

Filaggrin null alleles are not associated with hand eczema or contact allergy

A. Lerbaek,*† H. Bisgaard,‡ T. Agner,† K. Ohm Kyvik,§ C.N.A. Palmer¶ and T. Menné†

*National Allergy Research Centre, Gentofte Hospital, University of Copenhagen, Ledreborg Allé 40, 1, 2820 Gentofte, Denmark

†Department of Dermatology, Gentofte Hospital, University of Copenhagen, Denmark

‡Danish Paediatric Asthma Centre, Gentofte Hospital, University of Copenhagen, Denmark

§The Danish Twin Registry, Epidemiology, University of Southern Denmark, Odense, Denmark

¶Population Pharmacogenetics Group, Biomedical Research Centre, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, U.K.

Summary

Correspondence

Anne Lerbaek.

E-mail: anne.lerbaek@dadlnet.dk

Accepted for publication

31 July 2007

Key words

atopic dermatitis, contact allergy, filaggrin,

hand eczema

Conflicts of interest

None declared.

Background The filaggrin protein is a key component of stratum corneum and homo- or heterozygotes for the filaggrin variant alleles R501X and 2282del4 have varying degrees of impaired skin barrier. The variant alleles have repeatedly been shown to be associated with atopic dermatitis. Any possible association with hand eczema or contact allergy are unexplored.

Objectives To explore associations between the variant alleles, hand eczema, contact allergy and atopic dermatitis.

Methods In total, 183 adult individuals participated in a clinical examination of the hands, patch testing and filaggrin genotyping. Children without any evidence of atopic dermatitis from the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) study were used as controls. The χ^2 test was used for comparison of allele frequencies.

Results The majority (73%) had hand eczema, 25% had contact allergy and 14% had a diagnosis of atopic dermatitis. The association between atopic dermatitis and the filaggrin variant alleles was confirmed (odds ratio 3.5, $P = 0.015$). Allele frequencies in individuals with hand eczema or contact allergy were not statistically significantly increased.

Conclusion There is no association between the variant alleles and hand eczema or contact allergy.

Filament aggregating protein (filaggrin) is an essential component in the terminal differentiation of the epidermis and formation of the stratum corneum. Profilaggrin, the precursor of filaggrin, accumulates in the keratohyalin granules formed in the granular layers of the epidermis. During cornification keratin filaments in keratinocytes are aggregated by filaggrin, resulting in a flattening of the keratinocytes and eventually formation of the cornified cell envelope, which is crucial for the skin barrier function.¹ The skin barrier provides protection against water loss and penetration of chemicals, and infectious and allergenic agents.

Homozygotes or compound heterozygotes for the two loss-of-function mutations (null alleles) R501X and 2282del4 in the gene encoding filaggrin have a complete loss of filaggrin products and present clinically with ichthyosis vulgaris, characterized by dry scaly skin.² The mode of inheritance is semi-dominant with variable penetrance; heterozygotes may present with a mild form of ichthyosis vulgaris or without symptoms.

A number of studies have recently established a strong association between the filaggrin variant alleles and atopic dermatitis,³ in particular, atopic dermatitis associated with asthma and allergic rhinitis,^{3,4} IgE sensitization,⁵ early onset⁶ and persistence into adulthood.⁷

Hand eczema is a frequent, often chronic relapsing disease, with a heterogeneous aetiology, including irritant and allergic contact dermatitis, atopic dermatitis, mixed forms and minor groups with vesicular and hyperkeratotic hand eczema. Atopic dermatitis is one of the main risk factors for hand eczema.⁸ Genetic risk factors significantly influence the risk of developing hand eczema,⁹ even in the absence of atopic dermatitis.^{10,11} Genetic markers for hand eczema have not yet been identified and any possible association with the filaggrin variant alleles is unexplored. An impaired skin barrier facilitates the penetrance of allergens, but whether or not the presence of mutations in the gene encoding filaggrin is associated with an increased frequency of contact allergy is unknown.

