Susceptibility to hand eczema in high risk occupations: Contribution of genetic and environmental factors

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SUSCEPTIBILITY TO HAND ECZEMA IN HIGH RISK OCCUPATIONS

Contribution of genetic and environmental factors

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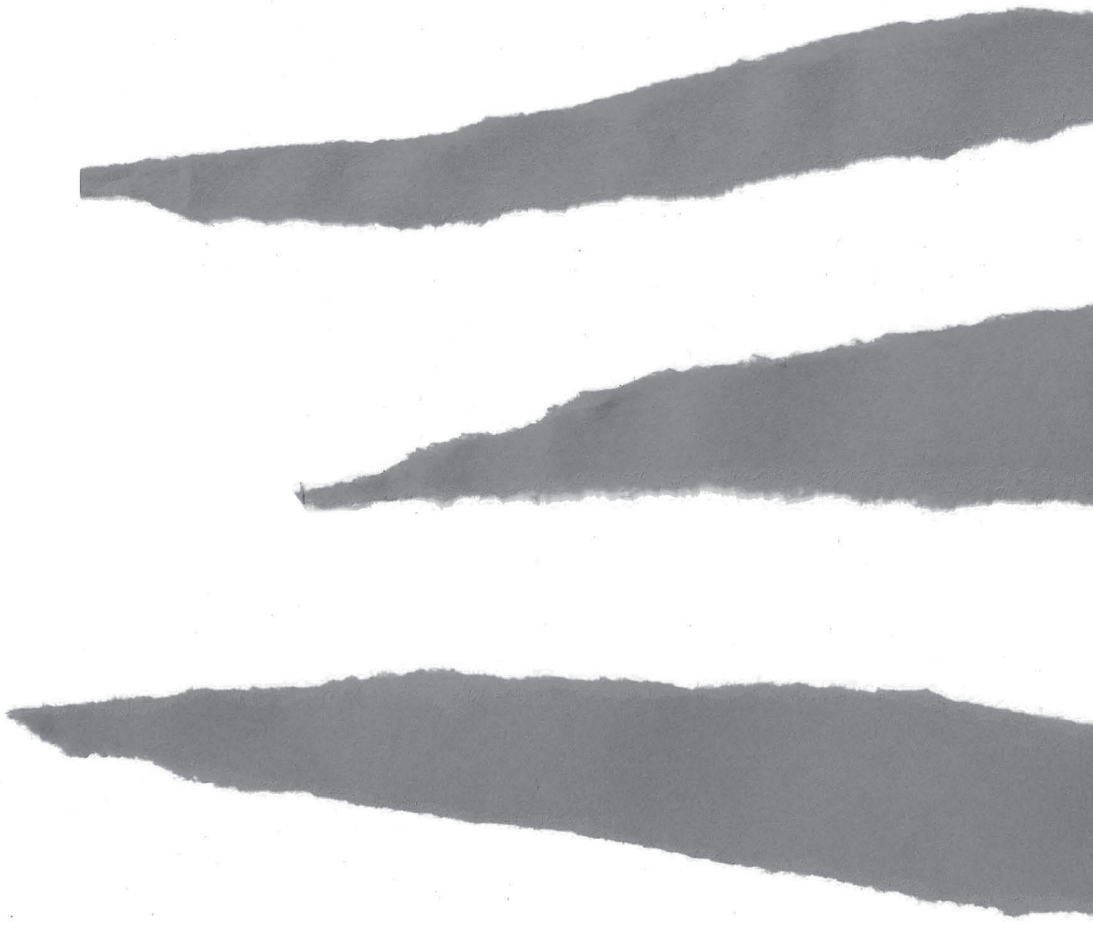

1 GENERAL INTRODUCTION

GENERAL INTRODUCTION

"Your skin. The most important 2m^2 of your life".

This was the slogan of a recent national campaign for the prevention of skin diseases in Germany [1]. The campaign was launched by a joint effort of the German statutory health and accident insurance institutions, and ran in the whole country during the years 2007 and 2008. Its purpose was to make people more aware of the unique protective function of the skin, its vulnerability to external stress such as contact with moisture, chemicals, mechanical irritation or UV-radiation, and the need for good skin care.

Indeed, the German slogan rightly stressed the importance of paying more attention to the skin, as it is one of the most remarkable organs of our body. The skin forms a barrier between our internal body and the environment – preventing body water loss and blocking entrance of pathogens, toxins, and UV-radiation – and it adds to our experience of that environment through sensation of touch, pressure, heat or cold. Further, it plays an important role in temperature regulation and production of vitamin D. Skin is also an immunological organ; it homes various cells of the immune system that form the first line of defense against pathogens. Thus, our skin comprises several barriers; it not only keeps our inside in, it also actively keeps the outside out.

CONTACT DERMATITIS

The skin is continuously exposed to various stressors which can lead to the damage of one or more of its barriers. Examples are irritants (e.g. chemicals) or allergens which are commonly encountered in the workplace. Although skin has a formidable capability of repairing itself, repetitive damage as e.g. in the occupational setting can lead to changes in the skin barrier. Contact dermatitis, which is one of the most common occupational diseases [2;3], occurs as a consequence of a misbalance in inflammatory response aiming at barrier repair after skin damage by external stimuli. If a skin irritating chemical or allergen penetrates the skin, an inflammatory response is usually initiated to get rid of the compound and stimulate repair of skin barrier damage. Normally, the skin recovers and the inflammatory response will be downregulated. In some cases, however, the state of inflammation proceeds into contact dermatitis, an inflammatory skin condition characterized by red, swollen and itchy skin, sometimes with scaling and formation of vesicles. Several subtypes of contact dermatitis can be discerned:

- *Allergic contact dermatitis* occurs when an allergenic compound penetrates the skin and elicits a specific immune response (sensitization and subsequent Type-IV reaction).
- *Acute irritant contact dermatitis* is caused by relatively major damage to the skin, usually the result of a short-time exposure, e.g. an accidental contact with a corrosive chemical.

- *Chronic irritant contact dermatitis* may develop when the skin is repeatedly exposed to one or multiple skin irritating factors that cause only minor damage (usually mild irritants such as soap or water, or mechanical irritation e.g. friction), but there is not enough time between subsequent exposures for the skin to completely recover. The effect of successive inflammatory reactions then accumulates until a threshold is reached and the clinical symptoms of irritant contact dermatitis become visible. If not treated timely, this type of contact dermatitis may develop into a chronic form.

Contact dermatitis often involves the hands. This is not surprising, as this skin area typically comes into contact with a wide range of compounds that are used at work or in the home environment.

OCCUPATIONAL CONTACT DERMATITIS

In certain occupational sectors, contact dermatitis is considerably more prevalent than in the general population. This is due to occupational exposure to a variety of chemical substances, allergens, or physical factors, e.g. solvents, metal salts, proteins, plants or animal dander, mechanical friction and "wet work" (exposure to a combination of water, soaps, detergents, disinfectants and occlusive gloves). The most common form of occupational contact dermatitis (OCD) is irritant contact dermatitis (ICD), accounting for 50-80% of OCD [4], and wet work is a major risk factor for this type of dermatitis. Well-known occupations where workers have an increased risk of developing OCD include hairdressing, nursing, cleaning, kitchen work, floristry, construction work (e.g. bricklaying) and mechanics [5;6].

Nevertheless, many workers regard their skin problems as 'part of the work' and neglect to seek medical help. As a consequence, OCD is generally underreported in official registries of occupational diseases [2]. Finland, Denmark and Germany have a system of compulsory reporting to national registers, and there OCD is reported with rates of 50-80 cases per 100,000 workers per year [2]. However, a recent questionnaire survey among Danish hospital employees revealed that only 12% of the healthcare workers with hand eczema were actually registered as having occupational hand eczema in the Danish National Board of Industrial Injuries Registry, illustrating substantial underreporting of OCD even among healthcare professionals, who may be expected to be attentive towards disease [7]. In the Netherlands, a voluntary registry is kept by the Netherlands Center for Occupational Diseases [6]. In 2009 the incidence of OCD reported in a related network of dermatologists was 6 cases per 100,000 workers per year, although also here the true incidence was suspected to be higher because of underreporting [8]. A recent review by Nicholson and colleagues [3] estimated that the incidence of OCD in industrialized countries lies between 11 and 86 cases per 100,000 workers per year. Several epidemiological studies have been conducted to assess the prevalence of OCD among workers and apprentices in high risk occupations (Table 1).

The one-year prevalence of OCD found in European surveys in the hairdressing, healthcare and metalworking sectors was about 20-30% and mild skin symptoms were present in up to 50% of the workers or apprentices [9-23]. In comparison, in the European population, the lifetime prevalence of hand eczema (a generic term which includes irritant and allergic contact dermatitis but also other eczematous lesions on the hands, like atopic dermatitis or hyperkeratosis [24]) is estimated to be 14%, the one-year prevalence is on average 10%, and incidence rates vary from 3.3 cases/1000 person-years to 8.8 cases/1000 person-years [25-27].

OCD can have considerable impact on a person's workability. In a cross-sectional survey of over 400 hand eczema patients from 10 different European dermatology patch test clinics, 28% of the patients reported sick leave because of their OCD and in 12.3% of the patients this sick leave had lasted for more than 5 weeks [26]. A retrospective interview survey among more than 600 Finnish workers who had been referred to the Finnish Institute of Occupational Health with OCD, conducted 7-14 years after diagnosis, revealed that 25% had become unemployed and another 35% had changed their occupation because of their disease. Only 40% of the patients had completely recovered from their OCD [28]. Among healthcare workers, OCD may lead to decreased compliance with hand hygiene, because applying soap or disinfectants (such as alcohol gel) on damaged skin can be painful, and to increased colonization with bacteria such as *Staphylococcus aureus*. Furthermore, OCD has been shown to impair quality of life also in the private and social environment [3;29].

The adverse effects that OCD has in both the personal and the work situation, together with the fairly high prevalence rates in high risk occupations, indicate that the often observed ignorance of skin symptoms – reflected by workers failing to take preventive measures and delaying to seek treatment as well as by health professionals underreporting OCD – is unjustified. Prevention of OCD as well as early diagnosis and appropriate treatment are important. Especially for work-related ICD, where there is a stepwise progression from mild irritation to chronic eczema threatening workability [30], early prevention is vital.

For the prevention of contact dermatitis, insight in the mechanisms and factors which contribute to its development is essential. In addition to the effects of environmental exposures, endogenous factors also play a role in this. For example, some individuals have an intrinsically reduced skin barrier, which will be discussed in more depth later in this chapter. An impaired skin barrier may lead to increased penetration of harmful substances into the skin and may even enable entrance of substances which would not have been able to penetrate across a healthy skin, for example, allergens with a large molecular size. Thus, persons with a reduced skin barrier are more susceptible to damaging effects due to environmental exposures, for example, when working in jobs with high exposure to skin irritants or allergens. Understanding of the key elements that are responsible for the composition and structure of the skin will contribute to a better maintenance of the skin barrier and prevention of contact dermatitis in the workplace.

Table 1. Literature overview of the prevalence of OCD among workers and apprentices in high risk occupations

Reference	Country	Study population	Design	Definition of outcome(s)	Susceptibility factors assessed
Smit and Coenraads 1993	The Netherlands	Nurses (N = 371)	Retrospective questionnaire survey covering 33 months of follow-up, among newly hired nurses.	Hand eczema based on reported symptoms[a].	Not reported in detail.
Schmid *et al.* 2005	Germany	Apprentice Nurses (N = 104)	Prospective cohort study with follow-up measurements at 1 year and at 3 years after the start of the study.	Hand eczema based on reported symptoms[b]. Self-reported hand eczema[c]. Hand eczema diagnosed by dermatological examination. Skin barrier function assessed by TEWL.	Skin barrier function assessed by baseline TEWL.
Smit *et al.* 1994	The Netherlands	Apprentice Nurses (N = 111)	Prospective cohort study; two 'waves' of inclusion with follow-up measurements after 1 year and after 2 years (only for the first wave).	Hand eczema based on a combination of self-reported symptoms[a] and dermatological examination. Skin barrier function assessed by TEWL.	Self-report of childhood eczema, dry skin, asthma and hay fever; patch testing; skin prick testing.
Jungbauer *et al.* 2004	The Netherlands	Nurses (N = 822)	Cross-sectional questionnaire survey.	Hand eczema based on reported symptoms[a].	Self-report of atopic dermatitis (based on localization and onset of reported eczema), dry skin, asthma, hay fever or chronic bronchitis
Flyvholm *et al.* 2007	Denmark	Healthcare personell in a hospital (N = 1125)	Cross-sectional questionnaire survey.	Self-reported hand eczema.	Self-report of atopic dermatitis, rhinitis, asthma

Exposure factors assessed	Results	Remarks
Not reported in detail.	Period prevalence (33 months): 28.8%. Overall incidence rate (0-33 months): 7.8/100 person-years. Incidence rate 0-3 months: 11.3/100 person-years.	83% of the newly hired nurses had already worked as a nurse before they were hired by the studied hospital.
Self-administered questionnaire including items on frequency of hand washing, hand disinfection products, glove use and use of skin care products.	Point prevalence of hand eczema assessed by dermatological examination increased from 21.2% at inclusion to 36.5% in the third year. The 1-year prevalence of hand eczema based on reported symptoms was 25.0% in the first year and 26.9% in the third year; the corresponding 1-year prevalence values of self-reported hand eczema were 36.5% and 43.3%, respectively. Incidence of symptom-based hand eczema was 13.5% in the first year and 17.3% in the third year; for self-reported hand eczema it was 6.7% and 4.8%, respectively.	Baseline TEWL was not a predictor for hand eczema, but presence of symptoms was significanlty associated with higher TEWL values during follow-up.
Self-administered questionnaire (taken at inclusion) including items on frequency of hand washing, hand disinfection products, glove use, contact with medicaments and use of skin care products.	Incidence rate: 19.8/100 person-years in the first year; 5.2/100 person-years in the second year.	Baseline TEWL was not a predictor for hand eczema. Hand eczema was associated with mucosal atopy (asthma/hay fever) and dry skin, but not with childhood eczema or positive patch or prick tests.
Not reported in detail.	Point prevalence: 14%. 1-year prevalence: 25%.	Dermatological consultation was offered to those who reported hand eczema. 46% of those invited accepted the invitation, of which 58% had present HE and another 30% had indications for HE in the past 12 months.
Questionnaire items on job description, use of protective gloves, hand washing, use of hand disinfectants, and use of skin care products.	Point prevalence: 8.7%. 1-year prevalence: 22.8%. The 1-year prevalence among different job groups varied between 7.9% - 32.1%, with the highest prevalences in nursing aids, nurses, and assistant nurses.	1-year prevalence of hand eczema was associated with female sex, younger age (< 40 vs > 40 years old), atopic dermatitis, rhinitis, asthma, use of protective gloves, and hand washing, but not with use of hand disinfectants.

Table 1. Literature overview of the prevalence of OCD among workers and apprentices in high risk occupations (*continued*)

Reference	Country	Study population	Design	Definition of outcome(s)	Susceptibility factors assessed
Smith *et al.* 2006	Korea	Apprentice nurses (N = 202)	Cross-sectional questionnaire survey.	Hand eczema based on a combination of self-reported symptoms[a].	Systemic allergic disease: asthma, allergic rhinitis, hay fever and latex allergy.
Smith and Leggat 2004	Australia	Apprentice nurses (N = 232)	Cross-sectional questionnaire survey.	Hand eczema based on a combination of self-reported symptoms[a].	Self-reported atopic dermatitis and allergy
Smit *et al.* 1994	The Netherlands	Apprentice hairdressers (N = 74)	Prospective cohort study with a follow-up time of 10 months (until the end of practical training)	Hand eczema based on a combination of self-reported symptoms[a] and dermatological examination. Skin barrier function assessed by TEWL.	Self-report of childhood eczema, dry skin, asthma and hay fever; patch testing; skin prick testing.
Uter *et al.* 1998, 1999	Germany	Apprentice hairdressers (N = 2352)	Prospective cohort study with follow-up measurements after 1 year and after 3 years ('POSH' study)	Dermatological examination of skin changes on the hands, following a definition based on morphology, localization and severity[e]	Self-report of family and personal history of atopy
John *et al.* 2000	Germany	Apprentice hairdressers (N = 66)	Prospective cohort study with follow-up time of 3 years	Clinical examination	Anamnesis with emphasis on atopy, history of flexural eczema, hand eczema, allergic rhinitis and asthma.
Roberts *et al.* 2006	Australia	Apprentice hairdressers (N = 195) and hairdressers (N = 184)	Crossectional survey	Dermatological examination, classifying the hands as 'normal', 'mild skin changes', or 'moderate or severe skin changes'[f],	Interview on past or present atopic dermatitis, skin problems on the hands, hay fever and asthma

Exposure factors assessed	Results	Remarks
Not reported in detail.	Prevalence of symptoms: scaling 19.3%, dryness 11.9%, irritation 6.9%, and redness 6.4%. Overall prevalence of HE: 10.4%. HE prevalence increased from 6.9% in the 1st year to 22.9% in 4th year.	Prevalence of HE was associated with presence of systemic allergic diseases, with increasing year of study and with living with a flatmate compared to living alone.
Self-reported previous nursing work and alcohol or tobacco intake	1-year prevalence: 18.5%. The 1-year prevalence increased from 10.8% in the first year to 17.0% in the second year and 27.4% in the third year.	The 1-year prevalence was associated with self-reported atopic dermatitis.
Self-administered questionnaire (taken at inclusion) including items on frequency of hand washing, hand disinfection products, glove use, contact with medicaments and use of skin care products.	Incidence rate: 32.8/100 person-years. Cumulative incidence over one year: 27.9% The incidence rate was highest in the first 6 months of practical training.	Dry skin was a significant susceptibility factor for developing hand eczema.
Self-administered questionnaire including questions on occupational tasks, skin protection, cleansing and skin care.	Point prevalence of skin changes: 35.4% at baseline, 47.5% after 1 year and 55.1% after 3 years. Period prevalence of mild skin changes: 46.5%. Period prevalence of hand eczema: 28.5%. Incidence rate of hand eczema: 36.7/100 person-years. The incidence rate declined after the first year of follow-up.	Wet work for more than 2 hours a day was a significant risk factor.
Questions about leisure activities, occupational tasks and skin protection habits, asked during clinical examination.	Incidence rate in the first year: 31.7/100 person-years. Incidence rate over 3 years: 21.1/100 person-years. Cumulative incidence: 29%.	The higher incidence in the first year was related to high wet work exposure.
Interview on use of gloves, job tasks, pre-vocational exposure and knowledge of skin hazards	In apprentice hairdressers, 28.0% had mild dermatitis and 4.7% had moderate to severe dermatitis upon clinical examination. In hairdressers, 17.4% had mild and 8.1 had moderate to severe dermatitis.	A self-reported history of atopy, especially atopic dermatitis, and female sex were associated with skin problems on the hands. Of the participants with clinically examined skin changes present, 34.9% considered themselves to have normal skin.

Table 1. Literature overview of the prevalence of OCD among workers and apprentices in high risk occupations (*continued*)

Reference	Country	Study population	Design	Definition of outcome(s)	Susceptibility factors assessed
Berndt *et al.* 1999, 2000	Switzerland	Metalworker apprentices (N = 201)	Prospective cohort study with follow-up measurements every 6 months, for a total period of 2.5 years ('PROMETES' study)	Dermatological examination of the hands; Hand eczema was defined as presence of at least one of the following: erythema and scaling, papules, excoriations, vesicles or exudation	Atopic skin diathesis as assessed during dermatological examination[g]
Funke *et al.* 2001	Germany	Apprentices in the car industry (N = 2078)	Prospective cohort study with follow-up measurements after 1 year and after 3 years ('PACO' study)	Dermatological examination with clinical assessment of hand eczema	Questionnaire used during dermatological examination; including items on atopic skin disease and history of hand eczema
Apfel-bacher *et al.* 2008	Germany	Workers in the car industry (N = 1494)	Additional follow-up of the 'PACO' cohort 10-16 years after the start of apprenticeship.	Self-reported skin symptoms followed by dermatological examination or telephone interview	Self-administered questionnaire including items on flexural eczema, hay fever, allergic asthma or rhinitis, dry skin and family history of eczema and allergic symptoms. In dermatological examination: atopic skin diathesis[g]

[a] Hand eczema was defined as having had two or more of the following (combinations of) symptoms: 1) Red and swollen hands or fingers 2) Red hands or fingers and fissures 3) Vesicles on hand or between fingers 4) Scaling hands or fingers with fissures 5) Itching hands or fingers with fissures, *plus* a duration of more than 3 weeks or recurrene of the symptoms.
[b] Hand eczema was defined as having had one or more of the following (combinations of) symptoms: 1) Red and swollen hands or fingers 2) Red hands or fingers and fissures 3) Vesicles on hand or between fingers 4) Scaling hands or fingers with fissures 5) Itching hands or fingers with fissures.

Exposure factors assessed	Results	Remarks
Exposure assessment based on diaries of job tasks in combination with job-specific exposure checklists formulated beforehand. Self-report of domestic exposures and frequency of hand washing and skin care.	Cumulative incidence: 23%. Incidence during the first 6 months of follow-up: 9%. Incidence during the last 6 months of follow-up: 3%.	A history of flexural eczema (but not atopic skin diathesis in general) was associated with higher risk of hand eczema. Apart from irritant exposure, mechanical friction was a risk factor for hand eczema. A lack of recovery time was also related to increased risk of hand eczema.
Questionnaire used during dermatological examination; including items on domestic exposure, exposure to irritants (task-based), skin cleansing and use of barrier creams	1-year cumulative incidence: 8.6% 3-year cumulative incidence: 14.1% Incidence was highest during the first 6 months of follow-up and declined thereafter.	Diagnosed hand eczema was predominantly of the irritant type (93.8%). Exposure was a relevant risk factor.
Not reported	Point prevalence: 9.4% Period prevalence: 21.0% Cumulative incidence since start of apprenticeship: 29.3% Cumulative incidence since start of employment (end of apprenticeship): 18.0%. In 40.0% of apprentices with hand eczema, hand eczema was persisting or recurrent during employment.	

[c] Self-reported hand eczema was defined as a positive answer to the question "Did you suffer from hand eczema in the past year?"
[d] TEWL: Transepidermal water loss.
[e] For detailed definition of skin changes, see Uter *et.al.* (1998) [31]
[f] Mild skin changes included dry skin; moderate to severe skin changes included redness, scaling, flaking, peeling, weeping and cracking.
[g] According to the score system proposed by Diepgen *et. al.* in 1991, as reported by Coenraads and Diepgen (1998) [32]

THE SKIN AND ITS BARRIER FUNCTION

Structure of the skin

From the inside to the outer surface, the skin consists of two primary layers: the inner layer or dermis, and the outer layer or epidermis. The dermis mainly consists of collagen fibers, elastic fibers and connective tissue and contains nerves, blood vessels, hair follicles and sweat glands. The epidermis forms the outer layer of the skin. It can be divided into several sublayers (Fig. 1). The main cell type in the epidermis is the keratinocyte. Other cells that are found in the epidermis include melanocytes (cells that produce pigment upon UV-radiation) and Langerhans cells (antigen-presenting cells). Keratinocytes divide in the basal layer or Stratum Basale, and then move up across the prickle layer (Stratum Spinosum) and granular layer (Stratum Granulosum) until they reach the horny layer (Stratum Corneum) [33]. Along the way they undergo multiple changes; their nucleus is digested and their shape changes from round to flat [33]. The dead, flattened keratinocytes that finally form the Stratum Corneum (SC) are called corneocytes or squame cells. The SC has an average thickness of 20 cell layers, but the thickness depends on body site, i.e. it is thinner at the eyelids and thicker at the soles of the feet [34]. Corneocytes are shed from the top layer of the SC in a process called desquamation. On average, one cell layer per day is shed [33]. As new

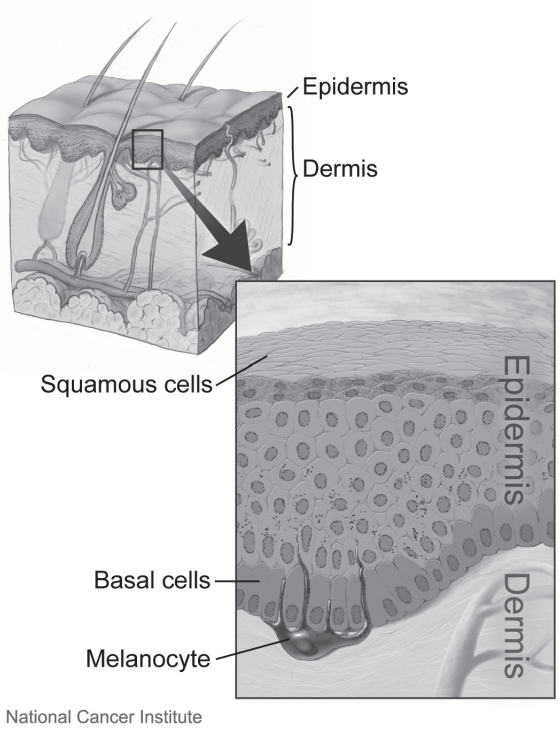

Fig. 1. Structure of the skin and different layers of the epidermis.(Credit: National Cancer Institute).

keratinocytes are continuously formed and migrate upwards while corneocytes are shed, the epidermis completely renews itself within a month [34].

Skin barrier function

The uppermost layer of the epidermis, the Stratum Corneum, is the key layer with regard to skin barrier function. The cells in the SC can be compared to a brick wall; the so-called 'brick and mortar' model [33;35]. In this model, the corneocytes are the bricks, and they are surrounded by a mortar consisting of intercellular lipid bilayers. The corneocytes are further connected to each other by proteins called desmosomes, which can be seen as the equivalent of iron rods that are passed through the bricks to increase the stability of a brick wall [36]. The resulting structure prevents water loss through the skin and blocks substances from diffusing into the skin (Fig. 2).

However, despite the physical barrier provided by the SC, some substances may still be able to cross the SC via the lipid bilayers (intercellular pathway) or, in the case of small hydrophilic compounds, through the corneocytes (transcellular pathway). In general, small molecular size (< 500 kDa) [37] and lipophilicity favor diffusion across the

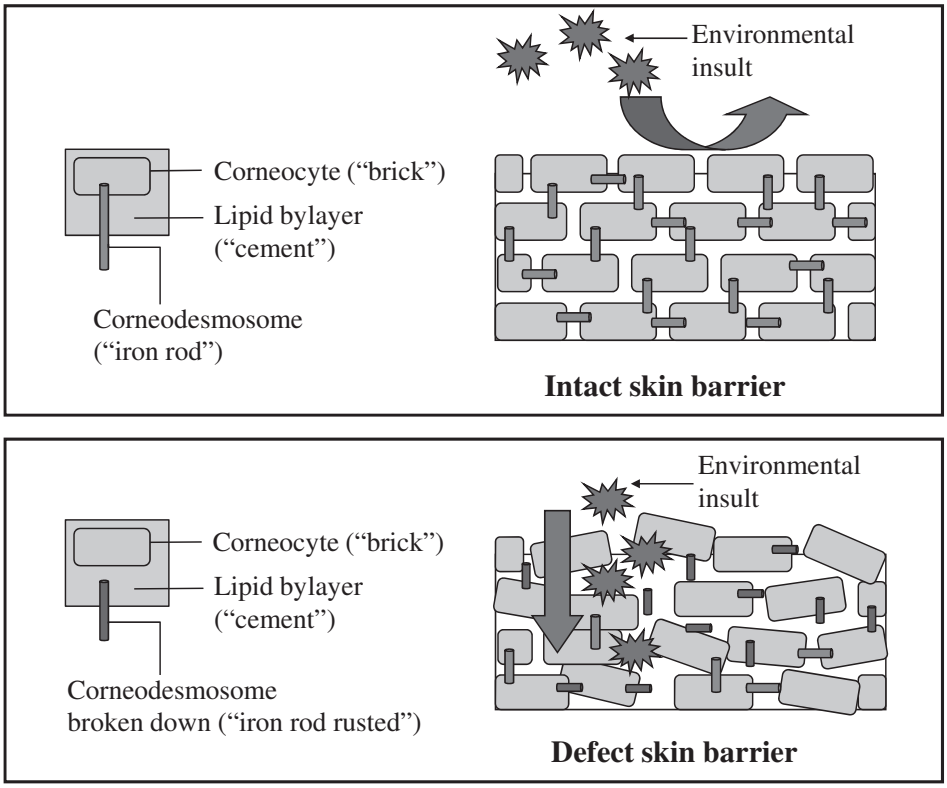

Fig. 2. 'Brick and mortar' model of the skin barrier in the stratum corneum

SC. Naturally occurring interruptions of the brick and mortar configuration, like hair shafts and sweat pores, may allow the entering of also larger molecules [38]. The skin barrier may be mechanically damaged, e.g. by scratching in individuals who suffer from an itchy skin condition such as atopic dermatitis (AD), a chronic inflammatory skin disease characterized by dry skin, pruritus, and erythematous lesions. In addition, various chemical and physical stressors are known to break down the skin barrier by causing disruption of the brick and mortar system, for example, detergents, oils and lubricants, and water. Water can cause excess hydration of the SC, causing the corneocytes to swell and increasing the permeability for foreign substances [30;39]. Exposure to detergents (soaps and surfactants) causes a rise in pH, which will enhance the activity of some pH-sensitive enzymes that are involved in the breakdown of corneodesmosomes during desquamation. Furthermore, detergents are able to solubilize the lipids in the lipid bilayers [36;38]. Oils and lubricants may also cause spatial disorganization of the lipids in the SC [30]. Both composition and organization of the lipids are important for skin barrier function [40]. Another potential damaging factor to skin barrier function is occlusion of the skin, for example by prolonged wearing of impermeable gloves. Skin occlusion affects hydration, temperature and pH of the skin [41], which all can influence the organization of the lipid bilayers essential for the barrier function. As a result, the permeability of the skin barrier increases. In addition, in case occlusive gloves are put on shortly after skin contact with irritants or allergens, occlusion prevents removal of substances from the skin surface (which would otherwise occur by evaporation or wiping), thus creating a 'reservoir' for prolonged exposure [41-43]. This may for example occur when a nurse puts on gloves shortly after washing her hands, while the skin is still moist. The presence of a reservoir effect is supported by several experimental studies showing that application of occlusive patches or chambers on irritant-exposed skin caused more severe damage of the skin barrier than occlusion of unexposed skin [41;43;44].

INDIVIDUAL SUSCEPTIBILITY TO IRRITANT CONTACT DERMATITIS

Atopic dermatitis

As stated before, although exposure to irritants is a prerequisite for developing ICD, some persons are more prone to develop this disease. The best-known and most firmly established susceptibility factor for the development of ICD is presence or previous presence of AD. The prevalence of AD is 10-20% in children and up to 10% in adults in the general European population [45-47]. The increased risk of developing work-related hand eczema for individuals with a history of AD has been recognized since decades [48-50], and recent population studies reported odds ratios of about 4 to 5 for the development of hand eczema in subjects with AD or childhood eczema [16;51;52]. The mechanisms through which AD predisposes to contact dermatitis in an environment with skin threatening factors are not completely clear. An immunological

pathogenesis is plausible; the cytokine milieu in the skin of AD patients is dominated by pro-inflammatory cytokines, so that contact with irritants may more easily lead to immune hyperreactivity. Furthermore, Langerhans cells are more active in AD skin [38;53]. Following the hypothesis that atopy (a genetic predisposition to develop allergic diseases) in general could be one of the causal factors for ICD, several studies have investigated associations between contact dermatitis and atopic features other than AD, like rhinitis, asthma, or allergy. However, results are contradictory and inconclusive although most of the studies conclude that respiratory atopy does not increase the risk for ICD [3;12;19;48;54]. Lately, the focus in AD etiology has shifted from immunological pathogenesis to defects in the skin barrier function. This was mainly caused by a breakthrough discovery that loss-of function mutations in the gene encoding for the epidermal protein *filaggrin* are a major risk factor for development of AD and for the development of other atopic diseases (e.g. asthma) in combination with AD. Several studies have convincingly shown that even in uninvolved skin of AD patients, the barrier function is less strong than that of healthy controls [6;55-57]. The mechanisms that underlay a skin barrier defect in AD are not completely understood, but aberrant composition and structure of lipid bilayers and proteins of the cornified skin envelope have been shown to play a role [38;40;58-60]. Another unresolved issue is whether a diminished skin barrier in AD is intrinsic and thus a primary event in the development of AD, or a consequence or event secondary to inflammation. Probably, as several mechanisms are operative, both factors contribute to a reduced skin barrier (the 'outside-inside-back to outside' paradigm) [45;61;62], which may at least partly explain why AD patients are more prone to develop contact dermatitis.

Filaggrin

One possible factor contributing to an impaired skin barrier function in AD is a decreased level of filaggrin in the skin. The main function of filaggrin is to aggregate keratin filaments in the transition of keratinocytes into corneocytes (hence the name filaggrin, short for 'filament aggregating protein'). This aggregation is essential for optimal development of the skin barrier, because it strengthens the 'brick and mortar' structure. Filaggrin is derived from profilaggrin, a very large insoluble molecule that is present in the granules of the Stratum Granulosum. During epidermal proliferation, profilaggrin is enzymatically dephosphorylated and cleaved into filaggrin monomers [34;63;64]. In the SC, filaggrin itself is degraded into several hygroscopic amino acids known as *natural moisturizing factors* (NMF). As the name suggests, NMF contribute to the hydration of the stratum corneum and inhibit water loss through the skin by attracting moisture. One of the NMF constituents, urocanic acid, also functions as an immunosuppressant, has antibacterial effects and protects against UV-radiation [65]. Furthermore, the presence of the acidic NMF helps to maintain a slightly acidic pH in the outer layer of the skin. Thus, a decreased amount of filaggrin not only leads to impaired skin barrier function in terms of structure, but also via decreased skin hydration (leading to dry skin) and changes in the skin surface pH. The latter is important because pH influences

the activity of various skin proteases (enzymes that are important for desquamation) and the release of inflammatory cytokines [64;66]. Furthermore, an elevated skin surface pH leads to increased bacterial colonization of the skin (especially by *Staphylococcus aureus*) which in turn may shift the skin immune system towards a Th2-mediated inflammatory response [45;67]. Superinfections and colonization with *Staphylococcus aureus* are one of the main features of AD [68;69]. Larger numbers of microorganisms have also been found on the skin of healthcare workers who were affected by contact dermatitis compared with non-affected colleagues [70-72].

