Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis

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ABSTRACT

Objective To investigate whether filaggrin gene defects, present in up to one in 10 western Europeans and North Americans, increase the risk of developing allergic sensitisation and allergic disorders.

Design Systematic review and meta-analysis.

Data sources Medline, Embase, ISI Science Citation Index, BIOSIS, ISI Web of Knowledge, UK National Research Register, clinical trials.gov, the Index to Theses and Digital dissertations, and grey literature using OpenSIGLE.

Study selection Genetic epidemiological studies (family, case-control) of the association between filaggrin gene defects and allergic sensitisation or allergic disorders.

Data extraction Atopic eczema or dermatitis, food allergy, asthma, allergic rhinitis, and anaphylaxis, along with relevant immunological variables relating to the risk of allergic sensitisation as assessed by either positive skin prick testing or increased levels of allergen specific IgE.

Data synthesis 24 studies were included. The odds of developing allergic sensitisation was 1.91 (95% confidence interval 1.44 to 2.54) in the family studies and 1.57 (1.20 to 2.07) in the case-control studies. The odds of developing atopic eczema was 1.99 (1.72 to 2.31) in the family studies and 4.78 (3.31 to 6.92) in the case-control studies. Three studies investigated the association between filaggrin gene mutations and allergic rhinitis in people without atopic eczema: overall odds ratio 1.78 (1.16 to 2.73). The four studies that investigated the association between filaggrin gene mutations and allergic rhinitis in people with atopic eczema reported a significant association: pooled odds ratio from case-control studies 2.84 (2.08 to 3.88). An overall odds ratio for the association between filaggrin gene mutations and asthma in people with atopic eczema was 2.79 (1.77 to 4.41) in case-control studies and 2.30 (1.66 to 3.18) in family studies. None of the studies that investigated filaggrin gene mutations and asthma in people without atopic eczema reported a significant association; overall odds ratio was 1.30 (0.7 to 2.30) in the case-control studies. The funnel plots suggested that publication bias was unlikely to be an explanation for these findings. No studies investigated the association between filaggrin gene mutations and food allergy or anaphylaxis.

Conclusions Filaggrin gene defects increase the risk of developing allergic sensitisation, atopic eczema, and allergic rhinitis. Evidence of the relation between filaggrin gene mutations and atopic eczema was strong, with people manifesting increased severity and persistence of disease. Filaggrin gene mutations also increased the risk of asthma in people with atopic eczema. Restoring skin barrier function in filaggrin deficient people in early life may help prevent the development of sensitisation and halt the development and progression of allergic disease.

INTRODUCTION

Atopic diseases, including eczema (atopic dermatitis), asthma, and allergic rhinitis have increased in prevalence in recent decades and now affect up to one in three children in economically developed countries. These conditions are responsible for appreciable morbidity and costs, both to people and to the state. The prevalence of these disorders varies worldwide and when coupled with data showing changes in prevalence over time points to the causal role of environmental factors. The key implication of this epidemiological evidence is that atopic allergic disorders should in principle be largely preventable.

The clinical cause of atopic disorders has been described as an atopic or allergic march. This concerns sensitisation to food or aeroallergens, or both, in early life, progressing to eczema and wheeze within the first two years of life, and often leading to chronic asthma, rhinitis, and other clinical manifestations of atopic allergy in later life.

Recent reports have suggested a key role of the protein filaggrin in maintaining an effective skin barrier against the environment. Mutations in the profilaggrin gene resulting in loss of function are common, being present in up to 10% of western European and North American populations. This is of potential importance given that rapid screening for filaggrin gene defects is now possible through analysis of cord or fetal blood specimens or, in older infants, using...
### Characteristics of included studies