The primary aim of this study was to investigate whether any relationship exists between the filaggrin variants R501X and 2282del4 and hand eczema. Secondly, any associations between the variant alleles and contact allergy and atopic dermatitis were explored.

Materials and methods

Study population

In 1996, a cohort of 6666 same-sex twin individuals born between 1953 and 1976 and living on Zealand or its neighbouring islands was drawn from the Danish Twin Registry and received a short questionnaire on hand eczema.⁹ A total of 5610 twin individuals responded. Twin pairs where one or both twin individuals had self-reported hand eczema or reported symptoms of hand eczema in the questionnaire and lived within 60 km of Copenhagen were invited to a clinical examination and patch test in 1997–1998.¹² A total of 1076 twin individuals participated. In 2005, all twin pairs where one or both had self-reported hand eczema in 1997–1998 were identified (659 individuals). Addresses were available for 605 twin individuals and they were invited by mail to a second clinical examination and patch test in 2005–2006 (Fig. 1). All individuals gave their written, informed consent

for participation in compliance with the principles of the Helsinki Declaration.

Hand eczema and atopic dermatitis

A diagnosis of hand eczema was based on a positive answer to a question on self-reported hand eczema (Have you ever had hand eczema?),¹³ given either in 1997–1998 or at the current examination. Fifteen individuals with self-reported hand eczema in 1997–1998 denied hand eczema at the present examination. Some of them had other diagnoses such as psoriasis and polymorphic light eruption on the hands. In case of present hand eczema, symptoms (scaling, erythema, vesicles, papules, fissures and oedema) were recorded. The U.K. Working Party's Diagnostic Criteria were used to define whether or not participants had ever had atopic dermatitis.¹⁴

Filaggrin genotyping

Venous blood samples or mouth swabs were collected from the twin individuals and kept at -80°C . DNA was prepared from blood samples and mouth swabs using QIAamp 96 DNA procedures (Qiagen GmbH, Hilden, Germany). Genotyping for R501X and 2282del4 was performed by TaqMan[®] allelic

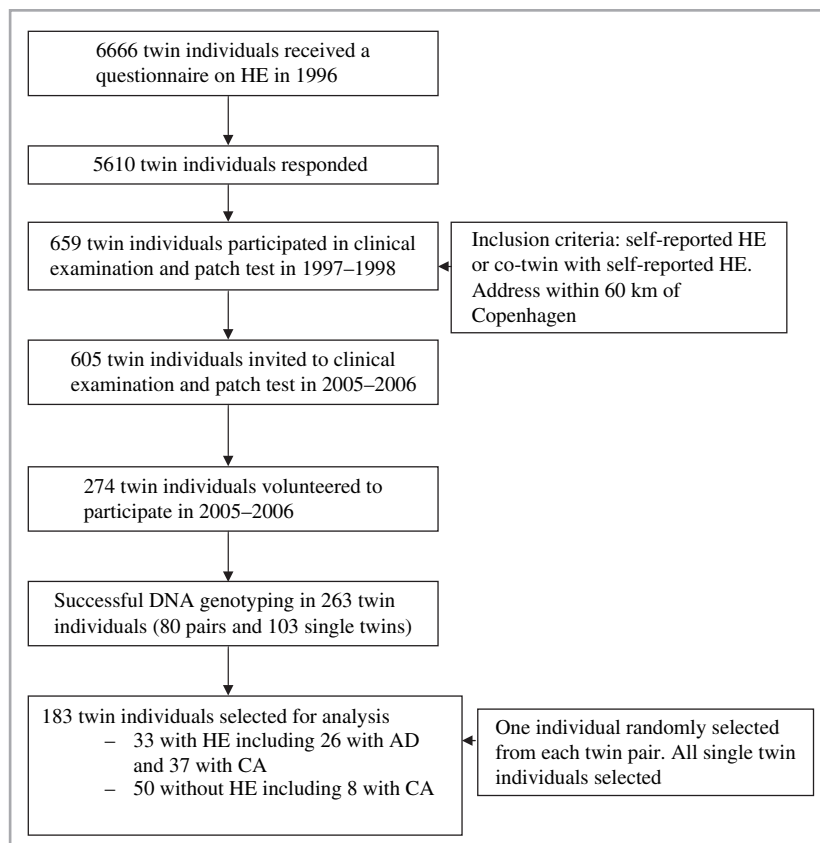


Fig 1. Flow diagram illustrating recruitment of the study population. HE, hand eczema; AD, atopic dermatitis; CA, contact allergy.