Loss-of-function mutations in the filaggrin gene (FLG)

Filaggrin is derived from profilaggrin, the production of which is encoded in the filaggrin gene (*FLG*). *FLG* is located within the epidermal differentiation complex on chromosome *1q21*, a dense cluster of genes involved in the terminal epidermal differentiation and formation of the stratum corneum [73]. It consists of a so-called N-terminal domain (important for calcium binding and nuclear localization) followed by 10, 11 or 12 nearly identical filaggrin repeats and a C-terminal domain, which is required for correct processing of the profilaggrin into filaggrin [65;74]. Although the large size of the gene and the highly repetitive DNA-sequence have made sequencing difficult for a long time, recent studies have identified a large number of loss-of-function mutations in the *FLG* gene leading to incomplete processing or even complete absence of filaggrin in the skin [74;75]. These mutations were first discovered in patients with ichtyosis vulgaris (mostly homozygous or compound heterozygous carriers of *FLG* loss-of-function mutations), an inheritable skin disease characterized by dry skin with fine scaling [76]. The two most common *FLG* mutations in European populations are 2282del4 and R501X, both of which are located on the first filaggrin repeat [64]. In total, over 40 different mutations have been described in European and Asian populations and in the general population of Western Europe the total prevalence amounts to 5-9% [77-83]. The impact of *FLG* loss-of-function mutations on skin barrier function has been demonstrated in experimental studies as well as in clinical studies involving ichtyosis vulgaris and AD patients. Using filaggrin deficient mice (*ft/ft* or "flaky tail" mice), Scharschmidt and colleagues showed that compared to wildtype mice, the *ft/ft* mice had abnormal barrier function, enhanced penetration of water-soluble tracers and haptens, and that they expressed reduced inflammatory thresholds to irritants as well as allergens. Exposure to low-dose hapten applications elicited a Th2 inflammatory response, which in turn worsened the barrier function [62]. Grüber and colleagues demonstrated that ichtyosis vulgaris patients had increased skin surface pH and a delayed barrier recovery after tape stripping as compared to controls with the wildtype genotype for *FLG*. Furthermore, ichtyosis vulgaris patients showed an increased permeability of a tracer substance and decreased corneocyte integrity in cultured skin cells compared to controls, indicating decreased barrier function [84]. Angelova-Fisher and colleagues performed tape stripping in AD patients and healthy, nonatopic controls, and showed that skin barrier integrity (assessed

by measurement of water loss from the skin) was significantly lower in AD patients carrying *FLG* mutations as compared to AD patients who were wildtype for *FLG* and controls [55]. Multiple epidemiological studies convincingly showed that *FLG* loss-of-function mutations were strongly associated with AD. Up to half of the individuals with moderate to severe AD carry one or more *FLG* mutations [74;77;85-88] and a recent meta-analysis revealed an OR of 3.4 (95% CI 2.7 – 4.2) for R501X and 2282del4 mutations in AD patients compared with controls [89].

Because it is evident that the amount of filaggrin in the skin influences skin barrier function, *FLG* loss-of-function mutations may also be a risk factor for ICD. However, studies focusing on *FLG* mutations and ICD are scarce. In 2009, Molin and colleagues [90] investigated *FLG* loss-of-function mutations in 122 German non-atopic patients with different subtypes of chronic hand eczema and compared them to 95 healthy controls. They found a positive association in the subgroup of patients diagnosed with a combination of irritant and allergic contact dermatitis, but not in the subgroup with ICD alone. However, the number of patients in this study was small (25 – 28 patients per subgroup). In 2010, Thyssen and colleagues [81] reported a cross-sectional study in which they genotyped R501X and 2282del14 polymorphisms in 3335 adults recruited from a random sample (n = 7931) of the Danish general population. The effect of *FLG* loss-of-function mutations on the prevalence of hand eczema – including ICD, AD and allergic CD – was significant in subjects with a history of AD (OR 3.0: 95% CI: 1.3 – 7.0), but not in subjects without AD (OR 0.8; 95% CI 0.4-1.7). A combined presence of AD and *FLG* loss-of-function mutation status yielded an OR of 3.2 (95% CI: 1.5 – 6.9).

Other factors affecting filaggrin levels in the skin and susceptibility to ICD

Apart from loss-of-function mutations, the amount of filaggrin in the skin can be influenced by other factors as well. One of these factors is variation in the amount of filaggrin repeats in the *FLG* gene, known as copy number variation (CNV). The repetitive part of the *FLG* gene may consist of 10, 11, or 12 filaggrin repeats. The more repeats are present, the more profilaggrin protein will be produced, which will eventually result in more filaggrin in the skin. Recently, Brown and colleagues showed in Irish AD patients compared with Irish population controls that CNV in the filaggrin gene also affect the risk of AD. The odds ratio for AD between a person with 20 filaggrin repeats (2x 10 repeats) and a person with 24 repeats (2 x 12) was 1.67. The CNV are common in the population: in the genotyped Irish population the allelic variant of 10 repeats was present in 33.9%; 11 repeats in 51.5% and 12 repeats in 14.6%. CNV appeared to influence the amount of urocanic acid, one of the breakdown products of filaggrin, in the stratum corneum of atopic dermatitis patients [63]. The expression of filaggrin in the SC may further be regulated by enzymatic activity, e.g. of enzymes responsible for the processing of profilaggrin into filaggrin [91] or for the breakdown of filaggrin into NMF and by the cytokine milieu in the skin. Studies in cultured keratinocytes have shown that filaggrin expression is reduced by the presence of pro-inflammatory cytokines like

interleukin (IL)-4, IL-13, IL-22, IL-25 and tumor necrosis factor (TNF)-α [92-95]. This may be one of the reasons why reduced levels of NMF – the breakdown products of filaggrin – are also found in the skin of AD patients without *FLG* loss-of-function mutations [65;96].

Susceptibility to ICD may also be influenced by genetic variation in genes involved in immunologic response. The G to A-transition on position 308 of the gene encoding TNF-α (*TNFA-308A*), which is related to increased production of TNF-α, has been associated with increased reactivity to skin irritation [97;98] and increased risk of ICD [99;100]. A protective effect of the variant *IL1A-889T* allele towards hand dermatitis was found in apprentices involved in activities with high risk of skin irritation [99]. Accordingly, the same research group reported that carriers of a variant *IL1A-889T* allele have a reduced amount of IL-1α in their stratum corneum [101]. It might be speculated that an intrinsic favourable cytokine balance reflected in a high IL-1RA /IL-1α ratio due to the reduced amount of IL-1α might result in a better resistance against skin irritants [4].

Recently, a genome-wide association study identified the gene ORMDL3 on chromosome 17q21 to be associated with asthma. The presumed function of this gene is regulation of sphingolipid synthesis and unfolded protein response in endoplasmatic reticulum. Because sphingosine is, together with ceramide, needed for skin barrier integrity, this ORMDL3 gene may also influence skin barrier function [102].

However, the investigation of these other genetic susceptibility factors falls beyond the scope of this thesis.

THE ROLE OF GENETIC SUSCEPTIBILITY IN THE PREVENTION OF OCCUPATIONAL CONTACT DERMATITIS

Knowledge of a worker's personal susceptibility to develop occupational disease may contribute to more effective, targeted prevention. The first, historic reference to genetic susceptibility screening was made as early as 1938 by a scientist named Haldane, who recognized that some potters developed bronchitis while others did not, and suggested that *"we could eliminate potter's bronchitis by rejecting entrants into the pottery industry who are congenitally disposed to it"*. Another historic example is the discovery made during the 1950s Korean war that a genetically determined deficiency of a certain enzyme (G6PD) caused some soldiers to develop acute hemolytic anaemia after taking antimalaria drugs [103]. Recent examples of research on genetic susceptibility to occupational disease include increased susceptibility to beryllium [104;105], polycyclic aromatic hydrocarbons (PAHs) [106], di-isocyanates [106], dust [107] and pesticides [107]. Personal susceptibility could be taken into account in job counseling to advice against high-risk professions for susceptible youngsters while they still have the opportunity to choose another vocational training program. Workers with increased susceptibility could be granted access to extra protective measures, e.g. personal protection equipment (like special gloves in case of OCD), or adjustment of their work tasks. This type of personal prevention is already being applied in some occupational sectors, for example, in Germany and in the Netherlands a prevention program exists in which nurses – being

at risk for developing OCD due to frequent wet work – undergo pre-employment examination including questions about AD and (history of) hand eczema symptoms as indicators of increased susceptibility to develop OCD. Susceptible individuals receive extra preventive measures and should be regularly followed-up by their occupational physician [108;109].

Possibly, genotyping for genes involved in skin barrier function, such as *FLG*, could improve the evaluation of susceptibility to OCD during various kind of screenings. *FLG* genotyping could also be deployed in diagnostics and targeted interventions or therapy for workers suffering from OCD.

However, before actually offering and applying such a genetic susceptibility test for OCD, several ethical issues need to be considered. The advantages of predictive, and especially genetic tests, have to be weighed against the disadvantages. Well-known disadvantages include the potential for discrimination, the shift of focus from a safe environment for all workers to selection of non-susceptible workers (which is against the priorities set by occupational health and safety professionals), the compromise of autonomy or social pressure to perform the test, the difficulty of informed consent in the face of complex risk knowledge and problems in risk communication. Besides, the clinical validity or predictive value of susceptibility tests is often difficult to assess, both on population and individual level, as it is dependent on many factors. Those factors are not only directly related to the test characteristics itself, but may also be related to the prevalence of the disease and the susceptibility factor in the population, the presence of other, un-tested, susceptibility factors in the population or in the individual and the extent of (future) exposure. Furthermore, the practical consequences of false-positive and false-negative results should be considered. These issues cannot be ignored when investigating the role of *FLG* genotyping in the prevention of OCD.

AIMS AND OUTLINE OF THIS THESIS

The primary aim of this thesis was to gain more insight into the contributions of *FLG* loss-of-function mutations, AD, and occupational exposure as risk factors for OCD. A secondary goal was to investigate whether it would be recommendable to include *FLG* genotyping in susceptibility screening programs for OCD in addition to the usual examination of present or past AD.

The thesis is outlined as follows:

Chapter 2 focuses on exposure to wet work as a risk factor for the development of ICD. In *Chapter 2.1*, the use of a newly developed sampler designed to quantify wet work exposure is evaluated among nurses. *Chapter 2.2* describes exposure to wet work and the occurrence of hand eczema during practical training periods among Dutch apprentice nurses in a prospective cohort study.

In **Chapter 3,** the influence of AD and *FLG* mutations as risk factors for OCD is studied. Two different study designs were used for this purpose: in *Chapter 3.1*, patients with chronic OCD are compared with apprentices in training for high risk occupations in

a case-control study; and in *Chapter 3.2*, the effects of *FLG* loss-of-function mutations, AD and exposure to wet work on the risk of hand eczema are described in the prospective cohort of Dutch apprentice nurses mentioned in Chapter 2.2.

Chapter 4 pays attention to the ethical implications of using *FLG* genotyping in susceptibility testing for OCD. A qualitative study design is applied to investigate the opinions of apprentice nurses – as a stakeholder group – on the use of a genetic test for susceptibility to hand eczema. The advantages and disadvantages of using such a test mentioned by the students are subsequently compared with international guidelines on genetic screening for susceptibility to occupational diseases.

Finally, **Chapter 5** gives a general discussion of the results, including recommendations for further research and for practice.

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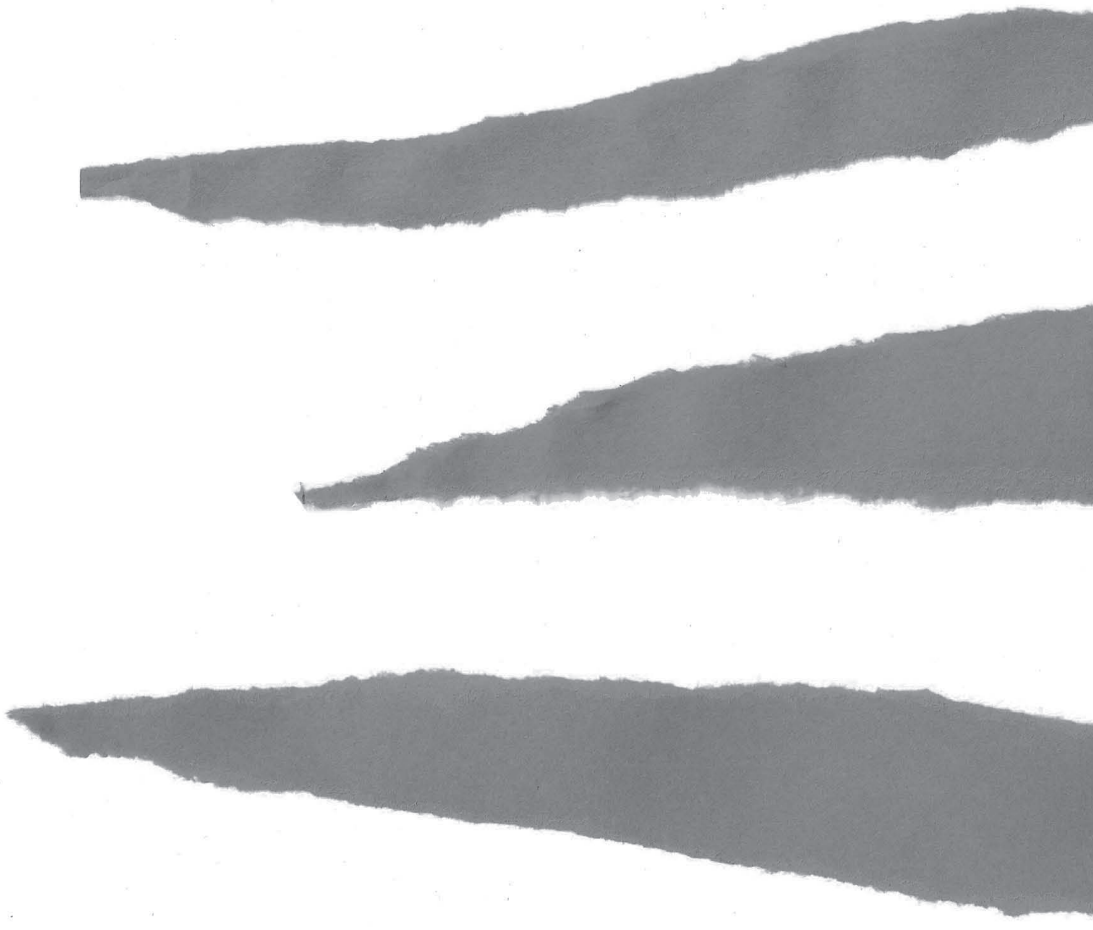

2

WET WORK

2.1

QUANTIFICATION OF WET-WORK EXPOSURE IN NURSES USING A NEWLY DEVELOPED WET-WORK EXPOSURE MONITOR

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ABSTRACT

Occupational contact dermatitis (OCD) is an important work-related disease. A major cause of OCD is 'wet work': frequent contact of the skin with water, soap, detergents, or occlusive gloves. The German guidance TRGS 401 recommends that the duration of wet work (including use of occlusive gloves) should not exceed 2 h day^{-1} and also the frequency of hand washing or hand disinfection should be taken into account. This highlights the need for a reliable method to assess duration and frequency of wet work. Recently, a wet work sampler has been developed by the University of Aberdeen. The sampler uses the temperature difference (ΔT) generated by evaporative cooling between two sensors: one sensor on the skin and a second one placed 2 mm above the skin. We have evaluated the use of this sampler in a health care setting, using direct observation as reference.

Twenty-six nurses wore the sampler on the volar side of the middle finger for ~2 h during their regular daily tasks, while being observed by a researcher. Sampler results were evaluated using various threshold values for ΔT to identify wet events of the hands.

The optimal ΔT to discern wet and dry skin differed considerably between individual nurses. Individual results yielded a median sensitivity of 78 and 62% and a median specificity of 79 and 68% for indicating wet skin and glove use, respectively. Overall, the sampler was moderately accurate for identifying wetness of the skin and less accurate for discerning glove use.

In conclusion, agreement between observed wet work and device-reported wet events in healthcare settings was not high and further adaptations and developments may be required.

INTRODUCTION

Occupational contact dermatitis (OCD) is a common work-related skin problem among healthcare workers. A major cause of OCD is 'wet work': frequent contact of the skin with water, soap, detergents, or the use of occlusive gloves. Wet work exposure is especially high in occupations showing a high prevalence of OCD, e.g. hairdressing, metalworking, food and cleaning industry and healthcare. Among nurses, the prevalence of OCD can rise to ~30% [1-3].

The risk of developing OCD increases with the total duration of exposure to wet work and the frequency of wet events. Already in 1981, Malten described irritant dermatitis as the result of a sequence of skin irritating events, each event taking place before the skin could recover from the previous event [4]. Jungbauer *et al.* (2004) argued that the risk of dermatitis may be more related to the frequency of exposure cycles rather than to the total duration of exposure, i.e. three exposure episodes of 10 min is more harmful than one exposure episode of 30 min [5]. This is especially relevant for nurses, whose exposure pattern is characterized by short but frequently recurrent wet episodes.

The German guidance TRGS 401 [6], which is the only existing guideline to regulate exposure to wet work, recommends that the total duration of wet work (including the use of occlusive gloves) should not exceed 2 h day^{-1} and that also the frequency of hand washing or hand disinfection should be taken into consideration. This highlights the need for a reliable method to assess duration and frequency of exposure to wet work. However, up to now, there are no suitable methods for measuring individual exposure to wet work. The most commonly used methods for this purpose are direct observations and questionnaires, but direct observations are expensive and time-consuming while questionnaires seem to be unreliable, as shown by Jungbauer *et al.* who studied the use of questionnaires for self-reporting of wet work exposure by nurses. They found that the respondents overestimated the duration of their wet work exposure by a factor of 2 (compared to direct observation), while the frequency of wet work episodes was underestimated, also by a factor of 2 [7]. Other common methods for dermal exposure assessment (e.g. absorbing patches or removal techniques) are not designed for measuring exposure to water and furthermore are unable to give information about the frequency or duration of wet-work exposure. Recently, a new wet-work exposure monitor has been introduced to resolve this problem [8]; preliminary results indicated that it may be a useful tool. However, it has not been validated in healthcare settings yet.

The aim of this study was to evaluate the performance of this new device in the daily practice of hospital nurses. We used direct observations as reference.

METHODS

Functioning of the sampler

As shown in Fig. 1, this instrument comprises two thermocouples mounted on a holder, which is worn on the finger, and linked to a data logger by wires. These wires are kept in place using Velcro wristbands and armbands, and the data logger itself may be worn

in a pocket, on the arm, or on the belt. The first thermocouple is positioned ~2 mm above the skin and records air temperature (*T*a), while the second one is located under the holder in contact with the skin and records skin temperature (*T*s). The temperature (degree Celsius) of the skin and the temperature above the skin are logged every 10 s.

The device is working on the basis of evaporative cooling. When the hand is dry, *T*a is usually lower than *T*s. Once the person immerses his/ her hand in water, both sensors are influenced by the liquid temperature, with the above-finger thermocouple responding more quickly, as shown in Fig. 2. This results in an increased difference in temperature between *T*s and *T*a. The higher the absolute difference, ΔT ($\Delta T = |Ts\text{-}Ta|$), the more likely that the skin is wet. When the skin is damp, water on the skin evaporates and causes the above-finger sensor to cool down, which also will result in an increase in ΔT. On contrary, the use of gloves will cause a decrease in ΔT due to the heat conduction in the closed environment inside the glove.

Observations

Sampling was performed in two different nursing wards in the Academic Medical Centre, Amsterdam. Twenty-six nurses from the departments of Internal Medicine & Infectious Diseases and Neurology participated in the study. Each nurse wore the device for a period of ~2 h while performing her regular daily tasks and was during that time observed by a researcher. The observer watched only one person at a time and recorded the time points of start and ending of wet work events accurately to the nearest second with use of a stopwatch. The main wet-work episodes included hand washing (with or without using soap), use of disinfective alcohol gel (hand alcohol) on the hands, use of occlusive gloves, and wetting of the hands not being hand washing (e.g. rinsing materials with tap water, using wet towels, helping patients with washing or bathing, etc.). In case the vision of the observer was blocked because of patient privacy

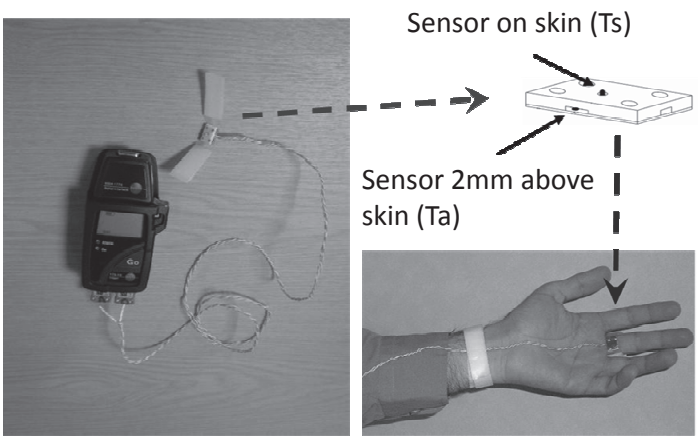

Fig. 1. Sampler for measuring wet work as designed by the University of Aberdeen.

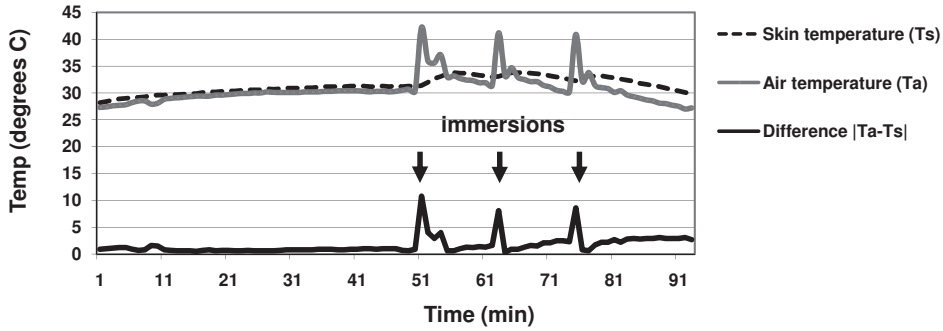

Fig. 2. Sampler result in an experimental setting (by courtesy of A.B., University of Aberdeen).

(e.g. body washing) or safety rules (e.g. work in quarantined rooms), the nurse was asked about her wet-work activities on return and these were recorded separately. Table 1 shows some typical wet-work activities and how these were recorded by observers.

Before the start of the observations, the timers of the wet-work sampler and the stopwatches were synchronized. The sensor, wire, and the data logger itself were disinfected by wiping them thoroughly with 70% alcohol in the laboratory and transferred to the nursing department in a plastic sealed bag. The sampler was worn permanently by the participants, including all hand hygiene or disinfection procedures performed by the participants.

Table 1. Rules for recording wet-work activities by hand with sensor during observation

Activity	Start	Stop	Comments
Hand washing	At first contact of hand/finger with water	At closing of the tap	Usually hands are dried with paper towel. If not so, write comment
Hand disinfection	At pressing the dispenser	At end of hand shaking or rubbing movement	
Glove use	Hand in glove	Glove off	When gloves are switched, count as new episode
Wet work	At first contact of hand/finger with water	At start of drying off the hands with towel or paper, or wiping them dry	Any contact with water that is not hand washing, is marked under wet work
Microwave-heated wash towels	Hand in wash towel	Hand out of wash towel	
Activities behind curtains	Curtain / door closed	Curtain / door opened	Ask participant about wet-work activities on return

Assessment of optimal threshold values for distinguishing wet skin and glove use

Two temperature readings (*T*s and *T*a) are produced by the sampler every 10 s. To account for the discontinuity of the data, a smoothing function was used: $\Delta T_{t,\,smoothed} = 0.7*\Delta T_t + 0.3*\Delta T_{t-1}$.

The smoothed ΔT values were rounded to the next quarter of a degree. By applying a threshold temperature value for ΔT above which the skin is qualified as 'wet', the classification of 'wet' or 'dry' can be assigned for each 10-s interval reading. A person had on average 620 (range: 255 – 876) parallel readings of ΔT and observations. Defining an optimal ΔT threshold value was done separately for each person in the dataset, as follows (for example, see Appendix as supplementary material available at *Annals of Occupational Hygiene* online): sampler readings were separated into wet, dry and gloved readings based on the corresponding observations. At each level of ΔT, the number of wet, gloved, and dry readings was counted. To find a threshold value for discriminating between wet and dry readings, the sensitivity and specificity of a certain ΔT threshold value was calculated using the corresponding proportion of correctly identified wet observations (sensitivity) and the proportion of correctly identified dry observations (specificity), respectively. This was done for each ΔT level in the range from 0 to 10°, rounded to a quarter of a degree. Subsequently, the 'true positive' (sensitivity) values were plotted against the 'false positive' (1-sensitivity) values in a reciever operating curve (ROC), each threshold value producing a different point on the ROC curve. The point on the ROC curve where the sum of sensitivity and specificity was maximal was regarded as the optimum threshold value for that person. As wearing of occlusive gloves results in a decrease instead of an increase in ΔT, two different threshold values were calculated: one comparing wet-work data to dry work data (after excluding observations with glove use), and the other comparing glove use data with dry work data (after excluding observations with wet work). To characterize the discerning capacity of the sampler, also the area under the ROC curve (AUC) was calculated. According to a guideline by Greiner *et al.*, an AUC between 0.9 and 1.0 means a highly accurate test, an AUC between 0.7 and 0.9 means moderate accuracy, an AUC between 0.6 and 0.7 means low accuracy, and an AUC of 0.5 means no discerning value (a non-informative test) [9].

RESULTS

Twenty-six nurses were observed during morning shifts. The mean duration of an observation was 107 min (range 42 - 167 min). The mean total duration of wet work performed in the observed time span was 25 min (22 ±14 % of the observation time). An overview of the observed wet-work activities and corresponding ΔT values is shown in Table 2.

The most common types of wet-work were the application of disinfectant alcohol gel on the hands and hand washing, occurring up to 13 and 9 times in 2 h of observation, respectively. Although most wet-work activities had a duration of only a few seconds, the majority were detected by the sampler, as illustrated in Table 2 and in Fig. 3. The

Table 2. Wet-work activities observed and corresponding median ΔT threshold values for 26 nurses

	Observed		Sampler
Wet-work activity	Duration of wetness (min:s)	Number of episodes per subject	ΔT
Hand washing	0:08 (0:01 – 1:36)	2 (0 – 9)	3.1 (0.1 – 11.5)
Hand alcohol	0:05 (0:01 – 0:10)	2 (0 – 13)	3.0 (0.0 – 8.4)
Other wet work	0:10 (0:01 – 6:24)	1 (0 – 4)	2.4 (0.0 – 8.6)
Gloves	5:23 (0:30 – 43:09)	3 (0 – 8)	0.9 (0.0 – 7.1)

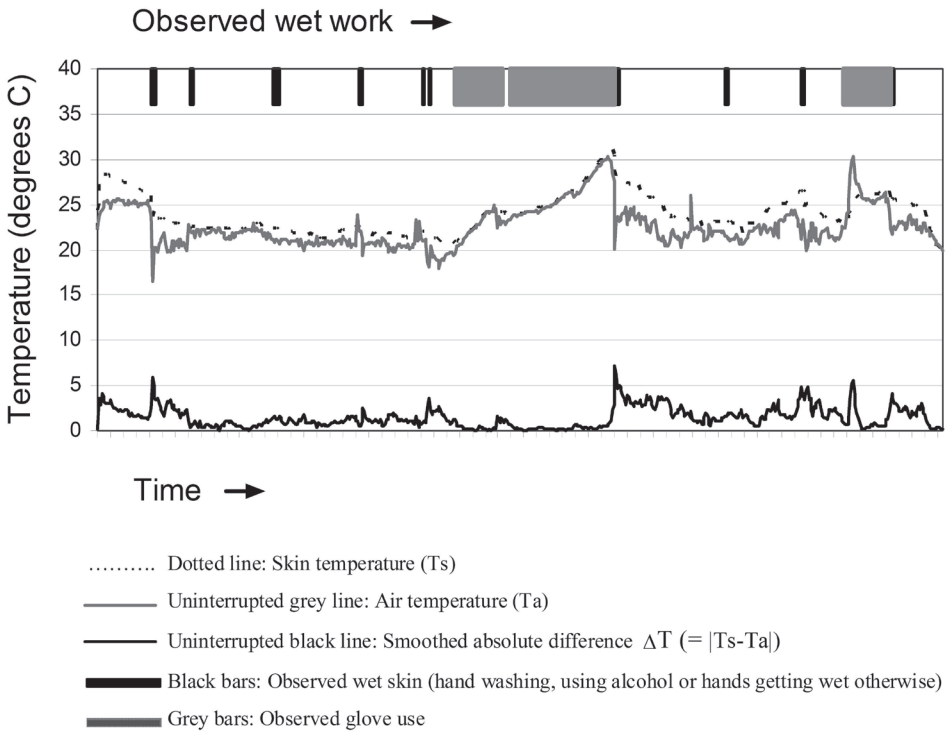

Fig. 3. Example of a 2-h sampling period using the wet-work sampler and personal observation in one nurse.

figure shows a typical example of a 2-h sampling output in which occurrence of wet skin is represented by black bars and the wearing of gloves is represented by grey bars. The straight grey line in the figure represents the air temperature *T*a, which deviates from the skin temperature (*T*s; dotted line) with downward peaks in case of contact with cold water or with upward peaks in case of contact with hot water. As can also be seen from the figure, with wearing gloves after some time, *T*a nearly equals *T*s. Besides, the occluded environment causes both *T*s and *T*a to rise. Usually, gloves were

used in relatively short tasks of ~5 min. In 25% of the occasions, gloves were worn for >10 min without changing pairs.

As described in the Methods section, a threshold value was calculated for each person separately using ROC analysis. The resulting median threshold values are shown in Table 3. The threshold value for ΔT, above which sampler readings were regarded as indicating 'wet skin', differed considerably between individual subjects, and the individual discerning value corresponding to the subject's optimal threshold varied between no discerning value at all (AUC <0.5; 1 out of 26 subjects) and highly accurate (AUC > 0.9; 8 out of 26 subjects). The same variation in results applied to the data regarding glove use. The performance of the sampler for discerning wet skin seemed slightly better than for discerning 'glove use', with a median sensitivity of 76 versus 63% and a median specificity of 79 versus 69%, respectively.

To study the performance of the sampler regarding the assessment of cumulative duration of exposure, the median threshold values from Table 3 were applied to the total dataset: all sampler readings with a $\Delta T > 2.25$ or < 1.25 were regarded as indicating wet skin and wearing gloves, respectively. Next, the frequency of exposure episodes was counted. For this, an extra criterion was applied, in order to evade false positives by coincidental fluctuation: sampler output was considered indicative of wet skin episodes only if 2 or more consecutive readings had a $\Delta T > 2.25$ and of glove use episodes only if 6 or more consecutive readings had a $\Delta T < 1.25$ (representing glove use of at least 1 min). The results are displayed in Table 4.

As shown from Table 4, application of the same median threshold value on every subject in the dataset decreased the sensitivity for discerning wet skin from 76 to 67% while the specificity rose from 79 to 86%. For discerning glove use the effect was opposite: sensitivity increased from 63 to 75% but specificity dropped from 69 to 52%.

Table 4 also shows that the exposure to wet work was overestimated by the sampler both for total duration and for frequency of exposure. On average, the total exposure times for wet skin and for wearing gloves were overestimated by a factor 3 and a factor 2, respectively. Regarding frequency of exposure, the majority of the exposure episodes for wet skin and glove use were classified correctly by the sampler. However, the number of false positives was substantial.

Table 3. Temperature threshold values for wet skin and glove use versus dry skin

	Wet skin versus dry skin ($N = 25$)			Wearing gloves versus dry skin ($N = 21$)		
	Median	IQR	Minimum - maximum	Median	IQR	Minimum-maximum
Threshold at ΔT	2.25	0.94	0.75 – 3.25	1.25	0.75	0.50 – 2.75
Sensitivity	76%	21.5	45 – 100%	63%	18.8	16 – 88%
Specificity	79%	13.8	41 – 99%	69%	14.5	52 – 87%
AUC	0.84	0.18	0.32 – 0.99	0.68	0.12	0.32 – 0.89

IQR, Interquartile Range.

Table 4. Performance of the sampler after application of the median threshold value for wet skin and glove use

	Wet skin (*N* = 25)		Wearing gloves (*N* = 21)	
Median	Median	Minimum-maximum	Median	Minimum-maximum
Sensitivity (%)	67	0 – 100	75	10 – 100
Specificity (%)	86	52 – 100	52	5 – 89
Total exposure time according to observations (min:s)	3:20	0:10 – 13:10	24:10	3:40 – 56:20
Total exposure time according to sampler (min:s)	15:50	0:10 – 44:30	56:50	12:20 – 131:00
Number of episodes observed by observer	6	1 – 14	3	1 – 8
Number of episodes correctly classified by sampler (%)	4 (71%)	0 – 9 (0 – 100%)	2 (75%)	1 – 6 (33 – 100%)
Number of episodes falsely classified as exposed by sampler (false positives)	8	1 – 22	7	1 – 15

DISCUSSION

According to the ROC analysis, the average performance of the sampler was moderately accurate for discerning wet skin (median AUC = 0.85) and less accurate for discerning glove use (median AUC = 0.67). Individual results yielded a median sensitivity of 78 and 62% and a median specificity of 79 and 68% for indicating wet skin and glove use, respectively. We note that these performances can only be obtained after the ΔT threshold of the subject involved has been determined. Using a group median value rather than individual threshold ($\Delta T > 2.25$ for wet skin and $\Delta T < 1.25$ for glove use) changed the median sensitivity to 67 and 75% and the median specificity to 86 and 52% for wet skin and glove use, respectively. This shift follows from the variation in the temperature difference between the two thermocouples on dry skin across different people.