| Trial, design, and setting | No of participants | Age (years) | Genotype | Outcome measures | Diagnostic method or criteria | Confounders | Quality |
|----------------------------|--------------------|-------------|----------|------------------|-------------------------------|-------------|---------|
| Barker et al 2007<sup>10</sup> (case-control, England): | | | | | | | |
| Cases from hospital dermatology clinics; population based controls (British ancestry): | 163 cases, 1463 controls | Cases 36.4 (range 16-82) | R501X and 2282del4 | Primary outcome persistent atopic dermatitis (present since early childhood) | UK Working Party definition as interpreted by experienced dermatologist | Matched for ethnicity | B |
| Bisgaard et al 2008<sup>19</sup> (cohort, Denmark and England): | 379 | NA | R501X and 2282del4 | Primary outcome age at onset of eczema; secondary outcomes allergic sensitisation: specific IgE >0.35KU/l to range of common inhalant and food allergens (environmental exposures included pet ownership) | Eczema (Hanifin and Rajka criteria) | Matched for ethnicity and age | B |
| Copenhagen Prospective Study on Asthma in Childhood (COPSAC): high risk birth cohort | 503 | NA | R501X and 2282del4 | Primary outcome age at onset of eczema; secondary outcomes allergic sensitisation and environmental exposure | Information on eczema collected by age 1 using interviewer administered validated questionnaire of International Study on Asthma and Allergies in Childhood; sensitisation assessed using skin prick tests | Matched for ethnicity and age | B |
| Brown et al 2008<sup>22</sup> (case-control, north west England): | 186 cases, 1035 controls | — | R501X, 2282del4, R2447X, S1247X, 3702delG, and 3673delC | Primary outcome early onset and persistent atopic dermatitis (present since early childhood) | Phenotypic characteristics as reported by Barker et al<sup>10</sup> | Matching for ethnicity, age, and sex | C |
| Brown et al (2008)<sup>19</sup> (case-control, north west England): | 190 cases, 599 controls | 7-9 | R501X, 2282del4, R2447X, S1247X, and 3702delG | Primary outcome mild to moderate atopic dermatitis; other outcomes raised total serum IgE level, early onset atopic dermatitis (2 years) | Atopic dermatitis (Hanifin and Rajka criteria) | Matched for ethnicity and age | B |
| Enomoto et al (2008)<sup>21</sup> (case-control and family study, Japan): | 376 cases, 923 controls | Cases mean 29.7 (range 16-64), controls mean 46.2 (range 19-78) | S2554X and 3321delA | Primary outcome atopic dermatitis; other outcomes raised total serum IgE level, early onset atopic dermatitis (2 years) | Eczema cases defined using UK diagnostic criteria and skin examination by dermatologist; cases of asthma and seasonal rhinitis defined by parental questionnaire | Matched for ethnicity and age | B |
| Participants from population cohort with DNA samples collected at birth, representing >85% of deliveries in one hospital, 1996-2003 | 105 families | Probands and siblings mean 13.3 (range 0.9-42) | S2554X and 3321delA | Atopic dermatitis | Hanifin and Rajka criteria | Unclear | B |
| Families from dermatology departments, University Hospital of Tsukuba, and several hospitals in Ibaraki | 406 families | Siblings mean 29 | R501X and 2282del4 | Atopic eczema, asthma with eczema, allergic rhinoconjunctivitis with eczema, and total IgE level | Diagnosed by same dermatologist, based on clinical examination and according to UK Working Party criteria; doctor diagnosed asthma or allergic rhinitis | Matched for ethnicity and adjusted for sex | B |
| Ekelund, et al (2008)<sup>24</sup> (family study, Sweden): | 178 cases, 210 controls | — | R501X and 2282del4 | Atopic dermatitis | Diagnosis by expert dermatologist or paediatric allergologist | Matched for ethnicity | C |
| Families from dermatology departments, Karolinska University Hospital and Danderyd Hospital, Stockholm | 30 cases, 39 controls | Cases mean 36.2 (SD 1.8), | R501X and 2282del4 | Atopic dermatitis | Diagnostic method unknown | | C |
| Trial, design, and setting | No of participants | Age (years) | Genotype | Outcome measures | Diagnostic method or criteria | Confounders | Quality |
|---------------------------|--------------------|-------------|-----------|-----------------|-------------------------------|-------------|---------|
| Disease funded Atopic Dermatitis and Vaccinia Network | | | | | | |
| Hubiche et al (2007) | (case-control, France): | 99 cases, 102 controls | Cases median 7 (range 2 months to 68 years) | R501X an- d2282delA | Atopic dermatitis | UK Working Party criteria, trained dermatologist did complete examination, registered doctor diagnosed asthma, disease severity using SCORAD, total IgE level using CAP system | Matched for ethnicity and age | B |
| Lerbaek et al (2007) | (case-control, Denmark): | 183 in cohort, 189 controls | Mean 41 (SD 6.6) | R501X and 2282delA | Primary outcome hand eczema; other outcomes contact allergy, atopic dermatitis | Hand eczema diagnosed on basis of positive answer to question, UK Working Party's criteria used to define whether participants had ever had atopic dermatitis, patch testing for contact allergy | Matched for ethnicity | C |
| Marenholz et al (2006) | (family study and case-control, Europe): | 490 families, including 903 children with eczema | Children mean 7.9 | R501X and 2282delA | Primary outcome atopic dermatitis (age at onset <2 years); subgroups —atopic dermatitis with asthma, atopic dermatitis with allergic rhinitis, other outcomes (raised specific IgE levels) | Eczema (Hanifin and Rajka criteria), children whose parents reported doctor diagnosed asthma or hay fever were defined as having asthma or allergic rhinitis, respectively; allergic sensitisation defined as presence of specific IgE to at least one tested allergen of ≥0.70 kU/L, severe to moderate disease defined by objective ECZ1 panel) | Matched for ethnicity | A |