discrimination assays (Applied Biosystems, Foster City, CA, U.S.A.) as previously described.^{3,15}

Patch testing

The ready-to-use True Test[®] system panel 1 and 2 (Mekos Laboratories A/S, Hillerød, Denmark) was sent by mail to all participants. Patches were placed on the back by the participants 3 days in advance of the scheduled examination and removed after 2 days. Reading of the patches was done on day 3 according to the International Contact Dermatitis Research Group guidelines.¹⁶

Control groups

At first, allele frequencies in twin individuals with hand eczema were compared with twin individuals without hand eczema, and likewise twin individuals with and without contact allergy and atopic dermatitis were compared. As the number of twin individuals without hand eczema and contact allergy was limited, allele frequencies in the twin subgroups with hand eczema and contact allergy were also compared with a group of 189 children without atopic dermatitis (91 male and 98 female), all born to Danish mothers with asthma. The children are currently being followed from birth in a prospective longitudinal follow-up study [the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) study].^{3,17} Atopic dermatitis in the COPSAC study was defined using the criteria of Hanifin and Rajka.¹⁸ Finally, in an analysis restricted to the subgroup of twins with hand eczema, allele frequencies in the subgroup with and without atopic dermatitis were compared.

Statistical analysis

When data on both twin individuals in a twin pair were available, one twin individual was randomly excluded from the analysis, thus leaving 183 twin individuals for analysis (Fig. 1). Allele frequencies were compared in subgroups of twins and in the COPSAC subgroup using the χ^2 test. Both variants were in Hardy–Weinberg equilibrium in the twin cohort, the twin subgroups and in the COPSAC subgroup. The χ^2 test was used in the drop-out analysis. SPSS version 11.0 was used for statistical analyses.

Results

A total of 274 twin individuals participated. DNA genotyping was successful in 263 individuals and 183 twin individuals (70 monozygotic, 103 dizygotic and 10 with unknown zygosity) were selected for analysis (see Statistical analysis). The following descriptive data apply to the 183 individuals. The mean age was 41 years (SD 6.6) and the male to female ratio was 64 : 119. The majority (73%) had had hand eczema and in this group, clinical signs of hand eczema were found in 41%. At least one positive patch test was detected in 25%; the

frequency in women and men was 32% and 11%, respectively. Nickel was the primary allergen responsible for sensitization (data not shown). A total of 14% had current or previous history of atopic dermatitis. All twin individuals with atopic dermatitis reported at least one episode of hand eczema. The frequency of atopic dermatitis in the hand eczema group was 20%.

The overall allele frequencies of R501X and 2282del4 in the twin cohort were 3% for both variants (yielding carrier frequencies of 7%). As there were no compound heterozygotes the combined carrier frequency was 13%. There were no homozygotes in the twin cohort. Highest allele frequencies were found in the twin subgroups with atopic dermatitis and contact allergy, 23% and 16%, respectively. Allele frequencies in the twin cohort, the twin subgroups and the COPSAC group are shown in Table 1.

No association between the phenotype with hand eczema and the two variant alleles was found. Also, no association between contact allergy (positive patch test) and the variant alleles could be demonstrated. The increased combined carrier frequency in individuals with atopic dermatitis did not reach statistical significance when compared with twin individuals without atopic dermatitis. Statistical results from the comparisons are displayed in Table 2.

Allele frequencies in the twin subgroups with hand eczema or contact allergy were not statistically significantly different from allele frequencies in the COPSAC subgroup of children without atopic dermatitis. Comparison of the twin subgroup with atopic dermatitis with the COPSAC subgroup reached statistical significance (see Table 2 for details).