About 70 - 75% of the observed episodes of wet skin or using occlusive gloves were recognized as such by the sampler. However, there was a large number of false-positive readings, so that the frequency of exposure was considerably overestimated. The sampler results also overestimated the total duration of wet skin by a factor of 3 and the total duration of glove use by a factor of 2.

The large inter-individual variation found in this study has probably hampered a good overall performance of the sampler. Characteristics of wet-work exposure in the observed hospital wards were diverse, varying between days according to the actual needs of the patients present and varying between individual nurses according to personal work habits. This makes it very difficult to find suitable threshold values for ΔT that can be applied to the whole population. Defining an optimal threshold on the individual level before each measurement, e.g. by letting a person perform a couple

of wet tasks before the start of his/her working shift, would thus be recommended when using the device for measuring nurses' wet-work exposure.

Furthermore, wet work in a nursing ward generally involves contact with water of different temperatures. In our study, we observed contact with cold water (13.5°C) as well as hot water (42.3°C); however, contact with lukewarm water of ~ 20°C was also common. The principle of detecting wet skin by the difference between skin temperature and air temperature obviously works better if the water temperature is significantly different from skin temperature, either cold or hot. Failure in detecting contact with lukewarm water may therefore have decreased the performance of the sampler in this study. Previously, the performance of this device has been studied in hairdressers and caterers, who were mostly exposed to warm water of about 40°C, and in florists, who mainly use cold water. These studies revealed similar discriminating power, with a median sensitivity of 63 - 81%, and a median specificity of 62 - 73% (A. Behroozy, unpublished data).

Regarding the use of occlusive gloves, the discerning value might be improved when counting only longer periods of glove wearing because it takes some time before a temperature equilibrium is reached inside the glove. In addition, one could reason that wearing gloves for a short time does not increase the risk of developing OCD; on the contrary, it protects the skin from exposure to irritants. Adverse effects on the skin may occur only after prolonged or repeated occlusion [10;11] or even only after occlusion following irritant exposure [12]. It would be of great importance to find the threshold of duration when glove use changes from primarily protection to a wet-work risk, for example in an experimental follow-up study using measurement of biophysical parameters in conjunction with the sampler. In the present study, we performed a second data analysis in which glove use was only counted if it lasted > 5 min. Against our expectations, this did not change the median threshold value and although the number of false positives decreased, it did not improve the overall performance of the sampler regarding the number of correctly identified episodes of exposure (data not shown).

One drawback in the design of this study may have slightly biased the results regarding sensitivity and specificity. Due to privacy and safety rules, the observer could not follow the nurse behind bed curtains or into quarantined rooms. Any wet-work activities that were performed behind closed curtains or in quarantined rooms were still recorded by the sampler, but could not be observed directly and therefore, the observations which we used as reference cannot be regarded as a real 'gold standard' here. However, since the nurses involved in such activities were asked about their exposure directly after return, this should not be a problem when looking at the number of exposure episodes in Table 3.

In conclusion, the sensitivity and specificity of this device for recognizing wet skin and glove use in the two sampled hospital wards was not high enough to promote its use for wet-work exposure estimation in nurses. On top of that, there were some practical issues: having the sensor on the finger with a velcro strap is not allowed in some departments for hygienic reasons, and the sensor holder caused irritation when hands were rubbed together (for hand washing or alcohol use). A further developed design with a completely smooth sensor holder and a smooth fix to the finger might

overcome some of these practical problems. On balance, though, we think that the pattern of exposure to wet work in hospital settings may be too complex for the use of this device to quantify wet-work tasks among nursing staff. However, it may be interesting to evaluate the performance of the sampler in other occupational groups with a more homogeneous wet-work exposure, for example the cleaning or catering industry.

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SUPPLEMENTARY FILE: EXAMPLE OF DEFINING THE OPTIMAL ΔT THRESHOLD VALUE FOR A PERSON

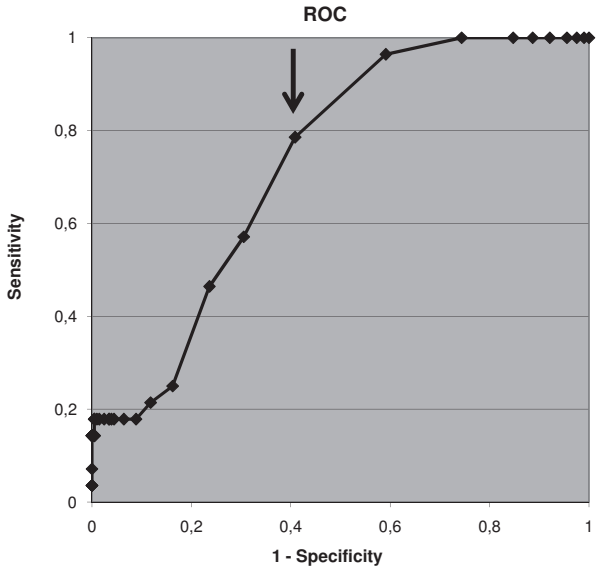

Fig. S1. ROC curve accompanying table A1. Every point on the curve represents a ΔT threshold value. The ΔT where sensitivity and specificity are maximal, i.e. the point closest to the upper-left corner of the graph, is the optimum ΔT threshold value for discerning 'wet skin' for this person. In this case, the optimum ΔT threshold was 2,25 degrees (arrow).

Table S1. Number of dry and wet sampler readings, sensitivity and specificity at different ΔT thresholds for discerning 'wet skin' for subject nr 308

ΔT	# readings *observed* as 'dry' at this ΔT	# readings *observed* as 'wet' at this ΔT	Total # of readings at this ΔT	# readings *classified* as 'dry' using this ΔT as threshold[1]	# readings *classified* as 'wet' using this ΔT as threshold[2]	Sensitivity (%)	Specificity (%)	1 – Specificity (%)	Sensitivity + Specificity (%)
0,00	2	0	2	0	231	100%	0%	100%	100%
0,25	3	0	3	2	229	100%	1%	99%	101%
0,50	4	0	4	5	226	100%	2%	98%	102%
0,75	7	0	7	9	222	100%	4%	96%	104%
1,00	7	0	7	16	215	100%	8%	92%	108%
1,25	8	0	8	23	208	100%	11%	89%	111%
1,50	21	0	21	31	200	100%	15%	85%	115%
1,75	31	1	32	52	179	100%	26%	74%	126%
2,00	37	5	42	84	147	96%	41%	59%	137%
2,25	**21**	**6**	**27**	**126**	**105**	**79%**	**59%**	**41%**	**138%**
2,50	14	3	17	153	78	57%	69%	31%	127%
2,75	15	6	21	170	61	46%	76%	24%	123%
3,00	9	1	10	191	40	25%	84%	16%	109%
3,25	6	1	7	201	30	21%	88%	12%	110%
3,50	5	0	5	208	23	18%	91%	9%	109%
	≋	≋	≋	≋	≋	≋	≋	≋	≋
9,50	0	0	0	230	1	4%	100%	0%	104%
9,75	0	0	0	230	1	4%	100%	0%	104%
10,00	0	1	1	230	1	4%	100%	0%	104%
Total	203	28	231						

Row with optimal temperature in **bold**.

[1] More specific: # readings classified as 'dry' at ΔT below the threshold ΔT.

[2] More specific: # readings classified as 'wet' at ΔT equal to or above the threshold ΔT.

2.2

WET WORK AND HAND ECZEMA IN APPRENTICE NURSES; PART I OF A PROSPECTIVE COHORT STUDY

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ABSTRACT

Background /Objectives

Environmental exposure and personal susceptibility both contribute to development of hand eczema. Here, we report an investigation on wet work exposure and its influence on the risk of developing hand eczema in apprentice nurses.

Methods

A prospective cohort study was performed among 721 Dutch apprentice nurses. Participants recorded wet work exposure and symptoms of hand eczema using specially designed diary cards.

Results

For 533 apprentice nurses, a follow-up time of 1 – 3 years was completed. Diary cards were supplied by 383 students. The 1-year period prevalence of hand eczema was 23% in the first year, 25% in the second year and 31% in the third year of follow-up. Eighty-one new cases of hand eczema developed, most of which occurred during the first year of follow-up. In approximately one-third of the participants, wet work exposure exceeded the national guidelines. Frequent hand washing during traineeships [odds ratio (OR) 1.5; 90% confidence interval (CI) 1.0 – 2.3], frequent hand washing at home (OR 2.3; 90% CI 1.5 – 3.7) and having a side job involving wet work (OR 1.6; 90% CI 1.0 – 2.4) were independent risk factors for hand eczema.

Conclusion

As a considerable number of apprentice nurses had already developed hand eczema during traineeships, more attention should be paid to skin protection in vocational education.

INTRODUCTION

Occupational hand eczema is one of the most common occupational diseases in industrialized countries; it usually is a manifestation of irritant or allergic contact dermatitis, irritant contact dermatitis being the most common form in the workplace, accounting for 50-80% of the cases [1]. A major cause of irritant contact dermatitis in the workplace is 'wet work', that is, frequent or long-lasting contact with water, soaps or detergents (e.g. during hand washing). Contact with disinfectants and prolonged wearing of occlusive gloves are also considered to be wet work [2]. Other well-known occupational exposures able to cause irritant contact dermatitis are oils, lubricants and solvents. In occupations with high exposure to wet work or to other skin threatening agents, for example nursing, hairdressing, the printing industry and the metalworking industry, the 1-year prevalence of hand eczema was reported to be between 20% and 30% [3-9]. In comparison, the 1-year prevalence found by (mainly Scandinavian) epidemiological studies among the general population between 18 and 69 years of age ranges from 9% to 14% [10].

Although skin exposure is a prerequisite for developing occupational hand eczema, the risk is influenced by personal susceptibility [1]. A well-known personal risk factor for the development of occupational hand eczema is the presence of atopic dermatitis, a chronic inflammatory skin disease whose main symptoms are dry skin, itching, and erythematous lesions [11;12]. A history of atopic dermatitis is currently used to identify susceptible individuals in occupational prevention programs in Germany and in the Netherlands [2;13]. Furthermore, recent findings suggest that loss-of-function mutations in the gene encoding for the epidermal protein filaggrin (*FLG*) increase the risk of contact dermatitis via impairment of the skin barrier [14-16]. Also, the effects of polymorphisms in genes encoding for inflammatory cytokines have been shown to be of influence [17;18]. Incorporation of the examination of these newly discovered polymorphisms into existing susceptibility screening or in health surveillance programs used in high-risk occupations may contribute to a better identification of susceptible individuals and to personalized prevention. However, the effects of susceptibility genes are difficult to assess quantitatively, owing to the complex interplay between genetic polymorphisms, atopic dermatitis, and occupational exposure. We therefore aimed to gain more insight in the relative effects of personal susceptibility factors and exposure by using a prospective cohort study design.

Apprentice nurses were chosen as the study population, for two reasons. First, a population of apprentices is not, or is minimally, affected by a 'healthy worker effect' as compared with a population of workers with long-term occupational experience, where some of the susceptible individuals may have left the occupation because of the development of hand eczema. Second, the nursing occupation was chosen because nurses are known to have a relatively high exposure to wet work and an associated increased risk of hand eczema. Owing to hygiene regulations, nurses frequently have to wash and disinfect their hands. Exposure to water, disinfectants, soaps and occlusive gloves is common, as is contact with latex or medication ingredients, which may cause allergic contact dermatitis [4;7;19-21]. The impact of hand eczema may be exceptionally

great in the healthcare sector, because nurses who have developed hand eczema have difficulties in maintaining hygiene standards; the application of disinfectant or soap to damaged skin can be painful, so affected nurses may try to lower the frequency of hand washing and disinfection. In addition, bacterial colonization (e.g. with *Staphylococcus aureus*) has been shown to be more prevalent on damaged skin than on intact skin [22-24]. Thus, prevention of hand eczema is important not only for the workers, but also for hospital hygiene and infection prevention.

Our goal was to examine the influence of both wet work exposure and personal susceptibility factors on the risk of developing hand eczema in a prospective cohort study among apprentice nurses. Here, we present the baseline characteristics of this cohort, the exposure to wet work during follow-up, and the occurrence of hand eczema in relation to exposure. The influence of personal susceptibility factors is described elsewhere [25].

METHODS

Subjects

Subjects were recruited from 15 different Dutch schools that prepare students for a career in nursing or care-giving with intermediate vocational education (six schools) or higher vocational education (nine schools). Students were eligible for participation if they had recently started a traineeship with a duration of at least 10 weeks, or were expecting to do so within the next few weeks. No further inclusion criteria were applied. The only exclusion criterion was the presence of chronic inflammatory disease (e.g. psoriasis and rheumatoid arthritis), because these diseases and their respective treatments may interfere with the inflammatory skin reactions related to hand eczema. After permission had been obtained from the schools' management boards, a school visit was organized, usually shortly before the start of the traineeships, during which the researchers informed the students about the study by means of a classroom presentation. Students were invited to participate in the study on a voluntary basis. A small gift was given for participation. Ethical approval was obtained from the Medical Ethical Committee of the Academic Medical Center, Amsterdam, The Netherlands.

Baseline Questionnaire

Students willing to participate filled in a questionnaire including items on demographic information, personal and family history of eczema, rhinitis, conjunctivitis and asthma, allergies and/or symptoms following exposure to dust, animals, pollen, foods, metals and wool, present or past skin diseases, presence of any chronic disease, medication use, present or past skin complaints regarding the hands or fingers, and exposure to wet work during previous jobs/apprenticeships, secondary jobs, and leisure or household activities. Atopy was defined as having experienced two or more of the following: symptoms following exposure to common allergens (respiratory or skin complaints after contact with animals, plants/pollens, dust, or food), rhinitis, conjunctivitis, or asthma.

Atopic dermatitis was assessed using the UK working party criteria 'questions only' definition [26], in which onset below 2 years of age was replaced by onset below 5 years of age as a proxy of 'childhood dermatitis'. This modification was made to increase sensitivity according to the rationale of the International Study of Asthma and Allergies in Childhood (ISAAC) [27]. To explore the existence of selection bias, during a subset of school visits to six of 15 schools the questionnaire was completed by all apprentices regardless of their participation in the study.

Wet work exposure during follow-up

During their traineeships, the apprentices regularly recorded the wet work activities that they performed by using diary cards specifically designed for this purpose. These diary cards were pocket-sized, folding cards of thick paper, with multiple checkboxes on which wet work activities could be ticked off directly during a shift (see Supplementary file and Table 3 for the different wet work activities included on the diary cards). The cards were carefully made and were piloted in a small number of student nurses to make them as user-friendly as possible. One card was used for one shift; either morning, late shift, or night shift. In addition, questions on possible secondary jobs were present on the reverse side of the diary card.

The diary cards were sent to the apprentices periodically by regular mail. A set of two cards at a time plus a return envelope was sent every 2-4 weeks, depending on the length of the traineeship. The apprentices were free to choose the specific day and shift on which they filled in the diary card. If no cards had been returned near the end of the traineeship, participants were contacted by email or telephone to retrieve information about their last traineeship and possible symptoms retrospectively.

Definition of outcome measures

Skin symptoms of the hands or fingers were recorded simultaneously with the exposure data on the reverse side of the diary cards. Participants were asked to indicate whether they had experienced any of redness, scaling, itch, fissures, vesicles or papules on the hands or fingers, and whether these symptoms had lasted for > 3 days. Following the classification for screening proposed by Vermeulen *et al*. [28], 'hand eczema' was defined as the presence of one or more of fissures and redness, fissures and itch, fissures and scaling, vesicles, or papules, plus a duration of > 3 days or recurrence (symptoms reported more than once).

As these criteria were developed for identifying cases of hand eczema in a working population, we were concerned that by using this definition, we would miss early-stage symptoms that may progress into hand eczema. Therefore, we used in addition a more lenient definition, 'mild hand eczema', defined as the presence of one or more of redness and itch, redness and scaling, itch and scaling, fissures and redness, fissures and itch, fissures and scaling, vesicles, or papules, all irrespective of duration or recurrence.

Without specification, (mild) hand eczema refers to any episode of (mild) hand eczema during follow-up. In some approaches, first episodes and recurrent episodes of hand eczema during traineeships are discerned, irrespective of the participant's history of hand eczema at inclusion. A first episode of hand eczema in a student without hand eczema history up to inclusion is equivalent to 'incident hand eczema'. As the exact date of onset of hand eczema was often not known (owing to the fluctuating course of the symptoms and spot-check-like sampling methods of the diary cards), the presence of hand eczema was treated as a binary variable during each traineeship. In the calculation of incidence rates, the length of a participant's traineeship was used as an equivalent for person-years in the denominator. Participants who had developed hand eczema during their first traineeship were excluded from the calculation of incidence rates during the second traineeship, etc.

Consultation of occupational physician by students with suspected hand eczema

Students who reported symptoms of hand eczema at inclusion or during follow-up received an invitation for a free consultation by an occupational physician specializing in dermatology problems. Depending on the student's preferences, either a visit or a telephone consultation was arranged. If possible, telephone consultations were supported by photographs of the skin symptoms provided by the student. The occupational physician diagnosed the skin condition according to a standardized protocol, taking into account morphology and indications for aetiology (atopic dermatitis, exposure to irritants and allergens). Possible diagnoses were:

- Irritant contact dermatitis, specified according to the presence or absence of concomitant atopic dermatitis,
- Allergic contact dermatitis, specified according to the presence or absence of concomitant atopic dermatitis,
- Atopic dermatitis,
- Combination of irritant-, and allergic contact dermatitis, specified according to the presence or absence of concomitant atopic dermatitis,
- Other.

If allergic contact dermatitis was suspected, the apprentice nurse was referred to a hospital dermatology department specializing in occupational dermatology and allergy (Department of Dermatology, VU University Medical Centre, Amsterdam, The Netherlands). In addition, the apprentices received advice on preventive measures and skin care.

Final questionnaire

At the end of the study period of 3 years, an email questionnaire was sent to all apprentices who were still participating in the study. This final questionnaire included

items on symptoms experienced during follow-up, localization, duration, recurrence and date of first occurrence of the symptoms, consultation of general practitioners or dermatologists, changes in hand hygiene behaviour as a result of symptoms, changes in hand hygiene behaviour as a result of participation in the study, use of protective hand cream, information on traineeships and side jobs, and smoking. Participants who did not respond to the email questionnaire after up to three reminders received a paper version.

Statistical analysis

Demographic characteristics, prevalence of atopic dermatitis, rhinitis or asthma and prevalence of hand eczema symptoms at baseline were compared between participants and non-participating classmates by calculating the percentage differences and 95% confidence intervals (CIs) based on a binomial distribution.

For the analysis of associations between exposure factors and the presence of (mild) hand eczema during follow-up, data from the exposure cards were used. For each apprentice, the data of each wet work activity from all cards of one traineeship were averaged, and the mean values per person were subsequently used in a mixed models design. Hand eczema is a disease with a fluctuating course, and the recovery time may be as short as a few days. Thus, the apprentice nurses would have time to recover from hand eczema in between traineeship periods. We therefore assumed that the probability of hand eczema developing in one traineeship does not depend on the exposure in previous traineeships. Each traineeship of one participant was therefore counted as a separate entity, and data from participants who entered a second or a third traineeship were entered as multiple records in the database. This, however, results in the problem that, regardless of susceptibility, participants who contributed data for multiple traineeships would have had more opportunities to develop hand eczema than those who had been followed for only one traineeship. Therefore, a mixed models design was used in the analyses, with participant identification number included as random effect (procedure GENLINMIXED in SPSS™). In such a mixed model, the within-subject correlation is taken into account. First, univariate mixed models were performed with each exposure factor separately as independent variable. Exposure factors showing a p-value of < 0.20 were included in a multivariate mixed model. In the univariate and multivariate mixed models, 90% CIs were used as these correspond to one-sided testing with $p < 0.05$.

RESULTS

Subject characteristics and follow-up

Between September 2008 and February 2011, a total of 728 apprentices signed up to participate in the study. Seven were excluded because of chronic inflammatory disease, leaving a cohort size of 721. The characteristics of the study population at

baseline are shown in Table 1. In addition, Table 1 includes a comparison of participants and non-participants in a subset of six schools. A history of atopic dermatitis, rhinitis, and asthma and lifetime prevalence of hand eczema were significantly more prevalent in participants. Reporting of hand eczema symptoms at baseline was significantly associated with a history of atopic dermatitis, rhinitis and asthma (data not shown). Associations between hand eczema and personal susceptibility factors are presented in Part II of this cohort study [25].

Fig. 1 presents a flow scheme of the study population in time. One hundred and eighty-eight participants (26%) were lost to follow-up shortly after they had completed the inclusion questionnaire, or were excluded from the analysis for another reason, for example because they had not performed any traineeship or had missing information about exposure or hand eczema symptoms during follow-up. Thus, 533 of the 721 included participants (74%) were followed for 1, 2 or 3 years. One hundred and fifty-nine apprentices quit their participation before the end of the study period: 62 for practical reasons (e.g. quit apprenticeship or changed career, went abroad, or entered a long period of only theoretical classes), 48 for motivational reasons (e.g. being too busy or not motivated to keep on filling in the diary cards)

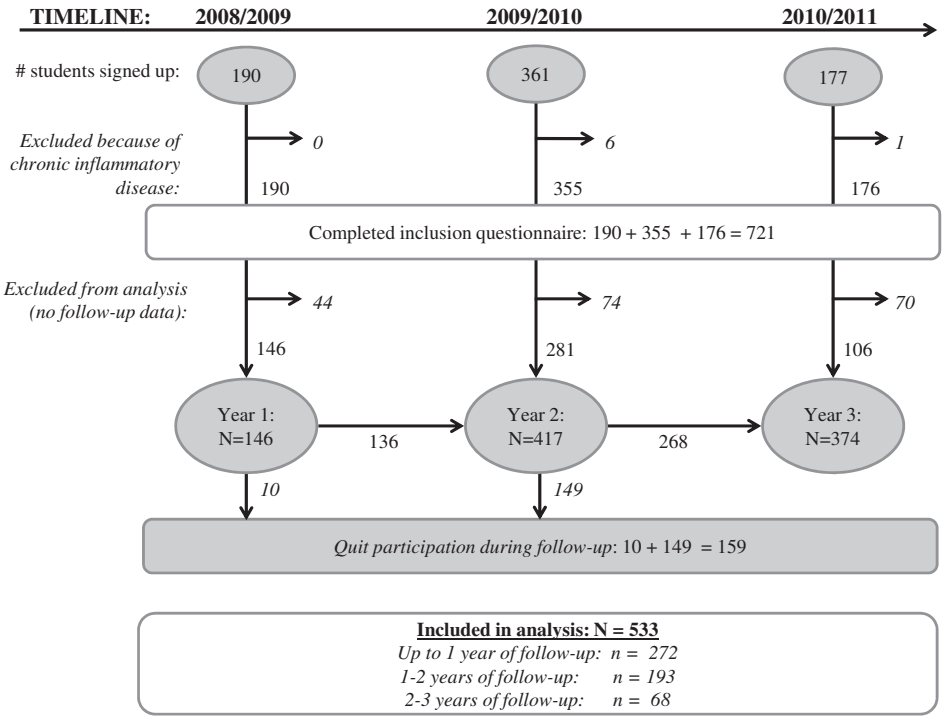

Fig. 1. Flow scheme of study population.

Table 1. Characteristics of the study population and comparison of participants with non-participants

	All participants included in cohort	Subset of six school visits where the questionnaire was filled in by all apprentices regardless of participation		
	n = 721	Willing to participate n = 106	Not willing to participate n = 99	Difference (± 95% confidence interval)
Female sex (%)	90	91	91	-1% (-9% – 7%)
Age at inclusion (years)[a] Median (25 - 75% percentile)	19.5 (18.3 – 20.9)			
Lifetime prevalence of atopic dermatitis (%)	24.3	25	13	11% (0.6% – 22%) *
Persistent atopic dermatitis (started in childhood and still present at time of inclusion) (%)	6	7	3	4% (-3% – 10%)
Eczema[b] with onset at < 5 years of age (%)	13	11	4	7% (-0.3% – 15%)
Eczema[b] with onset at > 18 years of age (%)	21	21	17	4% (-7% – 14%)
History of rhinitis (%)	46	55	34	20% (7% – 33%) *
History of asthma (%)	17	24	10	14% (3% – 24%) *
Symptoms caused by common allergens (dust, pollen, animals, food) (%)	44	44	33	11% (-2% – 24%)
Any symptoms on hands/fingers currently present (at the time of completing the questionnaire) (%)	24	20	18	2% (-9% – 12%)
Any symptoms on hands/fingers ever (currently present or in the past) (%)	54	49	37	12% (-2% – 25%)
Hand eczema currently present (at the time of completing the questionnaire) (%)	7	4	1	3% (-3% – 8%)
Hand eczema ever (currently present or in the past) (%)	16	15	6	9% (0.4% – 18%) *
General dry skin (%)	17	18	12	6% (-4% – 16%)

[a] Age at inclusion could not be calculated in the subset, because birth date was not known for non-participants.
[b] Eczema was defined as an itchy rash on the face, flexures, wrists/ankles, hands, or other locations.
* *p*-value of difference < 0.05.

and 49 for other (unspecified) reasons (Fig 1). There were no substantial differences in baseline characteristics between participants who completed one or more study years and those who were excluded from the analysis. However, of the participants with hand eczema during follow-up, 80% completed their participation until the end of the study period, as compared with 56% of those without hand eczema (chi-square, $p < 0.0001$).

Hand eczema during follow-up

Of those participants with sufficient follow-up information (n = 533), 285 (53%) reported any symptoms on their hands or fingers at any time during follow-up. The most commonly reported symptom was redness (reported by 38%), followed by itching (31%), scaling (25%), fissures (18%), papules (12%) and vesicles (7%). Of the 445 participants who had no history of hand eczema up to the time of inclusion, 81 (18%) developed hand eczema during the study. Most of these new cases occurred during the first traineeship, with an incidence rate of 36.7/100 person-years in traineeship. The incidence for the second and third year was combined because of the small number of participants in the third year; the incidence rate appeared to be 13.7/100 person-years in traineeship. The median duration of practical training was 20 weeks/year, with interquartile limits from 10 to 20 weeks. Calculation of incidence rates with the total period of follow-up per person instead of only the time in practical training resulted in incidence rates of 20.0/100 person-years and 8.5/100 person-years in the first year and second plus third year, respectively. Of the 88 participants with a history of hand eczema reported at inclusion, 47 (53%) reported hand eczema during their traineeship(s). Thirty-five of these 88 participants had hand eczema at the time of inclusion; 20 of them also reported hand eczema during their first traineeship. The period prevalence rates of mild hand eczema were 33% in the first year, 29% in the second year and 31% in the third year. The period prevalence rates of hand eczema were 21%, 25%, and 31%, respectively.

Figure 2 shows the period prevalence of hand eczema during the subsequent traineeships, differentiated by first and recurrent episodes of hand eczema. Approximately 15% of the participants who were followed during two subsequent traineeships (n=261) had hand eczema during both traineeships. In the subset of participants who were followed during three traineeships (n=68), a similar percentage had hand eczema during all three traineeships.

Less than one-third of the apprentices invited by the occupational physician accepted the invitation. The main reasons for declining were that the symptoms had resolved spontaneously, that symptoms could be controlled easily by using skin care products on their own initiative, that treatment had already been started by the subject's own general practitioner or dermatologist, or that the subjects just considered it to be 'not necessary'. A consultation was successfully arranged in 52 cases. Table 2 shows that 90% of the cases seen by the occupational physician were diagnosed with contact dermatitis.

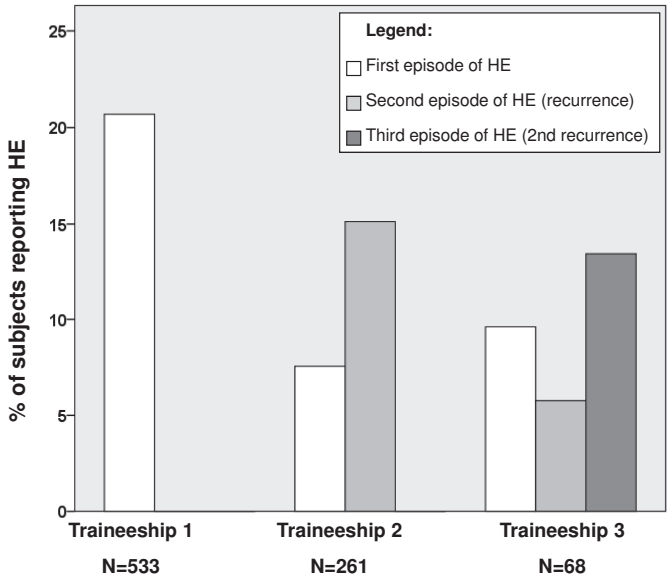

Fig. 2. Reported hand eczema (HE) during traineeships (period prevalence) in the first, second and third year of follow-up in a prospective cohort of apprentice nurses.

Table 2. Diagnoses of suspected skin symptoms in 52 apprentice nurses who were examined by a specialized occupational physician

Diagnose	n (%)	Remarks
Irritant contact dermatitis without atopy	21 (40)	-
Irritant and/or allergic contact dermatitis without atopy	5 (10)	Including 2 cases of protein contact dermatitis
Atopic dermatitis	2 (4)	-
Irritant contact dermatitis in combination with atopic dermatitis	14 (27)	Including 3 cases of irritant-provoked AD
Irritant and/or allergic contact dermatitis in combination with atopic dermatitis	7 (13)	Including 2 cases where atopic constitution was unclear
Other / unclear	3 (6)	-

Exposure to wet work in subjects with completed diary cards

A total of 2291 diary cards were returned, representing exposure data of 383 apprentices. Exposure-reponse relationships were studied in this subset of participants. Different types of wet work reported on the diary cards are shown in Table 3 and Fig. 3. The most reported type of wet work was hand washing, followed by the use of hand alcohol gel rubs, wearing of gloves, and other types of wet work such as contact with water and soap (e.g. when washing a patient) or disinfectants (e.g. when cleaning

medical equipment). The type of wet work exposure differed between healthcare sectors; for example, hand disinfection with alcohol gel rubs constituted a great deal of the exposure in hospitals, whereas in other sectors, hand washing was the most frequent wet work activity (Fig. 3).

Table 3. Exposure to wet work during one work shift as reported by 383 apprentice nurses

Wet work activity	% of apprentices reporting this activity minimally once a shift	Frequency per shift, median (25% - 75%)	Maximum frequency per shift
Hand washing			
water only	86	3 (1 – 5)	19
water and soap	99	6 (4 – 9)	21
Total	100	8 (6 – 12)	31
Hand disinfection (alcohol gel rub)			
Total	87	5 (2 – 11)	30
Wearing occlusive gloves			
With a duration of < 5 min per occasion	85	3 (1 – 4)	13
With a duration of 5-14 min per occasion	74	2 (1 – 3)	12
With a duration of 15-29 min per occasion	34	1 (1 – 2)	7
With a duration of > 30 min per occasion	11	1 (1 – 2)	4
Total (all occasions)	93	4 (2 – 6)	24
Contact with water (other than hand washing)			
With a duration of < 1 min per occasion	75	2 (1 – 3)	13
With a duration of 1- 14 min per occasion	64	2 (1 – 2)	10
With a duration of > 15 min per occasion	12	1 (1 – 2)	6
Total (all occasions)	89	3 (1 – 4)	24
Contact with soap or detergents (other than hand washing)			
With a duration of < 1 min per occasion	59	2 (1 – 3)	16
With a duration of 1-14 min per occasion	78	2 (1 – 3)	16
With a duration of > 15 min per occasion	23	1 (1 – 2)	8
Total (all occasions)	88	4 (1 – 5)	23
Contact with disinfectants (other than alcohol gel rubs)			
With a duration of < 1 min per occasion	44	2 (1 – 3)	16
With a duration of 1-14 min per occasion	21	2 (1 – 2)	16
With a duration of > 15 min per occasion	2	2 (1 – 2)	9
Total (all occasions)	52	2 (1 – 3)	27

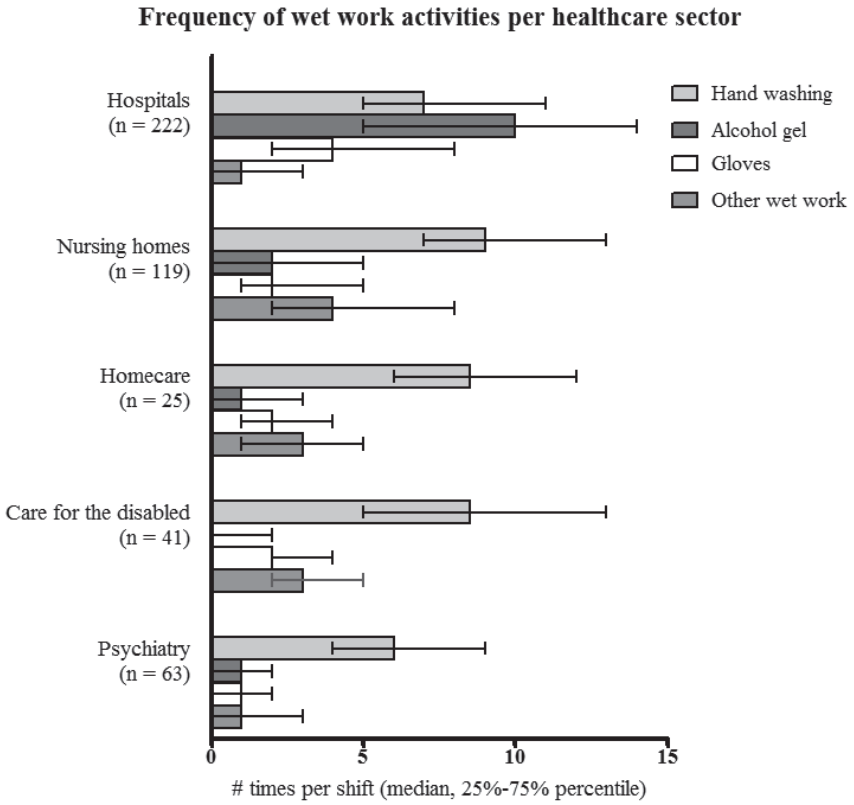

Fig. 3. Frequency of wet work (median and interquartile limits) reported by 383 apprentice nurses during 470 traineeships, stratified by healthcare sector. 'n' refers to the number of participants who worked in the healthcare sector concerned; the sum of n exceeds 383 because a number of apprentices participated in more than one traineeship.

