylang-Ylang). *Evid Based Complement Alternat Med*. 2015;2015:1-30.
121. de Groot AC, Schmidt E. Essential oils, part VI: sandalwood oil, ylang-ylang oil, and jasmine absolute. *Dermatitis*. 2017;28(1):14-21.
122. de Groot AC. Contact allergy to and other adverse effects of fragrances. *Dermatitis*. 2020;31(1):13-35.
123. Wuyts L, van Hoof T, Lambert J, Aerts O. Allergic contact dermatitis caused by aftershave creams containing *Glycyrrhiza inflata*. *Contact Dermatitis*. 2017;77(1):49-51.
124. O'Connell RL, White IR, White JML, McFadden JP. Liquorice extract in a cosmetic product causing contact allergy. *Contact Dermatitis*. 2008;59(1):52-52.
125. Numata T, Kobayashi Y, Ito T, Harada K, Tsuboi R, Okubo Y. Two cases of allergic contact dermatitis due to skin-whitening cosmetics. *Allergol Int*. 2015;64(2):194-195.
126. Nishioka K, Seguchi T. Contact allergy due to oil-soluble licorice extracts in cosmetic products. *Contact Dermatitis*. 1999;40(1):56.

127. Hourihane JO, Kilburn SA, Dean P, Warner JO. Clinical characteristics of peanut allergy. *Clin Exp Allergy*. 1997;27(6):634-639.
128. Scientific Committee on Consumer Safety. SCCS/1526/14 Opinion on peanut oil (sensitisation only). 2014. https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_155.pdf. Accessed October 16, 2019.
129. Olszewski A, Pons L, Moutete F, et al. Isolation and characterization of proteic allergens in refined peanut oil. *Clin Exp Allergy*. 1998;28(7):850-859.
130. Scientific Committee on Consumer Safety. SCCS/1534/14 Opinion on the safety of hydrolysed wheat protein IN cosmetic products. 2014. https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_160.pdf. Accessed October 9, 2019.
131. Kobayashi T, Ito T, Kawakami H, et al. Eighteen cases of wheat allergy and wheat-dependent exercise-induced urticaria/anaphylaxis sensitized by hydrolyzed wheat protein in soap. *Int J Dermatol*. 2015;54(8):e302-e305.
132. Paulsen E, Andersen KE. Colophonium and Compositae mix as markers of fragrance allergy: cross-reactivity between fragrance terpenes, colophonium and Compositae plant extracts. *Contact Dermatitis*. 2005;53(5):285-291.
133. Tanaka S, Matsumoto Y, Dlova N, et al. Immediate contact reactions to fragrance mix constituents and *Myroxylon pereirae* resin. *Contact Dermatitis*. 2004;51(1):20-21.
134. Mathias CG, Chappler RR, Maibach HI. Contact urticaria from cinnamic aldehyde. *Arch Dermatol*. 1980;116(1):74-76.
135. Wilkinson M, Gallo R, Goossens A, et al. A proposal to create an extension to the European baseline series. *Contact Dermatitis*. 2018;78(2):101-108.
136. Cheng J, Zug KA. Fragrance allergic contact dermatitis. *Dermatitis*. 2014;25(5):232-245.
137. Boussault P, Léauté-Labrèze C, Saubusse E, et al. Oat sensitization in children with atopic dermatitis: prevalence, risks and associated factors. *Allergy*. 2007;62(11):1251-1256.
138. De Paz Arranz S, Pérez Montero A, Remón LZ, Molero MM. Allergic contact urticaria to oatmeal. *Allergy*. 2002;57(12):1215.
139. Guin JD, Hoskyn J. Aggravation of rosacea by protein contact dermatitis to soy. *Contact Dermatitis*. 2005;53(4):235-236.
140. Shaffrali FC, Gawkrodger DJ. Contact dermatitis from soybean extract in a cosmetic cream. *Contact Dermatitis*. 2001;44(1):51-52.
141. Wantke F, Hemmer W, Götz M, Jarisch R. Contact dermatitis from jojoba oil and myristyl lactate/maleated soybean oil. *Contact Dermatitis*. 1996;34(1):71-72.
142. Dooms-Goossens A, Buyse L, Stals H. Maleated soybean oil, a new cosmetic allergen. *Contact Dermatitis*. 1995;32(1):49-51.
143. Delaunay J, Hacard F, Denery-Papini S, et al. Occupational immediate contact allergy to hydrolysed wheat protein after cosmetic exposure. *Contact Dermatitis*. 2018;78(4):291-292.
144. Airaksinen L, Pallasaho P, Voutilainen R, Pesonen M. Occupational rhinitis, asthma, and contact urticaria caused by hydrolyzed wheat protein in hairdressers. *Ann Allergy Asthma Immunol*. 2013;111(6):577-579.
145. Leheron C, Bourrier T, Albertini M, Giovannini-Chami L. Immediate contact urticaria caused by hydrolysed wheat proteins in a child via maternal skin contact sensitization. *Contact Dermatitis*. 2013;68(6):379-380.
146. Chinuki Y, Takahashi H, Dekio I, et al. Higher allergenicity of high molecular weight hydrolysed wheat protein in cosmetics for percutaneous sensitization. *Contact Dermatitis*. 2013;68(2):86-93.
147. Barrientos N, Vázquez S, Domínguez JD. Contact urticaria induced by hydrolyzed wheat protein in cosmetic cream. *Actas Dermosifiliogr*. 2012;103(8):750-752.
148. Chinuki Y, Kaneko S, Sakieda K, Murata S, Yoshida Y, Morita E. A case of wheat-dependent exercise-induced anaphylaxis sensitized with hydrolysed wheat protein in a soap. *Contact Dermatitis*. 2011;65(1):55-57.
149. Bouchez-Mahiout I, Pecquet C, Kerre S, Snégaroff J, Raison-Peyron N, Laurière M. High molecular weight entities in industrial wheat protein hydrolysates are immunoreactive with IgE from allergic patients. *J Agric Food Chem*. 2010;58(7):4207-4215.
150. Yagami A, Aihara M, Ikezawa Z, et al. Outbreak of immediate-type hydrolyzed wheat protein allergy due to a facial soap in Japan. *J Allergy Clin Immunol*. 2017;140(3):879-881.e7.
151. Pecquet C et al. Hydrolysed wheat protein: a new allergen in cosmetics and food. *Contact Dermatitis*. 2004;50(9):182-183.
152. Hann S, Hughes M, Stone N. Allergic contact dermatitis to hydrolyzed wheat protein in a cosmetic cream. *Contact Dermatitis*. 2007;56(2):119-120.
153. Livideanu C, Giordano-Labadie F, Paul C. Contact dermatitis to hydrolyzed wheat protein. *Contact Dermatitis*. 2007;57(4):283-284.
154. Codreanu F, Morisset M, Cordebar V, Kanny G, Moneret-Vautrin DA. Risk of allergy to food proteins in topical medicinal agents and cosmetics. *Eur Ann Allergy Clin Immunol*. 2006;38(4):126-130.
155. Varjonen E, Petman L, Mäkinen-Kiljunen S. Immediate contact allergy from hydrolyzed wheat in a cosmetic cream. *Allergy*. 2000;55(3):294-296.
156. Kanoh H, Banno Y, Nakamura M, Seishima M. Contact allergy to liquorice flavonoids: analysis with liquid chromatography-mass spectrometry. *Contact Dermatitis*. 2016;74(3):191-192.

How to cite this article: Bruusgaard-Mouritsen MA, Johansen JD, Zachariae C, Kirkeby CS, Garvey LH. Natural ingredients in cosmetic products—A suggestion for a screening series for skin allergy. *Contact Dermatitis*. 2020;83:251–270. https://doi.org/10.1111/cod.13550

Manuscript II. Bruusgaard-Mouritsen MA, Johansen JD, Garvey LH. Facial contact dermatitis caused by cosmetic-relevant allergens. Accepted for publication in *Contact Dermatitis* (August 31st 2021),

Title: Facial contact dermatitis caused by cosmetic-relevant allergens

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Manuscript details:
Word count for the Abstract: 200
Word count text: 4363
Tables: 4
Figures: 1

Acknowledgements
The authors gratefully acknowledge the contribution of the dedicated staff at the Department of Dermatology, Copenhagen University Hospital Gentofte. The authors are thankful to data manager Pao-Lung Tsai for data extraction for the current study.

Conflicts of interest
All authors declare no conflicts of interest.

Funding
This work was supported by grants from the Danish Environmental Protection Agency to The National Allergy Research Centre and Harboefonden. Both are gratefully acknowledged.

Abstract

Background: Facial allergic contact dermatitis caused by cosmetic products is common. New allergens in cosmetics continuously emerge.

Objectives: To investigate characteristics of patients with facial dermatitis between 2010-2019 including patch test results from cosmetic-related allergens and a new test series with cosmetic-relevant natural ingredients (CRNIs).

Methods: A retrospective study analyzing demographics, clinical characteristics according to MOAHLFA, and patch test results to 27 cosmetic-relevant allergens in facial dermatitis patients. A prospective study evaluating a screening test series with CRNIs in consecutive facial dermatitis patients for one year. These patients received a questionnaire for collecting extra characteristics e.g. concerning quality of life.

Results: Of 8740 tested patients, 2292 (26.2%) had facial dermatitis**.** Of these, 30.6% had cosmetic-induced facial dermatitis. The most common cosmetic-related allergens were fragrances and preservatives. The most common patch test positive CRNIs were hydroperoxides of limonene and linalool, and propolis. Potato and peanut were rare, but the most common prick test positive CRNIs, however without any relation to the use of cosmetic products. Facial dermatitis affected nearly all patients´ quality of life and caused limitations to their daily life.

Conclusion: Updated management and quick diagnosis of facial dermatitis is important to avoid negative impact on patients´ quality of life.

Keywords: Facial dermatitis, cosmetic products, natural ingredients, patch test, skin prick test

Introduction

The lifetime prevalence of facial dermatitis (FD) is around 10% in the general population.[1] Many FD patients present in dermatology clinics for diagnosis and treatment.[1] FD may have a negative impact on patients quality of life in addition to socioeconomic consequences.[2,3] Compared with hand dermatitis, surprisingly few studies have recently focused on the frequency, characteristics and potential impact of FD and FACD.[1,4,7] Cosmetics are probably the most common reason that FD patients present for patch testing, as allergic contact dermatitis is suspected.[7] Many ingredients in cosmetic products are contact allergens and have been shown to be common causes of facial allergic contact dermatitis (FACD), especially fragrances and preservatives.[4,8,9] However, investigations of FACD may have a certain complexity due to the many ingredients and potential allergens present in cosmetics, and the cause of the allergy may be overlooked. New allergens, such as natural ingredients, continuously emerge in cosmetic products and could potentially make investigation and causes of FD even more comprehensive as they may also give rise to contact urticaria and/or protein contact dermatitis in FD patients. Cosmetics labelled as "natural" with plant protein derivatives, e.g. wheat and oat, and animal-derived protein derivatives, e.g. milk, known with the potential to cause immediate-type allergy when ingested, are increasing in popularity as cosmetic ingredients.[10] Protein sensitization is commonly known to occur through gastrointestinal (food allergens) and respiratory (inhalation allergens) exposure, but also percutaneous sensitization from plant protein-containing cosmetics applied to the skin can occur, especially in an impaired skin barrier. Rarely, natural ingredients in cosmetic applications have also been described to cause severe immediate-type reactions.[11] Of all plant proteins commonly used in cosmetic products, wheat is probably the most well-known cause of non-severe and severe immediate-type allergic reactions caused by percutaneous exposure. Since 2009, more than 2000 cases of hydrolyzed wheat-induced allergic reactions following percutaneous exposure from use of a former popular facial soap with hydrolyzed wheat gluten have been described.[12]

In this study, we investigated characteristics of FD among consecutive patients suspected of FACD between 2010 and 2019. Further, we evaluated patch test results to 27 selected cosmetic-related allergens during this period. Lastly, we tested consecutive FD patients with a screening test series with natural ingredients during a one-year period to find out whether these ingredients could explain the cause of FD in more patients than is possible today, in order to optimize investigation and diagnosis.

Materials and methods

The study consisted of a retrospective database study and a prospective skin test study with natural ingredients present in cosmetic products.

Database study

The database study was a retrospective registry-based study of the characteristics and patch test results of patients with FD using retrospective data from The Clinical Database on Contact Allergy at the Department of Dermatology and Allergy, Copenhagen University Hospital Gentofte in Denmark. Data included consecutive patients ≥ 18 years of age; patch tested from January 2010 to December 2019. Patients, who were patch tested more than once, were included at first registration of FD or at first patch testing if without a diagnosis of FD. For patients diagnosed with FD, patch test results from 27 allergens identified as relevant for cosmetic products from European baseline and our department´s extended series were included (table 2).

Skin test study with natural ingredients

This was a prospective cohort study including consecutive patients ≥ 18 years of age; investigated for FD during a 12-month period from June 2020 to May 2021. All patients underwent standard allergy testing, and in addition a screening test series with cosmetic-relevant natural allergens was tested. The screening test series was recently developed by our research group. The screening test series was based on initially a market survey using an application named Kemiluppen (The Chemistry Magnifying Glass), which is a non-profit application helping consumers avoid problematic substances in cosmetic products. The application contains > 10,000 cosmetic products on the Danish market.[13] All cosmetic products were label-checked for common natural ingredients (defined as plant- or animal-derived). The market survey was followed by an extensive literature search in which the most common natural ingredients from the market survey were examined to determine how frequently they were described with the potential to cause allergic reactions as ingredients in cosmetics or other topically administered products.[11] Based on these investigations, a total of 21 ingredients were selected for a screening test series consisting of a patch test and a prick test series with naturally derived ingredients. The patients were also asked to complete a questionnaire on the first day of testing before any results were available.

Patch testing

Twenty-one natural contact allergens were used for patch testing. These were either available as standardized commercial patch test preparations purchased from commercial vendors or prepared at the laboratory at Gentofte Hospital. They consisted of 17 plant-derived allergens and 4 animal-derived allergens.[11] Patch testing was performed with allergens listed in table 3. There was a slight overlap between the allergens selected for this series and the retrospective database study of allergens of particular relevance for cosmetics. Besides from the addition of the 21 natural allergens to the standard patch test panel for patients with FD in this study, the patch test procedure itself did not differ for the patients in the database study. Patch testing was performed according to European guidelines.[14] Patients were patch tested with contact allergens mixed in petrolatum or aqua using aluminum 8-millimeter Finn® Chambers (SmartPractice, Phoenix, AZ, USA) taped to the upper back with Scanpor tape (Norgesplaster, Vennesla, Norway) for two days. Readings were done on day 2, day 3 or 4, and day 7. Positive allergic reactions were classified as +, ++ or +++ according to European guidelines published by the European Society of Contact Dermatitis (ESCD).[14] A doubtful reaction, irritant reaction or negative reading was classified as a negative response.

Skin prick testing

Ten natural allergens commonly used in cosmetic products and described in the literature with a potential to cause immediate-type allergy were used for skin prick testing.[11] These included cera alba, cera flava, milk, oat, peach, peanut, potato, propolis, soybean and wheat. They were either available as standardized commercial skin prick test preparations purchased from commercial vendors or prepared at the laboratory at Gentofte Hospital. To identify potential cross-sensitization bias from inhalational allergens to birch, grass and mugwort, all patients were skin prick tested with these. Skin prick testing was performed with prick test allergens listed in table 4. The skin prick test procedure itself did not differ for participants in the study compared to the prick test procedure they would otherwise have to complete as part of their diagnostic workup except for the addition of the 10 natural allergens. Skin prick testing was performed according to European guidelines using a lancet pressed through a drop of allergen extract.[15] Solid preparations were tested via prick-to-prick test. Histamine dihydrochloride 10 mg/ml was used as a positive control and saline as a negative control. Reactions were read at 15–20 minutes following application. The largest diameter of the wheal of each test was measured, a positive being a wheal of ≥ 3 mm.

Prospective questionnaire study

All patients in the skin test study with natural ingredients were asked to complete a questionnaire comprising 12 questions during their first clinic visit. The first part of the questionnaire concerned the patient´s facial dermatitis, possible exposures, causes and associated limitations of daily life. The patients were asked to identify skin symptoms from pictures of contact dermatitis and urticaria. The last part of the questionnaire was focusing on possible exposures to natural ingredients, symptoms associated with natural ingredients and the patient´s view on natural ingredients (see Table S1 in supplementary).

Covariates

Basic demographic characteristics (sex and age at time of patch testing) and clinical characteristics according to the MOAHLFA index (male; occupation; atopic dermatitis; hand; leg; face; age ≥ 40 years) were available for all patients.

Statistical analysis

Statistical analysis was performed using IBM SPSS version 25, R (R studio, version 3.6.1) and MS Office 365 Excel.

Demographic data, patch test results and final diagnoses were analyzed using descriptive statistics. The prevalence of FD was calculated as the proportions of patients with a F in MOAHLFA of all patch tested patients during 2010-2019. The prevalence of cosmetic-induced FD was calculated as proportions of FD patients with a cosmetic-relevant diagnosis (allergic contact dermatitis due to cosmetics and/or irritant contact dermatitis due to cosmetics). Factors associated with FACD and cosmetic-induced FD and estimation of odds ratios (ORs) and 95% confidence intervals (CI) were evaluated using univariate analysis and multivariate logistic regression. A p-value < 0.05 was considered statistically significant.

Permissions

The database study was approved by the Danish Data Protection Agency (reference: 2012-58-0004, international reference: HGH-2017-046, I-Suite number: 05630). Permission to collect data from The Clinical Database for Contact Allergy were given by the Danish Clinical Quality Program – National Clinical Registries. In May 2021, data were extracted from the database. All data were handled anonymously.

The skin test study was approved by the Danish Data Protection Agency (reference: P-2020-853) and the regional ethics committee (reference: H-19088990). Oral and written consent was obtained from all patients in the natural ingredient skin test study.