In the subanalysis restricted to twin individuals with hand eczema, comparison of the combined allele frequency in individuals with atopic dermatitis (23%) with the subgroup without atopic dermatitis (10%) was borderline statistically significant [odds ratio (OR) 2.6, 95% confidence interval 0.87–7.91, $\chi^2 = 3.072$, $P = 0.080$].

Drop-out analysis of the 274 twin individuals participating in the present study vs. those where one or both twin individuals had self-reported hand eczema in 1997–1998 (659 twin individuals) revealed no statistically significant difference regarding sex, zygosity, hand eczema status in 1997–1998, co-twins hand eczema status in 1997–1998, patch-test status or atopic dermatitis status (data not shown). Age was the only statistically significant factor influencing willingness to participate. When subdividing the twin individuals into three groups, 35% from the youngest age group and 41% in the middle group participated, whereas 50% from the oldest age group volunteered for the study ($P = 0.009$).

Discussion

The influence of genetic factors independent of atopic dermatitis on the risk of hand eczema was recently confirmed.¹⁰ Many candidate genes for atopic dermatitis have been proposed and investigated, but the filaggrin null alleles, R501X and 2282del4, were the first to be successfully replicated in a

Table 1 Frequency of filaggrin null alleles in the twin cohort and dependent on hand eczema (HE), contact allergy (CA) and atopic dermatitis (AD) status as well as in the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) cohort

Genotype	All (n = 183)	HE (n = 133)	No HE (n = 50)	CA (n = 45)	No CA (n = 136)	AD (n = 26)	No AD (n = 157)	HE and AD (n = 26)	HE and no AD (n = 107)	COPSAC ^a (n = 189)
R501X										
AA	171 (93.4)	126 (94.7)	45 (90.0)	42 (93.3)	127 (93.4)	23 (88.5)	148 (94.3)	23 (88.5)	103 (96.3)	182 (96.3)
Aa	12 (6.6)	7 (5.3)	5 (10.0)	3 (6.7)	9 (6.6)	3 (11.5)	9 (5.7)	3 (11.5)	4 (3.7)	7 (3.7)
aa	0	0	0	0	0	0	0	0	0	0
2282del4										
AA	171 (93.4)	123 (92.5)	48 (96.0)	41 (91.1)	128 (94.1)	23 (88.5)	148 (94.3)	23 (88.5)	100 (93.5)	180 (95.2)
Aa	12 (6.6)	10 (7.5)	2 (4.0)	4 (8.9)	8 (5.9)	3 (11.5)	9 (5.7)	3 (11.5)	7 (6.5)	9 (4.8)
aa	0	0	0	0	0	0	0	0	0	0
Combined frequency										
AA	159 (86.9)	116 (87.2)	43 (86.0)	38 (84.4)	119 (87.5)	20 (76.9)	139 (88.5)	20 (76.9)	96 (89.7)	174 (92.1)
Aa	24 (13.1)	17 (12.8)	7 (14.0)	7 (15.6)	17 (12.5)	6 (23.1)	18 (11.5)	6 (23.1)	11 (10.3)	14 (7.4)
aa	0	0	0	0	0	0	0	0	0	1 (0.5)

Numbers in parentheses are percentages.

AA, homozygous genotype for no R501X/2282del4; Aa, heterozygous genotype for R501X/2282del4; aa, homozygous genotype for R501X/2282del4; ^aCOPSAC: from the COPSAC study.¹⁷ Children without atopic dermatitis.

Table 2 Comparison of allele frequencies in different subgroups with the χ^2 test

	χ^2	OR (95% CI)	P-value
Comparison of individuals within the twin group (n)			
Twins with hand eczema (133) vs. twins without hand eczema (50)	0.047	0.9 (0.35–2.32)	0.828
Twins with contact allergy (45) vs. twins without contact allergy (136)	0.274	1.3 (0.50–3.34)	0.600
Twins with atopic dermatitis (26) vs. twins without atopic dermatitis (157)	2.640	2.3 (0.82–6.53)	0.104
Comparison of individuals in twin subgroups and individuals in COPSAC (n)			
Twins with hand eczema (133) vs. COPSAC (189)	2.048	1.7 (0.82–3.54)	0.152
Twins with contact allergy (45) vs. COPSAC (189)	2.477	2.1 (0.82–5.60)	0.116
Twins with atopic dermatitis (26) vs. COPSAC (189)	5.945	3.5 (1.21–9.99)	0.015