The German TRGS 401, the only existing guideline addressing wet work exposure that we know of, recommends that, if the total duration of wet work exposure exceeds 2 hr/day, protective measures should be taken [2], and a Dutch expert group involved in the definition of guidelines for occupational contact dermatitis stated that a frequency of wet work exposure of > 20 times/day is a risk factor for developing occupational contact dermatitis [29]. For 111 of the 383 participants (29%), the mean wet work exposure exceeded 2 hr/day, or the maximum frequency of hand washing or disinfection exceeded 20 times/day. The frequency of exceeding 2 hr/day of wet work and/or frequent hand washing or disinfection differed between healthcare sectors; 43% of the participants in hospital traineeships reported exposure exceeding these cut-offs, as compared with 17% of participants working in nursing homes, 12% of participants working in disabled care, 11% of participants working in homecare, and 6% of participants working in psychiatry. The difference between hospitals and each of the other sectors was statistically significant (all chi-square, $p < 0.0001$).

To study associations between different types of exposure and prevalence of hand eczema reported on the diary cards, generalized mixed models were used. There was some correlation between the different types of wet work, but not to a sufficient extent to disturb the regression approach (highest Spearman $r = 0.56$). In univariate analyses, the frequency of application of hand alcohol gel rubs, the frequency or duration of glove wearing and the frequency or total duration (all episodes summated) of contact with water only did not have a significant effect ($p > 0.20$), and only the frequency of hand washing, the frequency of contact with soap or detergents (other than hand washing) and the frequency of contact with disinfectants (other than hand alcohol gel rubs) were introduced in the multivariate analysis. In the multivariate analysis, only frequent hand washing (more than eight times per shift) was associated with hand eczema during traineeships [odds ratio (OR) 1.5; 90% CI 1.02 – 2.25], and there was a tendency for there to be an effect of contact with soap or detergents (more than four times per shift) (OR 1.5; 90% CI 0.97 – 2.30; Table 4). The effect of hand washing on mild hand eczema was similar to that on hand eczema (OR 1.6; 90% CI 1.16 – 2.32).

Table 4. Association of different types of wet work with prevalence of hand eczema during traineeships in 383 apprentice nurses (multivariate mixed model)

		Mild hand eczema during traineeships (207 episodes)		Hand eczema during traineeships (128 episodes)	
		Odds ratio (exp β)	90% confidence interval	Odds ratio (exp β)	90% confidence interval
Hand washing	> 8 times per shift versus < 8 times per shift[a]	1.6	(1.16 – 2.32)*	1.5	(1.02 – 2.25)*
Contact with soap or detergents, other than hand washing	> 4 times per shift versus < 4 times per shift[a]	1.3	(0.86 – 1.87)	1.5	(0.97 – 2.30)
Contact with disinfectants, other than alcohol hand rubs	> 2 times per shift versus < 2 times per shift[a]	1.1	(0.73 –1.71)	1.1	(0.69 – 1.79)

[a] Cut-off points for hand washing, contact with soap or detergents and contact with disinfectants were based on the median reported frequencies (see Table 3).
* $p < 0.10$.

Exposure outside of traineeships

Almost half of the participants (46%) reported having a job on the side, of whom 72% were working in healthcare, 15% were working in other sectors involving wet work (e.g. bars and restaurants) and 13% had side jobs not involving wet work. In univariate

analysis, work in healthcare, catering or other side jobs that involved wet work for > 8 hr/week was associated with prevalence of hand eczema during follow-up (OR 1.6; 90% CI 1.04 – 2.37) in comparison to absence or shorter duration of similar work. Hand washing at home > 10 times/day according to the inclusion questionnaire, which was reported by 17% of participants, was associated with hand eczema during follow-up (OR 2.3; 90% CI 1.42 – 3.64) in comparison to < 10 times/day. There was no substantial correlation between the frequency of hand washing at home and that in traineeships (Spearman $r = 0.14$).

Use of hand cream and changed behaviour regarding wet work exposure

Hand cream was used significantly more often by those who reported symptoms (e.g. itch and fissures) on the diary cards; 66% of participants without symptoms used hand cream, as compared with 84% of those with symptoms (chi-square, $p < 0.0001$). The mean frequencies of hand cream use per day were 1-10 times/day in the group without symptoms and 1-14 times/day in the group with symptoms. In the final email questionnaire, which was returned by 323 participants (response rate of 57%), the participants were asked whether they had changed their behaviour with respect to hand hygiene as a result of having symptoms. Of the 169 participants who reported any symptoms during follow-up in the final questionnaire (52%), 78 (46%) indicated that they had taken measures to reduce their symptoms. The most often reported measure was 'using more hand cream' (n=51), followed by attempts to reduce the frequency of hand hygiene activities or changing the type of hand hygiene products used (n=24), and wearing gloves more often (n=9). Eight participants had increased their use of hand alcohol gel rubs as alternative to washing with water and soap. In contrast, 11 participants had decreased their use of alcohol gel rubs, and instead used water and soap more often, because of the stinging sensation that they felt when using alcohol gels.

DISCUSSION

In this prospective cohort of Dutch apprentice nurses, we found that wet work exposure during traineeships varied between different healthcare sectors, and exceeded 2 hr/day and/or included hand washing or disinfection exceeding 20 times/day in 29% of participants. The 1-year period prevalence rates of hand eczema were 23% in the first year of follow-up, 25% in the second year, and 31% in the third year. Eighty-one new cases of hand eczema developed, most of which occurred during the first year of follow-up. In addition, ~15% of participants had recurrence of hand eczema during subsequent traineeships 1 or 2 years later. Frequent hand washing during traineeships (OR 1.5), frequent hand washing at home (OR 2.3) and having a side job involving wet work (OR 1.6) were risk factors for hand eczema during follow-up.

Other studies among nursing apprentices have reported 1-year prevalence rates ranging from 10% to 27% [7;30;31], which is in the range of our results. In a smaller prospective cohort study among Dutch apprentice nurses, a higher incidence rate was also found in the first year of follow-up (19.8/ 100 person-years) than in the second year (5.2/100 person-years) [8]. The same research group found 34 new cases of hand eczema over a follow-up period of (maximal) 33 months in a retrospective cohort of 371 newly employed nurses in a university hospital. The majority had developed the disease within the first 3 months of employment [32]. Several prospective cohort studies among apprentice hairdressers [5;8;9] and a survey among vocational trainees in a variety of high-risk occupations [33] have also indicated that the incidence of new hand eczema cases is highest shortly after the start of exposure to skin irritants.

To our knowledge, this is the first prospective study on hand eczema in which detailed information on exposure was collected at the individual level for a relatively large cohort. In our study, information on wet work exposure and skin symptoms was collected by means of diary cards, which were regularly sent to the participants. The advantage of the diary card method is that the information obtained supposedly reflects the daily exposure more accurately than retrospective questionnaires. Retrospective self-reporting tends to be influenced by recall bias, as shown by validation studies of questionnaires for self-reporting of wet work exposure among nurses [34;35]. The reported frequency of wet work activities on the cards in our study agreed with recent observational studies of wet work in hospital and geriatric nursing wards, in which the reported frequencies of hand washing, hand alcohol use and glove use were similar to our data [34-36].

The only wet work characteristic associated with (mild) hand eczema during the traineeships was the daily frequency of hand washing, with ORs of 1.6 and 1.5 for mild hand eczema and hand eczema, respectively. Furthermore, there was a tendency for other contact with soap or detergents to also increase the risk of hand eczema. The reported ORs are a fairly good approximation of the corresponding relative risks (hand eczema was reported for 24% of all traineeships observed, and the numbers of traineeships with high and low exposure were approximately equal). As 20 participants had hand eczema both at inclusion and during their first traineeships, it was unknown whether these cases of hand eczema had been caused by exposure during traineeship or were a continuation of pre-existing hand eczema. Repeated analysis excluding these 20 participants showed similar results, namely an increased risk of hand eczema conferred by hand washing and other contact with soap or detergents, but not by contact with disinfectants. These findings are in agreement with earlier epidemiological studies among hospital populations [4;37;38] and with experimental studies showing that soap and detergents are more damaging to the skin than, for example, alcohol-based hand disinfectants [39-41].

Diagnostic criteria constitute an important issue in epidemiological studies. In this study, we used a symptom-based definition of hand eczema. Previous validation studies have shown that symptom-based classifications tend to overestimate the prevalence of hand eczema, whereas self-reported hand eczema ('Do you have hand

eczema?') underestimates the prevalence [42;43]. Because we wanted to assess new cases of hand eczema, including mild cases of beginning hand eczema, we deliberately chose to use a symptom-based definition. As a result, the prevalence and incidence of hand eczema may have been slightly overestimated. It is reassuring to some extent that 90% of the 52 apprentices examined by a specialized occupational physician (a subset of approximately one-third of the apprentices classified as having hand eczema) were diagnosed as having irritant or allergic contact dermatitis. However, more than two-thirds of the apprentices invited for a consultation did not contact the physician. The most frequently mentioned reasons for declining the invitation (for example, symptoms had diminished with the use of hand cream on the subject's own initiative, or the subject did not consider it necessary to consult a physician) indicate that many apprentices considered their symptoms to be mild and not severe enough for them to consult a physician. A similarly low attendance rate was found by Jungbauer et al. [6], who invited 160 healthcare employees reporting symptoms of hand eczema in a questionnaire to consult a specialized occupational dermatology nurse. Less than half (46%) of the invited employees attended the consultation; the reasons for declining the invitation were not known, but it was suspected that individuals with milder complaints were less likely to attend the consultation.

One limitation of our study is that, as indicated in Table 1, subjects with atopic dermatitis, rhinitis and asthma were overrepresented in this cohort, as these atopic features were more common in participants than in non-participants (selection bias). Because atopic dermatitis is a known risk factor for hand eczema, this probably resulted in a higher proportion of participants with increased susceptibility to hand eczema, presumably leading to a slight overestimation of prevalence and incidence rates in apprentice nurses. Although the effects of exposure may be different in participants with atopic dermatitis and in participants without atopic dermatitis, the overrepresentation of atopic dermatitis in the group involved in exposure analysis (n=383) was so small (a frequency of 26% in the 383 participants, as compared with 19% in the unselected subsample of 205 apprentices in Table 1) that its influence on the effect size of the specific wet work activities can be regarded as negligible.

Apparently, having a history of atopic dermatitis does not seem to prevent youngsters from choosing occupations with high skin exposure. The impact of personal susceptibility factors on the risk of hand eczema in this cohort is described in more detail in Part II of this study [25].

Two recommendations for practice can be made on the basis of the results of this study. First, our results support the advice in the recent guideline on hand hygiene [44] to replace hand washing with hand disinfection using alcohol gel rubs where possible (i.e. if the hands are not visibly dirty) and promote the use of protective gloves. However, some apprentices with hand eczema appeared to prefer the use of water and soap over hand alcohol gel rubs, because of the stinging sensation that they experienced when applying the latter on their damaged skin. This indicates a need for education of vocational students on the effects of different hand hygiene procedures on the skin. Furthermore,

the guideline requires that alcohol gel rubs and suitable protective gloves be available at the workplace. Work sites in nursing homes, and especially in homecare, however, are not always equipped with proper hand disinfection products and the correct type of protective gloves, which hampers nurses' control over their own exposure.

Second, good access to and a positive attitude of vocational students towards consultation of an occupational physician should be promoted. Also individuals with mild hand eczema may benefit from a consultation with their occupational physician, because, apart from skin care advice, advice on working practices and exposure reduction is important in the case of occupational hand eczema, and this kind of counselling usually goes beyond what occurs in the consultation of general practitioners. Furthermore, timely measures for skin protection at the workplace may prevent mild symptoms from progressing into manifest hand eczema. Education of vocational students on this topic may help to increase attendance of consultations in the future.

In conclusion, this prospective cohort of Dutch student nurses has confirmed that, during vocational training, many apprentice nurses already have too much exposure to wet work and that they are at substantially increased risk of developing hand eczema. More attention should be paid in vocational education to the effects of wet work and to skin protection.

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SUPPLEMENTARY FILE: FOLDING-CARD FOR RECORDING WET WORK EXPOSURE AND SKIN SYMPTOMS

Hospital: Date: Department:

☐ Early shift ☐ Late shift ☐ Night shift

A. Hand washing

Water only	
Water + Soap / Shampoo / Showergel	
Water + Other, namely:	

B. Hand disinfection (alcohol gel rubs)

Hand alcohol gel rub	

C. Use of gloves

< 5 min	5 – 15 min	15 – 30 min	> 30 min

D. Use of hand cream / moisturizer

Hand cream	

E. Other work tasks *where your hands get wet*

	Very short (< 1 min)	1 – 15 min	> 15 min
Contact with water only			
Contact with water + soap / showergel / shampoo			
Contact with disinfectants			

F. Use of pre-heated wash towels

Pre-heated wash towels	

Don't forget to fill in the inside of the card !!

G. To be filled in at the end of your shift:

Did today's shift deviate from normal shifts regarding wet work?

- ☐ No
- ☐ Yes, I did **more wet work** than usual
- ☐ Yes, I used **gloves more often** / **longer** than usual
- ☐ Yes, I did **less wet work** than usual
- ☐ Yes, I used **gloves less often** / **shorter** than usual

During the past 2 weeks, has your skin been exposed to water or irritants *outside your traineeship*? (e.g. working in a bar, doing extra household chores, swimming)

- ☐ No
- ☐ Yes :

If yes, what did you do?

On how many days did you do it?

On average, how long did you perform 'wet work' per day?

Did you, since the last time you filled in a diary card, experience one or more of the following symptoms on your hands and/or fingers?

- ☐ No
- ☐ Yes, my hands or fingers clearly showed:
  - Redness ..... ☐
  - Scaling ..... ☐
  - Itching ..... ☐
  - Fissures ..... ☐
  - Vesicles ..... ☐
  - Bumps ..... ☐

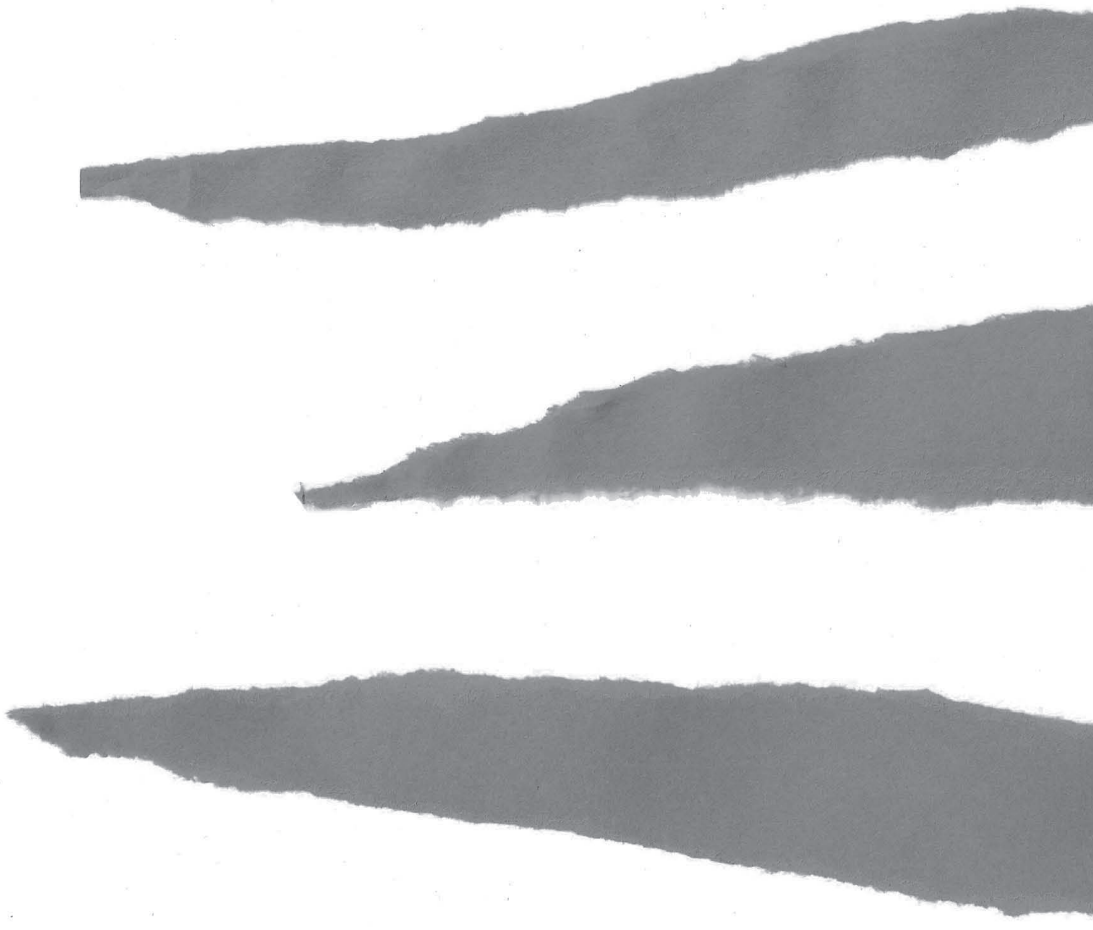
Did the symptoms last for more than 3 days?

- ☐ No
- ☐ Yes

Attention please:

- Did any unusual events occur today, which led to skin exposure to substances that are not mentioned on this diary card?
- Do you have skin complaints on your hands that are not mentioned in the above list?
- Or do you have other comments / remarks?

Then please note your remarks on the backside of this diary card!



3

SUSCEPTIBILITY

3.1

IMPACT OF ATOPIC DERMATITIS AND LOSS-OF-FUNCTION MUTATIONS IN THE FILAGGRIN GENE ON THE DEVELOPMENT OF OCCUPATIONAL IRRITANT CONTACT DERMATITIS

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ABSTRACT

Background

Atopic dermatitis (AD) and loss-of-function mutations in the filaggrin gene (*FLG*) are both associated with chronic irritant contact dermatitis (ICD). As *FLG* mutations also are a major risk factor for AD, it is not clear whether *FLG* mutations are an independent risk factor for ICD or whether the risk is mediated by AD.

Objectives

To investigate the relative contribution and interaction of *FLG* mutations and AD in German patients with occupational ICD and controls (vocational school apprentices).

Methods

A total of 634 patients and 393 controls were genotyped for R501X, 2282del4, R2447X and S3247X. Current or past flexural eczema was used as an indicator of AD.

Results

FLG mutations were found in 15.9% of the patients with ICD and 8.3% of the controls, with a crude odds ratio (OR) of 2.09 [95% confidence interval (CI) 1.33 – 3.28] for the combined genotype. The adjusted OR for *FLG* mutations, corrected for AD, was 1.62 (95% CI 1.01 – 2.58). Subjects with AD were at approximately three times higher risk to develop ICD (OR= 2.89; 95% CI 2.09 – 3.99). There was no evidence of an interaction between these two risk factors.

Conclusions

Our results indicate that both *FLG* mutations and AD increase the risk of ICD. Individuals with concurrent *FLG* mutations and AD are at the highest risk of developing ICD.

INTRODUCTION

Contact dermatitis (CD) is one of the most common occupational diseases in industrialized countries. In European surveys among workers and apprentices in 'high-risk' occupations, such as hairdressing, healthcare and metalworking, the 1-year prevalence varied between 20% and 30%, and mild skin symptoms were present in up to 50% of the workers or apprentices [1-7]. CD is commonly divided into allergic CD (ACD) and irritant CD (ICD), of which ICD is the more common in occupational settings [8]. ICD is caused by repeated exposure to irritants, for example soaps, detergents, disinfectants and water ('wet work') [9]. In addition to environmental factors, it is assumed that the risk of developing ICD is influenced by endogenous factors, of which atopic dermatitis (AD) is the most important. It has been estimated that AD increases the risk of developing ICD by a factor of between two and four [10;11]. In Germany and the Netherlands, a history of AD is used to identify susceptible individuals in prevention programs in high risk occupations [12;13]. However, the mechanisms by which AD influences the development of ICD are still largely unknown. Patients with AD have an impaired epidermal barrier function, even in uninvolved skin [14;15]. One possible factor contributing to an impaired skin barrier function in AD is a decreased level of the epidermal protein filaggrin, caused by loss-of function mutations in the filaggrin (*FLG)* gene. Filaggrin is important for the structure, function and hydration of the stratum corneum [16], which is the principal barrier of the skin. Reduced levels of filaggrin may lead to increased penetration of irritants and allergens through the skin, and subsequent inflammation [17-20]. *FLG* loss-of-function mutations are a major risk factor for AD; approximately 20-30% of patients with AD carry a *FLG* mutation [21-30]. However, skin barrier is also reduced in some patients with AD who do not carry *FLG* mutations [15], and the majority of heterozygous *FLG* carriers never develop AD [31].

So far it is unclear whether *FLG* mutations are an independent risk factor for ICD – e.g. due to a deficient skin barrier or an altered inflammatory status – or if they work through AD. It is also not clear how these mutations interact with the inflammatory processes that are characteristic of AD.

We previously studied the prevalence of the R501X and 2282del4 *FLG* mutations in 296 patients with ICD and a control group of 217 vocational school apprentices. We showed that *FLG* mutations doubled the risk for occupational ICD [32]. Here we present a continuation of that study, increasing the number of patients (n=634) and controls (n= 393). In addition to R501X and 2282del4, we genotyped our samples for two less common null mutations: R2447X and S3247X. Together these four different mutations constitute to more than 90% of the *FLG* mutations found in European populations [33]. The aim of the present study was to investigate the relative contribution and interaction of *FLG* mutations and AD to the risk of acquiring ICD.

METHODS

Study population

Ethical approval was obtained from the ethics committee of the University of Osnabrück.

Patients were recruited from a specialized clinic for treatment of occupational skin diseases, following a joint study protocol [34;35]. Between 2005 and 2011, all consecutive patients who presented with chronic CD of the hands for at least 3 months (either present at the time of examination or medically verified in the past), were of European descent, were at least 18 years of age and did not suffer from further chronic inflammatory diseases (e.g. rheumatoid arthritis, Crohn disease, systemic lupus erythematosus or psoriasis), as assessed by anamnesis, were asked to provide a DNA sample obtained by a buccal swab. A total of 634 patients fulfilling these inclusion criteria and having a primary diagnosis of ICD according to the dermatologists were included in the present study. For each patient, a full medical and dermatological history was taken, including information about sex, age, diagnosis, age of onset of CD and history of flexural eczema. The diagnosis of ICD was based on a patient's history, exposure to irritants, clinical distribution, presence of skin lesions and exclusion of other dermatologic entities, and patients having no clinically relevant type-IV-sensitization. Patients were patch tested to an extended range of allergens, including standardized and customized substances. All patients were tested at least with the European standard tray, and tests were conducted and read according to international guidelines [36]. Controls were recruited from vocational schools training students in high-risk occupations for hand eczema, e.g. hairdressing, nursing, metalworking, food and catering, or floristry. Of the 500 trainees asked to participate, 477 agreed. Of these, 84 were excluded because they were not of European descent or because they suffered from chronic inflammatory disease (e.g. rheumatoid arthritis or psoriasis). The remaining 393 trainees were in their second or third year of schooling. Following written informed consent, the controls were asked to complete a questionnaire including information about sex, age, and medical history, particularly with regard to the skin and to atopic symptoms (flexural eczema, rhinitis and asthma). Additionally, a subset of 245 students underwent a brief examination by an experienced dermatologist, who assessed present flexural eczema, anamnesis of childhood eczema and family history of rhinoconjunctivitis, allergic asthma and AD. Current or past flexural eczema in the patients, or self-reported flexural eczema in the controls was used as an indicator of AD.

Filaggrin genotyping

DNA material was obtained from buccal mucosa cells with buccal swabs (Geneticlab Diagnostic & Research, Pordenone, Italy). For each subject, two swabs were obtained and 2 ml lysis buffer (Puregene® Cell Lysis Solution, Gentra Systems, Minneapolis, MN, USA) was added to each swab to disrupt the cells and stabilize the DNA. Extraction and genotyping for R501X, R2447X and S3247X was performed by KBioscience (http://

www.kbioscience.co.uk). Genotyping was performed using the KASP single nucleotide polymorphism genotyping system (KBioscience), a homogeneous fluorescent resonance energy transfer-based system, coupled with competitive allele specific polymerase chain reaction (PCR). Blind duplicates and Hardy-Weinberg equilibrium tests were used as quality control tests.

R501X was genotyped by using the primer pair 5'-GAATGCCTGGAGCTGTCTCG-3' (C-allele) and 5'-CTGAATGCCTGGAGCTGTCTCA-3' (T-allele) with the common allele primer 5'-GCACTGGAGGAAGACAAGGATCG-3'. R2447X was genotyped by using the primer pair 5'-GAGTGCCTGGAGCTGTCTCG-3' (C-allele) and 5'-GAGTGCCTGGAGCTGTCTCA-3' (T-allele) with the common allele primer 5'-GAGGAAGACAAGGATCCCACCACA-3'. S3247X was genotyped by using the primer pair 5'-GTGTCTGGAGCCGTGCCTTG-3' (C-allele) and 5'-GGTGTCTGGAGCCGTGCCTTT-3' (A-allele) with the common primer 5'-CTTCCAGAAACCATCGTGGATCTGT-3'.

Genotyping for 2282del4 was performed by sizing a fluorescently labeled PCR fragment on an Applied Biosystems 3100 or 3730 DNA sequencer (Applied Biosystems, Foster City, CA, U.S.A.) as described previously [26;32].

Statistical analysis

The observed genotype frequencies were compared with the expected Hardy-Weinberg distribution by χ^2-test using an online calculator [37]. Differences in median age of onset of ICD among the patients were assessed with the Mann-Whitney U-test. To estimate the risk of disease conferred by a particular genotype, we calculated the odds ratios (ORs) with 95% confidence intervals (CIs) using χ^2-tests comparing the heterozygous and homozygous variant allele genotypes with the wildtype genotype. The effect of *FLG* loss-of-function mutations, AD and possible interaction effects were analysed using logistic regression with backwards selection of variables. The statistical analyses were performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, U.S.A.).

RESULTS

The demographic characteristics of patients and controls are shown in Table 1. The median ages of the patients and controls were 43 and 19 years, respectively. The median age at onset of ICD among the patients was 32 years, except for hairdressers and beauticians, who developed ICD on average at 19 years of age. The age at onset of ICD was significantly lower in patients with AD than in patients without AD (median age 25 vs. 37 years; $p < 0.0001$). *FLG* loss-of-function mutations did not influence the age of onset of ICD in the patients (data not shown).

The genotype distributions of the 2282del4, R501X, R2447X and S3247X polymorphisms observed in patients and controls did not deviate significantly from the Hardy-Weinberg equilibrium. *FLG* loss-of-function mutation prevalence and allele

Table 1. Demographic characteristics of patients and controls

		Patients[a]	Controls[a]
Total N		634	393
Sex, n (%)			
	Males	236 (38)	149 (38)
	Females	392 (62)	244 (62)
Age (years), median (25 - 75%)		43 (31 - 51)	19 (18 – 22)
Occupational Sector, n (%)			
	Hairdressing / Beauty	75 (12)	48 (12)
	Healthcare	252 (40)	95 (24)
	Cleaning	28 (5)	-
	Metalwork / Mechanics	122 (20)	128 (33)
	Construction	30 (5)	-
	Food and Catering	44 (7)	18 (5)
	Floristry and Gardening	14 (2)	-
	Other	64 (10)	100 (26)
Age at onset of irritant contact dermatitis (years), median (25 - 75%)		32 (22 – 43)	-

[a] Subgroup totals may not add up to the total N due to missing data.

frequencies for patients and controls are displayed in Table 2. *FLG* loss-of-function mutations were significantly more prevalent in patients with ICD compared with controls, with a crude OR of 2.09 (95% CI 1.33 – 3.28) for the combined carrier allele.

Table 3 shows the prevalence of *FLG* mutations and AD in patients and controls. A history of flexural eczema, used as an indicator of AD, was about twice as common among patients with ICD compared with controls (41.1% vs. 18.7%, respectively). Of the 245 controls who underwent a brief dermatological examination, 40 (16.3%) reported present or past flexural eczema in the questionnaire. Five of them revealed flexural eczema on the day of examination and another 33 were diagnosed with childhood flexural eczema according to their past medical history. *FLG* mutations were present in 13.6% of controls with a history of AD, compared with 7.4% of controls without a history of AD. Among patients, the carrier frequencies of *FLG* mutations were 22.6% and 10.7% in patients with or without a history of AD, respectively. Approximately 70% of the controls with *FLG* mutations - i.e. 6% of the total control population - had no history of AD.

Logistic regression analysis revealed that both *FLG* mutations and AD were significant risk factors for ICD; the effect of AD (OR 2.89) exceeded that of *FLG* (OR 1.61; Table 4). There was no significant interaction effect between FLG mutations and AD ($p = 0.67$).

Table 2. Genotype and allele frequencies among patients (N = 634) and controls (N = 393)

Polymorphism	Genotype	Patients n (%)[a]	Controls n (%)[a]	Odds ratio (95% confidence interval)
R501X	AA	587 (94.5)	350 (97.5)	
	Aa	34 (5.5)	9 (2.5)	
	aa	0 (0.0)	0 (0.0)	
	Wild-type allele	1208 (97.3)	709 (98.7)	1.00
	Mutation allele	34 (2.7)	9 (1.3)	2.25 (1.07 – 4.75) *
2282del4	AA	567 (90.6)	350 (95.1)	
	Aa	58 (9.3)	18 (4.9)	
	aa	1 (0.2)	0 (0.0)	
	Wild-type allele	1192 (95.2)	718 (97.6)	1.00
	Mutation allele	60 (4.8)	18 (2.4)	2.02 (1.17 – 3.49) *
R2447X	AA	599 (99.2)	345 (99.4)	
	Aa	5 (0.8)	2 (0.6)	
	aa	0 (0.0)	0 (0.0)	
	Wild-type allele	1203 (99.6)	692 (99.7)	1.00
	Mutation allele	5 (0.4)	2 (0.3)	1.44 (0.28 – 7.46)
S3247X	AA	608 (99.2)	357 (99.7)	
	Aa	5 (0.8)	1 (0.3)	
	aa	0 (0.0)	0 (0.0)	
	Wild-type allele	1221 (99.6)	715 (99.9)	1.00
	Mutation allele	5 (0.4)	1 (0.1)	2.94 (0.34 – 25.23)
Combined	AA	499 (84.1)	299 (91.7)	
	Aa	87 (14.7)	27 (8.3)	
	aa[b]	7 (1.2)	0 (0.0)	
	Wild-type allele	1085 (91.5)	625 (95.9)	1.00
	Mutation allele	101 (8.5)	27 (4.1)	2.09 (1.33 – 3.28) *

[a] Total number of subjects may differ between polymorphisms due to genotyping failures.
[b] Homozygous or compound heterozygous.
* Significant at $p < 0.05$.

DISCUSSION

In this study we confirmed the association between *FLG* loss-of-function mutations and the risk of developing ICD that we reported in our previous pilot study, which included only two *FLG* mutations, R501X and 2282del4, and was carried out on a

Table 3. Prevalence of atopic dermatitis (AD) and filaggrin gene (*FLG*) loss-of-function mutations in patients and controls

		Controls			Patients		
		FLG loss-of-function mutation			*FLG* loss-of-function mutation		
		No	Yes	Total	No	Yes	Total
History of AD[a]	No	237 (75.2%)	19 (6.0%)	256 (81.3%)	301 (52.6%)	36 (6.3%)	337 (58.9%)
	Yes	51 (16.2%)	8 (2.5%)	59 (18.7%)	182 (31.8%)	53 (9.3%)	235 (41.1%)
	Total	288 (91.4%)	27 (8.6%)	315 (100%)	483 (84.4%)	89 (15.6%)	572 (100%)

[a] AD was defined by current or past flexural eczema.

Table 4. Logistic regression model for the increased risk of developing irritant contact dermatitis due to atopic dermatitis (AD) and filaggrin gene (*FLG*) loss-of-function mutations

		AD[a]		*FLG loss-of-function mutation*	
	Total N[b]	No n (%)	Yes n (%)	No n (%)	Yes n (%)
Controls	393	308 (81.3%)	71 (18.7%)	299 (91.7%)	27 (8.3%)
Patients	634	361 (59.2%)	249 (40.8%)	499 (84.1%)	94 (15.9%)
Adjusted odds ratio (95% confidence interval)[c]		2.89 (2.08 – 4.03)*		1.61 (1.01 – 2.58)*	

[a] AD was defined by current or past flexural eczema.
[b] Subgroup totals may not add up to the total N due to genotyping failures and/or missing data on flexural eczema.
[c] Adjusted for AD and *FLG* mutations, respectively.
* Significant at $p < 0.05$.

smaller sample [32]. The crude OR for the combined mutant allele based on four polymorphisms (R501X, 2282del4, R2447X and S3247X) was 2.09 (95% CI 1.33 – 3.28), which is comparable with the OR of 1.91 found in our earlier investigation. Here, we show for the first time a significant association of ICD with *FLG* loss-of-function mutations, even if the analysis is adjusted for AD (OR 1.61; 95% CI 1.01 – 2.58). A history of AD increased the risk to develop ICD approximately threefold (OR 2.89; 95% CI 2.08 – 4.03). Thus, according to the regression model, concomitant presence of AD and *FLG* mutations would result in a 4.7-fold increased risk.