Results

Database study

Characteristics of patients with facial dermatitis

Between January 2010 and December 2019, 8740 patients were patch tested and 2292 patients (26.2%) were diagnosed with facial dermatitis (FD). Demographic data and clinical characteristics for the total and the facial study population are shown in Table 1 together with results of univariate analyses. There was no significant difference in age between the FD population and non-FD population (48.4 ± 16.7 years vs. 48.3 ± 16.9 years, P < 0.78). Female gender (OR 2.0, 95% confidence interval [CI] 1.8–2.3) and atopic dermatitis (OR 2.5, 95% CI: 2.3–2.8) were associated with a significantly higher risk of FD in multivariate logistic regression analysis compared to not having FD. Occupational dermatitis (OR 0.7, 95% CI: 0.6–0.8), hand dermatitis (OR 0.4, 95% CI: 0.30–0.39), and leg dermatitis (OR 0.6, 95% CI: 0.42–0.95) were associated with a significantly lower risk of FD. There was no significant association with age > 40 years (OR 1.1, 95% CI: 0.97–1.22).

Characteristics of patients with cosmetic-induced facial dermatitis

In 2292 patients diagnosed with FD, 701 (30.6%) were diagnosed with FD partly or fully caused by cosmetics. These comprised 637 patients (90.9%) with facial allergic contact dermatitis (FACD) due to cosmetics, 56 patients (8.0%) with facial irritant contact dermatitis due to cosmetics and 8 patients (1.1%) with both diagnoses (see flowchart in figure 1). Characteristics and results of univariate analysis can be found in Table 1. The cosmetic- induced FD population were significantly older than the non-cosmetic-induced FD population (50.1 ± 15.8 years vs. 47.7 ± 17.0 years, P < 0.01).

More females (87.6%) than males (12.4%) were diagnosed with cosmetic-induced FD and more patients with cosmetic-induced FD had an age above 40 years (72.2% vs. 46.6%). Female gender (OR 2.1, 95% CI: 1.6–2.7) and age above 40 years (OR 1.3, 95% CI: 1.0–1.6) were both associated with a significantly higher risk of cosmetic-induced FD in multivariate logistic regression analysis compared to not having non-cosmetic-induced FD. Atopic dermatitis was seen in 23.0% among

those with cosmetic-induced FD compared with 32.9% in those without, carrying a significantly lower risk (OR 0.6, 95% CI: 0.5–0.8). There was no significant association with hand dermatitis (20.0% vs. 22.6%) (OR 1.1, 95% CI: 0.9–1.4), leg dermatitis (1.0% vs. 1.3%) (OR 1.0, 95% CI: 0.4–2.5) or occupational dermatitis (12.8% vs. 8.6%) (OR 0.7, 95% CI: 0.5–1.0).

Out-come of patch testing in patients with facial dermatitis

In total, 701 patients were diagnosed with FD caused by cosmetics. Among these patients a significantly higher proportion had a positive patch test reaction to Fragrance mix I (FMI) (30.8% vs. 4.5%), Fragrance mix II (FMII) (13.7% vs. 2.0%), Methylisothiazolinone (MI) (9.4 vs. 4.1%), Methylchloroisothiazolinone/methylisothiazolinone (MCI) (7.7% vs. 3.7%) and colophonium (8.7% vs. 2.9%) compared with the non-cosmetic-induced FD population. Patch test results from the 27 cosmetic-relevant allergens are shown in table 2.

In total, 324 patients (46.2%) were allergic to fragrance allergens (FMI, FMII, balsam of Peru, hydroperoxides of limonene or linalool) and 119 patients (17%) to preservatives (MI, MCI/MI, formaldehyde, quaternium-15, diazolidinyl urea , IBPC, bronopol, imidazolidinyl urea, DMDM hydantoin and paraben Mix). An overlap was seen in 52 patients, comprising 52/324 (16%) of those with fragrance allergy and 52/119 (43.7%) of those with preservative allergy.

In 61 patients with positive patch test reactions to colophonium, 33 patients (54.1%) also had a positive patch test reaction to perfume related allergens where FMI was most common (22/33), followed by FMII (13/33), Balsam of Peru (9/33), hydroperoxides of limonene (9/33) and hydroperoxides of linalool (9/33). Nine patients (14.8%) with positive patch test reactions to colophonium also had a positive patch test reaction to a preservative-related allergen.

Skin test study with natural ingredients

A total of 87 patients with FD were eligible for patch testing with the extra series of natural ingredients. One patient declined participation and in 20 patients there were not room enough on the back following application of other patch test series. Hence, a total of 66 consecutive patients (62 females, 4 males) investigated for FD were included. The mean age was 47.94 ± 17.0 years (range 18-80 years).

Patch testing

All 66 patients included in this study were patch tested with the screening test series containing 21 selected natural allergens. Nine patients (13.6%) had a positive patch test to at least one allergen from the screening test series divided among five allergens. Clinically relevant exposure was investigated among cosmetic products used by the participating patients. Relevant exposures were found among four patients of which three were patch test positive to hydroperoxides of linalool and two patients were patch test positive to hydroperoxides of limonene from everyday products (cosmetics; soap, cream, massage oil, and detergent). Patch test results are shown in table 3.

Skin prick testing

All 66 patients included in this study were skin prick tested with the screening prick test series. Two patients were excluded due to dermographism. Twelve patients (18%) had a positive skin prick test to at least one allergen from the screening test series divided among five allergens. Seven patients were positive to potato, three patients to peanut and one patient to wheat. These patients were all cross-reactive to birch and grass. All patients skin test positive to birch, grass and/or mugwort were known with hay fever and/or atopic dermatitis. There was no detectable relevant exposure from currently used cosmetic products. Skin prick test results are shown in table 4.

Questionnaire

All 66 patients with FD completed the questionnaire.

Thirty-one patients (47%) reported current facial rash with 25 cases (80.6%) compatible with contact dermatitis and 6 cases (19.4%) with urticaria. Two of these six patients had positive SPT to birch and were known with allergic rhinoconjunctivitis to birch. Almost half of the patients (45.5%) were diagnosed with atopic dermatitis or hay fever previously.

Current symptoms among patients with current facial rash had lasted for either days (7.6%), weeks (9.1%), months (21.2%) or years (19.7%) while the duration was unknown in 42.4%.

The patients were also asked how long ago they experienced FD for the first time. First time symptoms were divided into < 6 weeks (4.5%), 6 weeks to 3 months (12.1%), 3-12 months (30.3%), > 1 year (50%) and “do not remember” (3.1%).

Twenty-nine patients (43.9%) additionally reported dermatitis elsewhere on the body; neck (24.4%), arms (24.2%), trunk/shoulders (18.2%), legs (18.2%), hands (16.7%), or feet (6.1%).

Forty patients (60.6%) suspected a cause of their dermatitis; cosmetics (50%), food (13.6%), others (13.6%), work-related exposure (12.1%), pharmaceuticals (4.5%) and/or botanical plants (3%). Of these,

24 patients (60%) suspected a specific product causing their FD. Thirteen patients were also patch tested with their own suspected product and four had a positive reaction to the suspected product (facial or hair products).

Quality of life and limitations in everyday life

Almost all patients (94%) experienced affected quality of life with 50% being affected much or very much. Of all patients, 62.1% experienced limitations to their everyday life.

The most common limitations to the everyday life or causes of affected quality of life were social limitation and itching. Other frequent causes were visual impairment due to swelling, sleep problems, pain and concerns about what the symptoms were and of the future. Some patients mentioned work-related limitations with poorer performances at work or a need to report sick, as well as the time-consuming perspective of having to go to the doctor for investigation.

Natural ingredients

In total, 43 patients (65.2%) preferred cosmetic products branded as "natural". Reported reasons for this was that natural cosmetics were healthier (65.2%), less allergenic (50%) and/or to be better for the environment (34.8%). Out of these 43 patients, 33 patients (77%) label checked the cosmetic products for natural ingredients. A total of 60.6% were aware of allergy to natural ingredients, however, only 6.1% expected they might be allergic to natural ingredients in cosmetic products.

Discussion

Database study

In this retrospective study, we investigated characteristics of patients diagnosed with facial dermatitis (FD) and cosmetic-induced FD between 2010 and 2019 in a single university clinic. We found that 26.2% of all patients had FD. Of these, 30.6% had FD caused by cosmetics. Other studies have reported the prevalence and found FD to be between 15.4-27,4%.[5,16,17] FD is known to be more common in women than men, which our data supports with more than 80% of the patients with FD being women. The number of women with cosmetic-induced FD was even higher with almost 90% being women. These results may be due to a more frequent use of cosmetic products and possibly an increased rate of seeking medical attention among women.[5,6,18,19]

We found that atopic dermatitis (AD) was associated with a significantly higher risk of FD. Thus, AD was more common in patients with FD compared with the non-facial population (29.9% vs. 17.0%). However, AD was associated with a significantly lower risk of cosmetic-induced FD including contact allergy to cosmetic relevant allergens in our study. Atopic dermatitis may increase risk of sensitization and susceptibility to irritants, especially in the facial skin exposed to a frequent use of various cosmetic products.[5] In a Swedish study from 2004, patients with present or previous atopic dermatitis reported significantly more adverse reactions to cosmetic and skin care products (37.3% vs 23.8%).[20] A few other reports have also confirmed that both atopic dermatitis and female gender give a significant increased risk of adverse reactions to cosmetic products.[6,18,20,21] It is possible that the patients in our study are selected and may have refrained from using e.g. fragranced cosmetics from an early age, or more likely that the diagnosis of irritant contact dermatitis to cosmetics has been underestimated in our study, due to lack of diagnostic criteria.

Few other studies have investigated the prevalence of cosmetic-induced dermatitis and found the prevalence between 24-31.9% comparable with our 30.6%.[14,18] In this study, we focused on patch test reactions to 27 common allergens in cosmetics and found that fragrance allergens comprised 46.2% of the positive reactions with FMI (30.8%) and FMII (13.7%) as the most common among those with cosmetic-induced FD. Fragrances are well known sensitizers and among the most common allergens in cosmetic products with increasing prevalence during recent years.[9] The current study demonstrate that these still are the most prominent cause of contact allergic reactions from cosmetic product and reflects the lack of regulation, sound risk assessment and prevention in this area.

Eight different preservatives were included in our patch test results. All together preservatives were responsible for 17% of positive patch test reactions with MI (9.4%) and MCI/MI (7.7%) as the common preservative allergens. In a Danish population-based study from 2001, the prevalence of contact dermatitis to cosmetic-related allergens had increased in women aged 20-55 years from 2.4% to 5.8% between 1990 and 1998.[23] Following this, an exceptional epidemic has occurred in most of the industrialized world due to the introduction of MI in cosmetic products in Europe since 2005. This epidemic has giving rise to both facial allergic dermatitis and hand dermatitis.[24–29] In Denmark, the prevalence of contact allergy to MI significantly increased from 1.5% in 2005 to 5.7% in 2013 where 41% of the patients with MI allergy were affected by FD.[30] A regulation was introduced in 2017 with the purpose of decreasing the prevalence. This has had some effect and in

2019 the number had decreased to 3.3%.[25] Other preservatives including formaldehyde and formaldehyde-releasers accounted for 0.3-6.7% of the positive patch test reactions in patients with cosmetic-induced FD.

Colophonium was the fifth allergen most frequently causing a positive patch test reaction (4.8%) and more commonly positive among the cosmetic-induced FD population (8.7%) compared with non-cosmetic-induced FD population (2.9%). Colophonium was, with FMI and FMII, in a recent study found to be among the eight most common allergens causing contact allergy in the general population.[31] Although colophonium is not regarded as a fragrance allergen, a statistically significant association has been found between colophonium and FMI.[32–34] Approximately 50% (33/61) of the patients with a positive patch test to colophonium were also patch test positive to at least one perfume-related allergen, thus, colophonium could possibly indicate a perfume allergy. While all patients allergic to FMI I and FMII showed a current clinical relevance, a current clinical relevance to colophonium was only found in approximately 50% of the patients. In a recent study carried out by our group investigating natural ingredients in cosmetic products on the Danish market, colophonium was found to be a very rare ingredient and included in less than 30 products out of more than 10,000 investigated cosmetic products (unpublished observation), however, many cases have been reported due to colophonium in epilating products.[11,35] Hence, this may represent cross-reactivity as other significant facial exposures to colophonium-containing products are unlikely.

Skin test study with natural ingredients

Currently, there is an increasing use of cosmetic products containing natural ingredients which consumers seem to relate to safety. Our group recently proposed a screening series containing natural ingredients, especially relevant for patients with FD.[11] To our knowledge, no other studies have developed a screening test series focusing exclusively on natural ingredients in commonly used cosmetic products.

In this study, 66 patients were patch tested and skin prick tested with this screening series. The most common patch test positive allergens were hydroperoxides of linalool (6.1%), propolis (4.5%) and hydroperoxides of limonene (3%). Hydroperoxides of linalool and limonene are known sensitizers, while recently more attention has been drawn to propolis and many other natural ingredients in cosmetic products as potential sensitizers.[36] The most common skin prick test positive allergens were potato (10.6%) and peanuts (4.5%). Only few patients were known with immediate-type allergy to these allergens upon exposure. Due to the sensitization with unknown clinical relevance in most of the skin prick test positive patients, it remains to be investigated if

these patients are at higher risk of developing allergic symptoms from cutaneous exposure in cosmetic products and therefore should be advised to avoid them. With the addition of natural ingredients in cosmetic products, new cosmetic-related natural allergens are expected to emerge. We expect that future awareness on natural ingredients may find an increase in prevalence of allergy to natural ingredients.

In our questionnaire study, 27 patients (40.9%) had current symptoms which had lasted for months to years, and 33 patients (50%) had experienced FD for the first time more than a year ago. Thus, FD is a chronic problem in many patients. Notably, irrespective of the period of FD, almost all patients (94%) reported affected quality of life with 50% being affected much or very much. In continuation of this, more than half (62.1%) experienced limitations to their everyday life following social limitation and itching causing sleep problems, pain and work-related limitations. Studies on facial dermatitis and quality of life has primarily been performed on atopic dermatitis patients with facial dermatitis. In these studies, facial dermatitis has been associated with low quality of life in atopic dermatitis patients especially within the areas of social activities and embarrassment.[37–39] Both of these areas are mentioned by the patients in our non-validated questionnaire as the primary cause of limiting their daily life.

Cosmetic products containing natural ingredients are commonly used by consumers.[11,36,40] In this study, 65.2% preferred cosmetic products branded as "natural" due to beliefs that they were healthier, less allergenic and/or better for the environment and many patients specifically went for this branding when buying cosmetic products. The high number of patients preferring natural cosmetic products is similar to the findings of an Italian questionnaire study where 48% of the patients used natural topical products.[36] In the current questionnaire study, cosmetics were suspected as the most common cause of FD by half of the responders while food, pharmaceuticals, work-related exposures, botanicals/plants and "others" were reported to a lesser extent. As patients may not suspect the natural ingredients as the cause of their dermatitis, it is important to test patients with the patient's own products containing these ingredients during investigation.[36]

There are some limitations to this study. The selected group of patients with FD in the database study did not exclusively consist of patients diagnosed with FD, as they may also present with hand dermatitis among others. The selection of specific cosmetic-relevant patch test allergens may have affected the true results of the widespread etiology of FD. Regarding the questionnaire study, in Denmark patients with milder FD is less commonly referred to a Dermatology Department and

more commonly seen by dermatologists in private practice. Therefore, the answers may represent patients with more severe FD.

Conclusion

In conclusion, many patients suffer from FD and undergo patch testing. A significant proportion of FD cases are caused by contact allergy to cosmetic ingredients, most prominently fragrance ingredients and preservatives. Also, among those tested with the natural ingredient series, fragrances stood out reflecting lack of regulatory control in this area. FD caused by undetected exposures can lead to reduced quality of life, as indicated by this study. A continuously updated facial or cosmetic series with relevant and emerging allergens, possibly including emerging natural allergens is crucial.

References

1. Glasser Heede N. *Epidemiology of Dermatitis - A Characterization of Genetic Predisposition and Personal Consequences PhD Thesis*. ISBN 978-87-92613-89-9; 2016.
2. Lorente AI, Miranda MT, Rojas CV, Almeida C, Mozo P, Lorente R. Facial dermatitis: epidemiological retrospective study and patch test. *J Eur Acad Dermatology Venereol*. 2019;33(1):e30-e32.
3. Boonchai W. What should be in the facial patch testing series for 2020? *Br J Dermatol*. 2021;184(1):13-13.
4. Rolls S, Owen E, Bertram CG, et al. What is in? What is out? Updating the British Society for Cutaneous Allergy facial series. *Br J Dermatol*. 2021;184(1):151-155.
5. Kasemsarn P, Iamphonrat T, Boonchai W. Risk factors and common contact allergens in facial allergic contact dermatitis patients. *Int J Dermatol*. 2016;55(4):417-424.
6. Garg T, Agarwal S, Chander R, Singh A, Yadav P. Patch testing in patients with suspected cosmetic dermatitis: A retrospective study. *J Cosmet Dermatol*. 2018;17(1):95-100.
7. Zirwas MJ. Contact Dermatitis to Cosmetics. *Clin Rev Allergy Immunol*. 2019;56(1):119-128.
8. Horton E, Uter W, Geier J, et al. Developing a cosmetic series: Results from the ESSCA network, 2009-2018. *Contact Dermatitis*. 2021;84(2):82-94.
9. Park ME, Zippin JH. Allergic Contact Dermatitis to Cosmetics. *Dermatol Clin*. 2014;32(1):1-11.
10. Pecquet C, Lauriere M, Huet S, Leynadier F. Is the application of cosmetics containingprotein-derived products safe? *Contact Dermatitis*. 2002;46(2):123.
11. Bruusgaard-Mouritsen MA, Johansen JD, Zachariae C, Kirkeby CS, Garvey LH. Natural ingredients in cosmetic products—A suggestion for a screening series for skin allergy. *Contact Dermatitis*. 2020;83(4):251-270.
12. Yagami A, Aihara M, Ikezawa Z, et al. Outbreak of immediate-type hydrolyzed wheat protein allergy due to a facial soap in Japan. *J Allergy Clin Immunol*. 2017;140(3):879-881.e7.
13. Danish Consumer Council THINK Chemicals | Forbrugerrådet Tænk Kemi. https://kemi.taenk.dk/english. Accessed September 25, 2021.
14. Johansen JD, Aalto-Korte K, Agner T, et al. European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice. *Contact Dermatitis*. 2015;73(4):195-221.
15. Heinzerling L, Mari A, Bergmann K-C, et al. The skin prick test-European standards. *Clin Transl Allergy*. 2013;3(1):3.
16. Li LF, Liu G, Wang J. Prevalence and 1-year outcome of facial allergic contact dermatitis in patients patch tested in a university hospital. *Contact Dermatitis*. 2007;57(3):187-190.
17. Katz AS, Sherertz EF. Facial dermatitis: patch test results and final diagnoses. *Am J Contact Dermat*. 1999;10(3):153-156.
18. Warshaw EM, Buchholz HJ, Belsito D V., et al. Allergic patch test reactions associated with cosmetics: Retrospective analysis of cross-sectional data from the North American Contact Dermatitis Group, 2001-2004. *J Am Acad Dermatol*. 2009;60(1):23-38.
19. Alani JI, Davis MDP, Yiannias JA. Allergy to cosmetics: a literature review. *Dermatits*. 2013;24(6):283-290.
20. Lindberg A, Tammela M, Boström Å, et al. Are adverse skin reactions to cosmetics underestimated in the clinical assessment of contact dermatitis? A prospective study among 1075 patients attending Swedish patch test clinics. *Acta Derm Venereol*. 2004;84(4):291-295.