OR, odds ratio; CI, confidence interval; COPSAC, Copenhagen Prospective Study on Asthma in Childhood: from the COPSAC study.¹⁷ Children without atopic dermatitis.

number of studies.¹⁹ The epidermal defect caused by the variants could possibly be of aetiological importance in other skin diseases, characterized by a compromised skin barrier or where a compromised skin barrier can be a trigger factor.

We investigated a possible association between the variant alleles and the phenotype with hand eczema. In this population-based, but selected group of individuals, with a high prevalence of hand eczema and atopic dermatitis, we found an overall combined carrier frequency of 13%. This is higher than other reported frequencies in the background population of between 8.8% and 9.6%.^{3,6,7} Two studies found considerably lower combined carrier frequencies in the control groups. Weidinger *et al.*²⁰ reported 6.1% and Marenholz *et al.*⁴ found only 5.1% carrying the variant alleles; however, in the latter study, a 'hyper-normal' control group was selected, as none of them had any allergy.

The twin subgroup with hand eczema did not exhibit a higher mutation frequency than the group without hand eczema; however, the (control) group without hand eczema

was small, limiting conclusions. Comparison with a subgroup of children without atopic dermatitis from a high-risk cohort of children born to mothers with asthma (COPSAC) also failed to find any statistically significant difference.

A defective skin barrier facilitates induction and elicitation of contact allergy. Kligman found increased rates of sensitization after pretreatment with an irritant or after combined exposure.²¹ Also, pretreatment of the skin with an irritant or combined exposure to an irritant and an allergen lowers the threshold for elicitation of contact allergy or increases the patch-test response.^{22,23} The impaired skin barrier caused by the variant alleles could possibly increase the risk of contact allergy. We did not find any statistically significant association between the variant alleles and contact allergy in the analysis of the total twin cohort or when compared with the COPSAC group.

The allele frequencies in twin individuals with hand eczema or contact allergy were increased compared with the COPSAC cohort, 13% and 16% vs. 7%, respectively. The negative

outcome of the association analyses may be due to insufficient power, and using a larger population-based control group may yield a different result. Thus a possible association between the variant alleles and hand eczema or contact allergy cannot be entirely excluded. Three of the seven individuals with a variant allele and contact allergy also had atopic dermatitis.

Contact allergy is diagnosed by means of patch testing. Some limitations apply to this study. Errors in the patch-test procedure cannot be entirely excluded, as participants applied and removed the patches themselves. Secondly, as the reading was done only on day 3, late reactions may have been missed. It has been demonstrated that between 3% and 8% of reactions become positive on day 6 or 7.^{24,25} Furthermore, individuals were only tested with 20 allergens, including the most frequent sensitizers. A negative test is obviously not a proof of absence of contact allergy. However, these standard allergens have been shown to detect 77–95% of all contact allergies in departments specialized in contact dermatitis.²⁶

In the twin subgroup with atopic dermatitis the combined carrier frequency was 23%; however, this was not statistically significantly different from the frequency in individuals without atopic dermatitis, even though the frequency in this group was much smaller (11%). A likely explanation is a lack of power, since the group with atopic dermatitis comprised only 26 individuals. We confirmed the association between atopic dermatitis and the filaggrin variant alleles when comparing with the COPSAC subgroup (OR 3.5, $P = 0.015$). In the subanalysis including only individuals with hand eczema, the comparison of subgroups with and without atopic dermatitis was borderline statistically significant. Thus the filaggrin null alleles could be a potential genetic marker for increased risk of atopic hand eczema (in individuals with atopic dermatitis). It has previously been shown that the risk of hand eczema increases with the severity of atopic dermatitis.⁸ This will need further investigation in a study including individuals with atopic dermatitis but without hand eczema.