We found *FLG* loss-of-function mutations in 15.9% of the ICD patients and in 8.3% of the controls. The *FLG* carrier frequency of 8.3% in our control group is in

agreement with a general prevalence of 7-10% in European populations [16]. Among cases with current or past AD, the prevalence of *FLG* loss-of-function mutations was 22.6%, which is in line with previously reported carrier frequencies of 21% for the four most common mutations in German adult patients with AD [23;30]. Theoretically, some selection bias could have occurred if more susceptible apprentices (e.g. with a history of AD) had chosen to avoid high risk occupations. However, as genotype distribution and prevalence of flexural eczema were similar to those reported in studies among the German general population, and dropout between the first and second year had been negligible, preselection of our control population was unlikely [38].

On the other hand, some recall bias could have occurred, as in our control group a history of flexural eczema, as a proxy for AD, was assessed by self-administered questionnaires. As in the patient group a history of flexural eczema was assessed by a standardized interview, we also performed examination by a dermatologist in a subset of controls. Self-reported history of flexural eczema correlated well with the dermatologist's conducted anamnesis. Furthermore, the prevalence of flexural eczema reported by our control subjects (19%) was in agreement with an earlier reported lifetime prevalence among German adolescent populations of 14 – 25% [39;40].

Another possible source of bias might be the age difference between controls and patients (median ages 19 and 43 years, respectively). Thus, we may have missed some cases of adult-onset AD in our control group. Epidemiologic data on late-onset AD are scarce, but some reports indicate that the proportion of patients with disease starting in adulthood is approximately 5% [40-42]. Therefore, we performed a second analysis with adjusted prevalence of AD in the controls (χ^2-test with Mantel Haenzel correction). Adjustment for age did not change the outcomes of the analysis.

To date, most studies investigating polymorphisms in *FLG* have focused on possible associations with AD, and only a few studies have addressed CD. In a 2009 pilot study Molin *et al.* [43] investigated two *FLG* loss-of-function mutations in 122 German nonatopic patients with different subtypes of chronic hand eczema (atopic hand eczema cases were excluded), and compared them to 95 control individuals of unknown origin. Marginally significant associations with *FLG* were reported for a subgroup of patients diagnosed with a combination of ICD and ACD, but not in the subgroup with ICD alone. However, several limitations limit the informative value of this study, such as the small sample size and the choice of the control population. In 2010, Thyssen *et al.* [44] performed a cross-sectional study genotyping R501X and 2282del4 in 3335 adults recruited from a random sample (n = 7931) of the Danish general population. The participants were patch tested and filled in a questionnaire addressing the presence of AD and hand eczema – including ICD, ACD, and atopic hand eczema – during the previous 12 months. *FLG* loss-of-function mutations were over-represented in cases of hand eczema in subjects with AD (OR 2.98; 95% CI: 1.27 – 7.01), but not in subjects without AD (OR 0.82; 95% CI 0.41 – 1.67). The combined presence of AD and *FLG* loss-of-function mutation status yielded an OR for hand eczema of 3.23 (95% CI 1.51 – 6.91).

The increased susceptibility to ICD in carriers of *FLG* mutations might at least partly be explained by barrier dysfunction, as demonstrated in patients with ichthyosis vulgaris without concomitant AD [17], in *FLG*$^{-/-}$ mice [45] and in infants with and without eczema [46]. Recently, we reported that patients with AD with *FLG* mutations had elevated levels of pro-inflammatory IL-1 cytokines [47], which might influence inflammatory response after exposure to irritating chemicals. A reduced threshold to inflammation from topically applied irritants has been shown in filaggrin-deficient ('flaky tail') mice [19]. On the other hand, patients with AD without *FLG* mutations also showed a deficient skin barrier and reduced expression of filaggrin break-down products [15;31;46;48]. Furthermore, filaggrin expression can also be reduced by *FLG* intragenic copy number variations [49], through downregulation by inflammatory cytokines [33;50] or by modulation of enzymatic processes [16]. The fact that in the present study AD had a stronger effect than *FLG* loss-of-function mutations indicates that other factors, e.g. immunological processes, may play a role in individual susceptibility to ICD next to an impaired skin barrier.

However, it has to be stressed that exposure to skin-irritating factors remains the major causative factor for ICD, and intrinsic factors such as AD and *FLG* mutations only modify the risk. Excessive environmental exposure to irritants and/or allergens, not only in the workplace but also at home (e.g. nickel), may even conceal the role of genetic susceptibility in epidemiological studies. Unfortunately, the design of our case-control study did not allow for including exposure as a risk factor for ICD. To gain more insight in the complex interplay between *FLG* loss-of-function mutations, atopic predisposition and exposure, a prospective cohort design would be preferable.

In summary, our results indicate that both *FLG* loss-of-function mutations and AD significantly increase the risk of ICD, with respective ORs of 1.61 and 2.89. Individuals with both *FLG* mutations and AD have an approximately four- to fivefold increased risk of developing ICD.

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3.2 FILAGGRIN LOSS-OF-FUNCTION MUTATIONS AND ATOPIC DERMATITIS AS RISK FACTORS FOR HAND ECZEMA IN APPRENTICE NURSES: PART II OF A PROSPECTIVE COHORT STUDY

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ABSTRACT

Background /Objectives

Environmental exposure and personal susceptibility both contribute to development of hand eczema. In this study, we investigated the effect of loss-of-function mutations in the filaggrin gene (*FLG*), atopic dermatitis and wet work exposure on the development of hand eczema in apprentice nurses.

Methods

Dutch apprentice nurses were genotyped for the four most common *FLG* mutations; atopic dermatitis and hand eczema history were assessed by questionnaire. Exposure and hand eczema during traineeships were assessed with diary cards.

Results

The prevalence of hand eczema during traineeships was higher among subjects with a history of hand eczema reported at inclusion. Hand washing during traineeships and at home increased the risk of hand eczema. After adjustment for the effects of exposure and *FLG* mutations, an odds ratio of 2.5 (90% confidence interval 1.7 – 3.7) was found for a history of atopic dermatitis. In this study, an increased risk of hand eczema conferred by *FLG* mutations could not be shown, but subjects with concomitant *FLG* mutations and atopic dermatitis showed the highest risk of hand eczema during traineeships.

Conclusion

A history of atopic dermatitis, a history of hand eczema and wet work exposure were the most important factors increasing the risk of hand eczema during traineeships.

INTRODUCTION

Hand eczema (HE), as a manifestation of contact dermatitis of the hands, is one of the most common occupational diseases in industrialized countries, and may account for up to 90% of all occupational skin diseases [1;2]. Skin exposure to irritants is a risk in occupations such as nursing, hairdressing and metalworking; in these occupations, 1-year prevalence rates of HE of up to 30% have been reported [3-7]. Although exposure is a prerequisite for the development of occupational HE, some workers are more susceptible than others. The best-known and firmly established susceptibility factor for the development of occupational HE is a history of atopic dermatitis (AD). The increased risk of developing occupational HE for individuals with a history of AD has long been recognized [8-10], and recent population studies reported up to fivefold increased risks [11-13]. One of the possible causes of the risk-enhancing effect of AD is an impaired skin barrier. Experimental studies have shown that the barrier function of the skin of patients with AD is reduced as compared with healthy controls, even in uninvolved skin areas [14-16]. The mechanisms that underlie reduced skin barrier in AD are not fully clear, but recent research suggests that the epidermal protein filaggrin might play an important role [17;18]. In the stratum corneum, filaggrin contributes to structural strength by aggregating the keratin filaments, and its breakdown products support hydration, pH balance, anti-bacterial defence and resistance to UV-radiation [18;19]. Several loss-of-function mutations have been identified in the filaggrin gene (*FLG),* resulting in reduced amounts or, in the case of homozygotes, in the absence of filaggrin in the skin. The summed prevalence of individuals who carry one or more of the most common *FLG* loss-of-function mutations in European populations is reported to be 7-10% [17;20-23]. The impact of these mutations on skin barrier function has been shown in animal models [24], in patients with ichthyosis or AD [14;25] and in 3-month old infants with and without eczema [26]. *FLG* loss-of-function mutations are strongly associated with AD; 16-44% of the individuals with moderate to severe AD carry one or more *FLG* mutations [20;22;27-29]. A recent meta-analysis revealed a more than 3-fold increased risk for developing AD in carriers of either one of the R501X or 2282del4 mutations [30]. Because filaggrin is important for the barrier function of the skin, it is plausible that *FLG* mutations as such can increase the risk for occupational HE. Indeed, recent case-control studies found an association between *FLG* mutations and occurrence of occupational HE [31-34]. In the aetiological relationship between *FLG* mutations and occupational HE, AD can be both an intermediate factor (as *FLG* mutations increase the risk of AD) and a co-determinant independent from *FLG*. In the present study, we aimed to gain more insight into the relative contributions of both *FLG* mutations and AD to the aetiology of occupational HE.

Knowledge of susceptibility factors could contribute to a more targeted prevention of occupational HE. In some countries, a history of HE and a history of AD are used to identify persons at risk in jobs with high skin exposure; susceptible workers are offered extra preventive measures and attention by their occupational physician [35;36]. It has not yet been investigated whether the predictive value of susceptibility screening

can be increased by adding a genetic susceptibility marker such as *FLG* mutations. Interestingly, 40% of *FLG* mutation carriers do not develop AD [19;37;38]. This subgroup will not be recognised as susceptible in current prevention programs. Another issue is that most of the studies that have explored the effect of *FLG* mutations on contact dermatitis [31-33;39-41] have not accounted for the extent of environmental exposure. Therefore, the relative contributions of *FLG* mutations and a history of or current AD, taking exposure into consideration, are still to be elucidated. We performed a prospective cohort study among apprentice nurses, who provided a DNA sample by buccal swab, filled in a questionnaire concerning symptoms of AD and atopy, and were consecutively followed up for 1-3 years, with regular monitoring of symptoms of HE as well as exposure to 'wet work' as assessed by diary cards. The general characteristics of this cohort, the exposure to wet work during follow-up and the occurrence of HE have been described in Part I of this study [42]. The present article describes the influence of *FLG* mutations, AD and exposure on the risk of HE in this cohort.

METHODS

Subjects

A detailed description of the study population and inclusion procedure is provided in Part I of this study [42]. In short, apprentices were recruited from 15 different Dutch vocational schools that prepare students for a career in healthcare (nursing or care-giving). Students were eligible for participation if they had recently started a traineeship with a duration of at least 10 weeks, or were expecting to do so within the next few weeks. The only exclusion criterion was the presence of chronic inflammatory disease (e.g. psoriasis or rheumatoid arthritis). Approval was obtained from the Medical Ethical Committee of the Academic Medical Center, Amsterdam, The Netherlands.

DNA sampling and genotyping

The four most common *FLG* loss-of-function mutations in European populations were genotyped: *R501X, 2282del4, R2447X* and *S3247X*. Subjects provided a buccal swab sample (Geneticlab Diagnostic & Research, Pordenone, Italy; http://www.geneticlab.it), and DNA material was obtained from buccal mucosa cells. For each subject, two swabs were obtained, and 2 ml of lysis buffer (Puregene® Cell Lysis Solution, Gentra Systems, Minneapolis, MN, USA) was added to each swab to disrupt the cells and stabilize the DNA. Extraction and genotyping for *FLG* mutations *R501X*, *R2447X* and *S3247X* was performed by KBioscience (http://www.kbioscience.co.uk). Genotyping was performed with the KASP single-nucleotide polymporphism genotyping system, a homogeneous fluorescence resonance energy transfer (FRET)-based system, coupled with competitive allele specific polymerase chain reaction (PCR). Blind duplicates and Hardy-Weinberg equilibrium tests were used as quality control tests. *R501X* was genotyped by using the primer pair GAATGCCTGGAGCTGTCTCG (C-allele)

and CTGAATGCCTGGAGCTGTCTCA (T-allele) with the common allele primer GCACTGGAGGAAGACAAGGATCG. *R2447X* was genotyped by using the primer pair GAGTGCCTGGAGCTGTCTCG (C-allele) and GAGTGCCTGGAGCTGTCTCA (T-allele) with the common allele primer GAGGAAGACAAGGATCCCACCACA. *S3247X* was genotyped by using the primer pair GTGTCTGGAGCCGTGCCTTG (C-allele) and GGTGTCTGGAGCCGTGCCTTT (A-allele) with the common primer CTTCCAGAAACCATCGTGGATCTGT. Genotyping for *2282del4* was performed by sizing a fluorescently labeled PCR fragment on an Applied Biosystems 3100 or 3730 DNA sequencer (Foster City, CA, USA) as described previously [32;43].

Questionnaires

At inclusion, participants filled in a questionnaire including items on eczema, rhinitis, conjunctivitis and asthma, allergies and/or symptoms following exposure to dust, animals, pollen, foods, metals and wool, present or past skin diseases, the presence of any other chronic disease, medication use, present or past skin symptoms on the hands or fingers, and exposure to wet work during previous jobs/apprenticeships, secondary jobs, and leisure or household activities. Atopy was defined as the presence of two or more of the following: symptoms following exposure to common allergens, rhinitis, conjunctivitis or asthma. AD was assessed according to a slightly modified version of the UK Working Party criteria 'questions only' definition, in which onset below 2 years of age was replaced by onset below 5 years of age as a proxy of 'childhood dermatitis'.

At the end of the follow-up period, an email questionnaire was sent to all participants still in the study. This final questionnaire included items on symptoms experienced during follow-up, consultation of general practitioners or dermatologists, changes in hand hygiene behaviour, the use of protective hand cream, information on traineeships and side jobs, and smoking.

Exposure and symptoms during practical training

During their traineeships, the students had to keep count of the wet work activities that they performed during several shifts, using special diary cards as described in detail in Part I of this study [42]. Skin symptoms on the hands were also recorded on the cards. If no cards had been returned near the end of the traineeship, students were contacted by email and/or telephone to retrieve information about the type of traineeship and possible symptoms retrospectively.

Following the classification for screening for HE symptoms proposed by Vermeulen *et al*. [44], HE was defined as the presence of at least one of the following combinations of symptoms: fissures and redness, fissures and itch, fissures and scaling, vesicles, or papules, plus duration of > 3 days or recurrence (symptoms reported more than once). As these criteria were originally developed for identifying cases of HE in a working population, we were concerned that, by using this definition, we would miss early-stage symptoms that may progress into HE. Therefore, we also used a more

lenient definition, 'mild HE', defined as the presence of at least one of the following symptoms or combinations: redness and itch, redness and scaling, itch and scaling, fissures and redness, fissures and itch, fissures and scaling, vesicles, or papules, all irrespective of duration or recurrence.

Without specification, HE refers to any episode of HE during follow-up. Some analyses comprise students without a history of HE in contrast to students with a history of HE. The latter refers to HE at any time before or at inclusion. A first episode of HE during follow-up in a student without a history of HE is equivalent to 'incident HE'.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics version 19 and Microsoft Excel™. In the subgroup analyses, *FLG* mutation carriers were compared with *FLG* wild-type individuals. No distinction was made between homozygous, compound heterozygous or heterozygous *FLG* mutation carriers, because the subgroup of homozygous or compound heterozygous carriers was too small for subgroup analysis to be performed. Because AD can be both an intermediate factor and an independent co-determinant in the aetiological relationship between *FLG* mutations and HE, we chose to use stratified analyses to study the effects on HE of *FLG* mutations and AD, each in the absence and in the presence of the other factor.

The relative risks (RRs) and confidence intervals (CIs) for the subgroup analyses of HE symptoms reported at inclusion were computed by using cross-tabulated results and applying the following formulas in Excel: RR = [*a/(a+b)*]/[*c/(c+d)*], with *a* and *c* being the numbers of HE cases in the 'exposed' and 'referent' groups, respectively; and 90% CI(RR) = exp{ln(RR) ± 1.645*SE[ln(RR)]}, in which SE[ln(RR)] = sqrt{*b*/[*a(a+b)*] + *d*/[*c(c+d)*]}. The 90% CI corresponds to one-sided testing with $p < 0.05$.

Analysis of the combined influence of susceptibility and exposure factors on the risk of HE during follow-up was performed with generalized linear mixed models in SPSS. HE is a disease with a fluctuating course, and the recovery time may be as short as a few days. Thus, the apprentice nurses would have time to recover from HE in between traineeship periods. We therefore assumed that the probability to develop HE in one traineeship does not depend on the extent of exposure or on having had HE in previous traineeships. Each traineeship was therefore counted as a separate entity, and data from subjects who entered a second or a third traineeship were entered as multiple records in the database. This, however, results in the problem that, regardless of susceptibility, subjects who contributed data for multiple traineeships would have had more opportunities to develop HE than those who had been followed for only one traineeship. Therefore, a mixed models design was used in the analyses, with participant ID included as random effect (procedure GENLINMIXED in SPSS™). In such a mixed model, the within-subject correlation is taken into account.

Analysis of wet work exposure in this cohort had revealed that a frequency of hand washing during practical trainings > 8 times per shift, hand washing at home > 10 times per day and working in a side job involving wet work for > 8 hr a week increased

the risk of HE (Part I) [42]. Therefore, these were included as binary variables in the multivariate mixed models to represent wet work exposure. Preceding this analysis, the mean frequency of hand washing in different healthcare sectors was assessed by linear mixed models with healthcare sector as fixed effect and subject ID as random effect (procedure MIXED in SPSS™). The mean frequency of hand washing during traineeships was lowest in psychiatry (7.0 times per shift), medium to high in homecare and hospitals (8.8 and 8.9 times per shift, respectively), and highest in care for the disabled and nursing homes (10.4 and 10.5 times per shift, respectively). The frequency of hand washing during traineeships was classified according to whether the traineeship was performed in psychiatry or in any other sector, which corresponds to a cut-off value of (supposedly) 8 times per shift. This classification was applied to all subjects.

Use of hand cream, exposure to wet work and number of subjects reporting HE during traineeships (Fig. 2) was compared between the four subgroups categorized by *FLG* and AD by use of the chi-square test.

RESULTS

Study population

The participation rate of the apprentices invited was ~50%. A total of 728 apprentice nurses completed the inclusion questionnaire. Seven apprentices were excluded because of chronic inflammatory disease. Some participants did not provide a buccal swab sample at inclusion, because they were aged < 18 years (in The Netherlands, these persons are only allowed to provide a DNA-sample with parental consent) and DNA sampling was postponed until parental consent was obtained or until they had turned 18 years during follow-up. Eventually, a total of 626 DNA samples were obtained, 596 of which were successfully genotyped for all four investigated *FLG* loss-of-function mutations (*R501X, 2282del4*, *R2447X* and *S3247X*). A further 150 participants were lost to follow-up shortly after completion of the inclusion questionnaire or quit the study before going through a traineeship (mostly because of changing career), leaving a total of 446 participants in whom to study the impact of susceptibility factors on the risk of developing HE during vocational training.

Genotype distributions and associations with atopic disease

Table 1 shows the genotype distributions. *FLG* mutations were present in 11.1% of the participants. Fifty-six individuals were heterozygous for one mutation, 2 were homozygous for *2282del4*, 3 were homozygous for *R2447X* and 1 was compound heterozygous for *2282del4* and *R501X*.

The genotype distributions were not in Hardy Weinberg-equilibrium for *2282del4, R2447X,* and the combined genotype. This was probably because of the relatively large number of homozygotes among subjects with AD, combined with a slight overrepresentation of subjects with AD in this cohort (see Part I) [42]. In participants

without AD (n=460), the genotype distributions for *2282del4* and the combined genotype were in Hardy Weinberg-equilibrium.

FLG mutations were associated with a history of AD (RR 1.8; 90% CI 1.37 – 2.35), especially with persistent AD starting before 5 years of age and still present at the time of inclusion (RR 2.6; 90% CI 1.43 – 4.67). There were also associations between *FLG* mutations and general dry skin (RR 2.5; 90% CI 1.82 – 3.38) and between *FLG* mutations and symptoms upon exposure to common allergens (RR 1.3; 90% CI 1.01 – 1.54). No association was found with rhinitis or asthma.

Table 1. Genotype distributions for the filaggrin gene (*FLG)* loss-of-function mutations *R501X, 2282del4, R2447X* and *S3247X* in apprentice nurses

FLG mutation	R501X	2282del4	R2447X	S3247X	Combined (four mutations)
Group size	608	614	610	607	596
AA, n (%)	587 (96.5)	576 (93.8)	604 (99.0)	605 (99.7)	530 (88.9)
Aa, n (%)	21 (3.5)	35 (5.7)	3 (0.5)	2 (0.3)	59 (9.9)
aa, n (%)	0 (0)	3 (0.5)	3 (0.5)	0 (0)	7 (1.2)
Total *FLG* carriers (Aa + aa), n(%)	21 (3.5)	38 (6.2)	6 (1.0)	2 (0.3)	66 (11.1)
Wild-type allele frequency (%)	98.3	96.7	99.3	99.8	93.9
Mutant allele frequency (%)	1.7	3.3	0.7	0.2	6.1

Symptoms of HE reported at inclusion

We used stratified analyses to investigate the effect of *FLG* mutations and AD on the occurrence of HE. The study population was divided into four groups: (i) participants without *FLG* mutations and with no history of AD (*FLG*-/AD-); (ii) participants with *FLG* mutations but with no history of AD (*FLG+*/AD-); (iii) participants without *FLG* mutations but with a history of AD (*FLG*-/AD+); and (iv) participants with both *FLG* mutations and a history of AD (*FLG+*/AD+). In a retrospective approach, we compared past and present symptoms of HE reported in the inclusion questionnaire between these four groups (Table 2). In total, 54% of all participants reported one or more skin symptoms, 13% had a history of HE, and 7% had HE at the time of inclusion. Regardless of *FLG* mutations, a history of AD conferred an increased RR for all investigated symptoms. Participants with concomitant *FLG* mutations and AD (*FLG+*/AD+) showed the highest symptom prevalence, and a significantly higher prevalence of scaling, fissures and current HE than the *FLG*-/AD+ subgroup. Among subjects without a history of AD, those who carried one or more *FLG* mutations (*FLG*+/AD-) did

not report more symptoms on the hands and fingers or HE before or at inclusion than those without *FLG* mutations.

Of the 7 homozygous or compound heterozygous apprentices among the *FLG* mutation carriers, 6 had a history of AD, 3 had HE at the time of inclusion, and another 3 reported a history of HE.

HE during traineeships: effects of AD, FLG mutations, and wet work

One to three years of follow-up was completed for 446 participants. One hundred and thirty participants (29%) reported HE on one or more occasions during their traineeships. Three hundred and fifty-nine subjects (81%) had no HE history up to the time of inclusion. Of these, 78 (22%) developed HE during their traineeships. Among the participants with a history of HE but no HE at the time of inclusion (n=52), 29 (56%) reported HE during one or more traineeships. Thirty-five subjects (8%) had HE at the time of inclusion. Mixed models analysis showed that, after adjustment for the effects of exposure, participants with a history of HE up to inclusion were at increased risk of developing HE during traineeships [odds ratio (OR) 4.5; 90% CI 2.96 – 6.98]. After taking into account AD history, the OR for having HE during traineeships for participants with a history of HE was 3.9 (90% CI 2.5 – 6.1). Twenty participants who had HE at the time of inclusion also reported HE during their first traineeship. Because, for these participants, it was unknown whether their HE was related to their traineeship or was a continuation of already existing HE, a second analysis was performed excluding

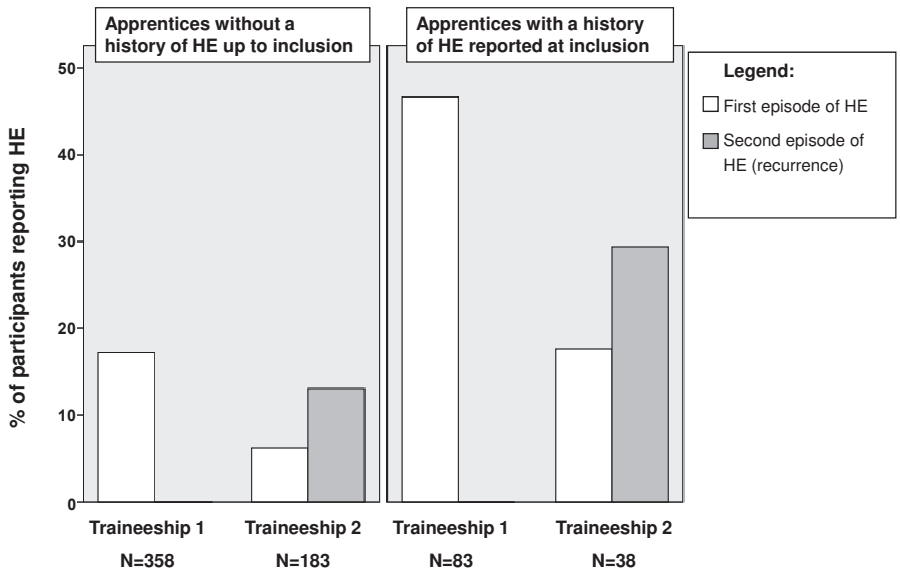

Fig. 1. Reported period prevalence of hand eczema (HE) during traineeships in the first and second year of follow-up in participants with or without a history of HE reported at inclusion.

Table 2. Number of apprentice nurses reporting having (or having had) symptoms of hand eczema at inclusion, and relative risk (RR) ratios for symptoms of hand eczema in four subgroups based on filaggrin gene (*FLG*) mutations and history of atopic dermatitis (AD)

Subgroup name:	*FLG*-/AD-	*FLG*+/AD-	*FLG*-/AD+	*FLG*+/AD+
Subgroup characteristics:	(Reference)			
FLG mutations[a]	No	Yes	No	Yes
History of AD[b]	No	No	Yes	Yes
	N = 405	N = 38	N = 125	N = 28
Reported symptoms on the hands/ fingers at inclusion:	n (%)	n (%)	n (%)	n (%)
Redness	84 (21)	7 (18)	60 (48)	16 (57)
Scaling	88 (22)	5 (13)	47 (38)	15 (54)
Itch	145 (36)	14 (37)	74 (59)	18 (64)
Fissures	91 (22)	11 (29)	46 (37)	15 (54)
Vesicles	48 (12)	0 (0)	32 (26)	8 (29)
Bumps	74 (18)	2 (5)	46 (37)	9 (32)
History of mild hand eczema[c]	163 (40)	13 (34)	81 (65)	19 (68)
History of hand eczema[d]	54 (13)	2 (5)	37 (30)	12 (43)
Mild hand eczema currently present	58 (14)	7 (18)	32 (26)	9 (32)
Hand eczema currently present	18 (4)	1 (3)	14 (11)	7 (25)

CI, confidence interval
[a] Carrier of one or more of the following loss-of-function mutations in *FLG*: *R501X, 2282del4, R2447X* or *S3247X*.
[b] A history of atopic dermatitis was assessed by questionnaire, with a slightly modified version of the UK Working Party criteria.
[c] One or more of the following combinations of symptoms: redness and itch, redness and scaling, scaling and itch, fissures and redness, fissures and itch, fissures and scaling, vesicles, or papules.

these participants, which resulted in an OR of 2.9 (90% CI 1.80 – 4.70). Fig. 1. shows the prevalence of HE in subjects with or without a history of HE reported at inclusion, divided into first and recurrent episodes of HE; it shows the high prevalence of HE during traineeships among participants with a history of HE at inclusion and the high recurrence rate for this group.

RR *FLG*+/AD- versus *FLG*-/AD-	RR *FLG*-/AD+ versus *FLG*-/AD-	RR *FLG*+/AD+ versus *FLG*-/AD-	RR *FLG*+/AD+ versus *FLG*+/AD-	RR *FLG*+/AD+ versus *FLG*-/AD+
RR (90%CI)	RR (90%CI)	RR (90%CI)	RR (90%CI)	RR (90%CI)
0.9 (0.50 – 1.59)	2.3* (1.85 – 2.89)	2.8* (2.01 – 3.77)	3.1* (1.48 – 6.51)	1.2 (0.87 – 1.62)
0.6 (0.30 – 1.22)	1.7* (1.35 – 2.21)	2.5* (1.78 – 3.42)	4.1* (1.68 – 9.88)	1.4* (1.01 – 2.01)
1.0 (0.71 – 1.48)	1.7* (1.40 – 1.95)	1.8* (1.39 – 2.32)	1.7* (1.06 – 2.88)	1.1 (0.84 – 1.41)
1.3 (0.83 – 2.01)	1.6* (1.28 – 2.09)	2.4* (1.72 – 3.31)	1.9* (1.01 – 3.39)	1.5* (1.03 – 2.06)
n.a.	2.2* (1.54 – 3.02)	2.4* (1.41 – 4.14)	n.a.	1.1 (0.64 – 1.94)
0.3* (0.09 – 0.91)	2.0* (1.55 – 2.61)	1.8* (1.08 – 2.85)	6.1* (1.43 – 26.10)	0.9 (0.53 – 1.43)
0.9 (0.58 – 1.25)	1.6* (1.39 – 1.87)	1.7* (1.33 – 2.13)	2.0* (1.19 – 3.30)	1.1 (0.82 – 1.33)
0.4 (0.12 – 1.25)	2.2* (1.63 – 3.02)	3.2* (2.12 – 4.87)	8.1* (1.98 – 33.52)	1.5 (0.95 – 2.21)
1.3 (0.71 – 2.33)	1.8* (1.30 – 2.46)	2.2* (1.37 – 3.68)	1.7 (0.74 – 4.12)	1.3 (0.75 – 2.10)
0.6 (0.11 – 3.14)	2.5* (1.44 – 4.42)	5.6* (2.91 – 10.87)	9.5* (1.24 – 72.89)	2.2* (1.13 – 4.40)

[d] One or more of the following combinations of symptoms: fissures and redness, fissures and itch, fissures and scaling, vesicles, or papules, plus duration of >3 days or recurrence (symptoms reported more than once).
* Significant at $\alpha < 0.05$.

The prevalence of HE during the first and second traineeship in the four subgroups of participants with or without AD and *FLG* mutations is shown in Fig. 2. Increased prevalence rates of HE were seen for the *FLG*-/AD+ subgroup and the *FLG*+/AD+ subgroup as compared with the *FLG*-/AD- control group. Both Figs. 1 and 2 are restricted to the first and second traineeships, because the number of participants

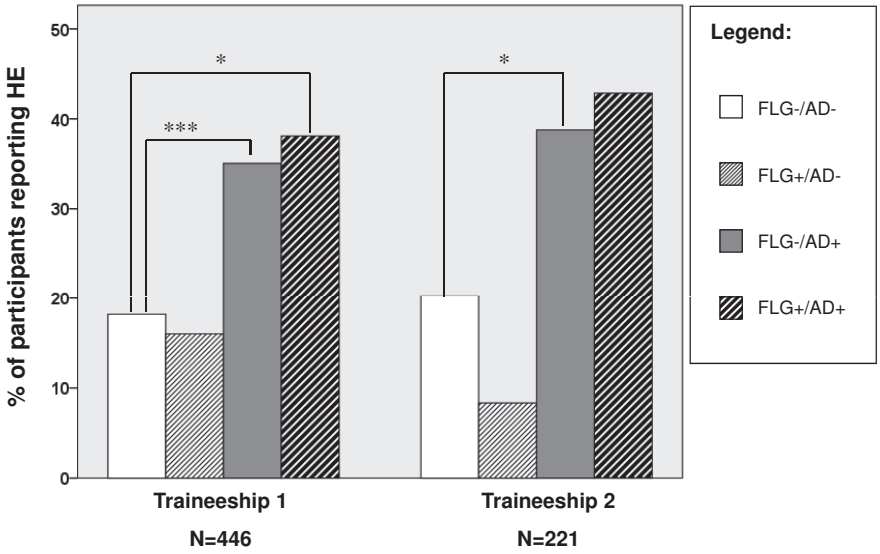

Fig. 2. Reported period prevalence of hand eczema (HE) during the first and second traineeships in four subgroups of participants. * $p < 0.05$, *** $p < 0.0001$.

that had completed a third traineeship was too small (n=57) for subgroup analysis to be performed.

The exposure did not differ appreciably between the four subgroups; the proportion of individuals who had traineeships in healthcare sectors with frequent hand washing (cut-off at > 8 times per shift) ranged from 63% to 71%.

The effects of AD, *FLG* mutations and exposure on the risk of developing HE during traineeships were calculated using a mixed models design. On the basis of the results of Part I of this study [42], the frequency of hand washing during traineeships, hand washing at home > 10 times a day and working in a side job involving wet work (e.g. healthcare, bars, or restaurants) were included in the models to account for wet work exposure. A first crude analysis resulted in an unadjusted OR of 1.1 (90% CI 0.7 – 2.0) for *FLG* mutations and an unadjusted OR of 2.8 (90% CI 1.9 – 4.1) for AD.

Table 3 shows the results of two multivariate mixed models including the four susceptibility subgroups together with exposure; in both models, the occurrence of (mild) HE in *FLG* wild-type participants without AD serves as the reference. Model 1 shows that, after adjustment for the effects of exposure, a history of AD and the combination of a history of AD and *FLG* mutations increased the risk of HE during vocational training with ORs, respectively, of 2.2 and 3.6. Taking into account the group sizes, the weighted OR of AD was 2.5 (90% CI 1.7 – 3.7). For mild HE, the corresponding ORs were both 2.1. For *FLG* mutations in participants without a history of AD, no effect could be shown (OR 0.7). Frequent hand washing during traineeships

(> 8 times per shift) and frequent hand washing at home (> 10 times per day) increased the risk of hand eczema, with ORs of 2.2 and 1.8, respectively.

Model 2 concerns HE during traineeships in participants who had been free from HE up to inclusion, and who had not been exposed to skin irritants before entering the study (e.g. previous education or career involving wet work or traineeships in previous school years, because the extent of that exposure could not be estimated). A similar tendency for an increased risk of HE was found for AD in combination with *FLG* mutations and for frequent hand washing at home.

As these result suggest that the influence of *FLG* mutations on HE differs between participants with and without AD, we investigated the existence of interaction in a model including *FLG*, AD, an interaction term between *FLG* and AD, and exposure. In the model including all participants (Model 1), no significant interaction effect could be shown [OR(interaction) 2.1; 90% CI 0.6 – 7.1]. In the model including only participants without previous HE or exposure (Model 2), a tendency (p = 0.08) for interaction was found [OR(interaction) 5.4; 90% CI 1.1 – 25.9].