21. Hafner M de FS, Rodrigues AC, Lazzarini R. Allergic contact dermatitis to cosmetics: retrospective analysis of a population subjected to patch tests between 2004 and 2017. *An Bras Dermatol*. 2020;95(6):696-701.
22. Davies RF, Johnston GA. New and emerging cosmetic allergens. *Clin Dermatol*. 2011;29(3):311-315.
23. Nielsen NH, Linneberg A, Menné T, et al. Allergic contact sensitization in an adult Danish population: two cross-sectional surveys eight years apart (the Copenhagen Allergy Study). *Acta Derm Venereol*. 2001;81(1):31-34.
24. The Scientic Committee on Cosmetic Products and Non-Food Products Intended for Consumers. Opinion Concerning Methylisothiazolinone (Colipa No. P94). https://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out_201.pdf Accessed August 24, 2021.
25. Havmose M, Thyssen JP, Zachariae C, Menné T, Johansen JD. The epidemic of contact allergy to methylisothiazolinone—An analysis of Danish consecutive patients patch tested between 2005 and 2019. *Contact Dermatitis*. 2021;84(4):254-262.
26. Lundov MD, Thyssen JP, Zachariae C, Johansen JD. Prevalence and cause of methylisothiazolinone contact allergy. *Contact Dermatitis*. 2010;63(3):164-167.
27. Lundov MD, Opstrup MS, Johansen JD. Methylisothiazolinone contact allergy - a growing epidemic. *Contact Dermatitis*. 2013;69(5):271-275.
28. Johnston GA. The rise in prevalence of contact allergy to methylisothiazolinone in the British Isles. *Contact Dermatitis*. 2014;70(4):238-240.
29. Urwin R, Wilkinson M. Methylchloroisothiazolinone and methylisothiazolinone contact allergy: a new 'epidemic.' *Contact Dermatitis*. 2013;68(4):253-255.
30. Schwensen JF, White IR, Thyssen JP, Menné T, Johansen JD. Failures in risk assessment and risk management for cosmetic preservatives in Europe and the impact on public health. *Contact Dermatitis*. 2015;73(3):133-141.
31. Alinaghi F, Bennike NH, Egeberg A, Thyssen JP, Johansen JD. Prevalence of contact allergy in the general population: A systematic review and meta-analysis. *Contact Dermatitis*. 2019;80(2):77-85.
32. Belloni Fortina A, Cooper SM, Spiewak R, Fontana E, Schnuch A, Uter W. Patch test results in children and adolescents across Europe. Analysis of the ESSCA Network 2002-2010. *Pediatr Allergy Immunol*. 2015;26(5):446-455.
33. Paulsen E, Andersen KE. Colophonium and Compositae mix as markers of fragrance allergy: Cross-reactivity between fragrance terpenes, colophonium and Compositae plant extracts. *Contact Dermatitis*. 2005;53(5):285-291.
34. Nardelli A, Carbonez A, Drieghe J, Goossens A. Results of patch testing with fragrance mix 1, fragrance mix 2, and their ingredients, and Myroxylon pereirae and colophonium, over a 21-year period. *Contact Dermatitis*. 2013;68(5):307-313.
35. Goossens A, Armingaud P, Avenel-Audran M, et al. An epidemic of allergic contact dermatitis due to epilating products. *Contact Dermatitis*. 2002;47(2):67-70.
36. Gilissen L, Huygens S, Goossens A. Allergic contact dermatitis caused by topical herbal remedies: importance of patch testing with the patients' own products. *Contact Dermatitis*. 2018;78(3):177-184.
37. Holm JG, Agner T, Clausen ML, Thomsen SF. Quality of life and disease severity in patients with atopic dermatitis. *J Eur Acad Dermatology Venereol*. 2016;30(10):1760-1767.
38. Boonchai W, Charoenpipatsin N, Winayanuwattikun W, Phaitoonwattanakij S, Sukakul T. Assessment of the quality of life (QoL) of patients with dermatitis and the impact of patch testing on QoL: A study of 519 patients diagnosed with dermatitis. *Contact Dermatitis*. 2020;83(3):182-188.

39. Blome C, Radtke MA, Eissing L, Augustin M. Quality of Life in Patients with Atopic Dermatitis: Disease Burden, Measurement, and Treatment Benefit. *Am J Clin Dermatol*. 2016;17(2):163-169.
40. Corazza M, Borghi A, Gallo R, et al. Topical botanically derived products: use, skin reactions, and usefulness of patch tests. A multicentre Italian study. *Contact Dermatitis*. 2014;70(2):90-97.

Tables

Table 1. Age and sex distribution of the total study population (n=8740) and the facial dermatitis study population (n=2266) and clinical characteristics, according to the MOAHLFA index (male; occupation; atopic dermatitis; hand; leg; face; age ≥ 40 years), for patients patch tested from 2010-2019.

		Total study population (n= 8740)			Facial dermatitis population (n=2292)		
	Total study population (n= 8740)	**Non-facial dermatitis population (n=6448)**	**Facial dermatitis population† (n=2292)**	**Non-facial dermatitis population versus facial dermatitis population**	**Non-cosmetic-induced facial dermatitis population (n=1591)**	**Cosmetic-induced facial dermatitis population‡ (n=701)**	**Non-cosmetic-induced facial dermatitis population versus cosmetic-induced facial dermatitis population**
Men	2730 (31.2)	2281 (35.4)	449 (19.6)	OR 0.4, 95% CI: 0.4–0.5	362 (22.8)	87 (12.4)	OR 0.5, 95% CI: 0.4–0.6
Women	6010 (68.8)	4167 (64.6)	1843 (80.4)	OR 2.3, 95% CI: 2.0–2.5	1229 (77.2)	614 (87.6)	OR 2.1, 95% CI: 1.6–2.7
Age ≥ 40 years	5949 (68.1)	4363 (67.7)	1586 (69.2)	OR 1.1, 95% CI: 1.0–1.2	1053 (46.6)	512 (72.2)	OR 1.4, 95% CI: 1.2–1.7
Facial dermatitis	2292 (25.9)	0 (0)	2292 (100)		1583 (100)	701 (100)	
Atopic dermatitis	1778 (20.3)	1093 (17.0)	685 (29.9)	OR 2,1 95% CI: 1.9–2.3	524 (32.9)	161 (23.0)	OR 0.6, 95% CI: 0.5–0.7
Hand dermatitis	3449 (39.5)	2949 (45.7)	500 (21.8)	OR 0.3, 95% CI: 0.3–0.4	360 (22.6)	140 (20.0)	OR 0.9, 95% CI: 0.7–1.1
Leg dermatitis	146 (1.6)	119 (1.8)	27 (1.2)	OR 0.6, 95% CI: 0.4–1.0	20 (1.3)	7 (1.0)	OR 0.8, 95% CI: 0.3–1.9
Occupational dermatitis	1835 (21.0)	1579 (24.5)	256 (11.2)	OR 0.4, 95% CI: 0.3–0.5	208 (12.8)	60 (8.6)	OR 0.7, 95% CI: 0.5–0.9

Data are presented as n (%). OR: Odds ratio. CI: Confidence interval.

† Facial dermatitis patients were defined by a "F" in MOAHLFA.

‡ The cosmetic-induced facial dermatitis population consisted of allergic (n=637) and irritant contact dermatitis (n=56), 8 patients had both diagnoses.

Table 2. Rates of positive patch test reactions in 2292 patients patch tested due to facial dermatitis.

Allergens	**Positive patch test results in facial dermatitis population (n=2292)**	**Non-cosmetic-induced facial dermatitis population (n=1591)**	**Cosmetic-induced facial dermatitis population (n=701)**	**Non-cosmetic-induced facial dermatitis population versus cosmetic-induced facial dermatitis population**
Fragrance mix m. Sorbitan sesquioleate (FMI) 8% pet (n=2277)	289 (12.7)	73 (4.5)	216 (30.8)	OR 9.2, 95% CI: 6.9–12.2
Methylisothiazolinone (MI) 0.2% aq (n=2270)	132 (5.8)	66 (4.1)	66 (9.4)	OR 2.4, 95% CI: 1.7–3.4
Fragrance mix II (FMII) 14% pet (n=2270)	129 (5.7)	33 (2.0)	96 (13.7)	OR 7.5, 95% CI: 5.0–11.3
Methylchloroisothiazolinone/methylisothiazolinone 3:1 in aq (MCI/MI) 0.02% aq (n=2272)	114 (5.0)	60 (3.7)	54 (7.7)	OR 2.1, 95% CI: 1.5–3.1
Colophonium 20% pet (n=2269)	108 (4.8)	47 (2.9)	61 (8.7)	OR 3.1, 95% CI: 2.1–4.6
Myroxylon Pereirae (Balsam of Peru) 25% pet (n=2270)	93 (4.1)	30 (1.9)	63 (9.0)	OR 5.1, 95% CI: 3.3–8.0
Hydroperoxides of Linalool 1% pet† (n=2251)	85 (3.7)	29 (1.8)	56 (8.0)	OR 4.7, 95% CI: 3.0–7.4
P-phenylenediamine 1% pet (n=2272)	81 (3.6)	40 (2.5)	42 (6.0)	OR 2.4, 95% CI: 1.5–3.8
Formaldehyde 2% aq (n=2276)	67 (2.9)	23 (1.4)	44 (6.3)	OR 4.6, 95% CI: 2.7–7.6
Hydroperoxides of limonene† 0.3% pet (n=2253)	57 (2.5)	18 (1.1)	39 (5.6)	OR 5.2, 95% CI: 3.0–9.1
Amerchol L 101/Lanolin 50% pet (n=2266)	30 (1.3)	12 (0.7)	18 (2.6)	OR 3.5, 95% CI: 1.7–7.3
Lanolin alcohol 30% pet† (n=2269)	19 (0.8)	7 (0.4)	12 (1.7)	OR 4.0, 95% CI: 1.6–10.1
2-Hydroxyethylmethacrylate (HEMA) 1% pet (n=620)	16 (2.6)	12 (0.7)	4 (0.5)	OR 1.0, 95% CI: 0.3–3.3
Quaternium-15 1% pet (n=2271)	16 (0.7)	6 (0.3)	10 (1.4)	OR 3.8, 95% CI: 1.4–10.6
Diazolidinyl urea 2% pet (n=2268)	15 (0.7)	4 (0.2)	11 (1.5)	OR 6.3, 95% CI: 2.0–20.0
Iodopropynyl butylcarbamate (IPBC) 0.2% pet (n=2265)	8 (0.4)	3 (0.1)	5 (0.7)	OR 3.8, 95% CI: 0.9–16.1
Sorbitan sesquioleate (20% pet) (n=2270)	8 (0.4)	1 (0.1)	7 (1.0)	OR 16.1, 95% CI: 2.0–131.0
Bronopol 0.5% pet (n=2268)	7 (0.3)	4 (0.2)	3 (0.4)	OR 1.7, 95% CI: 0.4–7.7
Imidazolidinyl urea 2% pet (n=2268)	7 (0.3)	2 (0.1)	5 (0.7)	OR 5.7, 95% CI: 1.1–29.6
Propyl gallate (1% pet) (n=2265)	6 (0.3)	2 (0.1)	4 (0.5)	OR 4.6, 95% CI: 0.8–25.1
DMDM hydantoin (2% aq) (n=2265)	5 (0.2)	0 (0.0)	5 (0.7)	-
Paraben mix (A) 16% pet (n=2267)	3 (0.1)	1 (0.1)	2 (0.3)	OR 4.6, 95% CI: 0.4–50.3
Monoethanolamine/Ethanolamine (2% pet) (n=2265)	3 (0.1)	1 (0.1)	2 (0.3)	OR 4.6, 95% CI: 0.4–50.5
Sorbic acid 2% pet (n=2267)	2 (0.1)	0 (0.0)	2 (0.3)	-
Chlorhexidine digluconate (0.5% aq) (n=2268)	1 (0)	1 (0.1)	0 (0.0)	-
Chlorhexidine diacetate (0.5% aq) (n=2268)	0 (0)	0 (0.0)	0 (0.0)	-
Ethylhexylglycerin (5% pet) (n=434)	0 (0)	0 (0.0)	0 (0.0)	-

Data are presented as n (%). OR: Odds ratio. CI: Confidence interval.

† Overlap between the allergens selected for this study of particular relevance for cosmetics and the extra series with selected natural allergens in cosmetic products.

Table 3. Patch test results from the screening patch test series containing 21 selected allergens in 66 patch tested patients.

Allergen	Manufacturer	Number (n) of positive reactions n=66
Hydroperoxides of linalool 1% pet	Chemotechnique	4 (6.1%), 95% CI: 1.7-14.8
Propolis 10% pet†	Allergeaze	3 (4.5%), 95% CI: 0.9-12.7
Hydroperoxides of limonene 0.3% pet	Chemotechnique	2 (3%), 95% CI: 0.4-10.5
Cera flava (yellow beeswax) "as is" †	Sigmaaldrich	1 (1.5%), 95% CI: 0.04-8.1
Cinnamic aldehyde w. Sorbitan sesquioleate 1% pet	Allergeaze	1 (1.5%), 95% CI: 0.04-8.1
Lanolin alcohol 30% pet†	Chemotechnique	0
Menthol 2% pet	Chemotechnique	0
Lemongrass oil 2% pet	Allergeaze	0
Cera alba (white bees wax) "as is"†	Sigmaaldrich	0
Eucalyptus oil 2% pet	Allergeaze	0
Lanolin as is†	Local Pharmacy, Region H, Denmark	0
Lavender oil 2% pet	Chemotechnique	0
Liquorize (glycyrrhiza root) 1% pet	Sigmaaldrich	0
Mentha piperita (peppermint oil) 2% pet	Allergeaze	0
Oat 10% aq	Prepared in house	0
Orange oil 2% pet	Allergeaze	0
Rose oil 2% pet	Chemotechnique	0
Sesquiterpenelactone mix 0.1% pet	Allergeaze	0
Tea tree oil, oxidixed 5% pet	Allergeaze	0
Wheat 10% aq	Prepared in house	0
Ylang-ylang oil 2% pet	Chemotechnique	0

Data are presented as n (%), (95% CI). CI: Confidence interval. Pet: petrolatum. Aq: Aqua. †Animal-derived allergens.

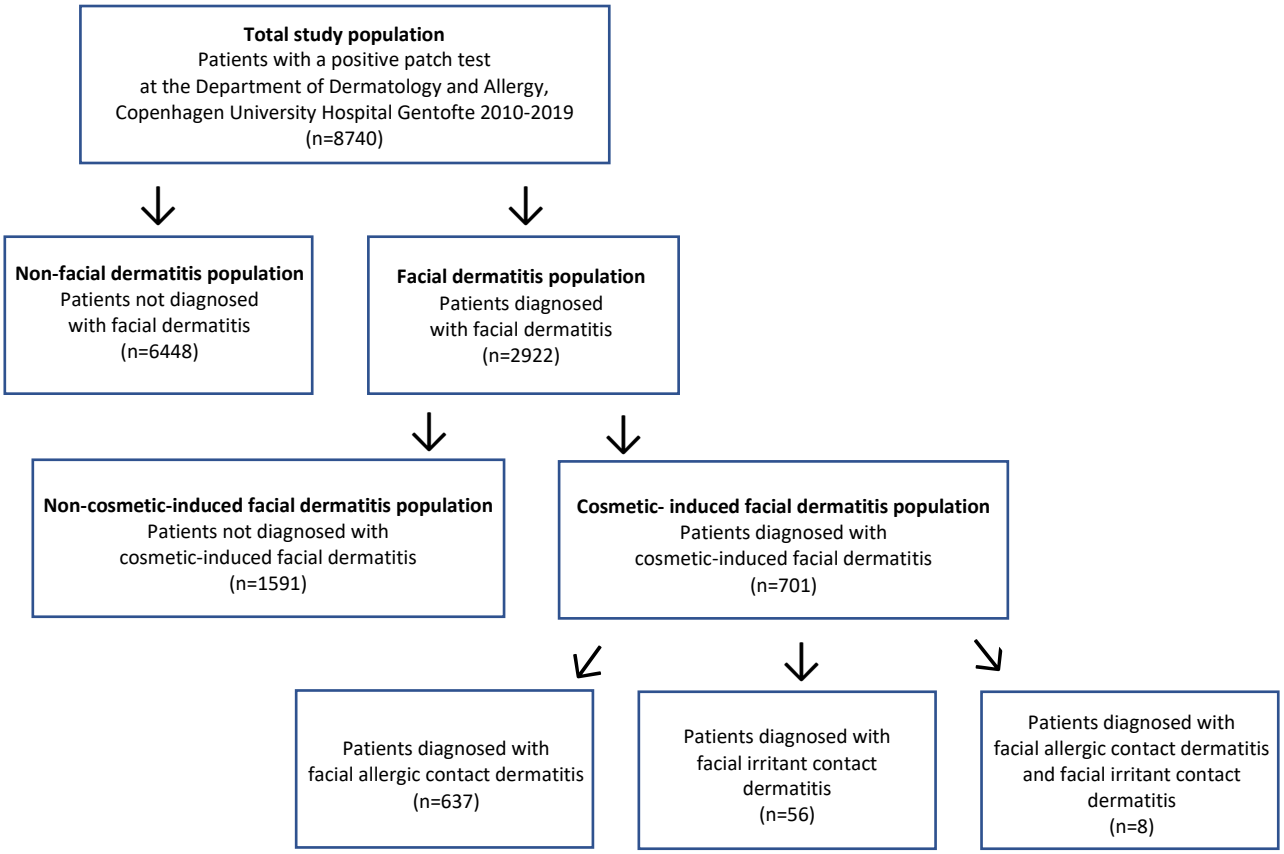
Table 4. Skin prick test results from the screening prick test series containing 13 selected allergens in 66 skin prick tested patients.