| German Multicenter Allergy Study (MAS) cohort; controls, repeatedly and consistently documented absence of signs and symptoms of allergic disease | 871 DNA samples, 189 cases with eczema, 321 controls | Allergy to any of 90 allergens | R501X and 2282delA | No explicit primary outcome; clinical outcomes atopic dermatitis, asthma with and without eczema, allergic rhinitis with and without eczema, other outcomes (raised specific IgE levels) | Doctor diagnosed eczema, parental report of eczema symptoms, or visible eczema at time of follow-up; asthma (≥1 wheeze episodes during past year at age 7 or 10) | Matching for age and ethnicity | B |
| Morar et al (2007) | (family study and case-control, London): | 148 families | Median 6.89 (SD 0.270) | R501X and 2282delA | No explicit primary outcome; clinical outcomes atopic dermatitis, asthma with atopic dermatitis; sensitisation outcome atopy (positive skin prick test response ≥3 mm than negative control, positive specific IgE, raised total serum IgE level, or any combination of these features) | Atopic dermatitis (Hanifin and Rajka criteria) defined by UK Working Party; for asthma and allergic rhinitis questions based on American Thoracic Society questionnaires; each family examined by doctor | Matched for ethnicity | B |
| Details as above (MCRC panel) | 278 families | Median 6.7 (SD 0.21) | R501X and 2282delA | Details as above | Details as above | Matched for ethnicity | B |
| Details as above (ECZ1 and MRCE panels) | 426 families | — | R501X and 2282delA | Details as above | Details as above | Matched for ethnicity | B |
| Cases and controls from families recruited from dermatology clinics at Great Ormond Street Hospital for Children | 426 families with 990 affected and unaffected children; 426 cases, 564 controls | — | R501X and 2282delA | No explicit primary outcome; clinical outcomes atopic dermatitis and asthma without atopic dermatitis | Atopic dermatitis (Hanifin and Rajka criteria) defined by UK Working Party | Matched for ethnicity | B |
| Nomura et al (2007) | (case-control, Japan): | 143 cases, 156 controls | — | R501X, 2282delA, 3702delG, 52554X, and 3321delA | Atopic dermatitis | Atopic dermatitis (Hanifin and Rajka criteria) | Matched for ethnicity | B |
| Nomura et al (2008) | (case-control, Japan): | 102 cases, 133 controls | — | S2554X, S2889X, 52554X, and 3321delA | Atopic dermatitis | Diagnosed by dermatologists using Hanifin and Rajka criteria | Matched for ethnicity | B |
| Trial, design, and setting | No of participants | Age (years) | Genotype | Outcome measures | Diagnostic method or criteria | Confounders | Quality |
|----------------------------|-------------------|-------------|-----------|------------------|-------------------------------|-------------|---------|
| Palmer et al (2006)w13 (case-control, Ireland, Scotland, Denmark): | | | | | | | |
| Cases from hospital dermatology clinics, control Irish population | 52 cases, 189 controls | | | RS01X and 2282del4 | Atopic dermatitis | Hanifin and Rajka criteria and UK working party definition, severity assessed using validated Nottingham eczema severity score | Matched for ethnicity | B |
| Cases from BREATHE study, Scotland; controls Scottish schoolchildren (both recruited from Tayside area, north east Scotland) | 604 cases, 1008 controls | | | RS01X and 2282del4 | Primary clinical outcome asthma; other outcome atopic dermatitis with asthma | Asthma diagnosed by doctors according to Scottish Intercollegiate Guidelines Network/British Thoracic Society diagnostic guidelines; in addition, parents were asked “Has your child ever had eczema or an itchy rash?” | Matched for ethnicity | B |
| Cases and controls from COPSAC | 142 cases, 190 controls | | | RS01X and 2282del4 | Clinical outcomes atopic dermatitis with and without asthma | Development of atopic dermatitis and asthma up to age 3 years monitored prospectively by clinical follow-up and diary card with complete follow-up data; atopic dermatitis (Hanifin and Rajka criteria) | Matching for ethnicity and age unclear | B |
| Palmer et al (2007)w11 (cross sectional, Scotland): | | | | | | | |
| Cohort from BREATHE study of childhood asthma | 874 | Mean 10.4 (SD 4.0), range 3-22 | RS01X and 2282del4 | Asthma severity: mean forced expiratory volume in one second/forced vital capacity (airway obstruction), prescribed drugs, British Thoracic Society asthma treatment step, rescue drug use, inhaled bronchodilator | Doctor diagnosed asthma, British Thoracic Society guidelines used for severity. Ratio of forced expiratory volume in one second to forced vital capacity used as measure of airway obstruction for severity, eczema status determined using question, does child have eczema? | Matching for ethnicity, age, and sex unclear | B |
| Rogers et al (2007)w6 (family study, North America): | | | | RS01X and 2282del4 | Asthma without atopic dermatitis, asthma with atopic dermatitis, asthma severity, sensitisation (atopy, total IgE level) | Diagnosis of asthma based on methacholine hyper-reactivity and ≥1 of following criteria for at least 6 months in year before recruitment: asthma symptoms at least twice a week, at least two uses a week of inhaled bronchodilator, or daily asthma drug; diagnosis of atopic dermatitis based on affirmative response to question, “Has your child ever had atopic dermatitis for 2 years?” | Matched for ethnicity and age | B |
| Ruether et al (2006)w15 (case-control and family study, northern Germany): | | | | RS01X and 2282del4 | Astopic dermatitis | Hanifin and Rajka criteria | Matched for ethnicity | B |
| Cases from dermatological outpatient ward, controls from PopGen Project | 272 cases, 276 controls | Cases median 10 | RS01X and 2282del4 | Clinical outcomes asthma without atopic dermatitis | | | |
| Families recruited from dermatological outpatient ward | 338 families | Median 10 | RS01X | Astopic dermatitis | Hanifin and Rajka criteria | Matched for ethnicity | B |
| Sandilands et al (2007)w3 (case-control, Ireland): | | | | | | | |
| Cases from hospital based paediatric dermatology clinic, controls children sampled by cheek swab at dispersed centres | 188 cases, 736 controls | Cases mean 4.9 | RS01X, 2282del4, 2447X | Astopic dermatitis | Dermatologist diagnosed moderate to severe atopic dermatitis using UK Working Party criteria | Matched for ethnicity | B |
buccal smears, at a reasonable although not insignificant cost (£<100; £117; $164).