Use of hand cream

Use of hand cream at least once a day was reported by 53% of the subjects in the *FLG*-/AD- subgroup. The usage of hand cream was significantly more frequent than this in the *FLG*-/AD+ subgroup (68%, chi-square, p = 0.04) and in the *FLG*+/AD+ subgroup (90%, p = 0.006), but not in the *FLG*+/AD- subgroup (62%, p = 0.190).

DISCUSSION

This study examined both genetic susceptibility and environmental exposure as risk factors for HE. Regarding AD, we found a distinct effect on HE with both the follow-up and the retrospective approaches. During follow-up, we found no indication of an increased risk of HE conferred by *FLG* loss-of-function mutations, although the apprentices with *FLG* mutations in addition to a history of AD had the highest OR of HE during traineeships (OR 3.6). With the retrospective approach, *FLG* mutations only had an effect on HE at inclusion in participants with AD. Frequent hand washing during traineeships (> 8 times per shift) or at home (> 10 times per day) increased the risk of HE during follow-up, with ORs of 2.2 and 1.8, respectively.

Our results confirm that a history of AD is associated with an increased risk of HE in high risk occupations, a finding that has been made in several epidemiological studies [45-49].

The fact that we could not show an effect of *FLG* mutations in the present study was unexpected. The high OR for HE during traineeships among participants with concomitant *FLG* mutations and AD is consistent with the results reported in our recent case-control study on occupational contact dermatitis patients and vocational students in training for high risk occupations. In that study, an effect of *FLG* mutations

Table 3. Multivariate mixed models including atopic dermatitis (AD), filaggrin gene (FLG) loss-of-function mutations and exposure to frequent hand washing as risk factors for hand eczema (HE) during traineeships

		Model 1		Model 2
		Mild HE during practical training	HE during practical training	HE during practical training in participants without previous wet work exposure and with no history of HE up to inclusion
No. of participants included		446	446	247
No. of exposure records		667	667	375
Factor		OR (expβ) (90% CI)	OR (expβ) (90% CI)	OR (expβ) (90% CI)
FLG mutations and AD	*FLG*: No AD: No	1.0 (reference)	1.0 (reference)	1.0 (reference)
	FLG: Yes AD: No	0.9 (0.4 – 1.8)	0.7 (0.3 – 1.7)	0.5 (0.1 – 1.9)
	FLG: No AD: Yes	2.1 (1.0 – 3.1)*	2.2 (1.4 – 3.4)*	1.4 (0.7 – 2.9)
	FLG: Yes AD: Yes	2.1 (1.0 – 4.0)*	3.6 (1.7 – 7.5)*	3.7 (1.0 – 13.5)
Frequent hand washing during traineeships (> 8 times per shift) [a]	Yes vs No	1.4 (0.9 – 2.3)	2.2 (1.2 – 4.2)*	1.4 (0.6 – 3.4)
Frequent hand washing at home (> 10 times per day)	Yes vs No	1.8 (1.2 – 2.8)*	1.8 (1.1 – 2.9)*	1.9 (0.9 – 4.2)
Working in a side job involving wet work for > 8 hr/ week	Yes vs No	1.6 (1.2 – 2.3)*	1.0 (0.7 – 1.5)	1.2 (0.6 – 2.1)

CI, confidence interval; OR, odds ratio.
[a] Based on the healthcare sector means of reported frequency of hand washing on exposure cards.
* $p < 0.05$.

irrespective of AD (OR 1.6; 95% CI 1.0 – 2.6) was found [34]. The cases in that study, however, had chronic and severe HE, as opposed to the apprentice nurses, who often had less severe HE, which might not become chronic. Possibly, higher exposure or a longer duration is needed to reveal an effect of *FLG* mutations.

In a recent cross-sectional population study, Thyssen *et al*. found that *FLG* mutations constituted a risk factor for HE in individuals with AD, but not in individuals without AD [33]. Our data also point to an interactive effect, although this was only indicated in part of the analyses (Model 2).

The absence of a significant effect of *FLG* mutations in this study could not be explained by differences in exposure or use of hand cream. A possible explanation

might be that some *FLG* mutation carriers are able to compensate reduced amounts of filaggrin in their skin via an as yet unknown mechanism, preventing them from developing AD as well as HE. This may partly explain the wide range in susceptibility to HE (OR 0.7 – OR 3.6) that we observed among *FLG* mutation carriers, which – in view of the observed tendency for interaction – is partly related to the absence or presence of AD. More research into skin barrier properties of *FLG* mutation carriers without AD may shed more light on this.

Irrespective of *FLG* mutations, a possible role of the filaggrin protein itself may be considered. Recently, one study investigated skin lipid composition, irritation response and the skin barrier in AD patients and controls, both with and without *FLG* mutations. No difference in stratum corneum lipid composition or increase in TEWL after a 24-h irritation test was found between *FLG* mutation carriers and *FLG* wild-type individuals [50]. Another recent study also found no difference in lipid composition and skin barrier function between AD patients with and without *FLG* mutations. However, there was a significant positive correlation between favourable lipid organization and skin barrier function with natural moisturizing factors (NMF) in the stratum corneum [51]. As NMF can be seen as a proxy for filaggrin expression [52;53], this might imply that filaggrin itself does play a role in the stratum corneum lipid composition and skin barrier function. Indeed, research among ichtyosis vulgaris patients carrying *FLG* mutations showed that filaggrin deficiency led to a paracellular defect in skin barrier function, caused by disrupted lipid bilayer organization and altered loading of lamellar bodies [25]. In addition to the loss-of-function mutations, several other factors, mostly associated with AD, can influence filaggrin levels in the skin. For instance, the expression of filaggrin may be downregulated by inflammatory cytokines, for example interleukin (IL)-4, IL-13, IL-22 and IL-25[37;54-56]. Also, Brown *et al*. have recently shown that the number of filaggrin repeats in the *FLG* gene may vary between 10, 11 or 12, and that these copy number variations are significantly associated with the risk of AD [57]. It might be speculated that variation in filaggrin expression caused by copy number variations may also play a role in susceptibility to occupational HE. Future studies investigating the role of filaggrin in occupational HE should consider the inclusion of copy number variations in their analysis.

Some limitations of this study need to be mentioned. First, because of the multiple traineeships in which participants were repeatedly at risk of HE, a mixed model analysis was used. We note that the ORs obtained from the models are an overestimation of the RRs. This especially applies to subgroups with a high prevalence of HE. Corresponding RRs can be calculated by using the estimated means obtained from the models. For example, in Model 1, the ORs for HE of 0.7, 2.2 and 3.6 for the *FLG+*/AD-, *FLG-*/AD+ and *FLG+*/AD+ subgroups correspond to RRs of, respectively, 0.7, 1.9 and 2.5.

Second, detailed information on wet work exposure was available for only 383 of the 446 participants who were followed-up in this cohort. Repeating the mixed models analysis in this subset of participants yielded similar results as when all subjects were included, which justifies the use of extrapolated exposure variables.

A third limitation is that, on the basis of the symptoms as reported, we were not able to distinguish between HE of the irritant, allergic or atopic type. Despite a similar clinical appearance, these subtypes of HE have different underlying mechanisms, and are probably not equally affected by genetic susceptibility factors. For example, *FLG* loss-of-function mutations have shown positive associations with irritant [32;40], but less so with allergic contact dermatitis [31;39]. Patch testing would be needed to differentiate between participants with irritant HE and those with allergic HE, but this was not feasible in our study. Among the 52 participants with reported symptoms of HE who were seen by the collaborating occupational physician, contact allergy was diagnosed in, at most, 23% (Part I) [42]. If we had been able to exclude the cases with contact allergy from our study, this would probably have shifted the ORs for HE resulting from *FLG* mutations a little away from 1. Also, we were not able to assess severity of HE on the basis of the self-reported symptoms. The use of hand cream by 90% of the participants in the *FLG+/*AD+ subgroup, however, suggests more severe HE in this subgroup. Possibly, a stronger effect of *FLG* mutations would have been found in association with severity of HE.

One of the underlying reasons for this study was to investigate whether adding *FLG* genotyping to the AD screening tool would improve the identification of susceptible individuals in high risk occupations. Our results do not convincingly indicate that this is the case. Even if the effect of *FLG* in subjects with a history of AD had been significant, the effect size would probably be too small for a substantial favourable effect on the predictive values of a screening procedure. Information about AD and HE history, as is currently asked for according to Dutch and German guidelines, is a feasible predictor the acquisition of occupational HE, as our results have confirmed. The results of our case-control study [34] and our present prospective cohort study show that those in the *FLG+/*AD+ subgroup are at the highest risk for occupational HE. Furthermore, occupational HE patients with concomitant AD and *FLG* mutations appeared to have a worse prognosis than *FLG*-/AD-, *FLG*-/AD+ or *FLG+*/AD- patients in a recent follow-up study [58]. New research might confirm that AD patients with *FLG* mutations are indeed substantially more susceptible to occupational HE than patients with AD without *FLG* mutations. If this is so, identifying *FLG* mutations among AD patients and advising avoidance of irritant exposure in such patients would be beneficial.

CONCLUSION AND RECOMMENDATIONS

A history of AD, a history of HE and wet work exposure were the most important factors increasing the risk of HE during traineeships. As our results confirmed that HE develops shortly after the start of exposure to wet work, even in traineeships, it is strongly recommended to start prevention programs as early as during vocational training, instead of at the time of employment. In addition, it would be interesting to further investigate skin barrier properties of *FLG* mutation carriers without AD, which may shed more light on the existence of possible mechanisms to compensate for reduced filaggrin in the skin.

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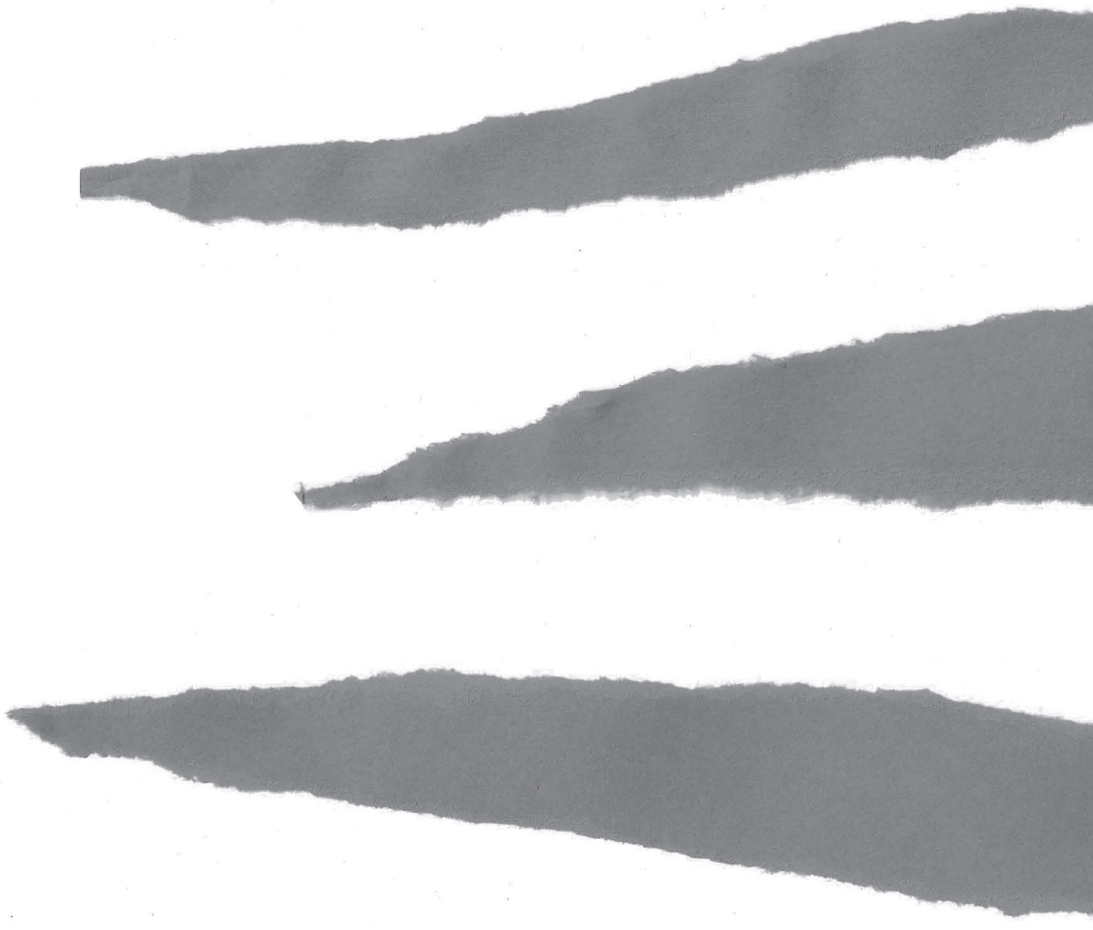

4 ETHICAL ISSUES

4.1

ETHICAL ISSUES OF GENETIC SUSCEPTIBILITY TESTING FOR OCCUPATIONAL DISEASES: OPINIONS OF TRAINEES IN A HIGH-RISK JOB

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ABSTRACT

Purpose

Genetic research has opened up possibilities for identification of persons with an increased susceptibility for occupational disease. However, regulations considering the ethical issues that are inevitably associated with the use of genetic tests for susceptibility for occupational diseases are scarce. We investigated whether opinions of an intended stakeholder group, that is, student nurses, are sufficiently addressed by existing recommendations.

Methods

Attitudes and opinions of Dutch student nurses toward a genetic test for susceptibility to occupational contact eczema were studied in a qualitative setup using focus groups, interviews and electronic questionnaires. The results were compared with guidelines and recommendations extracted from the literature.

Results

Sixty-nine percent of the student nurses said they would partake in a genetic test for susceptibility to occupational contact eczema when available. Concerns were expressed regarding the difficulty of interpreting test results, the utility of the test result in practice and the necessity of genetic tests for non-severe diseases. For the issue of privacy and confidentiality, the students expressed few worries and much confidence. The existing guidelines largely covered the students' opinions. Still, the data emphasized the need for good individual risk communication both before and after testing, taking into account that the test concerns susceptibility.

Conclusions

Comparing the students' statements with the issues addressed by the guidelines, we conclude that the guidelines should pay more attention to risk communication and practical advice accompanying the test results.

INTRODUCTION

Due to recent developments in genetic sciences, opportunities for identification of persons with a genetically determined increased susceptibility to occupational exposure have appreciably expanded. Genetic tests are increasingly accessible, for doctors, companies and the public. The increasing tendency to claim a "right to know", connected to a strong societal and political emphasis on individual responsibility for health, may further contribute to the demand for genetic susceptibility tests. Detection of susceptible persons at the workplace can improve prevention of occupational diseases, for example, through career counselling or timely application of personal protective measures. However, before actually offering and applying susceptibility tests for occupational diseases, several ethical issues need to be considered. Protective legislation addressing these ethical issues nevertheless is scarce. In 2008, the Dutch Council for Public Health and Healthcare reported that Dutch citizens are insufficiently protected against possible misuse of genetic test results [1]. In the USA workers are protected by the Genetic Information Nondiscrimination Act (GINA), in effect since 2009, which restricts the disclosure of genetic test results to employers and forbids genetic screening in the workplace [2]. European countries lack such legislation, except for Belgium, Finland and Austria, where the use of genetic screening in the work setting is forbidden [3;4].

Several guidelines and criteria for genetic screening have been formulated by different committees in Europe, some also focusing on its use in the workplace [5]. Most of these build on the authoritative World Health Organization (WHO) criteria for screening tests for early detection of disease as formulated by Wilson & Jungner [6]. Box 1 summarizes three different guidelines and recommendations on genetic testing for diseases in the workplace that have been published in the last decade. In 2002, MacDonald & Williams formulated six conditions that should be met before offering genetic testing to employees [7]. One year later, the European Group on Ethics in Science and New Technologies (EGE) [4] offered partly similar criteria for acceptability of genetic testing in the workplace. Both sets of criteria concern genetic testing for a variety of diseases, not exclusively those diseases caused by occupational exposure. In a 2006 report on the ethics of genetic screening in general, the British Nuffield Council stated that screening for occupational diseases should be contemplated only when certain conditions are fulfilled [5]. An extensive decision framework for genetic screening tests with 44 questions has been published in 2005 by the Office of Public Health Genomics of the US Centers for Disease Control and Prevention, addressing analytical validity, clinical validity, clinical utility and ethical issues: the "ACCE framework" [8;9].

The guidelines and recommendations mentioned above contain many valuable elements; however, for tests that concern susceptibility, some specific aspects need to be considered, for example, the complexity of estimating predictive value. Furthermore, more insight into the attitude of intended examinees in the scenario of tests for occupational diseases would be valuable, but to our knowledge, the number of studies addressing this topic is small [10-13].

The objective of this study was to make an inventory of opinions of student nurses regarding a genetic susceptibility test for occupational contact eczema. Further, we investigated whether these opinions are sufficiently addressed by existing recommendations.

Occupational contact eczema (OCE) is one of the most prevalent occupational diseases in western countries [14;15]. It can be caused by an allergic reaction or by mechanical or chemical irritation of the skin. Wet work, involving frequent contact with water and mild irritants such as soap, is a major cause of OCE mostly manifesting as hand eczema. Apart from exposure, individual susceptibility plays a significant role [16]. A well-known personal susceptibility factor is atopic dermatitis, an eczematous skin condition which is highly dependent on genetic predisposition [17;18]. Recent research has identified loss-of-function mutations in the filaggrin gene (*FLG*) as a major risk factor for atopic dermatitis [19]. Furthermore, these mutations are suspected to increase the risk of developing contact eczema as well [20-23]. In a recent case-control study, significant associations were found between OCE and atopic dermatitis and between OCE and *FLG* loss-of-function mutations with odds ratios of 2.89 and 1.61, respectively. Combined presence of *FLG* mutations and atopic dermatitis increased the risk of OCE approximately fivefold (Visser et. al., in preparation). In Germany and in the Netherlands, nurses, being at risk for developing OCE due to frequent wet work, are presently screened for increased susceptibility to develop OCE using history of atopic dermatitis as an indicator. Susceptible individuals receive extra preventive measures and are regularly followed-up by their occupational physician [24;25]. Possibly, *FLG* genotyping could improve the evaluation of susceptibility to OCE.

METHODS

Students in training to become a nurse were recruited from three schools for higher or intermediate vocational education, all located in Amsterdam, the Netherlands. The school institutional review boards agreed with the study protocol.

A detailed description of the recruitment process and data collection methods is presented elsewhere [26]. In short, a literature search was performed to identify factors that could influence decisions, beliefs or attitudes towards the use of genetic susceptibility tests. This resulted in a list of factors clustered in nine themes: "Emotions", "Expected effects of the disease", "Risk of developing the disease", "Expected use of test results", "Confidentiality and privacy", "Personal involvement" (e.g., having had the disease yourself, or knowing someone who has), "Social influences" (e.g., by family members, colleagues, media), "Principles and beliefs" (e.g., religious beliefs) and "Practical issues". Factors regarding test content were classified under the themes "Expected use of test results" and "Practical issues".

Consecutively, students were invited to voluntarily participate in a focus group, interview or electronic questionnaire, whichever involvement method they preferred.

Different recruiting techniques were used: a sample of student nurses participating in an ongoing cohort study (Visser et.al., in preparation) and studying in the Amsterdam area were invited by email. Posters were placed on school message boards and in cafeterias, and students were approached directly by 2-minute oral presentations in classes or in central study areas. During 2009/2010 a total of 5 focus groups (each 5 – 8 participants; a total of 33 participants) and 15 semi-structured interviews were held with student nurses. In addition, 32 students filled in an electronic questionnaire. The total number of participants was 80. Each student participated in only one involvement method. The percentage females was 80 %, and the mean age was 23 years (range: 17 – 45). The distribution of respondents over the first to the fourth educational year was 18, 19, 32 and 32%, respectively.

Focus groups, interviews and electronic questionnaires were set up following a similar standardized protocol. First, a brief introduction was given to the students about OCE ("hand eczema"), skin exposure and protection, and personal susceptibility [26]. Subsequently, the possibility of testing personal susceptibility with a genetic test was introduced followed by two questions: Question 1. "Would you use this test?" (possible answers: yes/ no/ doubt) and Question 2. "What are your motives for using or not using this test (according to you, what are the pros, cons and doubts)?" (open question). After all pros, cons and doubts brought up by the participants had been recorded, in all involvement methods the list with the factors extracted from the literature was introduced, clustered by theme. Respondents were asked whether (yes or no) and how or why (open question) these factors would influence their choice whether or not to use the test, and which (if any) other influential factors they could think of within the theme in question. Finally, after all themes had been discussed, participants individually prioritized which three themes they regarded as most important and which three themes they regarded least important.

RESULTS

In total, 55 out of 80 student nurses (69%) stated that they would take a genetic test for susceptibility to OCE if such a test was available. Eleven students (14%) stated they would *not* take such a test, and 14 (18%) doubted if they would take it or not.

The aim of this study was to identify the most relevant arguments in favor or against using the test expressed by the student nurses. Due to the character of the focus groups, which yielded data on group level instead of the individual level, the resulting opinions could not be referred to (numbers of) individual participants. Therefore, no information on the exact proportion of respondents that mentioned a certain opinion can be given. The arguments that were brought up will be reported below in a qualitative manner, clustered by theme.

At the end of the focus groups, interviews and questionnaire, the participants were asked to mark the three themes they considered most important and three themes they considered least important. Participants in the focus groups wrote their

Box 1. Three examples of existing recommendations and guidelines addressing genetic screening in the workplace

Criteria for offering genetic testing to employees, according to MacDonald & Williams-Jones (2002)

- A genetic test (for a specific condition) must be available which is highly specific and offers an acceptably low incidence of both false positives and false negatives; such a test must test for a gene that is sufficiently penetrant for the test result to have some important health implication.
- Testing should be carried out by an independent lab, and results of genetic tests should be given to workers directly, either by a geneticist or a genetic counselor; test results should be held confidential, and revealed to the employer only at the employee's request.
- Pre- and post-test genetic counseling must be available from a qualified health professional, and paid for by the employer, regardless of the outcome of the test.
- The gene being tested for must not be prominently associated with an identifiable and historically disadvantaged group.
- Where relevant, the employer must guarantee continued access to group insurance.
- The employer must ensure that if the employee chooses to reveal that she has tested positive, suitable policies are in place to ensure a reasonable degree of job security.

Conditions that must be fulfilled before genetic screening at the workplace can be considered, according to the European Group on Ethics in Science and New Technologies [EGE] (2003)

- The performance of the test is necessary for guaranteeing the protection of the employee's health and safety or those of third parties.
- The applicant or the employee should consent to the genetic test.
- There is scientifically proved evidence that the genetic test is valid and is the only method to obtain this information.
- The performance of the test does not prejudice the aim of improving conditions in the workplace.
- The principle of proportionality is respected regarding the motivations involved to perform the test.
- The principle of non-discrimination is not violated.
- The applicant or the employee should receive full information from an independent health professional on the testing procedure, the reasons for performing such tests, the potential outcomes and their implications and consequences, as well as the conditions of storing and access to data. They should also, if requested, be provided with access to independent legal counselling.

Conditions for genetic screening of employees for increased occupational risks, according to the British Nuffield Council on Bioethics (2006)

- There is strong evidence of a clear connection between the working environment and the development of the condition for which the screening is conducted.
- The condition in question is one which seriously endangers the health of the employee or is one in which an affected employee is likely to present a serious danger to third parties.
- The condition is one for which the dangers cannot be eliminated or significantly reduced by reasonable measures taken by the employer to modify or respond to the environmental risks.

prioritization on a separate sheet of paper, so that for this subject individual results could be obtained. Table 1 lists the themes with their relative importance given by 70 out of 80 students (10 questionnaire respondents declined to prioritize the themes). Some illustrative quotes are shown in Table 2.

Emotions (e.g., curiosity or anxiety)

The theme "Emotions", including curiosity and anxiety, was rated as important by many students in their prioritization of themes, especially by those who were in favor of partaking in the test. Several participants stated they were curious about their personal susceptibility, and would like to use the test to find out, or, in some cases, to confirm their own suspicions about their personal susceptibility (e.g., someone who already had contact dermatitis, or someone with a dark skin tone suspecting to be less susceptible). Also, knowledge of personal susceptibility would provide some kind of comfort. In contrast, a number of participants feared that the test would only lead to uncertainty and nervousness about developing OCE, especially in the case of a positive test result. They would rather not suffer from this stress, and instead just "wait and see".

Table 1. Student nurses' prioritization of themes representing considerations whether or not to use a genetic test for susceptibility to OCE

	% of respondents (n = 70) scoring this item as	
Theme	Important	Not important
Expected use of the test results	56	6
Expected effects of OCE	54	16
Emotions	50	16
Risk of developing OCE	40	20
Confidentiality and privacy	24	33
Personal involvement	21	23
Practical issues	20	41
Social influences	14	70
Principles and beliefs	4	61

Expected effects of the disease

The effect that OCE would have on work and private life was also a highly prioritized theme for those who were in favor of testing. These students stated that in addition to impaired work functioning for the affected person, for example, due to sensitive skin and pain, OCE may also lead to decreased hand hygiene compliance. This relates to the fact that most hand hygiene products, like disinfectant or alcohol gel, can be

Table 2. Arguments in favor of or against partaking in a genetic test for susceptibility to occupational contact eczema, expressed by student nurses

Theme	Illustrative argument(s): [+]: in favour of using the test; [-] : against using the test; [?]: questions or doubts
Emotions (e.g. curiosity, anxiety)	[+] *"I would rather be afraid of something I know, than of something that is uncertain. I want certainty".* [+] *"Everything you know is a bonus, everything you don't know is a lack of knowledge".* [+] *" I am curious: I want to know what eczema is, where it comes from, and what the consequences are".* [-] *"Life would be really boring if you already know what's going to happen"* [-] *"I can't cope with that, you'll keep worrying: when does it start, when will it happen to you! That uncertainty is terrible".*
Seriousness of the disease	[+] *"I would be ashamed if I suffered from serious hand eczema, that attracts attention, people will think you are dirty".* [+] *"If you don't feel well yourself, you cannot give good care".* [-] *"In general, hand eczema is not awful. It's a bit of a small issue. To screen everyone in the nursing profession is nonsense".*
Expected use of the test results	[+] *"I can use the test outcome to take extra preventive measures, wear gloves, use more hand cream. And there are plenty good preventive means".* [-] *"In principle, you are responsible* [for preventing OCE] *both before and after testing, and then I wonder: to what extend will the test make that responsibility grow, or decrease? I think the responsibility is your own anyway, and not with a test"* [-] *"When you see the first symptoms of hand eczema, you can always start to be more careful* then, *by using extra lotions etc."* [-] *"I know there are preventive measures, but it's not enough for me. How can I be sure that I don't get it, if I take the preventive measures? It's not like a pill, you have to pay so much attention all the time".* [-] *"I have doubts about the effectiveness of preventive measures. For example, using cotton gloves underneath other gloves, isn't that just too much effort and a waste of money?"*
Risk of developing OCE	[+] *"There is no certainty, but the risk is that high so I would want to know".* [-] *"If hand eczema would be very prevalent, I would be more willing to take the test".* [-] *"Even if the risk would be 1 in 2, I still think, if you never had any trouble with your skin, then I think the chance is lower to develop it eventually".* [-] *"1 out of 5 nurses develops hand eczema, so the chance is 1 out of 5 that that would be me, and if my risk is increased, the chance would only be higher that I would be that 1 out of 5. But 1 out of 5 already is quite a high chance. So that doesn't say very much".* [-] *"If you have an increased risk, I wouldn't put at stake the profession that you like to practice because* perhaps *you will get hand eczema".*
Confidentiality and privacy	[-] *"It should be well protected, especially with genetic information, because that not only concerns yourself but also your family".* [-] *"I am afraid that my DNA would be used for other tests without my permission".* [-] *"Nobody else should know my genetic makeup. It could be used against you, so it is important that this information is not accessible for others ".* [+] *"I really don't get why people are so scared about preserving their privacy, for example with the issue of electronic patient records. […] There is just the duty of professional confidentiality, and I assume that everyone who has access* [to the test results] *keeps to that".* [+] *"If you provide a DNA sample in a hospital, what can possibly go wrong?"* [+] *"I could use the test results as a kind of "proof" for my manager, in order to claim extra preventive measures, e.g. gloves, creams, soap. But not without my permission".*

Table 2. Arguments in favor of or against partaking in a genetic test for susceptibility to occupational contact eczema, expressed by student nurses (*continued*)

Theme	Illustrative argument(s): [+]: in favour of using the test; [-] : against using the test; [?]: questions or doubts
Principles and other considerations	[-] *"The end is lost; you may scan for anything these days".* [?] *"I worry that genetic research would make life manipulable and predictable. I can see the necessity of genetic research for serious illnesses, but shouldn't we draw a line somewhere?"* [?] *"People always want to have more information, but you never think about it beforehand what it does to you. You should think about it before you do the test, because afterwards, you cannot go back".*

painful when applied on damaged skin. Furthermore, colleagues would have to work extra shifts because of sick leave of the affected person. Because of their professional involvement, students felt responsible to maintain a healthy skin to be able to perform their work according to hygiene standards. They also mentioned that patients may not want to be treated by a nurse with hand eczema, because they think it is "dirty" or even infectious. As hand eczema is a disease that is directly visible and "cannot be hidden", these participants acknowledged a high impact of this condition in the social field. Other participants, however, thought that OCE is a non-severe, easy treatable disorder ("hand eczema is not cancer") and considered it to be "not serious enough to test for".

Expected use of the test results

The expected utility of the test results was prioritized as important by many participants who were in favor of testing, but it was also the theme with the highest prioritization rating for those who stated they did *not* want to be tested or for those who doubted. Participants who were in favor of testing said that knowledge of their susceptibility would motivate them to practice better skin care and avoid exposure where possible. On the other hand, some stated that skin care and preventive measures should be taken by everybody, not only by susceptible persons, and that a test therefore would have no added value. Taking protective measures was seen as "your own responsibility"; however, as this was used as an argument against testing by some participants, others used it as an argument in favor of testing.

Some participants expressed the concern that a positive test result might lead to over-protective behavior and "hypochondria", which would jeopardize the compliance to hand hygiene, or in the opposite case that people who find out to be *not* susceptible would become careless about their skin care and exposure or ignore the first symptoms of OCE leading to a delay in seeking treatment. Less than 10% of the participants mentioned that they would use the test results as an advisory component in their choice of professional training or career prospective.

Risk of developing OCE

The fact that a positive test result does not mean that a person will develop OCE with certainty and, the other way round, a negative test result is no guarantee for *not* developing OCE was considered a significant disadvantage. For this reason, a number of participants thought that undergoing a susceptibility test was pointless. At the presenting of the case, the students were told that the chance of acquiring OCE during an average nursing career is about 20%, or 1 out of 5, and that taking the test would inform you whether your *personal* risk would be higher or lower than 1 out of 5. This led to a diversity of reactions, such as

"On average 1 in 5, so, the chance that you get it is smaller than the chance that you don't get it",

"Considering how often we have to wash or disinfect our hands, I'm surprised that the probability is not higher",

or

"I think that is a lot, I had expected something like 1 out of 100".

Although the majority of the participants thought a prevalence of 20% was high, some regarded this as an argument against taking a test, because "the risk is high anyway". Nevertheless, others stated that information on an increased risk would still be relevant for them, even more so if that risk was high to begin with. Although many students acknowledged that they had difficulties interpreting the risk to develop OCE, the height of the risk was still a reasonably important factor in their considerations whether or not to take the test.

Confidentiality and privacy

A few participants emphasized the importance of protecting confidentiality of genetic test results; however, in general, questions about privacy and protection of personal test results were a relatively minor issue in the students' considerations. When specifically asked about it, most participants stated that they felt confident that such matters would be appropriately taken care of. Some thought that society's worries about privacy are exaggerated, referring to their experience that, at least in the medical setting, confidentiality is generally secured by the "oath of secrecy". Regarding the question of which (if any) parties should be allowed access to the test results, the majority allowed researchers and their general practitioner access to the test results and considered access by employers and insurance companies as unacceptable. However, some participants stated that disclosure of test results to employers also may have a positive effect: the employer could be convinced to supply personal protective equipment and skin care products. Disclosure of results to family members was a point of doubt, because on the one hand, they may benefit from this knowledge in view of preventive measures (this also applied to future offspring), but on the other hand, one may not want to raise unnecessary concern.

Personal involvement, Social influences, Principles and beliefs, Practical issues

Participants who had personal experience with hand eczema themselves, or had close relatives with hand eczema, were more inclined to take the test. This did not apply for participants who had seen the disease in friends or colleagues. The opinion of friends, colleagues and family members, or other social influence by, for example, school teachers, professional journals or the Internet was not regarded as influential by most participants. Nor was religion; only one respondent stated that she would not take the test because of religious constrains, while several other respondents who professed a religion said that taking a medical test would not interfere with their religious beliefs. A few participants expressed fundamental doubts about testing for OCE susceptibility, of which most thoughts were in the line of OCE being not "serious" enough to justify testing, or in the line of "what's next?" and "should we really want to know everything?".

As the case presented to the students was about a hypothetical test, practical issues like the logistics of the test method (e.g., self-test or clinical setting) and costs were not mentioned initially. When this theme was addressed by the researchers it was acknowledged to be influential but not decisive for their considerations.

DISCUSSION

Sixty-nine percent of the student nurses stated that they would agree to be tested for susceptibility to OCE. The most important arguments in favour of testing were curiosity and the possibility for preventive measures. On the other hand, concerns were expressed regarding the difficulty of interpreting test results, the utility of the test result in practice, and the usefulness of genetic tests for less serious diseases.

Most of the opinions expressed by the students are addressed in existing guidelines. In addition, many students mentioned the difficulty of risk interpretation and the need for practical advice accompanying the test results, elements that are hardly elaborated in the guidelines we reviewed. We will discuss the students' opinions in the light of existing guidelines, according to the themes mentioned before and in the order we consider as most appropriate to follow when choosing to offer a susceptibility test or not. Finally we will also briefly discuss test validity, which is an important condition, but was not a subject for the student nurses study.