Allergen	Manufacturer	Number (n) of positive reactions (n=66) N (%) (95% CI)
Potato (fresh food)	Prepared in house	7 (10.6%), 95% CI: 4.4-20.6
Peanut 1:20 G/V	ALK-Abelló	3 (4.5%),95% CI: 0.9-12.7
Milk 1:20 w/v	ALK-Abelló	2 (3%), 95% CI: 0.4-10.5
Peach 1:20 G/V	ALK-Abelló	2 (3%), 95% CI: 0.4-10.5
Wheat 1:10 w/v	prepared in house	1 (1.5%), 95% CI: 0.04-8.1
Cera alba “as is”	Sigmaaldrich	0
Cera flava “as is”	Sigmaaldrich	0
Oat 1:10 w/v	Prepared in house	0
Propolis 10% pet	Allergeaze	0
Soybean 1:20 w/v	ALK-Abelló	0
Birch	ALK-Abelló	18 (27.3%), 95% CI: 17.0-39.6
Grass	ALK-Abelló	18 (27.3%), 95% CI: 17.0-39.6
Mugwort	ALK-Abelló	5 (7.6%), 95% CI: 2.5-16.8

Data are presented as n (%), (95% CI). CI: Confidence interval. G/V: gram by volume. w/v: weight by volume. Pet: petrolatum.

Figure legends.

Figure 1. Flowchart of included patients in the database study.



Manuscript III. Bruusgaard-Mouritsen MA, Johansen JD, Garvey LH. Clinical manifestations and impact on daily life of allergy to polyethylene glycol (PEG) in ten patients. *Clin Exp Allerg*y. 2021 Mar;51(3):463-470.

Received: 5 August 2020 | Revised: 23 December 2020 | Accepted: 30 December 2020

DOI: 10.1111/cea.13822

ORIGINAL ARTICLE

WILEY

Clinical manifestations and impact on daily life of allergy to polyethylene glycol (PEG) in ten patients

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Funding information
Danish Environmental Protection Agency; National Allergy Research Centre and Kongelig Hofbundtmager Aage Bangs Fond

Abstract

Background: Polyethylene glycols (PEGs) are widely used as excipients in drugs, cosmetics and household products. Immediate-type allergy to PEGs including anaphylaxis is rare. The recent introduction of the mRNA-based COVID-19 vaccines has led to an increased focus on PEG as a possible culprit of allergic reactions to the vaccines. A low awareness of the allergenic potential of PEG among consumers, manufacturers and doctors leads to under-diagnosis and under-reporting of allergy to PEGs, putting patients at risk of repeated severe reactions.

Objectives: To investigate clinical manifestations, time to diagnosis and impact of a PEG allergy diagnosis on the daily life of patients diagnosed with allergy to PEG from 2010 to 2019.

Method: Ten patients diagnosed with allergy to PEG were included. Detailed clinical history was obtained, and allergy investigations had been performed at the time of diagnosis. All patients were contacted and asked to retrospectively complete a questionnaire about causes and impact on daily life of an allergy to PEG, scored on a likert scale (0–10) before and after diagnosis.

Results: Eight patients had experienced at least one anaphylactic reaction requiring adrenaline treatment. Anaphylaxis was primarily caused by antibiotic/analgesic tablets, depot-steroids, antacids and laxatives. Seven patients reported repeated reactions before diagnosis (median 3, range 2–6). Median time from first reaction to diagnosis was 20 months (range 2–120). None of the patients experienced severe allergic reactions after the diagnosis. Median likert score of the impact on daily life before diagnosis was 7 compared with 4 after diagnosis.

Conclusion and clinical relevance: The clinical manifestations of PEG allergy are often dramatic. Improved awareness about the clinical presentation and common culprits, clear product labelling and a standardized nomenclature is needed to ensure the timely diagnosis of PEG allergy to prevent repeated anaphylactic reactions with severe impact on patients' lives.

KEYWORDS
allergy, anaphylaxis, drug allergy, macrogol, PEG, polyethylene glycol

1 | INTRODUCTION

Polyethylene glycols (PEGs) or macrogols are widely used as excipients in pharmaceutical, cosmetic and household products. PEGs are added to optimize the properties of a product and are commonly used as tablet surface coatings, pill binders, lubricants, ointment and cream bases as well as in wound dressings, bone cement, dural sealants and in polymer-based drug delivery (PEGylated drugs). They are generally considered to have low toxicity and to be biologically inert. Although allergy to PEG is rare, immediate-type allergy, often with severe reactions including life-threatening anaphylaxis, has been described with increasing frequency in the past two decades following an increased focus on these 'hidden' allergens.[1-4] PEGs are not commonly found in food products. To date, no cases of allergic reactions to PEG in food products have been reported in the literature.

The recent introduction of the mRNA based COVID-19 vaccines has led to an increased focus on PEG as a possible culprit of allergic reactions to the vaccine.[5] PEGs are difficult to avoid due to the widespread use, and patients with PEG allergy are at particular risk of re-exposure due to the lack of a standardized nomenclature and insufficient product labelling.[1,2,6]

PEGs are polymers of ethylene oxide. In cosmetic products, PEGs are described by the average number of ethylene oxide units, for example, PEG 100.[1,2] In drugs and other pharmaceutical products, PEGs are described by the total molecular weight of the number of ethylene oxide units, and the synonym macrogol is often used. As the molecular weight of ethylene oxide is 44 g/mol, macrogol 4400 g/mol is calculated as 100 × 44 = 4400. Consequently, PEG 100 and macrogol 4400 is the same compound but named differently depending on the product. However, nomenclature is inconsistent and varies between countries. PEG molecular weights range from around 200 to 50,000 g/mol.[1,7]

In addition to being named differently depending on product type, PEGs have numerous other synonyms as described in a review by Wenande et al in 2016.[1] The non-standardized nomenclature combined with low awareness of the allergenic potential of PEGs among consumers, manufacturers and doctors leads to under-diagnosis and under-reporting of PEG allergy, putting patients at risk of experiencing repeated severe reactions before the diagnosis is made.[1] Patients with PEG allergy may also have cross-reactions to PEGylated drugs and structurally similar polymers, such as polysorbates and poloxamers.[1,8,9]

Beside the risk of repeated life-threatening anaphylactic reactions, patients are also at risk of substandard care due to fear of anaphylaxis on introducing new treatments. The impact on patients´ daily life has not been previously investigated, and the aim of this study was to investigate clinical manifestations, time to diagnosis and the effect on patients´ daily life in patients with PEG allergy. To our knowledge, this is the largest series of PEG allergic patients reported so far.

2 | METHODS

2.1 | Study population

A total of twelve patients were diagnosed with PEG allergy at the Allergy Clinic, Department of Dermatology and Allergy at Copenhagen University Hospital Gentofte during a nine-year period from 1 September 2010 to 31 August 2019. One patient had died, making 11 patients eligible for inclusion in the study. Patients were diagnosed with PEG allergy between 3 weeks to 8 years prior to the study start.

Oral and written informed consent was obtained from all patients. The study was approved by the regional ethics committee with number H-17021145.

2.2 | Clinical patient data

All patients had been under the care of the last author since diagnosis, and detailed information on clinical history, culprit agents and investigation and test results was obtained from patient records.

2.3 | Questionnaire

The questionnaire comprised 11 questions about exposure to PEGs, suspected causes, self-reported allergy symptoms, and the impact on their daily life scored retrospectively on a likert scale (0–10) before and after diagnosis, where 0 was no impact on daily life and 10 was severe impact on daily life. Furthermore, the patients were asked what they perceived to be the most important information from the healthcare professionals when diagnosed with PEG allergy.

The questionnaire was sent to 11 patients diagnosed with allergy to PEG between October 2017 and November 2019 (see Table S1).

3 | RESULTS

One patient declined participation; thus 10 patients were included (6 males, 4 females). The median age was 35 years (range 18–64 years). Two patients had pollen-induced rhinoconjunctivitis, and one patient was allergic to cat dander. The remaining patients had no other allergies prior to their diagnosis with allergy to PEG.

None of the patients had heard about PEG allergy before diagnosis, although one patient had a suspicion towards excipients after anaphylaxis to two different drugs. Nine patients reported either knowing or suspecting which products had caused their reactions (Table 1), and eight patients reported more than one product causing reactions. Healthcare products, but also cosmetic products and hygiene products (razors, toothpaste and dental floss) were reported as possible causes.

TABLE 1 Products suspected by PEG allergic patients to induce allergic symptoms.

Oral medication (analgesics, antacids, antibiotic tablets)
Injections
Shaving products (razors and shaving gel)
Cream/ointment
Mouth hygiene products (toothpaste, mouth wash, dental floss)
Hand soap
Laxatives
Hair products (shampoo, hair colouring)
Make-up and make-up remover
Vaseline used in connection with tattooing
Cough medicine
Epoxy used in workplace
Cleaning agent

In total the ten patients reported 31 reactions. Seven patients reported repeated reactions (median 3, range 2–6) before diagnosis. For all patients, median time from first reaction to diagnosis was 20 months (range 2–120 months). All patients experienced immediate hypersensitivity symptoms within 10 minutes of exposure and 15 reactions fulfilled the criteria for anaphylaxis with skin symptoms, combined with angioedema, respiratory or circulatory compromise. The most common symptoms were urticaria, itching, flushing, general discomfort, angioedema, breathlessness, burning sensation and fainting. Eight patients had experienced at least one episode of anaphylaxis requiring adrenalin treatment, before diagnosis. The most severe reaction was a case of perioperative cardiac arrest (Table 2). Main products confirmed to have caused anaphylaxis are shown in Table 3. Three patients were misdiagnosed with penicillin allergy, idiopathic anaphylaxis and chronic idiopathic urticaria, respectively, before the correct diagnosis of PEG allergy.

After the diagnosis was made, four patients reported accidental re-exposure to PEG mainly in cosmetic products (hand soap, shampoo, cream, toothpaste, razors and shaving gel) but also pharmaceutical products (tablets, steroid cream, hormone injections during fertility treatment). All four patients reported milder reactions with itching, and three patients reported transient swelling of the face. One patient with pre-existing asthma reported breathlessness. Symptom onset was within 0–10 minutes in all patients. None of the patients needed treatment with adrenaline or hospitalization.

Median likert score on impact on daily life before diagnosis was 7 (range 0–10) compared with 4 (range 0–8) after diagnosis, scored retrospectively. After diagnosis, seven patients still reported limitations to their daily life. Some patients experienced periods of stress and anxiety either before or after diagnosis, as a result of the overwhelming fear of a new anaphylactic reaction due to the widespread use of PEG.

All patients were aware of the use of PEGs in drugs and other pharmaceutical products and potential exposure on contact with the health care sector. Nearly all patients were aware of the use of PEGs in cosmetics and checked for PEGs in cosmetic products before using them. All patients reported informing about their allergy when in contact with hospitals, general practitioners, specialists, dentists and hairdressers.

Four patients had experienced work- or school-related exposure from soap, cream and cleaning products. None of the patients had to quit their job or school due to the allergy.

All patients specified the importance of receiving sufficient information about allergy to PEG when their diagnosis was made. The most valuable information from the patients´ point of view was information on which products contain PEGs as well as how to check if a product contains PEGs, for example, by reading the package insert and/or the product information on the Danish Medicines Agency homepage. The fact that numerous products contain PEG was also valuable information. The patients additionally emphasized the importance of receiving an allergy warning card, follow-up appointments and the possibility of continued access to advice from a doctor at the Allergy Clinic. Most of the patients have had several contacts to the department asking advice about medications they were prescribed or treatments they needed after the diagnosis was made. Also, in cases of hospital admissions or need for treatment, for example, surgical procedures, chemotherapy or fertility treatment, advice has been sought by health care personnel treating the patients. Some patients expressed a need for a PEG-containing product database and an online forum or focus group with other patients diagnosed with PEG allergy.

4 | DISCUSSION

We present the detailed clinical manifestations and culprit agents for ten patients diagnosed with allergy to PEGs. We also found that allergy to PEG has severe impact on the patients´ daily life with some improvement after diagnosis. However, more than half of the patients continued to experience some limitations to their daily life.

Before diagnosis, 80% of the patients had experienced one or more episodes of anaphylaxis requiring adrenalin treatment. This is consistent with the findings in the review by Wenande et al., where 76% of identified cases experienced anaphylactic reactions with more than half being caused by laxatives or bowel preparations.[1] In this study, all patients´ initial allergic reactions were caused by exposure to pharmaceutical drugs and products. The main products causing anaphylaxis were oral medication such as analgesic tablets, antibiotic tablets, antacids and laxatives followed by injections of depot-steroids. Several other studies and case reports have also described initial allergic reactions to PEGs caused by exposure to pharmaceuticals, especially laxatives and depot-steroid injections.[1,9-12] None of the patients had ever experienced symptoms from foods or vaccines.

Due to widespread use of PEG in over-the-counter medication such as analgesics, antacids and laxatives, most patients in our cohort had several allergic reactions to PEGs before diagnosis. Similar findings have been described in numerous case reports.[2,12-17] The median time to diagnosis was two years, emphasizing the difficulty

TABLE 2 Clinical manifestations, culprits and test results from 10 patients with PEG allergy.

Age at time of diagnosis	Sex	Culprit agents	Symptoms	PEG skin prick test (SPT) results at time of diagnosis	Other test results
27	F	Intramuscular Depo-medrol ® steroid injection (PEG 3350) Balancid Novum ® reflux tablet (PEG 6000) Effexor ® antidepressant tablet (PEG400) Vaseline prior to tattooing (PEG unknown MW) Helosan ® cream (PEG−100 stearate)	Anaphylaxis: Immediate onset facial erythema, pruritus, dizziness, hoarseness, vomiting Anaphylaxis: Immediate palmo-plantar pruritus, vomiting, dizziness Generalized pruritus Localized urticaria in tattooed area, generalized urticaria and palmo-plantar pruritus Erythema, pruritus	SPT positive for Depo-medrol ® (PEG 3350), Carbamid ® skin cream (PEG 1500), Helosan ® skin cream (PEG 100-sterate), chewable Balancid Novum ® tablet (PEG 6000), PEG 3350 (10%) and PEG 6000 (50%) SPT negative for ethylene glycol (100%) and diethylene glycol (100%)	Direct and indirect basophil histamine release test: positive for Balancid Novum tablet suspension (PEG 6000), PEG 3350 10% and PEG 6000 100%.
63	F	Intraarticular Depo-medrol ® steroid injection (PEG 3350)	Anaphylaxis: Palmo-plantar pruritus, generalized urticaria, hypotension	SPT positive for PEG 3000 (50%) and PEG 6000 (50%) SPT negative for ethylene glycol (100%), diethylene glycol (100%), polysorbate 80 (20%) and Solu-medrol ® (100%)	Direct basophil histamine release test: positive for PEG 3000 and PEG 6000, negative for Depo-medrol
36	M	Vepicombin ® tablet (PEG 6000) Vepicombin ® tablet during drug provocation (PEG 6000) Burana ® Ibuprofen tablet (PEG 6000) Mucoangin ® throat lozenge (PEG 6000) Xerodent ® oral tablet (PEG 6000) Balancid Novum ® reflux tablet (PEG 6000) Sensodyne ® Dental floss (PEG 6000) Xerodent ® oral tablet (PEG 6000)	Anaphylaxis: generalized pruritus, feeling warm, hypotension and unconsciousness Anaphylaxis: generalized pruritus, feeling warm and hypotension Anaphylaxis Generalized discomfort, pruritus and feeling warm Generalized discomfort, pruritus and feeling warm Generalized discomfort, pruritus and feeling warm Oral tingling, localised oedema, pruritus and nausea Dyspepsia, itching of mouth, generalized pruritus, discomfort	SPT positive for PEG 6000 (50%), PEG 20.000 (0,01%) and poloxamer 407 (10%) SPT negative for ethylene glycol (100%), diethylene glycol (100%), PEG 300 (100%), PEG 3000 (50%), polysorbate 20 (100%) and polysorbate 80 (20%)	Oral provocation with Vepicombin Novum 1 MIE (PEG 6000): positive (anaphylaxis: immediate onset respiratory distress, itching in mouth, plantar pruritus, hypotension. Tryptase: 17.4 µg/l (baseline 3.77µg/l) Negative IgE for penicillin Oral provocation with Primcillin granules (without PEG) negative Direct basophil histamine release test: negative for PEG 300, PEG 3000, PEG 6000 and Polysorbate 80.
32	M	Accell Connexus ® DBM putty (Poloxamer 407) during hand surgery for scaphoid fracture	Anaphylaxis: cardiac arrest immediately post-operatively	SPT positive for polysorbate 80 (20%), poloxamer 407 (10%) and Bone Accell Connexus ® containing poloxamer 407 SPT negative for ethylene glycol (100%), diethylene glycol (100%), PEG 300 (100%), PEG 3000 (50%) and PEG 6000 (50%)	Direct basophil histamine release test: positive for Accell Connexus bonematrix
52	M	Unidentified perioperative exposure during coronary stent insertion	Anaphylaxis: Dyspnoea, hoarseness, oral angioedema and truncal urticaria	SPT positive for PEG 3000 (50%), PEG 6000 (50%), PEG 20.000 (0,1%) and poloxamer 407 (10%) SPT negative for ethylene glycol (100%), diethylene glycol (100%), PEG 300 (100%), polysorbate 20 (100%) and polysorbate 80 (20%)	