In contrast to many of the previous hospital-based studies on the association between atopic dermatitis and the filaggrin variant alleles, this study was population-based. Thus, our group with atopic dermatitis may represent a greater spectrum of disease severity including phenotypes with milder symptoms, compared with earlier studies. Weidinger *et al.* actually demonstrated that the 2282del4 mutation and the combined genotype were statistically significantly associated with a more severe phenotype (SCORAD > 31).²⁰

The U.K. Working Party's Diagnostic Criteria have been thoroughly validated, although mostly, but not entirely in children. In this study the possibility of recall bias is considerable. Possibly, mild cases with symptoms restricted to early childhood have been forgotten and thus missed. Such a misclassification could change the results in both directions depending on whether or not those in question were carriers of the variant alleles and whether or not they had hand eczema.

A well-defined phenotype is essential in genetic association studies. In this study the diagnosis of hand eczema was based on a question on self-reported hand eczema. A similar question has been validated and has shown high specificity, but less sensitivity.²⁷ In the present context a high specificity is preferable. Furthermore the diagnosis could be confirmed in 41% of cases due to visible signs of hand eczema at the examination.

The COPSAC subgroup of children without atopic dermatitis was chosen as a secondary control group due to the limited number of twin individuals without hand eczema and due to availability. A more suitable control group would be an adult group without hand eczema and contact allergy, matched on sex and atopy status.

Individuals with hand eczema comprise a very heterogeneous group both regarding aetiology, severity and prognosis. Finding a single candidate gene influencing all subtypes may turn out to be a difficult task, even though the inflammatory processes may be similar. Two cytokine gene polymorphisms have been identified as being of importance for the development of allergic contact dermatitis: IL16-295 and TNFA-308.^{28,29} The latter was also present in increased frequency in individuals with a low irritation threshold and may thus also be a marker of increased risk of irritant contact dermatitis.³⁰

In conclusion, no association between the filaggrin null alleles and hand eczema or contact allergy overall could be demonstrated; however, insufficient power is a consideration. The association between atopic dermatitis and the filaggrin variant alleles was confirmed in the comparison of the individuals with atopic dermatitis and the COPSAC subgroup without atopic dermatitis. In the subanalysis, including only individuals with hand eczema, an almost statistically significant increased frequency in individuals with atopic dermatitis was seen. This suggests that the filaggrin null alleles in future could be used as a potential marker for increased risk of hand eczema in patients with atopic dermatitis. However, this hypothesis needs further investigation.

Acknowledgments

We thank Aage Vølund PhD for assistance with the statistical analyses and Simon Lee BSc for his assistance in the genotyping assays. The study was supported by research grants from Aage Bangs Foundation and King Christian the 10th Foundation.

References

- 1 Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. *Nat Rev Mol Cell Biol* 2005; **6**:328–40.
- 2 Smith FJ, Irvine AD, Terron-Kwiatkowski A *et al.* Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 2006; **38**:337–42.
- 3 Palmer CN, Irvine AD, Terron-Kwiatkowski A *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006; **38**:441–6.