Seriousness of the disease

Some of our students questioned if OCE is serious enough to test for. This corresponds to "seriousness of the disease" mentioned in all existing guidelines. Adverse effects on work performance and effects on quality of life should also be considered here. In the scenario of career counseling, milder diseases can be accepted as precondition to allow the offering of susceptibility testing than in the scenario of a pre-employment examination where selection of workers may be the consequence.

Expected use of the test result and possibilities for preventive measures

Most of the thoughts on utilizing the test results to take preventive measures expressed by the students were related to means of exposure reduction in the workplace. The remark of several student nurses that protective measures should be taken by everyone, regardless of susceptibility, corresponds with the view expressed by several authors that susceptibility testing should not shift the focus from exposure reduction in the workplace to selection of individual workers [2;3;7;27-29]. Measures to reduce exposure on workplace level always come first, following the so called occupational hygiene strategy.

Effective preventive measures must be available for those who test "susceptible", for example extra personal protective equipment and an educational program or a change of work tasks. However, even if preventive measures are available, they may not be recognized as an argument in favor of testing by (part of) the target group. Some of the students stated that they would not change their skin protection behavior anyway. Two earlier studies investigating the willingness to take a genetic test among young adults have found similar results. Harel *et al.* (2003) used a questionnaire survey among 361 high school students (16-18 years) to assess whether or not they would be interested to take a genetic test for hypercholesterolemia, for breast cancer and for Tay Sachs disease [30]. One of the most important arguments against testing for hypercholesterolemia was "I would not act on the results anyway". In a recent interview study among 33 American college students, only one-third of the participants stated that they would be interested in taking a genetic test for susceptibility to lung cancer and that they considered a "positive" result as an extra motivation to quit smoking [31].

Interpretation of risks and communication of test results

The difficulty of risk interpretation and the need for practical advice accompanying the test results is lacking in most recommendations about genetic testing for occupational diseases. MacDonald & Williams-Jones recommend genetic counselling, but do not specify which components this counselling should include. Risk interpretation is difficult, especially for susceptibility tests as these only provide a *change in probability* to develop a disease. Even if risk information is well communicated, the individual's interpretation of personal risk may be confused by non-rational lines of reasoning, such as "binary thinking", where people perceive any risk – regardless of its actual size – in a binary (yes/no), not a graduated (probabilistic) way [32;33]. The European Group on Ethics expressed that researchers, policy makers and companies implementing susceptibility tests should beware of misinterpretation of risk estimates too [4]. Furthermore, perception of risk is known to be dependent on personal context, including emotions, and people may exaggerate or downplay risks regardless of the numerical probability they assigned to it [33;34]. The influence of subjective values and emotions on risk interpretation should not be underestimated. Adding practical

advices to the test results will promote adequate behaviour and may help to prevent, for example, hypochondria and the "carte blanche effect"[2;7;35].

Voluntary consent

It was mentioned by several student nurses that the test should be offered on a voluntary base and that it should be up to the tested individual to disclose the results to their employer or not. It can be questioned, however, to what extent genuine voluntary testing can be achieved in a workplace setting[2;3;29]. Workers may fear that refusing to take the test may have negative consequences for their position. The employer should ensure that participation is voluntary and that there are no consequences for those who refuse to be tested. Preferably, the test should be executed by an independent organization, so that feelings of mistrust among workers are prevented.

Confidentiality and privacy

Worries about privacy and confidentiality were expressed by only a few student nurses; many respondents stated that they were confident that such matters would be properly taken care of. Furthermore, whereas most ethicists are worried that employers may abuse test results for employee selection, a few of our participants came up with the idea of using a positive test result to convince employers to supply extra protection. The confidence in privacy protection may reflect the optimistic views of a relatively young population that has not (yet) experienced situations where privacy may be violated. Similarly, in the study of Harel *et al.* (2003)[30] only a quarter of the students agreed with the statement "I am worried that results will be misused by my insurance company / employer". In contrast, privacy and confidentiality issues were the main concerns expressed in three recent American surveys, i.e. among beryllium workers and their relatives[13], employees of a university research centre and a national laboratory[12] and unionized workers[36]. The workers in the study of Brandt-Rauf *et al*. (2011)[36] distrusted even their own physicians, despite their oath of secrecy. The fact that most occupational physicians have to protect the workers as well as the interests of the companies they work for often gives rise to a suspicious attitude among workers towards their occupational physicians[37]. The arrangement of privacy and confidentiality issues, including who has access to the results, should be clearly communicated in the test information.

Test validity

In our study of the student's opinions, the validity of the test was not a subject for investigation. However, because of the specific character of genetic susceptibility tests, the test validity –in the widest sense- deserves attention.

Analytical validity (reliability) for genetic tests will approach 100%. The clinical validity of susceptibility tests, however, is difficult to assess. For presymptomatic tests of present disease, the error can be expressed relatively simple by the "predictive"

value of a positive or negative test result. For susceptibility tests, the error in the prediction of – future – disease is more complex, as it contains unknown elements that often cannot be assessed: the extent of future exposure, the presence of non-tested susceptibility or protection factors, and interactions between these factors, including the tested gene. This holds especially for predicting the disease risk for an individual.

Nevertheless, before offering a test one should estimate, using some assumptions, the predictive values on group level. For example, if in a group of 100 workers the frequency of the susceptibility gene is 10%, the relative risk of acquiring the disease in carriers of the susceptibility gene versus non-carriers is 3, and the lifetime incidence of the disease at the prevailing level of exposure is 20% for the whole group, the probability of developing disease in carriers and non-carriers can be calculated as 50% (5/10) and 17% (15/90), respectively, see Table 3.

As the incidence of occupational diseases depends on the extent of exposure and on the presence of not tested susceptibility factors, the probability of disease can be estimated from data stemming from another population only with due consideration of these factors. This should be considered for every new application. Furthermore, in this example 50% of the carriers will not develop disease while 17% of non-carriers will still develop disease. The importance of the 50% healthy positives and 17% diseased negatives should be considered (in tests of presymptomatic disease the corresponding groups would be called false-positives and false-negatives; however in susceptibility testing there is no such "false"). Healthy positives may experience unnecessary worry or even unjust exclusion from jobs. Diseased negatives would also have occurred without testing; their presence points to a low efficiency of the execution of the test in this population, that is, they limit the preventable fraction.

The practical consequences of the presence of healthy positives or diseased negatives will differ depending on the type of disease, the characteristics of the target population and available treatment or prevention options with different kinds of benefits and harms. In the case of a susceptibility test for hand eczema, for example, a healthy person with a "susceptible" test result would receive unneeded extra skin care resulting in unnecessary costs, whereas a person who would falsely test "non-susceptible" would receive only standard skin care where extra skin care would be needed. In this case, the consequences of being a "diseased negative" could be more serious than that of being a "healthy positive". For tests for diseases requiring invasive treatment or complete elimination of exposure (which could result in job loss) this can be the other way round.

This study presented opinions concerning ethical aspects of the use of a genetic susceptibility test for OCE among student nurses. As our participants were recruited by convenience sampling in a single city, the results cannot be extrapolated to other populations without considerations. Nevertheless, we confirmed that most opinions expressed by a student nurse stakeholder group as described are covered by the existing guidelines on genetic testing in the workplace.

Table 3. Frequency of occupational disease depending on the presence of a susceptibility trait

Susceptibility trait	Disease will develop (at continuing exposure)	Disease will not develop (at continuing exposure)	
Present ("positives")	5	5	10
Not present ("negatives")	15	75	90
All	20	80	100

Example: 100 subjects, carrier frequency = 10%, relative risk of disease = 3, cumulative disease incidence = 20%.

Comparing the students' statements with the issues addressed by the guidelines, we conclude that the guidelines should pay more attention to risk communication and practical advice accompanying the test results. In our opinion, the following key elements should be considered for the choice to offer a test or not: (1) validity of the test, including analytical reliability, frequency of the trait, relative disease risk of the trait, and expected disease frequency, (2) the seriousness of the disease, and (3) the possibilities for prevention and related benefits and harms. Three additional elements related to the implementation of the test should be considered: (4) risk communication and need for practical advice accompanying the test results, (5) voluntary consent and (6) privacy and confidentiality. Guideline development can be organized by public health officials, associations of occupational physicians or other occupational health care providers. Our data emphasize the need for good individual risk communication both before and after testing, taking into account that the test concerns susceptibility.

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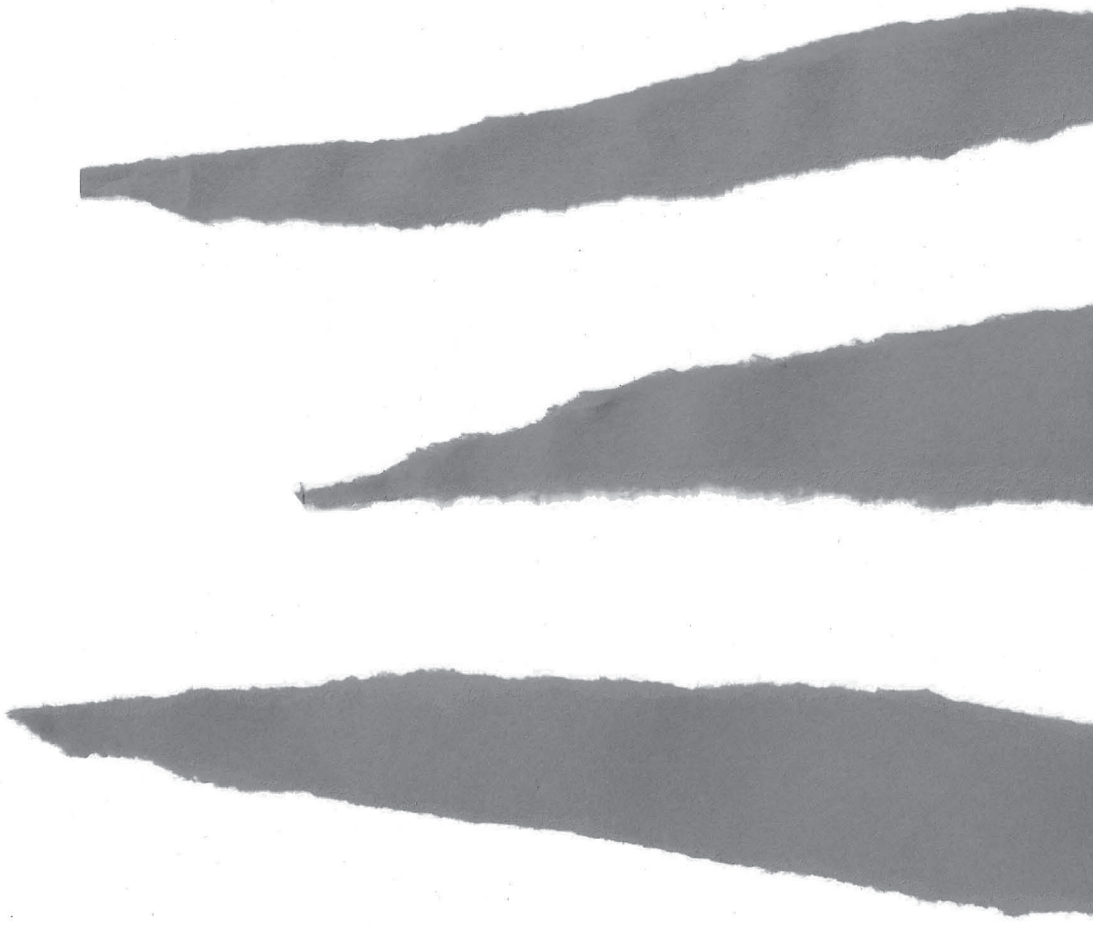

5

GENERAL DISCUSSION

GENERAL DISCUSSION

This thesis primarily aimed to investigate the contributions of loss-of-function mutations in the filaggrin gene (*FLG*), atopic dermatitis (AD), and occupational exposure to development of contact dermatitis in high risk jobs. An underlying thought was the question whether *FLG* genotyping would be feasible to use in susceptibility screening programs for occupational contact dermatitis (OCD). Furthermore, we investigated the attitude of apprentice nurses toward susceptibility testing. In this chapter, the main results are summarized, and interpretations of the results and some methodological considerations are discussed. Finally, recommendations for practice and further research are given.

MAIN FINDINGS

On the basis of the studies described in this thesis, we have found that:

- *Apprentice nurses are at substantial risk of developing hand eczema already during traineeships (Chapter 2.2).* In the cohort study, the 1-year period prevalence of HE was 21% - 31% during follow-up. Among participants without a history of hand eczema, 18% developed hand eczema during their traineeship.
- *Regarding exposure, frequent hand washing during traineeships was the main risk factor for hand eczema in apprentice nurses (OR = 2.2), while the use of hand alcohol gel rubs and occlusive gloves did not increase the risk. In addition to occupational exposure, hand washing at home was a significant risk factor (OR = 1.8) (Chapter 2.2).*
- *A history of AD increases the risk for OCD (Chapter 3.1 and 3.2).* In the case-control study of occupational irritant contact dermatitis (ICD) the OR for AD, adjusted for *FLG* mutations, was 2.9. In the prospective cohort study of hand eczema the OR for AD, adjusted for wet work exposure and *FLG* mutations, was 2.5.
- *Adjusted for AD, FLG mutations increased the risk for OCD in the case-control study, but not in the prospective cohort study (Chapter 3.1 and 3.2).* In the case-control study an increased risk of occupational ICD conferred by *FLG* mutations alone was found (OR=1.6). In the prospective cohort study, *FLG* mutations in absence of AD had no effect on the risk of hand eczema.
- *Individuals with concomitant FLG mutations and AD appear to have the highest risk of developing OCD (Chapter 3.1 and 3.2).*
- *The opinion of apprentice nurses towards genetic testing for susceptibility to hand eczema is incompletely covered by existing guidelines on genetic screening for susceptibility to occupational diseases (Chapter 4).* Issues missing in the guidelines were: (1) the difficulty with interpreting risk information and (2) the need for practical advice accompanying test results.

METHODS USED TO ASSESS WET WORK EXPOSURE, ATOPIC DERMATITIS AND HAND ECZEMA

In addition to the methodological issues discussed in the respective studies, the following issues deserve some extra attention:

Measurement of wet work exposure

Because of the relevance of wet work exposure in the development of OCD, we aimed to perform a thorough exposure assessment in our cohort study. Several exposure measurement techniques are available for this purpose, each with their own advantages and disadvantages. The use of observations or questionnaires (self-report) are two common methods for the assessment of wet work exposure. Observations of the subjects performing wet work are time-consuming, which makes them unsuitable for large cohorts, and have the drawback of inducing behavioral changes in the workers observed. On the other hand, self-report can be subject to recall bias. Some more objective techniques exist for the measurement of dermal exposure, e.g. absorbing patches, rinse or wipe techniques, but none of these are suitable for sampling exposure to water. Another challenge for the assessment of wet work exposure is its complexity. According to the German TRGS guideline, the total duration of wet work should not exceed 2 hours a day [1], and a Dutch expert group recommended that the total number of wet work events (all added together) should not exceed 20 times a day [2]. In order to uphold these guidelines, both the cumulative duration *and* frequency of contact with irritants (including water) should be measured as well as the use of occlusive gloves [1;3]. The wet work sampler described in Chapter 2.1 seemed promising in this respect, but unfortunately, it appeared not to be suitable in assessing wet work exposure in nurses. In our cohort study, we therefore chose to use diary cards. Earlier studies focusing on wet work exposure in nurses have found that when using questionnaires, nurses tend to overestimate the duration and underestimate the frequency of their wet work activities [4;5]. In our study, the participants were asked to fill in the cards on several days of each traineeship period to obtain more reliable estimates in the case of fluctuations in wet work activities. The exposure estimates obtained by using the diary cards showed good agreement with the data from other observational studies among (apprentice) nurses [4;6-8], suggesting that our results were reliable at least on a group level.

Case definition for AD

The definition of AD in research is still a point of debate. In preceding studies that investigated AD as a risk factor for OCD, diverging criteria have been used. In several studies, the categorization of AD was based on a positive answer on questions like "Have you ever had atopic dermatitis?" or "Has a physician ever told you that you had atopic dermatitis?", whereas in other studies, symptom-based definitions were used, usually including the presence or a history of flexural eczema, asthma, allergic rhinitis,

or general allergic symptoms like hay fever [9-14]. In our prospective cohort, we used a set of criteria based on the UK Working Party Diagnostic Criteria [15] "Question-only" version, whereas in our case-control study we based the diagnosis of AD on the current or past presence of 'flexural eczema'. Despite this difference in the case definition of AD, the prevalence of AD among both the Dutch and German apprentices (24% and 19%, respectively) is in the range of what has been found in other epidemiological studies [16-19].

Case definition for hand eczema

As opposed to our case-control study, where the diagnostics was based on clinical examination, questionnaires and exposure history, our prospective cohort study used a symptom-based definition to assess self-reported hand eczema. Although we initially intended to have a clinical examination of all suspected hand eczema cases, from practical reasons this turned out to be impossible. Based on the reported symptoms, we could not classify the hand eczema as irritant, allergic or atopic. As *FLG* mutations are reported to have little influence on allergic contact dermatitis [20-24], the presence of allergic hand eczema cases may have influenced the observed low association between *FLG* mutations and hand eczema in our cohort.

ROLE OF FLG LOSS-OF-FUNCTION MUTATIONS AND AD IN SUSCEPTIBILITY TO OCD

Previous studies have convincingly shown that present or past AD increases the risk of developing OCD [9;10;25-27], an effect that was confirmed in our case-control study as well as in our prospective cohort study. The mechanisms by which AD modifies the risk for OCD is not clear yet. A general enhanced immune reactivity in AD skin may explain part of the increased susceptibility to OCD. Another explanation might be an impaired skin barrier function, which is a major hallmark of AD. Skin barrier failure in AD may be due to reduced amounts of filaggrin, but it can also be caused by other factors, for example, an impaired organization and structure of the skin lipids, altered enzyme activity involved in desquamation, or changes in the levels of the proteins of tight junctions and the cornified envelope [28-35]. Intrinsic filaggrin deficiency is not only dependent on *FLG* mutations but also on copy number variations in the *FLG* gene [36]. Reduced levels of filaggrin can also be a secondary effect of disease itself, as the Th2-mediated cytokine milieu in AD skin has been shown to suppress the expression of filaggrin [37-40]. It is likely that in heterozygous carriers of *FLG* mutations, the levels of filaggrin are further decreased by the processes related to AD, which might explain the highest susceptibility of the individuals with concomitant AD and *FLG* mutations in our studies.

In the case-control study, we found a small but significant effect of *FLG* mutations on the risk of OCD, adjusted for the history of AD. In the cohort study, we did not

find a distinct effect of *FLG* mutations on the risk of hand eczema. This might partly be explained by the differences in disease status between these two studies (chronic, rather severe irritant contact dermatitis versus early symptoms of hand eczema of all subtypes, which may have included atopic and allergic hand eczema), as discussed earlier. Our two studies agree in the finding that individuals with concomitant AD and *FLG* mutations had the highest risk of OCD. A large cross-sectional study among the general population in Copenhagen found a similar result: *FLG* mutations were associated with hand eczema in subjects who also had AD (OR=2.98; 95% CI 1.27 – 7.01) but not in subjects without AD (OR=0.82; 95% CI 0.41 – 1.67) [13].

In the studies described in this thesis, the contribution of *FLG* mutations and AD to the development of OCD each was calculated while adjusting for the other risk factor, a common procedure to eliminate confounding. However, AD is not only an independent risk factor for OCD, but is also – in part of the subjects with *FLG* mutations – an element in the etiological pathway from *FLG* to OCD. Thus, the etiological contribution of *FLG* mutations may be somewhat underestimated in our studies. *FLG* mutations are associated with more severe AD [41-45] but on the other hand, less than half (approximately 40%) of the *FLG* carriers develops AD [46;47]. Thus it seems that in addition to *FLG* mutations, some extra stimulus is needed to predispose for AD and subsequently OCD. The exact nature of these internal or external stimuli is as yet unknown, but a specific cytokine milieu and environmental exposure to allergens or irritants at a young age may be involved. It might also be that part of the *FLG* mutation carriers somehow are able to compensate for the reduced filaggrin levels in their skin via yet unknown mechanisms, which enable them to (partly) restore their skin barrier function and protect them against developing OCD as well as AD. More research into skin barrier properties of this subgroup of *FLG* carriers and into possible predisposing stimuli is warranted, as it might open up possibilities to better protect individuals from AD and OCD.

SOCIAL IMPLICATIONS

Predicting the risk of OCD

One underlying reason behind the investigations in this thesis was the question whether *FLG* genotyping can be used as a test for susceptibility to OCD, in addition to assessment of the presence of the known risk factor AD. Different answers to this question may apply according to the precise aims and context for which *FLG* genotyping would be used. Possible scenarios could be, for example: 1) use as part of the diagnosis in OCD-patients aiming at more targeted prevention and therapy, 2) use as part of a pre-employment medical examination, or 3) use in career or educational counseling.

In the first scenario, as part of diagnosis, *FLG* genotyping can add to the understanding of the individual disease aetiology and can influence treatment and prevention measures. Recently, it has been found that topical application of recombinant filaggrin restores filaggrin levels in the skin of *FLG*-deficient ('flaky tail') mice and restores their Ichtyiosis Vulgaris phenotype towards normal skin [48]. This may

be a starter to the development of new topical treatment aimed at restoring filaggrin levels in the *FLG* mutation carriers.

In the second and third types of intervention, *FLG* genotyping is used as a tool to assess *susceptibility*, which implies that some considerations are needed in addition to the criteria for *diagnostic* tests. One reason is that a positive result on a susceptibility test does not mean that the disease will occur with certainty; the probability of the development of the disease is influenced also by other factors than susceptibility, such as the 'background' risk of the disease in the population and the exposure characteristics. As discussed in **Chapter 4**, the positive predictive value of a test is one of the key criteria to select and decide on the implementation of a screening test. It displays the probability that a person will develop the disease, given a positive test. Also for a screening test with a high positive predictive value, however, the decision on application of the test depends on the context in which it would be used: the supposed gain due to more targeted prevention has to be balanced against the efforts and costs of testing and ethical issues associated with using the test, such as potential exclusion from the job for people with a positive test result and potential violation of privacy or confidentiality. Furthermore, the possibilities for and the effectiveness of preventive measures that can be taken upon a positive test result may differ per situation.

In pre-employment medical examination according to the Dutch OCD guideline, individuals with AD in combination with chronic hand eczema are regarded as unfit for working in jobs with frequent wet work exposure. Individuals with AD without a history of hand eczema are advised to pay extra attention to skin care and are monitored by the occupational physician [49]. Our results indicate that among individuals with AD, the subgroup with concomitant AD and *FLG* mutations have a substantially increased risk of developing OCD. Moreover, a longitudinal follow-up of the OCD patients from our case-control study revealed that AD+/*FLG*+ patients had more persistent disease associated with the worst prognosis and the lowest rate of return to their job [50]. These prognostic data emphasize that this particular group should receive special attention in the pre-employment medical examination.In the scenario of education or career counseling, it might be considered to advise only the most susceptible, AD+/*FLG*+, individuals to avoid high-risk jobs, while non-carriers with a history of AD can pursue a career in a high-risk job when desired – provided that they take extra preventive measures. As already mentioned in **Chapter 4**, good risk communication – including practical advices – is of crucial importance in this scenario. Ideally, the expectations and perceptions of the intended target groups regarding susceptibility testing should be surveyed, so that risk communication can be tailored for each group concerned. A qualitative study by our group has shown that the best method to achieve this is by using interviews or focus groups [51].

Prevention of OCD: perceptions, behavior and possible interventions

In addition to the measured effects of exposure and susceptibility factors, our prospective cohort study has yielded some other interesting results regarding

behavior of the apprentice nurses towards wet work exposure and skin protection and regarding dealing with skin complaints.

Through communication with study participants during our prospective cohort study, we have noticed that many students were not aware of the skin damaging effects of wet work. For example, most apprentices did not know that hand washing results in more skin damage than disinfection with alcohol gel. Some of them actually perceived the opposite, because the alcohol may give a stinging or burning sensation, especially on already compromised skin. The idea that the use of alcohol gel rubs is worse to the skin than the use of water and soap seems to be a frequently occurring misconception; two German questionnaire surveys revealed that 60-70% of the nurses regarded hand alcohol gel rubs as more damaging to the skin than hand washing [52]. In addition, students working outside hospitals in e.g. nursing homes or homecare sometimes did not have access to alcohol gel rubs at their workplace, so they had to use water and soap. The same applied for the availability of protective gloves.

Another observation from our cohort study revealed that the majority of apprentices who reported hand eczema did not consult the occupational physician, even if they were actively invited to an easily accessible, free consultation by telephone. The invitation included the message that the occupational physician would give professional advice about skin care and protection. Still, approximately two-third of the students who were offered such a consultation did not make use of it. Especially if the symptoms were relatively mild, they were regarded as 'not worth bothering a physician with'. Among healthcare employees with self-reported hand eczema who were invited to consult a specialized occupational dermatology nurse, a slightly higher attendance rate of 46% was observed [27]. The importance of skin protection measures is often not recognized by workers, even if they already have OCD [53]. Such a rather careless attitude towards OCD symptoms is undesirable, because early intervention can prevent the progression from mild symptoms to severe, chronic OCD.

Obviously, the first step in the prevention of OCD is education. Students pursuing a career in healthcare or other high risk occupations should be informed on the effects of skin exposure and the importance of adequate skin care and early recognition of symptoms, especially if they have a history of AD. This means that knowledge on occupational exposure, skin care and prevention of OCD should be disseminated to vocational schools and become an integral part of the curriculum. The role of personal susceptibility should be brought under attention among (prospective) students while they still have the option to choose between different specializations; educational or career counsellors should play a more proactive role in this. Furthermore, education should not stop after finishing the vocational training; workers should be reminded of the importance of preventive measures and should be stimulated to report early skin symptoms to their occupational physician or occupational health nurse. Employers in healthcare should be educated about the skin damaging effects of different hand hygiene measures, the costs associated

with hand eczema (e.g. in terms of sick leave, but also increased risk of infections) and adequate prevention measures. As skin irritation forms a major reason for noncompliance with hand hygiene rules [52;54;55], which is estimated to cause one-fifth of healthcare associated infections [56], implementation of the Dutch OCD guideline will kill two birds with one stone. Not only hospitals, but also nursing homes and similar healthcare institutions should receive this information, so that the availability of alcohol gel rubs and protective gloves can be promoted.

Education and training as part of secondary and tertiary prevention programs (aiming at improving skin condition in workers with OCD) has already been shown successful according to a few intervention studies in Germany [57-59] and Denmark [60]. However, to our knowledge, studies describing interventions aiming at primary prevention of OCD are lacking. It would be recommendable to develop educational programs for primary prevention and assess their effectiveness in longitudinal studies.

CONCLUSIONS AND RECOMMENDATIONS

In summary, the results of this thesis lead to the following conclusions and recommendations:

Conclusions

1. Apprentice nurses are at substantial risk of developing hand eczema during traineeships; the most important exposure factor is frequent hand washing at the workplace as well as at home.
2. Adjusted for AD, *FLG* mutations significantly increased the risk of chronic irritant OCD (OR=1.6) in our case-control study, but had no effect on the risk of hand eczema in apprentice nurses. Individuals with concomitant *FLG* mutations and AD appear to have the highest risk of OCD.
3. Guidelines on genetic screening for susceptibility to occupational diseases show a deficiency concerning risk communication and the need of practical advice accompanying the test results for the individuals undergoing genetic screening.

Recommendations for further research

1. More research is needed into skin barrier function of *FLG* mutation carriers without AD, to reveal possible compensatory mechanisms.
2. Future studies should further investigate the relative risk for OCD conferred by *FLG* mutations. In view of the relatively small number of *FLG* carriers among the population, multicenter studies of starting employees in high risk occupations are to be preferred.
3. Intervention studies should be set up on the effects of primary prevention of OCD by education and training programs, preferably embedded in a comprehensive preventive program.

Recommendations for practice

1. Education and encouragement to prevent hand eczema should be intensified, not only for workers but also for vocational students, giving attention to alternatives for the use of water and soap, to skin care, and to early recognition of signs and symptoms. Occupational and regulatory health professionals, employers and vocational schools should facilitate exposure reduction measures, promote skin care and give attention to high risk groups.
2. Including *FLG* genotyping in addition to the anamnesis of AD as susceptibility screening for OCD in all applicants for a high risk job is not recommended. However, *FLG* genotyping of individuals with AD may aid in diagnosis and more tailored therapy and prevention. In view of the high risk of OCD in AD+/*FLG*+ individuals, renouncing from entering a high exposure job may be considered for this group.
3. In the process of implementing any screening tool, attention should be paid to difficulties with interpreting risk information by the person undergoing the test and to practical advice accompanying the test results. The best way to prepare such an intervention is by deploying focus groups or interviews with stakeholders.

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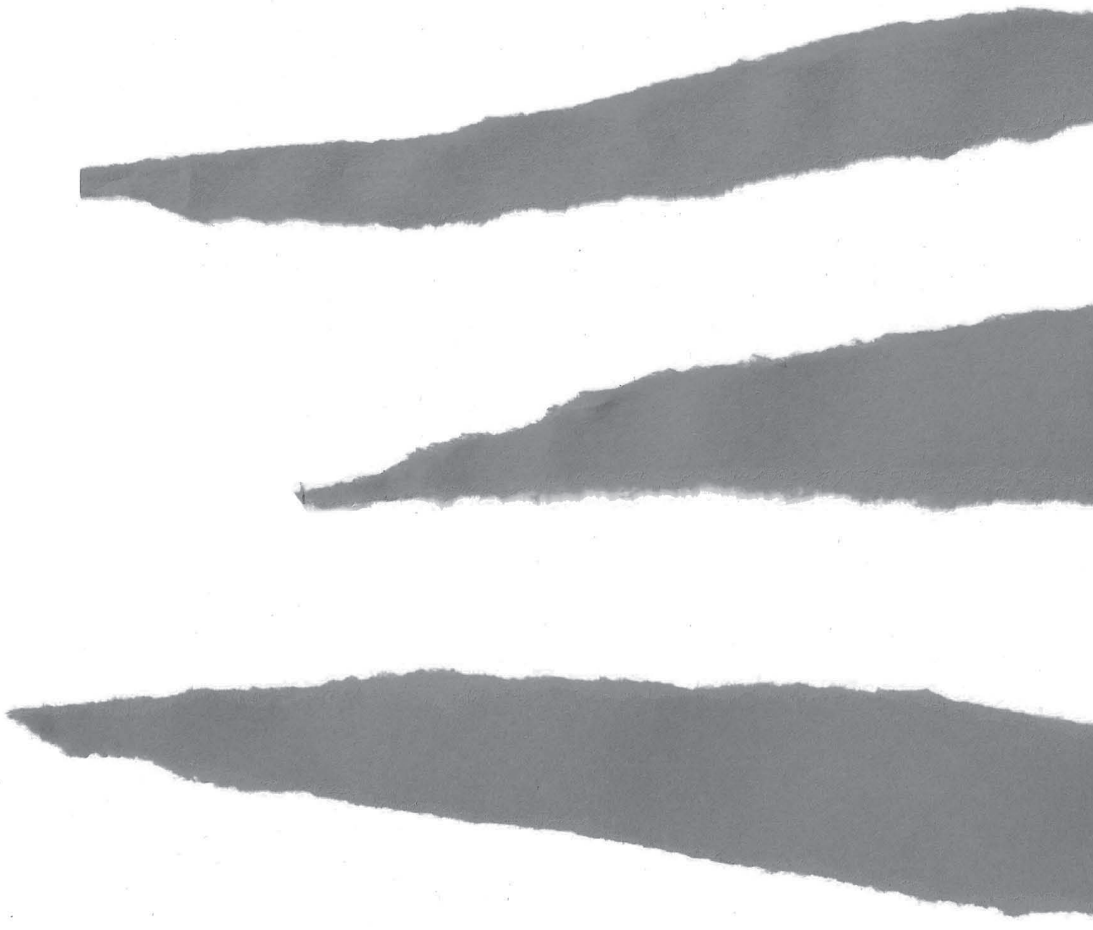

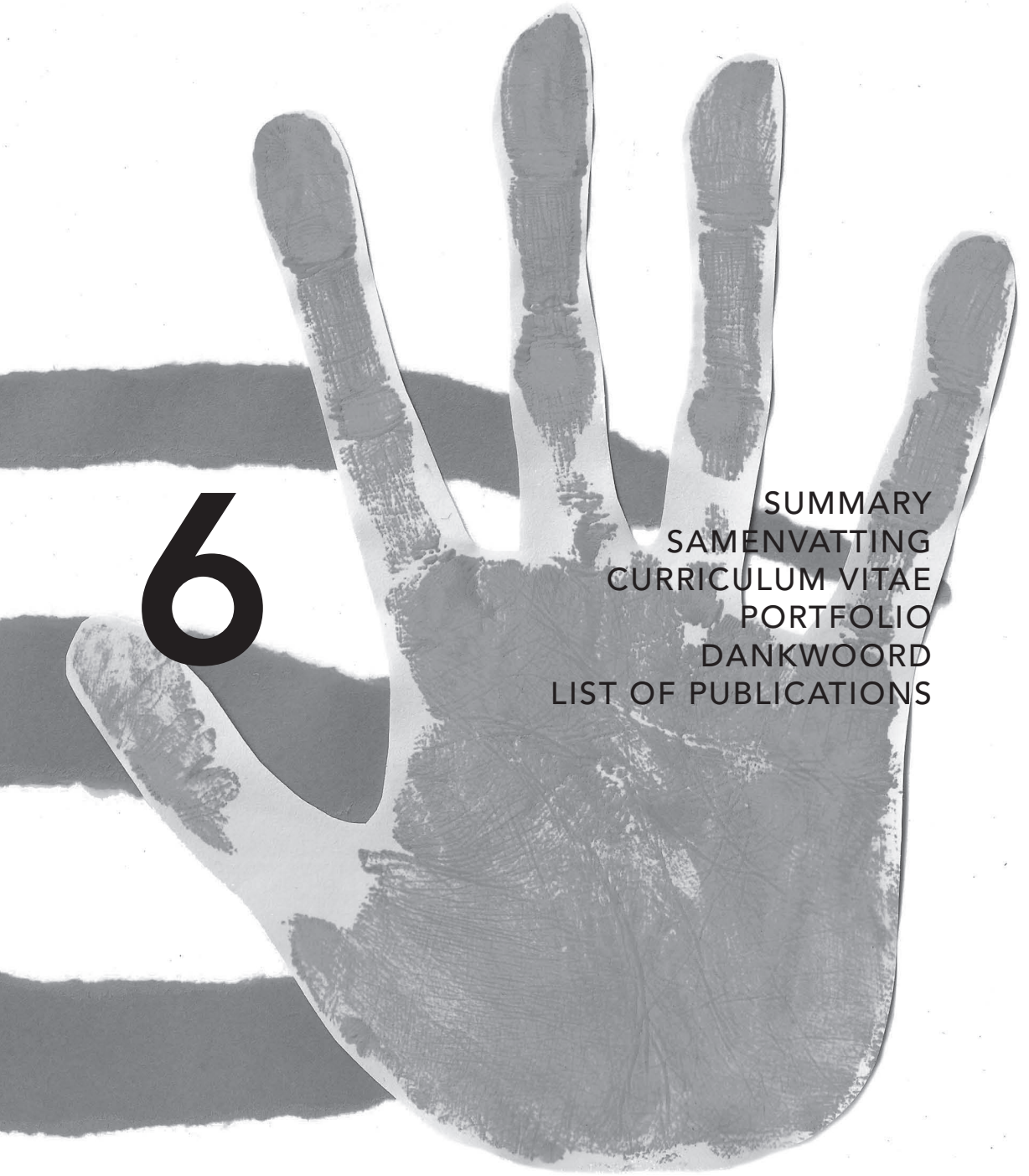

6

SUMMARY
SAMENVATTING
CURRICULUM VITAE
PORTFOLIO
DANKWOORD
LIST OF PUBLICATIONS

SUMMARY

General introduction

Occupational Contact Dermatitis (OCD) is a highly prevalent work-related disease, that is induced by skin exposure to irritants or allergens. In the workplace, irritant contact dermatitis (ICD) is the most common form of OCD. High risk occupations are in health care, hairdressing, the food sector and the metal industry. OCD tends to become chronic; persistent OCD often results in impaired quality of life and loss of work ability.