(Continues)

TABLE 2 Continued

Age at time of diagnosis	Sex	Culprit agents	Symptoms	PEG skin prick test (SPT) results at time of diagnosis	Other test results
30	M	Migea ® analgesic tablet (PEG 6000) Burana ® Ibuprofen tablet (PEG 6000) Panodil Zapp ® analgesic tablet (PEG 6000)	Immediate onset generalized pruritus and urticaria, palmo-plantar swelling Anaphylaxis: Immediate onset generalized pruritus and urticaria, palmo-plantar swelling, oropharyngeal swelling and urticaria Urticaria on chest	SPT positive for PEG 3000 (50%), PEG 6000 (50%), PEG 20.000 (0,01%) and poloxamer 407 (10%) SPT negative for ethylene glycol (100%), diethylene glycol (100%), PEG 300 (100%), polysorbate 20 (100%) and polysorbate 80 (20%)	Oral provocation with acetylsalicylic acid: negative
37	M	Vepicombin ® penicillin tablet (PEG 6000) Intraarticular Depo-medrol ® steroid injection (PEG 3350)	Anaphylaxis: Immediate onset burning sensation in mouth, pruritus and generalized urticaria, pallor, sweating, palmo-plantar swelling Anaphylaxis: Immediate onset itching of mouth and tongue, itching at the injection site, back pain, dizziness, sweating, fainting, respiratory distress	SPT positive for PEG 300 (100%), PEG 3000 (50%), PEG 6000 (50%), polysorbat 80 (20%) and poloxamer 407 (10%) SPT negative for ethylene glycol (100%) and diethylene glycol (100%)	
22	F	Vepicombin ® penicillin tablet (PEG 6000) Balancid Novum ® reflux tablet (PEG 6000) Movicol ® laxative (PEG 3350) Panodil ® analgesic tablet (PEG and polysorbate 80)	Periorbital swelling and palmar swelling, pruritus Immediate onset pruritus, periorbital swelling and palmar swelling Anaphylaxis: Immediate onset itching of tongue, generalized pruritus, palmo-plantar swelling, angioedema Anaphylaxis: Abdominal pain, dizziness, feeling faint, angioedema, tingling in the fingers	SPT positive for PEG 6000 (50%) and poloxamer (407 10%) SPT negative for ethylene glycol (100%), diethylene glycol (100%), PEG 300 (100%), PEG 3000 (50%) and polysorbate 80 (20%)	
16	M	Movicol ® laxative (PEG 3350) Dulcosoft ® laxative (PEG 4000) Dispropan ® intraarticular steroid injection (PEG 3350)	Immediate onset urticaria, conjunctival redness, tachycardia, hypertension Abdominal pain, diarrhoea Anaphylaxis: Generalized urticaria, dizziness, discomfort, chest pain, weakness, feeling faint	SPT positive for PEG 6000 (50%) and poloxamer 407 (10%) SPT negative for ethylene glycol (100%), diethylene glycol (100%), polysorbate 20 (100%) and polysorbate 80 (20%)	Direct basophil histamine release test: negative for movicol
33	F	Intraarticular Depo-medrol ® steroid injection (PEG 3350) Intraarticular Depo-medrol ® steroid injection (PEG 3350) Sanex ® bodylotion (PEG 4400)	Anaphylaxis: Immediate onset itching of gums and face, angioedema including tongue, palmo-plantar pruritus, feeling of throat tightening, bronchospasm, feeling faint Generalized erythema, pruritus Pruritus, urticaria	SPT positive for PEG 3000 (50%), polysorbate 80 (20%) and poloxamer 407 (10%), Depo-medrol (PEG 3350) and Sanex bodylotion SPT negative for ethylene glycol (100%), diethylene glycol (100%), PEG 300 (100%) and polysorbat 20 (100%)	

Products	Number of patients experiencing anaphylaxis
Depot-steroid injections	5
Analgesic tablets (specific formulations of paracetamol and ibuprofen)	3
Antibiotic tablets (same specific formulation of penicillin)	3
Bone cement and unknown product in perioperative setting	2
Antacids (calcium carbonate-magnesium hydroxide)	1
Laxatives (macrogol)	1

TABLE 3 PEG-containing products confirmed to have caused anaphylaxis in the study cohort. Some patients experienced anaphylaxis to more than one product.

in making the diagnosis of PEG allergy. In many cases the diagnosis of PEG allergy is made by a convincing clinical history of severe allergic reactions to two or more structurally unrelated PEG-containing products, confirmed by a positive skin prick test with PEG in one or more concentrations, for example, PEG 300, PEG 3000 and PEG 6000. Oral provocation and intradermal testing are not routinely recommended due to the risk of inducing severe allergic reactions.[1]

None of the patients in our study were aware of PEGs or PEG allergy until the diagnosis was made during allergy investigations. Importantly, only one of the healthcare professionals who had been treating the patients for their allergic reactions suspected PEG as the culprit. Several patients were misdiagnosed with idiopathic anaphylaxis, urticaria or allergy to the active ingredient in drugs, for example, penicillin prior to the diagnosis. Other case reports have shown similar cases of patients with misdiagnoses.[13,17,18]

When PEG allergy is suspected, several actions should be taken in order to help the patient (Table 4). In general, investigation, diagnosis and follow-up of patients with allergy to PEGs is challenging due to the lack of standardized test method for PEGs and the need for special expertize and comprehensive follow-up by an allergist with specific knowledge about PEG allergy.[13] Most patients in this cohort have had several contacts to the Allergy Department, after the diagnosis was made, asking advice about medication they were prescribed. In addition, on many occasions, health care professionals have sought advice on how to avoid exposure to PEG during various medical treatments or surgical procedures.

One example of special challenge regarding medical treatment among PEG allergic patients in our department has been cancer treatment. A former patient with PEG allergy was initially refused treatment of his cancer with chemotherapy by the attending physicians, who had no knowledge about PEG allergy and considered the risk of allergic reactions to be too high. After intervention by an allergist from our department, the planned treatments were reviewed for PEG content and relevant treatment without PEG could be given uneventfully. The patient later had an urticarial reaction to a bandage where PEG was used on the surface to increase absorption. A special challenge in cancer treatment is that chemotherapy drugs are often PEGylated. PEGylation is the conjugation of PEGs to drugs, leading to prolonged half-life in plasma and less immunogenicity without compromising the clinical efficacy.[19,20] This example shows that treating patients with PEG allergy can be challenging, complicated and demanding on resources.

When diagnosed with PEG allergy, the patients are advised to inform the local pharmacist to get assistance in ensuring that any medicine or over-the-counter product is PEG-free. As the PEG content may vary in different formulations of the same drug, and even in different doses of the same formulation, each individual drug needs to be checked for PEG. Also, new parallel imports of drugs appear frequently for over-the-counter drugs such as paracetamol, and the excipients may vary. For those reasons it is not possible to compile a list of 'safe' PEG-free drugs even though the patients requested it, as it may give a false sense of security. Thus, patients are encouraged to identify their own safe products for over-the-counter or prescription medicine and to bring their own medication, for example, pain killers in case of hospitalization. Some drug groups almost all contain PEG, and in Denmark it is difficult to find proton pump inhibitors or contraceptive pills without PEGs. Importantly, most antihistamines contain PEGs which have implications for treatment of allergic symptoms.[21]

Some patients suggested that an online forum or focus group with other patients diagnosed with PEG allergy could be useful for exchanging experiences. Currently, a few patient-initiated online forums exist with PEG allergic patients helping and supporting each other. Investigations with these types of patient interventions have not been described in the literature to date.

Almost half of the patients in this cohort experienced accidental re-exposure and reactions to PEG-containing products despite great efforts to avoid it. Re-exposures were mainly caused by everyday cosmetic products used on the skin such as soap, shampoo and razors, and none of the patients experienced serious reactions to this type of re-exposure. The most popular razors on the market have a gel pad containing PEGs. In several of our patients, using these led to instant localized redness, itching and/or urticaria. Shaving using a razor with a PEG-containing gel pad may be an important entry source through microlacerations in the skin, which could play a role in sensitization to PEGs.

To the best of our knowledge, this is the largest cohort of patients with PEG allergy reported so far. Yet, a cohort consisting of only ten patients is also a limitation to the study. The questionnaire is not validated, and patients have been included with

TABLE 4 PEG allergy recommendations

Suspect PEG allergy in patients with: Repeated, severe allergic reactions/anaphylaxis to ≥2 structurally different drugs/products. A history of reaction with a drug/compound containing polysorbate 80/poloxamer 407 Severe allergic reaction/anaphylaxis to drugs where sensitization to active ingredient has been excluded on testing (e.g., antibiotics, analgesics) Severe unexplained allergic reactions in connection with surgery/invasive procedures Severe allergic reactions to depot injections, antacids and PEG based laxatives
Investigate and diagnose patients with skin prick testing containing a standardized panel of PEGs[8]
Provide patients with detailed information about their allergy
Educate patients in manually checking labels for cosmetic products and pharmaceutical drugs
Provide patients with an allergy warning card, follow-up appointments and continued access to advice from an allergist with special knowledge about PEG allergy
Take initiatives to address the insufficient product labelling and the need for a standardized nomenclature in cosmetic and pharmaceutical products

varying frequencies up to eight years from first symptoms and diagnoses, increasing the risk of recall bias primarily on the question about impact on daily life. Data on clinical manifestations were collected from the medical notes from the initial consultation and diagnostic work-up, and these data will help increase awareness about clinical presentations in PEG allergy patients and potential culprits.

Although only a limited number of patients with PEG allergy have been diagnosed until now, the frequency is expected to rise with increased awareness of this allergy.[1,20]

In conclusion, we provide details of the clinical manifestations on ten patients with confirmed allergy to PEG, which will be helpful for health care workers unfamiliar with allergy to PEGs. Since both the mRNA based COVID-19 vaccines first on the market from Pfizer/BioNTech and Moderna contain PEG 2000, there is now great interest in learning more about this rare allergy. Until now, the diagnosis of PEG allergy has often been delayed, leading to repeated, severe allergic reactions significantly affecting daily life of patients. PEGs are difficult to avoid due to the widespread use causing a high risk of inadvertent re-exposure. However, once the diagnosis has been made, several actions of importance to the PEG allergic patient can be taken to help them avoid potentially life-threatening re-exposure to PEG-containing products, thereby improving the daily life for these patients.

ACKNOWLEDGEMENTS

This work was supported by grants from the Danish Environmental Protection Agency to the National Allergy Research Centre and Kongelig Hofbundtmager Aage Bangs Fond and is gratefully acknowledged.

CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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REFERENCES

1. Wenande E, Garvey LH. Immediate-type hypersensitivity to polyethylene glycols: a review. *Clin Exp Allergy*. 2016;46(7):907-922.
2. Jover Cerdá V, Rodríguez Pacheco R, Doménech Witek J, Marco de la Calle FM, de la Sen Fernández ML. Immediate hypersensitivity to polyethylene glycols in unrelated products: when standardization in the nomenclature of the components of drugs, cosmetics, and food becomes necessary. *Allergy Asthma Clin Immunol*. 2019;15(1):1-5.
3. Antolin-Amerigo D, Sánchez-González MJ, Barbarroja-Escudero J, Rodríguez-Rodríguez M, Á'varez-Perea A, Alvarez-Mon M. Allergic reaction to polyethylene glycol in a painter. *Occup Med*. 2015;65(6):502-504.
4. Calogiuri G, Foti C, Bilo MB, Garvey LH. Macrogols: a misleading cause of drug hypersensitivity diagnosis. *Clin Immunol Endocr Metab Drugs*. 2016;4(1):9-13.
5. Garvey LH, Nasser S. Anaphylaxis to the first COVID-19 vaccine – is polyethylene glycol (PEG) the culprit? *Br J Anaesth*. 2020;S0007-0912(20)31009-6. https://doi.org/10.1016/j.bja.2020.12.020. Online ahead of print.
6. Wenande EC, Skov PS, Mosbech H, Poulsen LK, Garvey LH. Inhibition of polyethylene glycol-induced histamine release by monomeric ethylene and diethylene glycol: a case of probable polyethylene glycol allergy. *J Allergy Clin Immunol*. 2013;131(5):1425-1427.
7. Knop K, Hoogenboom R, Fischer D, Schubert US. Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. *Angew Chem Int Ed Engl*. 2010;49(36):6288-6308. https://doi.org/10.1002/anie.200902672.
8. Bruusgaard-Mouritsen MA, Jensen BM, Poulsen LK, Johansen JD, Garvey LH. Optimizing investigation of suspected allergy to polyethylene glycols. Submitted 2020.
9. Stone CA, Liu Y, Relling MV, et al. Immediate hypersensitivity to polyethylene glycols and polysorbates: more common than we have recognized. *J allergy Clin Immunol Pract*. 2019;7(5):1533-1540.e8.
10. Gökay SS, Çelik T, Sari MY, Ekinci F, Yildizdaş RD, Yilmaz HL. Urticaria as a rare side effect of polyethylene glycol-3350 in a child: case report. *Acta Clin Croat*. 2018;57(1):187-189.
11. Lee SH, Hwang SH, Park JS, Park HS, Shin YS. Anaphylaxis to polyethylene glycol (Colyte®) in a patient with diverticulitis. *J Korean Med Sci*. 2016;31(10):1662-1663.
12. Wylon K, Dölle S, Worm M. Polyethylene glycol as a cause of anaphylaxis. *Allergy Asthma Clin Immunol*. 2016;12(1):67.
13. Brandt N, Garvey LH, Bindslev-Jensen U, Kjaer HF, Bindslev-Jensen C, Mortz CG. Three cases of anaphylaxis following injection of a depot corticosteroid with evidence of IgE sensitization to macrogols rather than the active steroid. *Clin Transl Allergy*. 2017;7:2.

14. Wenande E, Kroigaard M, Mosbech H, Garvey LH. Polyethylene glycols (PEG) and related structures: overlooked allergens in the perioperative setting. *A&A Case Rep*. 2015;4(5):61-64.
15. Shah S, Prematta T, Adkinson NF, Ishmael FT. Hypersensitivity to polyethylene glycols. *J Clin Pharmacol*. 2013;53(3):352-355.
16. Jakubovic BD, Saperia C, Sussman GL. Anaphylaxis following a transvaginal ultrasound. *Allergy Asthma Clin Immunol*. 2016;12(1):3.
17. Sellaturay P, Nasser S, Ewan P. Polyethylene glycol-induced systemic allergic reactions (anaphylaxis). *J Allergy Clin Immunol Pract*. 2020;S2213-2198(20)31007-2. https://doi.org/10.1016/j.jaip.2020.09.029. Online ahead of print.
18. Co-Minh HB, Demoly P, Guillot B, Raison-Peyron N. Anaphylactic shock after oral intake and contact urticaria due to polyethylene glycols. *Allergy*. 2007;62(1):92-93.
19. Liu Y, Smith CA, Panetta JC, et al. Antibodies predict pegaspargase allergic reactions and failure of rechallenge. *J Clin Oncol*. 2019;37(23):2051-2061.
20. Sahiner UM, Tolga Yavuz S, Gökce M, et al. Anaphylactic reaction to polyethylene-glycol conjugated-asparaginase: Premedication and desensitization may not be sufficient. *Pediatr Int*. 2013;55(4):531-533.
21. Berndt K, Kuske M, Beissert S, Spornraft-Ragaller P, Bauer A. Non-IgE-dependent hypersensitivity to macrogol 6000. *J Ger Soc Dermatol*. 2018;16(4):479-481.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Bruusgaard-Mouritsen MA, Johansen JD, Garvey LH. Clinical manifestations and impact on daily life of allergy to polyethylene glycol (PEG) in ten patients. *Clin Exp Allergy*. 2021;51:463–470. https://doi.org/10.1111/cea.13822

Manuscript IV. Bruusgaard-Mouritsen MA, Jensen BM, Poulsen LK, Johansen JD, Garvey LH. Optimizing investigation of suspected allergy to polyethylene glycols. *J Allergy Clin Immunol.* 2021 May 27:S0091-6749(21)00825-3.

Optimizing investigation of suspected allergy to polyethylene glycols

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Background: Polyethylene glycols (PEGs) are polymers of varying molecular weight (MW) used widely as excipients in drugs and other products, including the mRNA vaccines against coronavirus disease 2019. Allergy to PEGs is rare. Skin testing and graded challenge carries a high risk of inducing systemic reactions.

Objective: We evaluated skin prick test (SPT) results and *in vitro* reactivity over time to different MW PEGs and assessed cross-sensitization patterns in PEG allergy.

Methods: Ten patients with previously diagnosed PEG allergy underwent SPT twice with PEGs 26 months apart. Lower MW (PEG 300, 3000, 6000) were tested, followed by PEG 20,000, in stepwise, increasing concentrations. Cross-sensitization to polysorbate 80 and poloxamer 407 was assessed. SPT was performed in 16 healthy controls. *In vitro* basophil histamine release (HR) test and passive sensitization HR test were performed in patients and controls.

Results: Patients previously testing positive on SPT to PEG 3000 and/or 6000 also tested positive to PEG 20,000. Patients with a longer interval since diagnosis tested negative to lower MW PEGs and positive mainly to higher concentrations of PEG 20,000. Three patients developed systemic urticaria during SPT. Eight patients showed cross-sensitization to poloxamer 407 and 3 to polysorbate 80. All controls tested negative. *In vitro* tests showed limited usefulness.