Lack of expression of the protein filaggrin has been shown to predispose to the development of ichthyosis vulgaris and, more recently, atopic eczema or dermatitis.6,7 The filaggrin gene resides on human chromosome 1q21 within the epidermal differentiation complex, a region that also harbours genes for several other proteins that are important for the normal barrier function of the epidermis.8 The primary function of filaggrin seems to be to aggregate the epidermal cytoskeleton to form a dense protein-lipid matrix thereby regulating permeability of the skin to water and external particles such as allergens.9

Information about the association between filaggrin gene defects and allergic disorders is accruing rapidly. The initial focus was atopic eczema, which has been investigated in several studies, but to what extent do filaggrin gene defects and allergic disorders is accruing rapidly. The initial focus was atopic eczema, which has been investigated in several studies, but to what extent do filaggrin gene defects increase this risk, and what impact, if any, do they have on the risk of developing other allergic disorders?10-13 From first principles, these impact, if any, do they have on the risk of developing other allergic disorders?10-13 From first principles, these...
example, suggested by a study involving a murine model of atopic eczema, which found that dysfunction of the skin barrier not only enhances sensitisation to allergens but also leads to systemic allergic responses such as increased IgE levels and airway hyperreactivity.\(^\text{14}\) These observations support the idea that absorption of allergens through the skin of patients with atopic eczema may predispose to the development of other allergic conditions.