In addition to environmental exposure, the development of OCD is influenced by personal susceptibility. The uppermost layer of the skin, the Stratum Corneum, forms an effective barrier against penetrance of chemical and biological agents and prevents water loss from the skin. Some individuals have an intrinsically impaired skin barrier, for example, individuals with atopic dermatitis (AD). AD is a chronic inflammatory skin disease characterized by dry skin, pruritus, and erythematous lesions, often including flexural eczema. In the European population, AD is prevalent in 10-20% of children and up to 10% in adults, and it is a firmly established risk factor for ICD. Furthermore, it has recently been discovered that 'filament aggregating' epidermal protein *filaggrin* has an important function in the skin barrier. Loss-of-function mutations in the filaggrin gene (*FLG*), present in approximately 7-10% of the Western European population, result in a decreased amount of filaggrin in the skin and increase the susceptibility to AD and, possibly, to ICD.

The primary purpose of this thesis was to study the contribution of *FLG* loss-of-function mutations, AD, and occupational exposure in the causation of OCD. A second goal was to explore whether it would be recommendable to include *FLG* genotyping in susceptibility screening programs for OCD, in addition to the usual examination of present or past AD. In this context, also the attitude of apprentice nurses towards genetic susceptibility screening for OCD was studied.

Wet work

'Wet work', i.e. frequent contact with water, soap, detergents, or prolonged use of occlusive gloves, is a major cause of OCD. The German guidance TRGS 401 recommends that the duration of wet work should not exceed 2 h/day. This highlights the need for a reliable method to assess duration and frequency of wet work.

In Chapter 2.1, the use of a recently developed wet-work sampler was evaluated in a healthcare setting, using direct observation as reference. The sampler uses the temperature difference, generated by evaporative cooling, between a sensor on the skin and a second one 2 mm above the skin. Twenty-six nurses wore the sampler on the volar side of the middle finger for approximately 2 hours during their regular daily tasks, while being observed by a researcher. Sampler results were evaluated using various threshold values for the temperature difference to identify wet events of the hands. The optimal temperature difference to discern wet and dry skin

varied considerably between individual nurses. Individual results yielded a median sensitivity of 78 and 62% and a median specificity of 79 and 68% for indicating wet skin and glove use, respectively. As agreement between observed wet work and device-reported wet events was not high, further developments are required.

In Chapter 2.2, wet work exposure and its influence on the risk of developing hand eczema were investigated in a prospective cohort study among Dutch apprentice nurses, who were starting their practical training in different healthcare sectors, e.g. hospitals and nursing homes. Participants recorded wet work exposure and symptoms of hand eczema using specially designed diary cards. Seven hundred and twenty-one apprentices were included; for 533 participants, a follow-up time of 1–3 years was completed. Diary cards were supplied by 383 participants.

The 1-year period prevalence of hand eczema was 23% in the first year of follow-up, 25% in the second year and 31% in the third year. Eighty-one new cases of hand eczema developed, most of which occurred during the first year of follow-up. In approximately one-third of the participants, wet work exposure exceeded the Dutch national guideline of 2h/day or 20 wet work events/day. Frequent hand washing during traineeships [odds ratio (OR) 1.5; 90% confidence interval (CI) 1.0–2.3], frequent hand washing at home (OR 2.3; 90% CI 1.5–3.7) and having a side job involving wet work (OR1.6; 90% CI 1.0–2.4) were independent risk factors for hand eczema.

Influence of AD and FLG mutations

In Chapter 3.1, the contribution of *FLG* mutations and AD on the risk of OCD was investigated in German patients with severe, chronic irritative OCD and in controls (vocational school apprentices). 634 Patients and 393 controls were genotyped for the most common *FLG* mutations (R501X, 2282del4, R2447X and S3247X). Current or past flexural eczema was used as an indicator of AD.

FLG mutations were found in 16% of the patients with OCD and in 8% of the controls, with a crude OR of 2.1 (95% CI 1.3–3.3) for the combined genotype. The OR for *FLG* mutations, adjusted for AD, was 1.6 (95% CI 1.0–2.6). Subjects with AD had an OR, adjusted for *FLG* mutations, of 2.9 (95% CI 2.1–4.0). There was no evidence of interaction between these two risk factors.

In Chapter 3.2, the contribution of *FLG* mutations and AD, together with wet work, on the development of hand eczema was studied in a prospective cohort study in Dutch apprentice nurses. At inclusion, history of AD and hand eczema were assessed by questionnaire, and genotyping was performed for the four most common *FLG* mutations. Exposure and hand eczema prevalence during traineeships were assessed with diary cards.

The prevalence of hand eczema during traineeships was higher among subjects with a history of hand eczema at inclusion. Hand washing during traineeships and at home increased the risk of hand eczema (OR=2.2 and OR=1.8, respectively). Adjusted for the effects of exposure and *FLG* mutations, an OR of 2.5 (90% CI 1.7–3.7) was

found for AD. Subjects without a history of AD showed no increased risk of hand eczema conferred by *FLG* mutations, but subjects with concomitant *FLG* mutations and AD had an OR of 3.6 (90% CI 1.7-7.5), adjusted for wet work exposure.

Opinion of apprentice nurses on genetic testing for susceptibility to OCD

Genetic research has opened up possibilities for identification of persons with an increased susceptibility for occupational disease. However, regulations considering the ethical issues that are inevitably associated with the use of genetic tests for susceptibility for occupational diseases are scarce. The question is whether the opinions of intended stakeholder groups are sufficiently addressed by existing recommendations.

In **Chapter 4**, attitudes and opinions of Dutch student nurses as a stakeholder group toward a genetic test for susceptibility to OCD were studied in a qualitative setup, using focus groups, interviews and electronic questionnaires. The results were compared with guidelines and recommendations extracted from the literature.

Sixty-nine percent of the student nurses said they would partake in a genetic test for susceptibility to OCD when available. The main arguments in favour of testing were curiosity about one's susceptibility and the intention to take preventive measures based on the test result. Concerns were expressed regarding the difficulty of interpreting test results, the utility of the test result in practice and the necessity of genetic tests for non-severe diseases. For the issue of privacy and confidentiality, the students expressed few worries and much confidence that this would be well organized. The existing guidelines largely covered the students' opinions. Still, two important issues were missing in the guidelines, namely: (1) the need for good risk communication considering difficulties with interpreting risk information, and (2) the need for practical advice accompanying test results.

General discussion

In **Chapter 5** the main findings of the thesis are discussed. The most important finding is that *FLG* mutations mainly appear to increase the risk of OCD in the presence of concomitant AD. It seems that some extra stimulant may be needed to predispose for AD and OCD in *FLG* mutation carriers. It might also be possible that some *FLG* mutation carriers are able to compensate for the reduced amount of filaggrin in their skin, counterbalancing their predisposition to develop AD and OCD.

The usefulness of *FLG* genotyping will depend on the context in which it would be used. Possible scenarios are diagnosis and treatment, pre-employment medical screening, and career counselling.

Many apprentices in our cohort study were hardly aware of the health risks associated with wet work exposure and had a rather careless attitude towards skin protection. This stresses the importance of education.

On the basis of the results of this thesis, the following conclusions and related recommendations are presented:

1. Apprentice nurses are still at substantial risk of developing hand eczema during traineeships. Education and encouragement to prevent hand eczema should be intensified, giving attention to alternatives for the use of water and soap and to skin care. The effectiveness of such activities should be assessed.
2. Adjusted for AD, *FLG* mutations significantly increased the risk of chronic irritant OCD (OR=1.6) in our case-control study, but had no distinct effect on the risk of hand eczema in apprentice nurses. Individuals with concomitant *FLG* mutations and AD appear to have the highest risk of OCD.
3. Including *FLG* genotyping in addition to the anamnesis of AD as susceptibility screening for OCD in all applicants for a high risk job is not recommended. However, *FLG* genotyping of individuals with AD may aid in diagnosis and more tailored therapy and prevention. Furthermore, in view of the high risk of OCD in AD+/*FLG*+ individuals, renouncing from entering a high exposure job may be considered for this group.
4. Guidelines on genetic screening for susceptibility to occupational diseases show a deficiency concerning risk communication and the need of practical advice accompanying individual test results. The guidelines should be improved in this respect. In the preparation of such a screening, these elements can be elaborated by deploying focus groups or interviews with stakeholders.

SAMENVATTING

Achtergrond

Contacteczeem is een veel voorkomende beroepsziekte, die veroorzaakt wordt door blootstelling aan huidirriterende stoffen zoals zepen en desinfectiemiddelen, of allergenen zoals nikkel. Veelvuldig contact met water en zeep – zogenaamd "nat werk" – is een belangrijke oorzaak van contacteczeem. Nat werk komt vooral voor in beroepen waarin vanwege hygiëne-eisen vaak de handen moeten worden gewassen, zoals in de verpleging en bij de voedselbereiding, en in beroepen waarin veel contact met water en chemicaliën is, zoals in de kappers- en schoonmaakbranche. Ook het langdurig dragen van vloeistofdichte handschoenen wordt onder nat werk gerekend, omdat de huid hierdoor wordt 'afgesloten', wat kan leiden tot een verstoorde vochtbalans in de huid.

Blootstelling is een essentiële factor in de ontwikkeling van contacteczeem, maar daarnaast speelt ook persoonlijke gevoeligheid een belangrijke rol. De meest bekende persoonlijke risicofactor voor het krijgen van contacteczeem is atopische dermatitis (AD), een erfelijke huidaandoening die meestal op jonge leeftijd voor het eerst optreedt en gekenmerkt wordt door eczeem aan de buigzijde van de ellebogen en knieën. In Westerse landen is AD één van de meest voorkomende huidziekten; het komt bij ongeveer 20% van de kinderen en ongeveer 10% van de volwassenen voor. Mensen die AD hebben, of dit als kind hebben gehad, lopen een verhoogd risico op contacteczeem als zij gaan werken in beroepen met veel nat werk. De Nederlandse Vereniging voor Arbeids- en Bedrijfsgeneeskunde (NVAB) beveelt daarom aan dat werknemers met (een voorgeschiedenis van) AD bij indiensttreding in bijvoorbeeld ziekenhuizen extra worden begeleid door de bedrijfsarts.

Een belangrijk verschijnsel bij AD is een verstoorde huidbarrière, waardoor de huid minder goed bestand is tegen contact met irriterende stoffen. De huidbarrière wordt gevormd door het bovenste laagje van de huid, de hoornlaag of Stratum Corneum. De specifieke structuur en kenmerken van het Stratum Corneum zorgen voor een effectieve barrière tegen het doordringen van chemische en biologische stoffen, terwijl tegelijkertijd het verlies van vocht vanuit de huid wordt tegengegaan. Recent onderzoek heeft uitgewezen dat het eiwit *filaggrine* een belangrijke rol speelt in de huidbarrière. Ook is aangetoond dat er verscheidene mutaties kunnen voorkomen in het gen dat codeert voor de aanmaak van filaggrine (*FLG*), die leiden tot een verminderde hoeveelheid of zelfs afwezigheid (bij homozygoten) van functioneel filaggrine in de huid. *FLG* mutaties komen voor bij ongeveer 10% van de Europese bevolking en zijn sterk gerelateerd met AD: ongeveer 40% van de mensen met *FLG* mutaties ontwikkelt AD. Degenen met een mutatie in het *FLG* gen maar zonder AD worden niet opgemerkt als gevoelig in het bestaande preventieprogramma voor contacteczeem. Maar vanwege hun tekort aan filaggrine is het waarschijnlijk dat zij wel een verminderde huidbarrière hebben, en daardoor lopen zij wellicht ook een verhoogd risico op contacteczeem.

Het hoofddoel van dit onderzoek was daarom meer inzicht te krijgen in de rol van *FLG* mutaties als risicofactor voor contacteczeem, vergeleken met de bekende risicofactoren AD en blootstelling aan nat werk. Een tweede doel was te onderzoeken of het testen op *FLG* mutaties een nuttige aanvulling zou kunnen zijn op de bestaande screening op AD in het preventieprogramma voor contacteczeem.

Nat werk

In **Hoofdstuk 2** wordt ingegaan op blootstelling aan nat werk als risicofactor voor contacteczeem. De Nederlandse richtlijn voor de preventie van contacteczeem hanteert een grens voor de blootstelling aan nat werk, die gebaseerd is op de Duitse richtlijn TRGS 401: de totale duur van nat werk mag niet meer dan 2 uur per dag bedragen, en het totaal aantal keer handen wassen of desinfecteren mag niet meer dan 20 keer per dag zijn. Een objectieve meetmethode die zowel de duur als de frequentie van nat werk handelingen kan meten is echter nog niet voorhanden. **Hoofdstuk 2.1** evalueert het gebruik van een sampler om nat werk te meten bij verpleegkundigen. De sampler, die ontwikkeld is door het Institute for Occupational Medicine (IOM) in Edinburgh, Schotland, meet vochtigheid op basis van de temperatuur van twee sensoren: één sensor die direct op de huid zit, en een tweede sensor die 2 mm boven de huid zit. Deze sensoren registreren elke 10 seconden de temperatuur. Als de huid in contact komt met water (of een andere vloeistof), ontstaat er een temperatuurverschil tussen de twee sensoren. Wanneer dit temperatuurverschil boven een bepaalde drempelwaarde komt, is dat een indicatie dat de huid nat is; wanneer het onder een bepaalde ondergrens komt, is het een indicatie dat handschoenen worden gedragen. Het gebruik van deze sampler werd getest op twee verpleegafdelingen. Zesentwintig verpleegkundigen droegen de sampler gedurende 2 uur tijdens hun normale dienst, terwijl zij tegelijkertijd werden geobserveerd door een onderzoeker. De temperatuurverschillen gemeten door de sampler werden vervolgens vergeleken met de geobserveerde handelingen van de verpleegkundigen. Voor iedere verpleegkundige werd een individuele optimale drempelwaarde berekend waarboven het gemeten temperatuurverschil 'nat werk' aanduidde, en een tweede drempelwaarde voor het aanduiden van het dragen van vloeistofdichte handschoenen. Dit resulteerde in een gemiddelde sensitiviteit van 78% en een gemiddelde specificiteit van 79% voor het aanduiden van nat werk. Dit betekent dat de sensor nat werk in 78% van de gevallen correct aanwees en droog werk in 79%. Voor het aanduiden van handschoengebruik werd een sensitiviteit van 62% en een specificiteit van 68% gehaald. Op basis van de resultaten werd geconcludeerd dat de sampler niet voldoet om nat werk te meten bij verpleegkundigen. Een mogelijke reden hiervoor is dat verpleegkundigen zeer diverse nat werk handelingen uitvoeren, waaronder veel contact met desinfecterende alcoholgel en contact met lauwwarm water.

In **Hoofdstuk 2.2** wordt de blootstelling aan nat werk en het voorkomen van handeczeem beschreven in een prospectief cohortonderzoek onder Nederlandse leerling-verpleegkundigen. In totaal namen 721 leerling-verpleegkundigen deel aan het onderzoek, waarvan er 533 succesvol gevolgd zijn voor een periode van tussen

de 1 en 3 jaar. Tijdens hun stages noteerden de deelnemers regelmatig op speciaal ontworpen dagboekkaartjes hoe vaak zij nat werk handelingen deden. Daarnaast vulden ze op de kaartjes in of zij bepaalde klachten aan hun handen of vingers hadden gehad, die duidden op handeczeem. Ook konden zij klachten van handeczeem rechtstreeks melden bij de onderzoekers. Deelnemers die – actief of via de kaartjes – symptomen van handeczeem meldden, kregen een consult aangeboden met een gespecialiseerde bedrijfsarts. In het eerste jaar van follow-up rapporteerde 23% van de deelnemers handeczeem, in het tweede jaar was dit 25% en in het derde jaar 31%. Van de deelnemers die tot aan het begin van het onderzoek klachtenvrij waren geweest ontwikkelde 18% handeczeem tijdens hun stage, waarbij de meeste gevallen zich voordeden binnen één jaar na het begin van de stage. Bij ongeveer een derde van de deelnemers was de blootstelling aan nat werk tijdens de stages hoger dan de richtlijn van 2 uur per dag of 20 handelingen per dag. Het effect van verschillende nat werk handelingen (bijvoorbeeld handen wassen, gebruik van handalcohol, gebruik van handschoenen) op het voorkomen van handeczeem werd onderzocht in een statistisch model (multivariaat mixed model). Risicofactoren voor het krijgen van handeczeem waren het meer dan 8x per dag handen wassen tijdens de stage [odds ratio[1] (OR) 1.5; 90% betrouwbaarheidsinterval (CI) 1.0 – 2.3], het meer dan 10x per dag handen wassen thuis (OR 2.3; 90% CI 1.5 – 3.7) en het werken in een bijbaan met nat werk (bijvoorbeeld in de zorg of in de horeca) voor tenminste 8 uur per week (OR 1.6; 90% CI 1.0 – 2.4). Overige nat werk handelingen, zoals het gebruik van handalcohol gel of het dragen van handschoenen, hadden in dit onderzoek geen invloed op het risico op handeczeem. Dit resultaat is in overeenstemming met de literatuur; eerdere experimentele onderzoeken hebben aangetoond dat blootstelling aan alcoholgels minder schadelijk is voor de huid dan blootstelling aan water en zeep.

Contact met water en zeep komt dus duidelijk naar voren als de meest relevante blootstellingsfactor binnen nat werk, en handhygiënerichtlijnen adviseren dan ook om indien mogelijk handalcohol gel te gebruiken in plaats van water en zeep. Veel leerling-verpleegkundigen in het cohortonderzoek bleken hier niet van op de hoogte te zijn. Daarnaast bleek dat slechts ongeveer een derde van de uitgenodigde deelnemers de uitnodiging voor een consult met de bedrijfsarts accepteerde. Eén van de meest genoemde redenen om niet op de uitnodiging in te gaan was dat de klachten volgens de betreffende deelnemer niet ernstig genoeg waren.

AD en FLG mutaties als risicofactoren voor contacteczeem

In Hoofdstuk 3 wordt de invloed van persoonlijke risicofactoren bestudeerd.

Hoofdstuk 3.1 beschrijft een patiënt-controle onderzoek waarbij patiënten met chronisch, werkgerelateerd contacteczeem zijn vergeleken met leerlingen

[1] Een Odds Ratio (OR) is een gebruikelijke schatter van het relatief risico (RR), de verhouding van het risico op ziekte tussen een blootgestelde groep en een controlegroep. De RR is vaak iets kleiner dan de OR.

van verschillende beroepsopleidingen als controles. Dit onderzoek is uitgevoerd in samenwerking met de Universiteit van Osnabrück, Duitsland. De vier meest voorkomende *FLG* mutaties onder de West-Europese bevolking (R501X, 2282del4, R2447X en S3247X) werden geanalyseerd in DNA uit wangslijmmonsters van 634 patiënten uit twee gespecialiseerde klinieken in Osnabrück en Hamburg, en van 393 controles. Huidige of vroegere aanwezigheid van eczeem in de elleboog- of knieholtes werd gebruikt als aanwijzing voor AD. Zestien procent van de patiënten en 8% van de controles waren drager van één of meer *FLG* mutaties. De ongecorrigeerde OR voor de 4 mutaties bijeengenomen was 2.1 (95% CI 1.3 – 3.3). Gecorrigeerd voor AD was de OR voor *FLG* mutaties 1.6 (95% CI 1.0 – 2.6). Omgekeerd was de OR voor AD, gecorrigeerd voor *FLG* mutaties, 2.9 (95% CI 2.1 – 4.0). Er werd geen interactie gevonden tussen deze twee risicofactoren. Hieruit werd een OR van 4.7 afgeleid voor personen die zowel *FLG* mutaties als AD hebben.

Hoofdstuk 3.2 beschrijft de invloed van AD en *FLG* mutaties op het risico op handeczeem in het eerder genoemde prospectief cohortonderzoek onder Nederlandse leerling-verpleegkundigen. Bij intrede in het onderzoek vulden de deelnemers een vragenlijst in over o.a. huidige of vroegere aanwezigheid van AD, allergieën en klachten van handeczeem, en werd een wangslijmmonster afgenomen voor analyse van de vier meest voorkomende *FLG* mutaties. Zoals eerder beschreven, rapporteerden de deelnemers regelmatig hun nat werk en eventuele symptomen van handeczeem via speciaal ontworpen dagboekkaartjes. Deelnemers die al eens handeczeem hadden gehad in het verleden, hadden meer kans om opnieuw handeczeem te krijgen tijdens hun stage. Dit gegeven bevestigt het recidiverende karakter van handeczeem. De invloed van nat werk, AD en *FLG* mutaties op het voorkomen van handeczeem werd onderzocht in een multivariaat mixed model. Veelvuldig handen wassen tijdens de stages en/of thuis verhoogde het risico op handeczeem in dit model met ongeveer een factor 2. Deelnemers met (een voorgeschiedenis van) AD hadden een verhoogde kans op het krijgen van handeczeem tijdens de stage, met een OR, gecorrigeerd voor de effecten van handen wassen en *FLG* mutaties, van 2.5 (90% CI 1.7 – 3.7). Bij deelnemers die een *FLG* mutatie hadden, maar geen AD, was geen verhoogd risico op handeczeem te zien. Deelnemers die zowel een *FLG* mutatie als (een voorgeschiedenis van) AD hadden, liepen echter wel een verhoogd risico; gecorrigeerd voor het effect van handen wassen was de OR 3.6 (90% CI 1.7 – 7.5).

Uit deze studies blijkt dat *FLG* mutaties met name een verhoogd risico op contacteczeem lijken te vormen als er óók AD aanwezig is. Wellicht is er een extra stimulus nodig om het risico op zowel AD als contacteczeem te verhogen bij degenen die drager zijn van een *FLG* mutatie. Het zou misschien ook zo kunnen zijn, dat sommige mensen met *FLG* mutaties hun tekort aan filaggrine in de huid op één of andere manier kunnen compenseren, zodat hun gevoeligheid voor het ontwikkelen van AD en contacteczeem niet verhoogd wordt. Over mogelijke biologische mechanismen hiervoor is echter nog niets bekend.

Testen op FLG mutaties als indicatie voor gevoeligheid voor contacteczeem?

Inzicht in genetische risicofactoren zou gebruikt kunnen worden voor een betere identificatie van gevoelige werknemers in hoogrisico beroepen. Het gebruik van genetische informatie om te 'screenen' op verhoogde gevoeligheid roept echter ook ethische en maatschappelijke vragen op. In **Hoofdstuk 4** wordt daar aandacht aan besteed Hiervoor is door middel van interviews, focusgroepen en vragenlijsten onderzoek gedaan naar de mening van leerling-verpleegkundigen – als toekomstige belanghebbenden – over hun bereidheid tot deelname aan een genetische test op gevoeligheid voor handeczeem. De voor- en nadelen die zij noemden zijn vergeleken met wat er in internationale richtlijnen wordt geadviseerd over genetisch screenen op gevoeligheid voor beroepsziekten. Meer dan de helft (69%) van de ondervraagde studenten antwoordde dat zij gebruik zouden willen maken van een genetische test op gevoeligheid voor handeczeem, wanneer die hen aangeboden zou worden. De belangrijkste argumenten vóór het gebruiken van zo'n test waren nieuwsgierigheid naar de eigen gevoeligheid en de verwachting preventieve maatregelen te kunnen nemen op basis van de testresultaten. Argumenten tegen het testen waren voorziene problemen met het interpreteren van de testresultaten (met name van de hoogte van een risico), twijfels over het praktische nut van de testresultaten ('ik zou er toch niets mee doen') en de mening dat handeczeem als ziekte niet ernstig genoeg is om genetisch op te testen. De meeste deelnemers gaven aan zich geen zorgen te maken over privacy en vertrouwelijkheid van de testresultaten. De meeste argumenten die door de leerling-verpleegkundigen werden genoemd, worden ook behandeld in de bestudeerde richtlijnen. Twee belangrijke punten ontbreken echter: het belang van goede risicocommunicatie, met aandacht voor het interpreteren van risico's, en de behoefte aan praktische adviezen ter ondersteuning van de testresultaten.

In de algemene discussie (**Hoofdstuk 5**) wordt verder ingegaan op de vraag of het testen op *FLG* mutaties van nut zou kunnen zijn voor de preventie van contacteczeem. Dit is afhankelijk van de context waarin de test gebruikt zou worden. Mogelijke gebruiksscenario's omvatten het gebruik van de test voor verbeterde diagnose en gerichte behandeling van bestaand contacteczeem, het testen als onderdeel van de (medische) keuring van nieuwe werknemers in risicoberoepen, en het gebruik van de test als onderdeel van een opleidings- of beroepskeuzeadvies.

Conclusies en aanbevelingen

De resultaten van dit proefschrift hebben geleid tot de volgende conclusies en aanbevelingen:

1. Leerling-verpleegkundigen lopen nog steeds een aanzienlijk risico om handeczeem te ontwikkelen tijdens hun stages. Meer voorlichting is nodig ter preventie van handeczeem, met name over de effecten van verschillende nat werk handelingen op de huid, verzorging van de huid, en het tijdig reageren op klachten van

handeczeem. Hiervoor zullen voorlichtingsprogramma's ontwikkeld moeten worden, en de effectiviteit van deze programma's zal moeten worden geëvalueerd.

2. Gecorrigeerd voor het effect van AD verhoogden *FLG* mutaties het risico op contacteczeem in de patiënt-controle studie (OR 1.6), maar hadden geen duidelijk effect op het risico voor handeczeem in de cohortstudie onder leerling-verpleegkundigen. Personen met beide risicofactoren hadden in beide studies het hoogste risico om contacteczeem te krijgen.
3. Het testen op *FLG* mutaties als aanvulling op de anamnese van AD om gevoeligheid voor contacteczeem te bepalen bij werknemers in risicoberoepen in het algemeen, wordt niet aanbevolen. Echter, bij personen met AD kan *FLG* genotypering bijdragen aan een meer gerichte diagnose, therapie (indien OCD ontstaat), en preventie van contacteczeem. Gezien het hoge risico op contacteczeem voor personen met *FLG* mutaties in combinatie met AD, valt het te overwegen om het werken in een risicoberoep af te raden voor deze groep.
4. In bestaande richtlijnen voor genetisch testen op gevoeligheid voor beroepsziekten ontbreekt risicocommunicatie en de aanbeveling om praktische adviezen op te nemen in de rapportage van testresultaten. Dit zijn belangrijke punten, die zouden moeten worden toegevoegd aan dit soort richtlijnen. In de voorbereiding van de implementatie van een test op gevoeligheid voor een bepaalde (beroeps) ziekte kan de invulling van deze punten worden uitgewerkt door focusgroepen of interviews te houden met de betrokkenen.

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CURRICULUM VITAE

Maaike Johanna Visser werd geboren op 23 november 1981 in Leeuwarden. Na het behalen van haar VWO-diploma aan het Thomas à Kempis College te Zwolle ging zij in 1999 Milieuhygiëne studeren in Wageningen. Voor haar afstuderen deed zij achtereenvolgens onderzoek naar decentrale afvalwaterzuivering, blootstelling aan endotoxinen tijdens schoonmaakwerkzaamheden op rioolwaterzuiveringsinstallaties, en blootstelling aan endotoxinen en allergenen bij proefdierwerkers. Dit laatste onderzoek voerde zij uit aan het Karolinska Instituut te Stockholm, Zweden. Hiervoor ontving zij in 2006 de prof. Quanjerprijs van het Astma Fonds voor beste stageverslag. Naast haar doctoraaldiploma behaalde zij in 2005 het diploma voor de Master of Science "Toxicology and Environmental Health" aan de Universiteit Utrecht. Na haar afstuderen werkte zij twee jaar bij het Institute for Risk Assessment Sciences (IRAS) te Utrecht, aan diverse projecten op het gebied van milieu, arbeid en gezondheid. In januari 2008 startte zij met haar promotieonderzoek aan het Coronel Instituut voor Arbeid en Gezondheid in het Academisch Medisch Centrum (AMC) te Amsterdam, onder begeleiding van dr. S. Kezic, dr. M.M. Verberk, prof. dr. F.J.H. van Dijk en prof. dr. J.D. Bos. De resultaten hiervan zijn te vinden in dit proefschrift. Tijdens haar promotietraject volgde zij tevens de Postdoctorale Opleiding Toxicologie. Momenteel werkt zij als onderzoeksmedewerker bij het Coronel Instituut.

PORTFOLIO

Name PhD student: Maaike Visser
PhD period: January 2008 – July 2012

Name PhD supervisor: Prof. dr. F.J.H. van Dijk / Prof. dr. J.D. Bos

1. PhD training

	Year	Workload (Hours/ ECTS)
General courses		
AMC World of Science	2008	20 / 0.7
Clinical Data Management	2008	6 / 0.2
Specific courses		
Genetic Epidemiology	2008	30 / 1.1
Practical Biostatistics	2008	40 / 1.1
Advanced Biostatistics	2010	60 / 2.1
Kwalitatief Onderzoek in de Gezondheidszorg	2008	54 / 1.9
DNA Technology	2010	60 / 2.1
Postgraduate Education in Toxicology		
Molecular Toxicology	2009	42 / 1.5
Cell Toxicology	2009	42 / 1.5
Medical, Forensic and Regulatory Toxicology	2009	84 / 3.0
Organ Toxicology	2009	42 / 1.5
Immunotoxicology	2010	42 / 1.5
Pathobiology	2010	42 / 1.5
Risk Communication	2010	42 / 1.5
Mutagenesis and Carcinogenesis	2011	42 / 1.5
Ecotoxicology	2011	82 / 3.0
Laboratory Animal Science	2011	42 / 1.5
Seminars, workshops and master classes		
NSPOH mini-symposium 'Baas over eigen gezondheid?'	2010	6 / 0.2
NSPOH symposium 'Health Check Mania'	2011	6 / 0.2
Presentations		
Presentations research meetings Coronel Institute (6)	2008 – 2013	84 / 3.0
Presentations at (inter)national conferences (4)	2009 – 2013	56 / 2.0
Presentations for scientific working groups and Health and Safety Executives (4)	2008 – 2013	56 / 2.0

(Inter)national conferences		
NVvA Symposiom 'Arbeidshygiëne, een waardevol specialisme'	2008	8 / 0.25
European Society of Contact Dermatitis conference 2008, Estoril, Portugal	2008	16 / 0.5
Occupational and Environmental Exposures of Skin to Chemicals conference 2009, Edinburgh, UK	2009	24 / 0.75
Occupational and Environmental Exposures of Skin to Chemicals conference 2011, Toronto, Canada	2011	24 / 0.75
Other		
Participation in COST-Action 'Skin barrier and Atopic Diseases (SKINBAD)':		
Scientific meetings	2009 – 2013	16 / 0.5
Co-organizing of Training school 'Skin Bioengineering Techniques', Amsterdam, The Netherlands	2011	28 / 1.0
Participation in Training school 'Immunology and Genetics of Atopic Dermatitis', Split, Croatia	2012	16 / 0.5

2. Teaching

	Year	Workload (Hours/ ECTS)
Tutoring, Mentoring		
Teaching and supervision of medical students writing a paper	2008, 2010	56 / 2.0
Student coaching / mentoring	2008, 2009	56 / 2.0

LIST OF PUBLICATIONS

Publications included in this thesis

Ethical issues of genetic susceptibility testing for occupational diseases: opinions of trainees in a high-risk job.
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Clinical course of occupational irritant contact dermatitis of the hands in relation to filaggrin genotype status and atopy.
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Revealing barriers and facilitators to use a new genetic test: comparison of three user involvement methods.
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Impact of tumour necrosis factor-α polymorphisms on irritant contact dermatitis.
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Ind Health. 2009 Oct;47(5):469-78. Review.