Conclusions: Skin test reactivity to PEG can decrease over time, but titrated SPT with increasing concentrations of PEG 20,000 can be diagnostic when lower MW PEGs test negative. To avoid systemic reactions, stepwise SPT is mandatory. (J Allergy Clin Immunol 2021;■■■:■■■-■■■.)

Key words: *Drug allergy, anaphylaxis, polyethylene glycol, PEG, macrogol, skin prick test, basophil histamine release, COVID-19 vaccine*

Abbreviations used
COVID-19: Coronavirus disease 2019
HR: Histamine release
MW: Molecular weight
PEG: Polyethylene glycol
PMA: Phorbol 12-myristate 13-acetate
PS: Passive sensitization
SPT: Skin prick test

Polyethylene glycols (PEGs) or macrogols are hydrophilic polymers of varying molecular weight (MW) used as excipients in many different products, including drugs and cosmetics.[1] PEGs have recently gained renewed interest because PEG 2000 is an excipient in the BioNTech/Pfizer and Moderna mRNA vaccines against coronavirus disease 2019 (COVID-19). Two cases of anaphylaxis in the first days of vaccination in the United Kingdom directed the suspicion against PEGs.[2] Allergy to PEGs is rare, but an increasing number of patients have been diagnosed over the past 2 decades.[1] A review by Wenande and Garvey[1] identified 37 case reports of PEG allergy between 1977 and 2016. In the United States, there are approximately 4 PEG-associated cases of anaphylaxis caused by laxatives per year, and the US Food and Drug Administration has registered 133 reports associating PEG with anaphylaxis since 1989.[3] The true prevalence of PEG allergy is unknown but is suspected to be significantly underreported, and a rise in the incidence of PEG allergy is expected as a result of the continued extensive use and increased focus on this hidden allergen.[1,3,4]

PEGs are synthesized by polymerization of ethylene oxide and addition of water, and they vary in MW and chain length. Low MW PEGs are viscous, clear liquids, while high MW PEGs are waxy, white solids.[1,5] There is potential for cross-sensitization to structurally related derivatives sharing the same chemical groups as PEG.[3,5-8] Other excipients, such as polysorbates derived from pegylated sorbitan or poloxamers comprising a trimer consisting of 1 moiety of polypropylene glycol surrounded by 2 moieties of PEGs, have been reported to show cross-sensitization (Fig 1).[1,9] Cross-sensitization is likely underestimated and rarely investigated; its clinical significance is not clear.

Diagnosing patients with PEG allergy is challenging. They often present with repeated, severe allergic reactions/anaphylaxis to structurally different drugs/products, and PEGs are rarely suspected. When suspected, performing a skin prick test (SPT) with a panel of different MW PEGs is the recommended investigation, although systemic reactions have been reported on SPT.[1,10] Intradermal testing and graded challenge with PEG-containing products should only be performed with caution as a result of the relatively

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This work was supported by grants from the Danish Environmental Protection Agency to the National Allergy Research Centre and Kongelig Hofbundtmager Aage Bangs Fond.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication January 4, 2021; revised May 7, 2021; accepted for publication May 19, 2021.

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0091-6749

https://doi.org/10.1016/j.jaci.2021.05.020

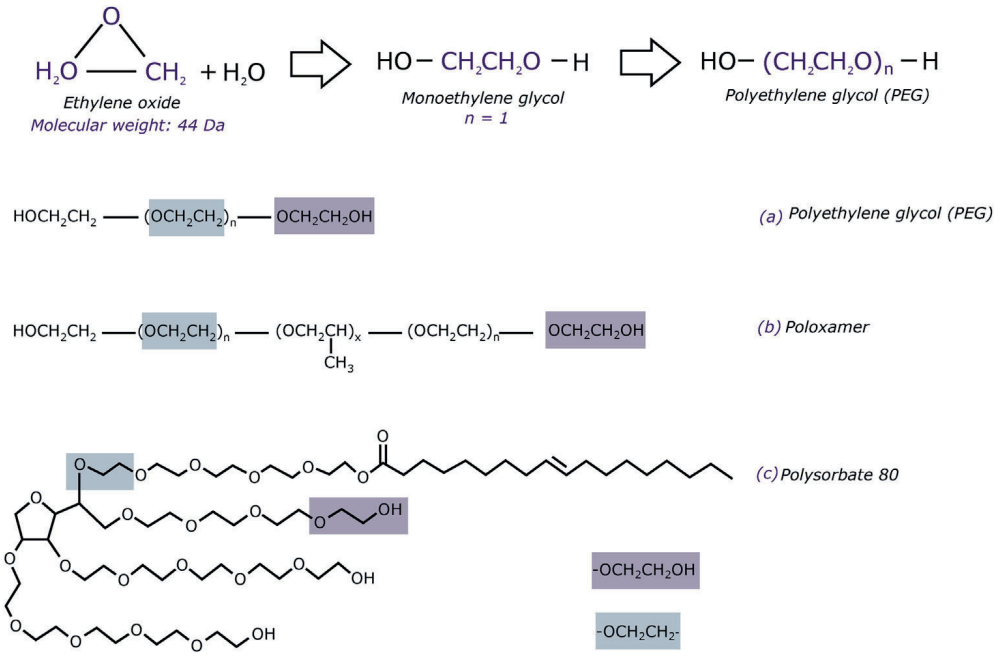

FIG 1. Molecular structure and polymerization of PEG and the derivatives poloxamer and polysorbate 80, which share 2 chemical moieties, $-(OCH_2CH_2)-$ and $-OCH_2CH_2O$. Image reproduced with permission from *Clinical & Experimental Allergy* from Wenande and Garvey.[1]

high risk of inducing anaphylaxis.[1,3,7,10-15] Skin test reactivity may decrease over time, showing negative results on titrated SPT with recommended low MW (PEG 300, 3000, 6000) despite a strong clinical suspicion, putting patients at risk of inadvertent reexposure if the diagnosis is not confirmed.

To date, there is limited knowledge about skin test reactivity over time, cross-sensitization to structurally related polymers, and supplemental diagnostic tests. In this study, therefore, we evaluated skin test reactivity over time and cross-sensitization patterns in 10 patients with confirmed allergy to PEG. We investigated whether titrated SPT with increasing concentrations of a PEG 20,000 MW can increase diagnostic sensitivity of SPT in PEG allergy. Further, because a reliable *in vitro* test would minimize the risk to patients, we assessed the basophil histamine release test with and without passive sensitization (PS). We present an investigation algorithm that is based on the study findings.

METHODS

Study design

The study included SPT results and histamine release test results from the time of diagnosis and initial allergy assessment. In addition, prospective testing was performed with a PEG SPT series developed for the study, as well as blood samples at 2 different time points 26 months apart. Blood samples were analyzed with histamine release test with and without PS.

Written and oral informed consent was obtained from all patients as well as participants in the control group. The study was approved by the regional ethical committee (file H-17021145).

Patients

Twelve patients were diagnosed with PEG allergy at the Allergy Clinic at Gentofte Hospital from September 2010 to August 2019. The diagnosis was made at the initial assessment by a history of 1 or more allergic reactions to PEG-containing products combined with a positive SPT to 1 or more low MW PEGs.

In the current study, we included 10 patients diagnosed with PEG allergy aged ≥18 years at the time of inclusion in the study. One patient had died, and another declined participation.

We included 8 patients diagnosed until 2017, who consented to participate twice, with a second visit 2 years later in 2019. One patient later declined the second visit. We consecutively invited patients newly diagnosed with PEG allergy between 2017 and 2019. Two patients were included after 2017, and these only participated once in the study.

Control group

The control group comprised 16 healthy, nonallergic individuals matched for age and sex who had a blood sample drawn and were tested once with the study PEG SPT series.

Skin prick testing

PEGs and derivatives were prepared in sterile water at the Laboratory of Medical Allergology, Gentofte Hospital, Hellerup, Denmark (see Table E1 in this article's Online Repository at www.jacionline.org). SPT was performed stepwise one concentration at a time with 20 minutes' observation between each step. The PEG SPT series developed for the study comprised the following: lower MW PEGs: PEG 300 (100%), PEG 3000 (50% wt/vol), PEG 6000 (50% wt/vol), polysorbate 80 (20% wt/vol), and poloxamer 407 (10% wt/vol). These were tested first in stepwise fashion. If only local reactions occurred on testing, SPT was performed stepwise with PEG 20,000 in concentrations of 0.01%, 0.1%, 1%, 10%, and 20% (wt/vol) until a positive response was reached. Three patients with very strong local responses or systemic urticaria to lower MW PEG were not tested with PEG 20,000 for ethical and safety reasons. SPT was performed on the forearm with a positive control with histamine 10 mg/mL and a negative control with saline. Duplicate testing was performed if the test was negative. The control subjects were tested with all components in duplicate. A positive reaction was defined as a wheal diameter of ≥3 mm.

Blood sampling and histamine release tests

Prospective blood samples were drawn before SPT at both visits. Histamine release test were performed on the day of blood sampling on 10 PEG-allergic patients and 16 healthy controls using the method previously described by Larsen et al.[16] PEG 300, PEG 3000, PEG 6000, PEG 20,000, poloxamer 407, polysorbate 80 (Sigma-Aldrich, St Louis, Mo), anti-IgE (KPL, Gaithersburg, Md), and phorbol 12-myristate 13-acetate (PMA) + ionomycin (both from Sigma-Aldrich) were all tested in 6 concentrations. The percent HR (%HR) equals released histamine of stimuli divided by maximum histamine release induced by PMA + ionomycin stimulation. Histamine release >10% was considered positive if found in 2 consecutive concentrations. If anti-IgE response was <10%, the test was considered inconclusive (see this article's Methods section in the Online Repository at www.jacionline.org).

RESULTS

Six men and 4 women participated in the study. The median age was 35 years (range, 18-64 years). Three patients had a history of allergic rhinoconjunctivitis; none had a history of reactions to food, PEG-free drugs, venoms, or vaccinations. None of the patients had received a vaccination containing polysorbate 20, polysorbate 80, or PEG since diagnosis.

For all patients, median time from first reaction to PEG until diagnosis was 20 months (range, 2-120 months). Median time from diagnosis to first study visit was 30 months (range, 1-86 months).

The most common PEG exposures were oral medications, such as analgesic tablets, antacids, antibiotic tablets and laxatives, and depot steroid injections. Table I lists the culprit agents. The mean number of reactions before diagnosis was 3 (range, 2-6). Eight patients had at least 1 reaction fulfilling the criteria for anaphylaxis and requiring epinephrine. Clinical patient data are reported in detail elsewhere.[17]

Skin prick tests

In 9 patients, the diagnosis was made at initial allergy assessment by a positive SPT to PEG 3000 and/or PEG 6000 (Table I). Patient 4, who experienced cardiac arrest after insertion of poloxamer 407–containing bone cement during hand surgery, only tested positive on SPT with the PEG derivatives poloxamer 407 and polysorbate 80 at initial allergy assessment 1 month after his reaction. He later tested positive to PEGs of varying MW at the first study visit three and a half years later.

All patients previously testing positive to PEG 3000 and PEG 6000, and who lost reactivity to these concentrations over time, still tested positive on PEG 20,000. Patients with a longer interval since diagnosis (patients 1, 2, 5, and 8) tested negative to lower MW and positive only to the higher concentrations of PEG 20,000 (Table I). Three patients (patients 6, 7, and 10) were not tested with PEG 20,000 in the study because they developed systemic urticaria during SPT with lower MW PEG. Patient 6 had tested positive for PEG 20,000 0.01% at the time of diagnosis. Symptoms were treated successfully with PEG-free oral antihistamines in all 3 patients.

All 16 participants in the control group tested negative in all SPT concentrations in duplicate.

Changes in SPT reactivity to PEG over time. In 7 patients, reactivity decreased over time, with loss of reactivity to the lower MWs. Decreased reactivity median time was 41 months (range, 26-82 months). In patients 3 and 9, reactivity increased over time after 26 and 16 months, respectively. In patient 10, who was newly diagnosed, reactivity did not change over 9 months.

Cross-sensitization. Eight patients showed cross-sensitization to PEG derivatives (all 8 to poloxamer 407 and 3 to polysorbate 80) at some point between diagnosis and last study visit. Patients 1 and 2, who had the longest delays since diagnosis (7 and 4 years, respectively), tested negative to both derivatives during the study. Neither had been tested with poloxamer 407 and polysorbate 80 before, as we had not been aware of the potential for cross-sensitization at the time of their diagnosis.

Histamine release test

Blood samples from patients or healthy controls were investigated for basophil reactivity when stimulated with PEGs. Four patients had a HR test performed at the time of diagnosis, and 2 patients (patients 1 and 2) tested positive to relevant MW PEGs, while 2 tested negative. Patients 1 and 2, who had tested positive in HR test at diagnosis but who tested negative at the first study visit, remained positive on SPT.

At the first study visit, 2 of 10 patients tested positive. Patient 7 tested positive for a relevant MW PEG, while patient 4 only showed partial concordance between SPT and HR test (see Fig E1 and Table E2 in this article's Online Repository at www.jacionline.org). Four patients (patients 3, 5, 6, and 10) had inconclusive tests, most likely due to nonreleasing basophils, a phenomenon found in 10% to 20% of the general population where basophils are found to be unresponsive (ie, histamine is not released) when using IgE-dependent stimuli.[18] Three of these patients (patients 3, 6, and 10) had systemic urticarial reactions during SPT in this study. Four patients had a negative HR test to PEG and PEG derivatives despite having a positive skin test. HR test was not performed at the second study visit.

PS histamine release test

To circumvent the problem of nonreleasing basophils, the technique of PS was used, where blood from blood donors with releasing basophils was passively sensitized with IgE from patients or healthy control serum. PS HR was performed at both study visits. Only 1 patient (patient 1) showed a positive response of doubtful clinical relevance. Six patients had negative results and 3 patients had inconclusive results on samples from the first study visit. Seven patients who had a blood sample analyzed at the second visit all tested negative (Table E2).

HR and PS HR were negative in all tests in all 16 controls.

DISCUSSION

In this study of 10 patients with PEG allergy, which is to our knowledge the largest cohort of PEG-allergic patients reported to date, we found that SPT reactivity to PEGs may decrease over time, but that the diagnosis can still be made by SPT with higher MW PEGs. All patients who had lost skin test reactivity over time to low MW PEG (PEG 3000 and/or PEG 6000) tested positive to PEG 20,000 in varying concentrations. In 7 patients, reactivity decreased over time with loss of reactivity to a lower MW PEG, while reactivity increased over time in 2 patients and remained stable in 1 patient. The 2 patients (patients 1 and 2) with the

TABLE I. SPT results over time in 10 patients with confirmed PEG allergy

Patient no., year of diagnosis	Age, sex	Culprit agents	Latest reaction to diagnosis (months)	SPT at diagnosis (SPT 0)	SPT 0 to SPT A (months)	SPT at first study visit (SPT A) 2017*	SPT at second study visit (SPT B) 2019
1, 2010	28, F	Intramuscular Depo-Medrol (methylprednisolone acetate) injection (PEG 3350) Balancid Novum (magnesium hydroxide) reflux tablet (PEG 6000) Effexor (venlafaxine) antidepressant tablet (PEG 400)	7	PEG 300 **PEG 3350 (+)** **PEG 6000 (+)** Poloxamer and polysorbate not tested	82	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01-10 **20% (+)** Poloxamer 407 Polysorbate 80	Declined
2, 2014	63, F	Intra-articular Depo-Medrol (methylprednisolone acetate) injection (PEG 3350)	2	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** Poloxamer and polysorbate not tested	46	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01-10 **20% (+)** Poloxamer 407 Polysorbate 80	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01 0.1 **10% (+)** Poloxamer 407 Polysorbate 80
3, 2014	37, M	Vepicombin (phenoxymethylpenicillin) antibiotic tablet (PEG 6000) Vepicombin (phenoxymethylpenicillin) antibiotic tablet during drug provocation (PEG 6000) Burana (ibuprofen) tablet (PEG 6000) Mucoangin (ambroxol) throat lozenge (PEG 6000) Xerodent (sodium fluoride) oral tablet (PEG 6000) Balancid Novum (magnesium hydroxide) reflux tablet (PEG 6000) Sensodyne Dental floss (PEG 6000)	4	PEG 300 PEG 3000 **PEG 6000 (+)** **PEG 20,000** **0.01% (+)** Poloxamer 407 **(+)** Polysorbate 80	45	PEG 300 PEG 3000 **PEG 6000** **PEG 20,000** 0.01 **0.1% (+)** Poloxamer 407 **(+)** Polysorbate 80	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** Poloxamer 407 **(+)** Polysorbate 80
4, 2014	33, M	Accell Connexus DBM putty (poloxamer 407) during hand surgery	1	PEG 300 PEG 3000 **PEG 6000** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	41	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** **PEG 20,000** 0.01 **0.1% (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01 **0.1% (+)** Poloxamer 407 Polysorbate 80
5, 2014	53, M	Unidentified perioperative exposure during coronary stent insertion	5	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** **PEG 20,000** 0.01-10 **20% (+)** Poloxamer 407 **(+)** Polysorbate 80	39	PEG 300 PEG 3000 **PEG 6000 (+)** **PEG 20,000** 0.01-10 **20% (+)** Poloxamer 407 Polysorbate 80	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01 0.1 **1% (+)** Poloxamer 407 Polysorbate 80
6, 2016	30, M	Migea (telfenamic acid) tablet (PEG 6000) Burana (ibuprofen) tablet (PEG 6000) Panodil (paracetamol) Zapp tablet (PEG 6000)	5	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** **PEG 20,000** **0.01% (+)** Poloxamer 407 **(+)** Polysorbate 80	20	PEG 300 **PEG 3000 (+)** Polysorbate 80 *Systemic urticaria*	PEG 300 PEG 3000 **PEG 6000 (+)** Polysorbate 80 *Systemic urticaria*

(Continued)