- 4 Marenholz I, Nickel R, Ruschendorf F *et al.* Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. *J Allergy Clin Immunol* 2006; **118**:866–71.
- 5 Weidinger S, Illig T, Baurecht H *et al.* Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. *J Allergy Clin Immunol* 2006; **118**:214–19.
- 6 Stemmler S, Parwez Q, Petrasch-Parwez E *et al.* Two common loss-of-function mutations within the filaggrin gene predispose for early onset of atopic dermatitis. *J Invest Dermatol* 2006; **127**:722–4.
- 7 Barker JN, Palmer CN, Zhao Y *et al.* Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. *J Invest Dermatol* 2006; **127**:564–7.
- 8 Rystedt I. Hand eczema and long-term prognosis in atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1985; **117**:1–59.
- 9 Bryld LE, Agner T, Kyvik KO *et al.* Hand eczema in twins: a questionnaire investigation. *Br J Dermatol* 2000; **142**:298–305.
- 10 Lerbaek A, Kyvik KO, Mortensen J *et al.* Heritability of hand eczema is not explained by comorbidity with atopic dermatitis. *J Invest Dermatol* 2007; **127**:1632–40.
- 11 Lerbaek A, Kyvik KO, Ravn H *et al.* Incidence of hand eczema in a population-based twin cohort – genetic and environmental risk factors. *Br J Dermatol* 2007; **157**:552–7.
- 12 Bryld LE, Hindsberger C, Kyvik KO *et al.* Risk factors influencing the development of hand eczema in a population-based twin sample. *Br J Dermatol* 2003; **149**:1214–20.
- 13 Susitaival P, Flyvholm MA, Meding B *et al.* Nordic Occupational Skin Questionnaire (NOSQ-2002): a new tool for surveying occupational skin diseases and exposure. *Contact Dermatitis* 2003; **49**:70–6.
- 14 Williams HC, Burney PG, Hay RJ *et al.* The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol* 1994; **131**:383–96.
- 15 Sandilands A, Terron-Kwiatkowski A, Hull PR *et al.* Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet* 2007; **39**:650–4.
- 16 Frosch PJ, Menne T, Lepoittevin JP. *Contact Dermatitis*, 4th edn. Berlin: Springer Verlag, 2006.
- 17 Bisgaard H. The Copenhagen Prospective Study on Asthma in Childhood (COPSAC): design, rationale, and baseline data from a longitudinal birth cohort study. *Ann Allergy Asthma Immunol* 2004; **93**:381–9.
- 18 Halkjaer LB, Loland L, Buchvald FF *et al.* Development of atopic dermatitis during the first 3 years of life: the Copenhagen Prospective Study on Asthma in Childhood cohort study in high-risk children. *Arch Dermatol* 2006; **142**:561–6.
- 19 Morar N, Willis-Owen SA, Moffatt MF *et al.* The genetics of atopic dermatitis. *J Allergy Clin Immunol* 2006; **118**:24–34.
- 20 Weidinger S, Rodriguez E, Stahl C *et al.* Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. *J Invest Dermatol* 2006; **127**:724–6.
- 21 Kligman AM. The identification of contact allergens by human assay. II. Factors influencing the induction and measurement of allergic contact dermatitis. *J Invest Dermatol* 1966; **47**:375–92.
- 22 McLelland J, Shuster S, Matthews JN. 'Irritants' increase the response to an allergen in allergic contact dermatitis. *Arch Dermatol* 1991; **127**:1016–19.
- 23 Pedersen LK, Haslund P, Johansen JD *et al.* Influence of a detergent on skin response to methyldibromo glutaronitrile in sensitized individuals. *Contact Dermatitis* 2004; **50**:1–5.
- 24 Saino M, Rivara GP, Guarrera M. Reading patch tests on day 7. *Contact Dermatitis* 1995; **32**:312–13.
- 25 Jonker MJ, Bruynzeel DP. The outcome of an additional patch-test reading on days 6 or 7. *Contact Dermatitis* 2000; **42**:330–5.
- 26 Menne T, Dooms-Goossens A, Wahlberg JE *et al.* How large a proportion of contact sensitivities are diagnosed with the European standard series? *Contact Dermatitis* 1992; **26**:201–2.
- 27 Meding B, Barregard L. Validity of self-reports of hand eczema. *Contact Dermatitis* 2001; **45**:99–103.
- 28 Reich K, Westphal G, König IR *et al.* Association of allergic contact dermatitis with a promoter polymorphism in the IL16 gene. *J Allergy Clin Immunol* 2003; **112**:1191–4.
- 29 Westphal GA, Schnuch A, Moessner R *et al.* Cytokine gene polymorphisms in allergic contact dermatitis. *Contact Dermatitis* 2003; **48**:93–8.
- 30 Allen MH, Wakelin SH, Holloway D *et al.* Association of TNFA gene polymorphism at position -308 with susceptibility to irritant contact dermatitis. *Immunogenetics* 2000; **51**:201–5.