We undertook a systematic review and meta-analysis to investigate the relationship between filaggrin gene mutations, allergic sensitisation, and development of a range of atopic allergic disorders—namely, food allergy, eczema, asthma, allergic rhinitis, and anaphylaxis.

**METHODS**

We considered as eligible for inclusion any type of genetic epidemiological study in humans of all ages and ethnic groups that investigated the association between filaggrin gene defects and allergic sensitisation or allergic disorders. Case studies were excluded. The clinical outcome measures of interest were atopic dermatitis or dermatitis, food allergy, asthma, allergic rhinitis, and anaphylaxis, along with relevant immunological variables relating to the risk of allergic sensitisation as assessed by either positive skin prick testing or increased levels of allergen specific IgE.

**Search strategy**

We searched Medline, Embase, ISI Science Citation Index, and BIOSIS databases from their inception to 31 December 2008. Our searches were not restricted by language, age, sex, or publication type. Search terms were “filaggrin”, OR “profilaggrin”, OR “1q21”, OR “epidermal differentiation complex”, OR “R301X”, OR “2282del4”, OR “3321delA”, OR “S2554X” AND “allergy”, OR “asthma”, OR “food allergy”, OR “atopic dermatitis”, OR “eczema”, “rhinitis”, OR “anaphylaxis”, OR “sensitisation”, OR “epidermal dysfunction” (see web extra appendix 1 for the detailed search strategy). We checked the bibliographies of included studies for additional studies, supplemented with a citation search of references using ISI Web of Knowledge.

Using the UK National Research Register, clinical trials.gov, and the Index to Theses and Digital dissertations we searched for details of unpublished, ongoing studies. We also used OpenSIGLE to search for grey literature.

**Study selection, quality assessment, data extraction, and statistical analysis**

Two reviewers scanned the identified articles on the basis of the title, key words, and abstract (when available). Articles were rejected on the initial screen if they failed to meet our inclusion criteria. When a title or abstract could not be rejected with certainty, the reviewers obtained a full text copy of the article for evaluation. The full text versions of all relevant articles identified by a search of references were also obtained. Two reviewers then assessed the eligibility of studies for inclusion in the review. Any disagreements were resolved by discussion.