TABLE I. *(Continued)*

Patient no., year of diagnosis	Age, sex	Culprit agents	Latest reaction to diagnosis (months)	SPT at diagnosis (SPT 0)	SPT 0 to SPT A (months)	SPT at first study visit (SPT A) 2017*	SPT at second study visit (SPT B) 2019
7, 2017	37, M	Vepicombin (phenoxymethylpenicillin) tablet (PEG 6000) Intra-articular Depo-Medrol (methylprednisolone acetate) injection (PEG 3350)	3	**PEG 300 (+)** **PEG 3000 (+)** **PEG 6000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	1	**PEG 300 (+)** **PEG 3000 (+)** **PEG 6000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	PEG 300 **PEG 3000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)** *Systemic urticaria*
8, 2017	22, F	Vepicombin Novum (phenoxymethylpenicillin) tablet (PEG 6000) Balancid Novum (magnesium hydroxide) tablet (PEG 6000) Movicol (macrogol) laxative (PEG 3350) Panodil (paracetamol) tablet (PEG and polysorbate 80)	1	PEG 300 PEG 3000 **PEG 6000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	1	PEG 300 PEG 3000 **PEG 6000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	PEG 300 PEG 3000 PEG 6000 **PEG 20,000** 0.01 0.1 **1% (+)** Poloxamer 407 Polysorbate 80
9, 2018	16, M	Movicol (macrogol) laxative (PEG 3350) Dulcosoft (macrogol) laxative (PEG 4000) Diprospan (betamethasone) intra-articular injection (PEG 3350)	27	PEG 300 PEG 3000 **PEG 6000 (+)** **PEG 20,000** 0.01 **0.1% (+)** Poloxamer 407 **(+)** Polysorbate 80	16	PEG 300 **PEG 3000 (+)** **PEG 6000 (+)** **PEG 20,000** 0.01 **0.1% (+)** Poloxamer 407 **(+)** Polysorbate 80	Newly diagnosed, second study visit not possible
10, 2019	33, F	Intra-articular Depo-Medrol (methylprednisolone acetate) injection (PEG 3350) Intra-articular Depo-Medrol (methylprednisolone acetate) injection (PEG 3350) Sanex body lotion (PEG 4400)	5	PEG 300 **PEG 3000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)**	8	PEG 300 **PEG 3000 (+)** Poloxamer 407 **(+)** **Polysorbate 80 (+)** *Systemic urticaria*	Newly diagnosed, second study visit not possible

Positive (+) SPT results are indicated in boldface.
*Patient 9 in 2018 and patient 10 in 2019.

longest time interval (7 and 4 years, respectively) since diagnosis lost reactivity to the lower MW PEGs and tested positive only to the higher concentrations (10-20%) of PEG 20,000. Two patients (patients 5 and 8) with shorter time intervals (2 to 3 years) since diagnosis had also lost reactivity to lower MW PEGs but tested positive to the lower concentrations (0.1-1%) of PEG 20,000. This indicates that SPT with increasing concentrations of a PEG 20,000 can be used to increase diagnostic sensitivity of SPT even if there is a long delay between clinical reaction and allergy assessment. Despite careful stepwise SPT with increasing concentrations, 3 patients developed systemic urticaria during testing, even with lower MW PEG (PEG 3000), thus confirming that SPT with PEGs can be hazardous to patients with a history of severe allergic reactions if not performed with stepwise increasing concentrations.[1,10]

Severe systemic reactions on intradermal and provocation testing with PEGs have been repeatedly reported, so avoiding these test modalities would be a safer option.[1,3,7,10-14] On the basis of the results of our study, we suggest an investigation algorithm that is based on a titrated stepwise approach of SPT only (Fig 2). If results of SPT with lower MW PEGs are negative and clinical suspicion of PEG allergy is strong, we suggest to test PEG 20,000 in increasing concentrations using a stepwise approach. We believe that this approach will minimize the need for more hazardous test modalities such as intradermal test and graded challenge, which we only recommend if clinical suspicion is strong and the full algorithm has shown negative results. In patients with a low pretest probability of PEG allergy, such as patients with a history of reactions to several drugs not consistently containing PEGs, we only perform SPT with low MW PEGs, poloxamer 407, and polysorbate 80 without the stepwise approach. In our study, 5 patients tested positive only to PEG 20,000 at the last study visit, although they had previously tested positive to lower MW PEGs. If these patients had been referred with a long delay since their initial reaction, SPT with lower MW PEGs could have turned out falsely negative, and intradermal test or graded challenge might have been performed, putting the patients at risk of experiencing systemic reactions on testing.

Because many health care professionals are unfamiliar with the clinical presentation of PEG allergy, we provide in Table II a list of clinical scenarios where allergy to PEGs should be suspected. Because the mRNA COVID-19 vaccines contain PEG 2000, it is important that patients with suspected allergy to PEGs are investigated before vaccination, and we have included PEG 2000 in the new algorithm for this reason.

Another important finding of this study is that if lower MW SPT tested positive, PEG 20,000 would also test positive, suggesting that allergenicity increases with increasing MW[10]

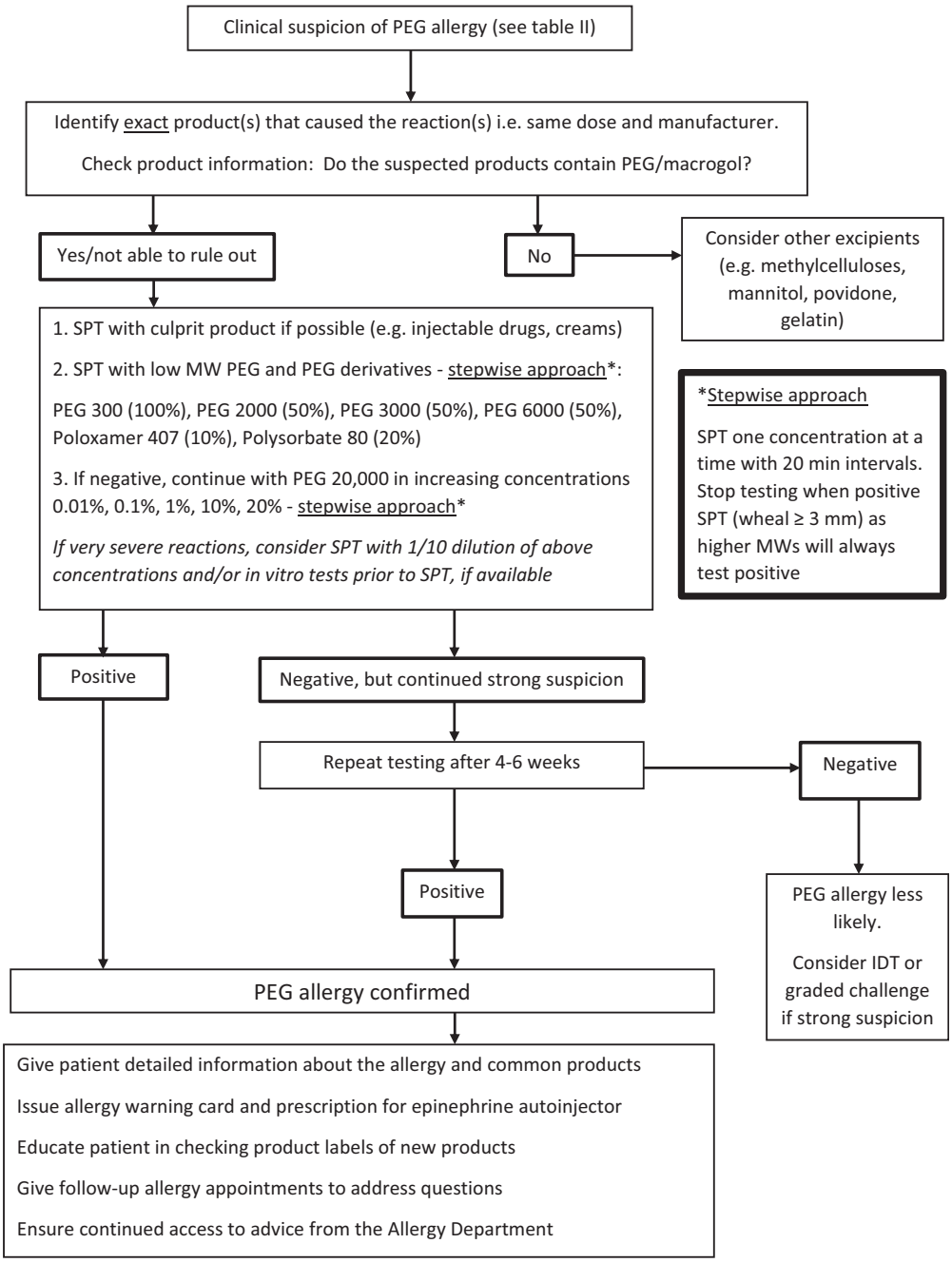

FIG 2. Investigation algorithm for patients with suspected PEG allergy. A stepwise approach should always be used in patients with severe reactions and strong suspicion of PEG allergy. In patients with milder reactions and weak suspicion of PEG allergy, several tests can be performed simultaneously after individual risk evaluation.

TABLE II. Clinical history where allergy to PEG should be considered[17]

- Repeated, severe allergic reactions/anaphylaxis to ≥2 structurally different drugs/products (eg, tablets, depot injections, antacids, PEG-based laxatives).
- Severe allergic reactions to only some formulations, or doses, of same generic drug.
- Severe allergic reaction to drugs, where allergy to the active ingredient has been excluded on testing (eg, antibiotics, analgesics).
- Severe allergic reaction to drugs containing PEG or PEG derivatives (polysorbate 80, poloxamers).
- Severe allergic reaction to vaccines containing PEG (mRNA vaccines) or PEG derivatives (polysorbate 80, poloxamers).
- Severe allergic reaction to PEGylated drugs, where allergy to the active drug is excluded.
- Severe unexplained allergic reactions in connection with surgery or invasive procedures.

and that there is no upper threshold for positivity.[1] This means that if SPT is positive to PEG 3000, further testing with higher MW PEGs is not necessary and may put the patient at risk of a systemic reaction. We have included this important information in the algorithm. Whether the threshold on SPT translates into a threshold for clinical reactivity has not been confirmed. Other groups have suggested that a lower threshold can be identified and that patients can use products with PEGs of MWs testing negative on SPT or challenge.[3,10] In our center, however, we adopt the more cautious approach of warning against PEGs of all MWs even if lower MWs test negative. This is supported by the results of the current study, where 2 patients showed an increase in reactivity by testing positive on lower MW at the second study visit. This may be explained by unknown accidental re-exposure to PEG. There is a high risk of accidental re-exposure, as PEGs are widely used in daily life and in the health care setting. It is possible that minor asymptomatic exposure—for example, from soaps, creams, cosmetics, or tablet coatings containing PEG—can be enough to maintain or increase allergenic reactivity. Finally, a lack of standardized labeling and the possibility of admixture with other MW PEGs means that the MW stated on drugs and other products cannot always be trusted.[1,19]

Recommendations for investigation of patients with suspected hypersensitivity to PEG are generally based on experiences from very few patients, making it difficult to assess specificity and sensitivity of individual tests. However, on the basis of negative SPT results for all MWs and concentrations in 16 healthy controls in this study, as well as negative SPT results with PEG 300, PEG 3000, PEG 6000, polysorbate 80, and poloxamer 407 in 314 non–PEG-allergic patients investigated as part of routine allergy assessment in our clinic during 2012-19, the specificity of our SPT series with PEG is likely to be high.

Although SPT is the recommended investigation when diagnosing patients with PEG allergy, even this procedure, generally considered very safe for most other allergens, may lead to systemic allergic reactions if performed with too high concentrations in highly reactive patients, such as those with severe reactions or when testing takes place soon after the allergic reaction. In our study, 3 patients developed systemic urticaria during SPT but responded quickly to treatment with oral antihistamines not containing PEG. This emphasizes the need for a stepwise approach. Indeed, using this protocol, we have never induced anaphylaxis on SPT. We suggest that a 1/10 dilution of our recommended concentrations may be used initially in patients with a strong suspicion of PEG allergy and/or severe or recent reactions. Because of the risk of systemic reactions, testing should always be performed in a specialized setting with equipment and expertise in treating immediate-type allergic reactions. It should be ensured that antihistamine tablets without PEG are available for treating early symptoms.[10] On the Danish market, presently only a single oral antihistamine is PEG-free.

Cross-sensitization between PEGs and structurally related polymers have only been rarely investigated.[1,3] Eight patients showed cross-sensitization to PEG derivatives in our study, all 8 to poloxamer 407 and 3 to polysorbate 80. Patient 4 is the only patient in this study with a history of a clinical reaction to poloxamer 407. Another patient from our clinic diagnosed with PEG allergy, who had died before this study, did have a clinical reaction to polysorbate 80.[7]

Some patients showed a decrease in skin test reactivity to these other polymers over time, while others maintained their reactivity. The clinical relevance of cross-sensitization is unknown and urgently needs elucidating because polysorbate 80 is used in many drugs[3] and vaccines, including some of the upcoming vaccines against COVID-19.

To our knowledge, the skin test reactivity over time in patients with PEG allergy has not been previously investigated. It is not known whether allergenic reactivity remains dormant until reactivated by re-exposure or if it can disappear permanently. In this study, some patients (patients 2, 4, and 5) only had a single reaction, and they seemed to be less reactive on SPT.[17] It may be that they can tolerate limited exposure to PEG. Others have had repeated severe reactions with years in between and may never lose their reactivity. Although there may be individuals who truly lose sensitization to PEG, the risk of severe reactions on re-exposure means that until more affirmative information is available to prove otherwise, in our clinic, we tell patients that PEG allergy covers all MW PEGs and is for life.

If a reliable *in vitro* test for allergy to PEG were available, the risk of inducing systemic reactions on SPT or other test modalities would be eliminated. However, such a test is presently not available. For other allergens, *in vitro* test reactivity can decrease or even be lost over time with lack of exposure; this has been shown for IgE to chlorhexidine, ethylene oxide, and penicillin.[20-23] It has been suggested that PEG allergy is caused primarily by an IgE-mediated mechanism.[4,24] An assay for detecting anti-PEG IgE has been reported to show promising results in a small cohort of patients.[25]

In this study, we investigated the direct HR test with and without PS. HR testing is used in some centers in Denmark and shows good results for allergy to things like chlorhexidine, peanut, and pollen.[16,26,27] Previously our group published promising results on HR and PS for PEG in a single patient (patient 1) on blood sampled close to the clinical reactions.[4] On testing in this study 82 months later, this patient had lost reactivity. In the current study, direct HR was only positive in 2 patients, and another Danish group showed similar results.[15] One of these patients had been diagnosed just 1 month previously and with proper titration of the PEG substances; direct HR test may have a place in patients where investigations take place within a few months of exposure. Four patients showed inconclusive results, likely due to nonreleasing basophils, a well-known limitation of this test.[18] In the present study setting, HR test with PS was negative in all patients and was not considered helpful in the diagnosis of allergy to PEG. Improved *in vitro* diagnostic tests for patients with allergy to PEG (and structurally related derivatives) therefore remains to be developed. In addition the potential for improving the sensitivity of biologic tests, a serologic assay identifying specific IgE to PEGs would be useful.[4,25] However, at the moment, no commercially validated specific IgE assay for PEGs or structurally related polymers is available.

In general, optimization of safe diagnostic tests is of great importance to PEG-allergic patients and health care personnel. However, there are still many gaps in the current knowledge. The rarity of the allergy and its unknown true prevalence make it difficult to describe the epidemiology and future prognosis. In addition, there is a potential lack of generalizability across health care systems and countries. The pathway to sensitization is unknown, and basic immunologic mechanisms remain to be identified. There is only limited experience with allergy investigation, primarily based on SPT, but data on positive and negative predictive value are sparse. Skin test reagents are not standardized, and the role of intradermal and *in vitro* testing remain to be

defined. Total avoidance of PEG causes considerable stress to patients in terms of the large number of products they need to avoid. Developing a safe method for determining a lower threshold for reactivity, thereby potentially allowing exposure to small amounts of PEG, would be helpful. Not much information is available on cross-reactivity patterns with polysorbate 80 and other polymers; this should be addressed because the BioNTech/Pfizer and Moderna mRNA COVID-19 vaccines contains PEG 2000, while most of the other available non-mRNA vaccines contain varying amounts of polysorbate 80. There is therefore an urgent need to identify a COVID-19 vaccine that can be used safely in PEG-allergic patients.

In conclusion, we have presented novel results of skin test reactivity to PEGs over time and cross-sensitization patterns in 10 patients with allergy to PEGs. On the basis of our experience as well as the results of this study, we suggest an optimized investigation algorithm for patients with suspected allergy to PEGs that is based on titrated stepwise SPT with PEGs of increasing MW, utilizing the fact that higher MW PEGs are likely to test positive even after many years. We therefore minimize the need for other tests that carry a high risk of inducing anaphylaxis. Cross-sensitization between PEGs and poloxamer 407 and polysorbate 80 is common, but the clinical implications remain unknown. Although *in vitro* tests would be the safest option for patients, we confirm the findings of others that *in vitro* testing so far has limited use in the investigation of allergy to PEGs.

Clinical implications: An algorithm using a stepwise approach of skin prick testing to polyethylene glycols (PEGs) of increasing molecular weights and concentrations can be used to diagnose allergy to PEGs.

REFERENCES

1. Wenande E, Garvey LH. Immediate-type hypersensitivity to polyethylene glycols: a review. Clin Exp Allergy 2016;46:907-22.
2. Garvey LH, Nasser S. Anaphylaxis to the first COVID-19 vaccine: is polyethylene glycol (PEG) the culprit? Br J Anaesth 2021;126:e106-8.
3. Stone CA, Liu Y, Relling MV, Krantz MS, Pratt AL, Abreo A, et al. Immediate hypersensitivity to polyethylene glycols and polysorbates: more common than we have recognized. J Allergy Clin Immunol Pract 2019;7:1533-40.e8.
4. Wenande EC, Skov PS, Mosbech H, Poulsen LK, Garvey LH. Inhibition of polyethylene glycol–nduced histamine release by monomeric ethylene and diethylene glycol: a case of probable polyethylene glycol allergy. J Allergy Clin Immunol 2013;131:1425-7.
5. Calogiuri G, Foti C, Bilo MB, Garvey LH. Macrogols: a misleading cause of drug hypersensitivity diagnosis. Clin Immunol Endocr Metab Drugs 2016;4(1):9-13.
6. Yamasuji Y, Higashi Y, Sakanoue M, Katsue H, Kawai K, Arai N, et al. A case of anaphylaxis caused by polyethylene glycol analogues. Contact Dermatitis 2013;69:183-5.
7. Wenande E, Kroigaard M, Mosbech H, Garvey LH. Polyethylene glycols (PEG) and related structures: overlooked allergens in the perioperative setting. A A Case Rep 2015;4:61-4.
8. Badiu I, Guida G, Heffler E, Rolla G. Multiple drug allergy due to hypersensitivity to polyethylene glycols of various molecular weights. J Investig Allergol Clin Immunol 2015;25:368-9.
9. Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. Vol 23, Pharmaceutical research. Amsterdam: Springer; 2006. p. 2709-28.
10. Sellaturay P, Nasser S, Ewan P. Polyethylene glycol–induced systemic allergic reactions (anaphylaxis). J Allergy Clin Immunol Pract 2021;9:670-5.
11. Borderé A, Stockman A, Boone B, Franki AS, Coppens MJ, Lapeere H, et al. A case of anaphylaxis caused by macrogol 3350 after injection of a corticosteroid. Contact Dermatitis 2012;67:376-8.
12. Oyarzabal NA, Longo Areso N, Bernedo Belar N, Popolizio IG, Arregui AV, Balza de Vallejo OV. Anaphylactic shock due to allergy to macrogol 4000 contained in SonoVue®. Case Rep Clin Med 2017;06:143-7.
13. Antón Gironés M, Roan Roan J, de la Hoz B, Sánchez Cano M. Immediate allergic reactions by polyethylene glycol 4000: two cases. Allergol Immunopathol (Madr) 2008;36:110-2.
14. Dewachter P, Mouton-Faivre C. Anaphylaxis to macrogol 4000 after a parenteral corticoid injection. Allergy 2005;60:705-6.
15. Brandt N, Garvey LH, Bindslev-Jensen U, Kjaer HF, Bindslev-Jensen C, Mortz CG. Three cases of anaphylaxis following injection of a depot corticosteroid with evidence of IgE sensitization to macrogols rather than the active steroid. Clin Transl Allergy 2017;7:2.
16. Larsen LF, Juel-Berg N, Hansen KS, Clare Mills EN, van Ree R, Poulsen LK, et al. A comparative study on basophil activation test, histamine release assay, and passive sensitization histamine release assay in the diagnosis of peanut allergy. Allergy Eur J Allergy Clin Immunol 2018;73:137-44.
17. Bruusgaard-Mouritsen MA, Johansen JD, Garvey LH. Clinical manifestations and impact on daily life of allergy to polyethylene glycol (PEG) in ten patients. Clin Exp Allergy 2021;51:463-70.
18. Nguyen KL, Gillis S, MacGlashan DW. A comparative study of releasing and nonreleasing human basophils: nonreleasing basophils lack an early component of the signal transduction pathway that follows IgE cross-linking. J Allergy Clin Immunol 1990;85:1020-9.
19. Povoski SP, Davis PD, Colcher D, Martin EW Jr. Single molecular weight discrete PEG compounds: emerging roles in molecular diagnostics, imaging and therapeutics. Expert Rev Mol Diagn 2013;13:315-9.
20. Opstrup MS, Poulsen LK, Malling HJ, Jensen BM, Garvey LH. Dynamics of plasma levels of specific IgE in chlorhexidine allergic patients with and without accidental re-exposure. Clin Exp Allergy 2016;46:1090-8.
21. Opstrup MS, Malling HJ, Krøigaard M, Mosbech H, Skov PS, Poulsen LK, et al. Standardized testing with chlorhexidine in perioperative allergy—a large single-centre evaluation. Allergy Eur J Allergy Clin Immunol 2014;69:1390-6.
22. Opstrup MS, Mosbech H, Garvey LH. Allergic sensitization to ethylene oxide in patients with suspected allergic reactions during surgery and anesthesia. J Investig Allergol Clin Immunol 2010;20:269-70.
23. Blance M, Garcia J, Vega JM, Miranda A, Carmona MJ, Mayorga C, et al. Anaphylaxis to penicillins after non-therapeutic exposure: an immunological investigation. Clin Exp Allergy 1996;26:335-40.
24. Bommarito L, Mietta S, Nebiolo F, Geuna M, Rolla G. Macrogol hypersensitivity in multiple drug allergy. Ann Allergy Asthma Immunol 2011;107:542-3.
25. Zhou ZH, Stone CA, Jakubovic B, Phillips EJ, Sussman G, Park J, et al. Anti-PEG IgE in anaphylaxis associated with polyethylene glycol. J Allergy Clin Immunol Pract 2021;9:1731-3.e3.
26. Garvey LH, Krøigaard M, Poulsen LK, Skov PS, Mosbech H, Venemalm L, et al. IgE-mediated allergy to chlorhexidine. J Allergy Clin Immunol 2007;120:409-15.
27. RefLab. Standard/non-standard materials. Available at: https://reflab.dk/allergy-testing/histamine-release-test/standard-materials/. Accessed June 26, 2020.

METHODS

Histamine release (HR) tests were performed on the day of blood sampling on 10 polyethylene glycol (PEG)-allergic patients and 16 healthy controls. Blood was drawn before skin prick test (SPT) on the study day. On the day of blood sampling, blood was centrifuged, and plasma replaced with piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES) buffer (RefLab, Copenhagen, Denmark). Glass fiber–coated microtiter plates (RefLab) were added 50 μL diluted blood and 50 μL stimulant (polyclonal goat anti-human IgE [VWR International, West Chester, Pa], PMA, and ionomycin [both from Sigma-Aldrich, St Louis, Mo], or PEG 300, PEG 3000, PEG 6000, PEG 20,000, poloxamer 407, or polysorbate 80 [Sigma-Aldrich]) in 6 concentrations. The plates were incubated for 60 minutes at 37°C, and released histamine was determined by making o-phthaldialdehyde–histamine fluorescent complexes, which were quantified on a Histareader (RefLab).

To perform the passive sensitization (PS) HR test, fresh buffy coat blood obtained from the local blood bank (Rigshospitalet, Copenhagen, Denmark) was added 10 pg/mL recombinant human IL-3 (Trichem, Skanderborg, Denmark) and stored overnight at 8°C. The buffy coat blood was washed with PIPES buffer followed by ice-cold stripping buffer (RefLab) to remove IgE from donor basophils. IgE-stripped cells were then incubated with serum for 1 hour at 37°C, and the cell suspension (25 μL) and stimulants (25 μL) were added to glass fiber–coated microtiter plates, with released histamine quantified as described above.

Percent HR (%HR) was calculated as the released histamine of stimuli divided by maximum HR induced by PMA + ionomycin stimulation. Participants who had a %HR of <10% to anti-IgE stimulation were designated as nonreleasing. Those with nonreleasing basophils did respond to PMA + ionomycin (Fig E1). Participants not responding to 2 consecutive PEG doses with ≥10% basophil reactivity were considered nonreacting. A test was regarded conclusive if the basophils reacted to anti-IgE or PEG stimulation, and inconclusive if the participant was both nonreleasing and nonreacting.

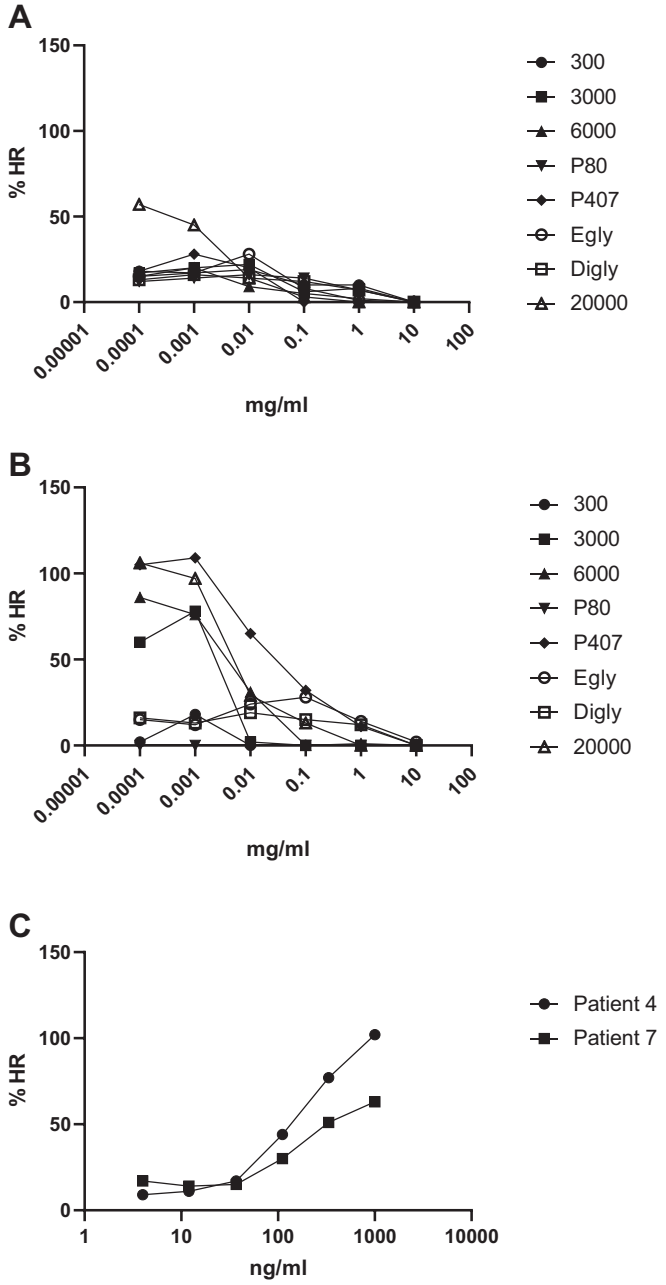

FIG E1. Direct basophil HR tests in response to PEG 300, PEG 3000, PEG 6000, PEG 20,000, poloxamer 407 or polysorbate 80, ethylene glycol, and diethylene glycol for **(A)** patient 4 (positive for PEG 20,000), **(B)** patient 7 (positive for PEG 3000, PEG 6000, PEG 20,000, and poloxamer 407), and **(C)** anti-IgE for patients 4 and 7 at first study visit in 2017. Unfortunately, the 10-fold dilution range of PEGs from 10 mg/mL to 0.1 ug/mL was not sufficiently long; in some cases, it only allowed demonstration of a positive response at the lowest concentrations (ie, at right part of the bell-shaped dose–response curve normally seen for HR). For logistical reasons, it was not possible to repeat the experiments using higher dilutions.

TABLE E1. Detailed procedure for preparing solutions for SPT for PEG, poloxamer 407, and polysorbate 80 at the Laboratory for Medical Allergology, Allergy Clinic, Gentofte Hospital, Denmark

Compound	Manufacturer product no.*	Dilution	Amount for 10 mL solution	Production method†
PEG 300	81162	No dilution	10 mL	PEG 300 is used undiluted; just form 10 mL aliquots of the solution.
PEG 3000	03997	50% (wt/vol)	5 g	For PEG 3000, PEG 6000, and poloxamer 407, weigh the appropriate amount and transfer to a 15 mL tube containing 5 mL sterile water. Poloxamer is difficult to dissolve, so always add sterile water before the compound. Tighten the lid and seal with parafilm. Place the tube on a tube rotator at 37°C for 2 hours. Ensure that the compound is dissolved. If not, leave the tube on the rotator at 37°C until dissolved. Centrifuge the tubes (500 × *g*, 5 minutes, 20°C), adjust the volume to 10 mL with sterile water, and vortex the suspension to ensure correct mixing.
PEG 6000	03394	50% (wt/vol)	5 g	
Poloxamer 407	16758	10% (wt/vol)	1 g	
PEG 20,000 (average molecular weight)	81300	0.01-20% (wt/vol)	2x2 g	For PEG 20,000, twice the amount is made up because more is needed for serial dilutions. Weigh 4 g and transfer to a 50 mL tube containing 14 mL sterile water. Tighten the lid and seal with parafilm. Place the tube on a tube rotator at 37°C for 2 hours. Ensure that the compound is dissolved. If not, leave the tube on the rotator at 37°C until dissolved. Centrifuge the tubes (500 × *g*, 5 minutes, 20°C), adjust the volume to 20 mL with sterile water, and vortex the suspension to ensure correct mixing. *Prepare 4 new tubes for serial dilution:* ● 10% PEG 20,000: Mix 8 mL of 20% PEG 20,000 with 8 mL sterile water. ● 1% PEG 20,000: Mix 2 mL of 10% PEG 20,000 with 18 mL sterile water. ● 0.1% PEG 20,000: Mix 2 mL of 1% PEG 20,000 with 18 mL sterile water. ● 0.01% PEG 20,000: Mix 2 mL of 0.1% PEG 20,000 with 18 mL sterile water. Vortex each dilution to ensure correct mixing before preparing the next dilution step. You will end up having more than 10 mL in the final solution.
Polysorbate 80	P1754	20% (v/v)	2 mL	Pipette 2 mL into a 15 mL tube containing 8 mL sterile water. Vortex the suspension to ensure correct mixing.

Once solutions are made following this procedure, they will stay in solution at room temperature. Solutions are transferred to a sterile vial for multiple use. Solutions are used for 6 months, but no studies have been done on stability or sterility. Solutions should be presumed to be nonsterile and should only be used for SPTs. The compounds used are classified as laboratory chemicals. Use of these substances for SPT may be subject to local legislation and is at the responsibility of the doctor ordering the test.

*Sigma-Aldrich (Sigmaaldrich.com).

†Description of method for production of 10 mL solution (transfer into sterile vials for multiple use and store at room temperature).

TABLE E2. Direct HR test and PS test results in 10 patients with confirmed PEG allergy

Patient no.	HR 0	HR A	PS 0	PS A	PS B
1	PEG 3350 PEG 6000	−	PEG 3350 PEG 6000	Poloxamer 407	NT
2	PEG 3000 PEG 6000	−	NT	−	−
3	−	(−)	NT	−	−
4	−	PEG 20,000	NT	−	−
5	NT	(−)	NT	−	−
6	NT	(−)	NT	−	−
7	NT	PEG 3000 PEG 6000 PEG 20,000 Poloxamer 407	NT	−	−
8	NT	−	NT	(−)	−
9	−	−	NT	(−)	NT
10	NT	(−)	NT	(−)	NT

Only positive results are provided in full. For HR, results are shown from initial reaction (0) and study visit A. For PS, results are shown for study visit A and study visit B. All patients were tested with PEG 300, PEG 3000, PEG 6000, PEG 20,000, poloxamer 407, or polysorbate 80 in 6 concentrations at study visit A and B. 0 indicates time of diagnosis; A, first study visit; and B, second study visit; and − indicates negative; (−), inconclusive; and NT, not tested.

10. Appendices

Appendix I: Questionnaire used in PART 1, manuscript II

Appendix II: Questionnaire used in PART 2, manuscript III

Appendix I

Table S1. Questionnaire about facial dermatitis and natural ingredients in cosmetic products.

1a. Do you have a facial rash today?
Yes; No
1b. If yes in 1a. Is it contact dermatitis or urticaria? (Pictures representing contact dermatitis and urticaria available for the patient).
1c. If yes in 1a. For how long have you had your facial rash?
Days; weeks; months; years; do not remember
2. How long ago did you experience facial dermatitis for the first time?
< 6 weeks; 6 weeks to 3 months; 3-12 months, > 1 year; do not remember
3a. Do you have dermatitis elsewhere on your body?
Yes; No
3b. If yes in 3a. Where?
Neck; arms; stomach/back/shoulders; legs; hands; feet
4a. Do you know what caused you facial dermatitis?
Yes; No
4b. If yes in 4a. What caused your facial dermatitis?
Cosmetics; food; others; work-related exposure; pharmaceuticals; botanical plants; others
5a. Do you suspect a specific product causing your facial dermatitis?
Yes; No
5b. If yes in 5a: Which product caused it? _____
6a. Has your facial rash affected your quality of life?
Yes; No
6b. If yes in 6a: How much has your facial rash affected your quality of life?

Very much; much; some; little; no
7a. Has your facial rash caused limitations to you everyday life?
Yes; No
7b. If yes in 7a. In what way has the facial rash limited you? _____
8a. Do you prefer cosmetic products branded as “natural”?
Yes; No
8b. If yes in 8a. Why?
Healthier; less allergenic; better for the environment; other; do not know
9. Do you check whether there are natural ingredients in a cosmetic product before you use it?
Yes; No
10. Do you know that it is possible to be allergic to natural ingredients?
Yes; No
11. Do you suspect that you are allergic to natural ingredients?
Yes; No
12. Have you previously been diagnosed with atopic dermatitis or hay fever?
Yes; No

Appendix II

Table A1. Questionnaire about PEG allergy

1. Had you heard about allergy to PEGs before your clinical investigation and diagnosis?
Yes; No
2. Did you suspect that you had allergy to PEGs before you had the diagnosis?
Yes; No
3a. Do you know which product caused your allergic reactions to PEGs?
Yes; No
If yes in 3a. Which product caused the allergy?
Tablets; laxatives; suppositories; wound bandages; catheter lubricant/ultrasound gels; medicine through the vein; creme/ointment; hair products; make-up or make-up remover; shaving products; mouth hygiene products (toothpaste, dental floss, mouthwash); other:_____
4. Which symptoms led to clinical investigation?
Itching skin; burning sensation; redness; rash; angioedema; allergic shock; breathing difficulties; feeling unwell; fainting; other: _____
5. Which information about PEG allergy was most important to you? _____
6a. Have you been exposed to PEGs since the allergy was diagnosed?
Yes; No
6b. How many times?
1; 2; 3; Don't know
6c. When was your last allergic reaction? _____
6d. If yes in 6a: Which product caused it? _____

Herlev og Gentofte Hospital

PhD Thesis 2021

ISBN nr. 978-87-93624-97-9