Drawing on published guidelines for the quality assessment of genetic epidemiological studies, we developed a customised checklist for assessing the quality of the studies, considering the key variables of participant selection, validity of the approach to genotyping, population stratification, and other statistical considerations (see web extra appendix 2).\(^\text{15-16}\) We contacted the authors of studies for missing information. On the basis of our assessment and prioritising the importance of internal validity, we classified studies as being of high, medium, or poor quality depending on the overall risk of drawing biased conclusions.

Data on the participants, study design, genetic polymorphisms, outcome measures, and associations were extracted using a customised data extraction sheet. We used Review Manager 4.2 (Cochrane Collaboration) and Comprehensive Meta-analysis, version 2 to analyse the data. As the two common filaggrin gene mutations R301X and 2282del4 are believed to have equivalent biological effects most of the studies analysed for a combined genotype effect along with an analysis of the two most common filaggrin gene mutations. In our meta-analyses we initially focused on the effects of these two common mutations separately and then combined.

We evaluated an overall estimate for the different outcomes for case-control studies and for family studies separately.\(^\text{15-16}\) When necessary in case-control studies we calculated the odds ratios for filaggrin carriers compared with non-carriers. We used the normal approximation of the Mantel-Haenszel statistic. When there was a zero in the contingency table we added 0.5. To calculate the confidence intervals for family studies we used the formula standard error \((\log \text{odds ratio})\) $=\sqrt{\frac{1}{T+1}+\frac{1}{U}}$ (see web extra appendix 3 for a fuller explanation).

**Fig 1 | Selection of studies**

| Papers not relevant (n=196) |
|-----------------------------|
| Retrieved for more detailed evaluation after scanning titles and abstracts (n=123) |
| Papers excluded (n=95): |
| Not a genetic epidemiology study (n=42) |
| Duplicate publication (n=5) |
| Case study (n=1) |
| Other topic (n=18) |
| Animal study (n=3) |
| Congress report (n=12) |
| Other (n=14) |
| Potentially appropriate papers (n=28) |
| Excluded as only reported as abstracts (n=4) |
| Articles included in systematic review (n=24) |
We used random effects modelling to pool the odds ratios. Study heterogeneity was investigated using the I² statistic. If heterogeneity was detected, we investigated this by undertaking subgroup analyses when possible, focusing on the impact of study quality and disease severity as explanatory factors. Possible publication bias was assessed graphically using funnel plots. When it was not appropriate or possible to undertake meta-analyses, we described the data using a narrative approach.

RESULTS

Overall, 24 of 319 identified papers were eligible for inclusion. No studies were identified that investigated the association between filaggrin gene defects and the risk of developing food allergies or anaphylaxis. Figure 1 describes the study selection process, and the table lists the characteristics of the included studies.

Sensitisation

Two case-control analyses and seven familial analyses (reported in four papers) investigated the association between filaggrin gene defects and allergic sensitisation (see web extra table A).

Case-control studies

Pooled data from the two case-control studies and seven familial analyses gave an overall odds ratio for the combined genotype of 1.57 (95% confidence interval 1.20 to 2.07; fig 2). Heterogeneity was significant (P=0.001) but could not be investigated owing to the small number of studies.

Family studies

One family study analysed two different family panels and presented results for each panel as well as for the panels combined. One family study reported P values only and therefore was not included in the meta-analysis.

Pooled data for the combined genotype from the other family studies gave an overall odds ratio of 1.91 (1.44 to 2.54; fig 2). Heterogeneity was significant (I²=72.20; P<0.001). The funnel plot showed a symmetrical inverted shape, suggesting there was no major publication bias (see web extra fig A).

The overall odds ratio for the filaggrin gene mutation R501X was 2.47 (1.70 to 3.59; fig 2) and for the filaggrin gene mutation 2282del4 was 2.25 (1.85 to 2.75; fig 2). Heterogeneity was not significant (P=0.11 and P=0.83, respectively).

Atopic eczema and atopic dermatitis

Twenty case-control analyses and eight familial analyses investigated the association between filaggrin gene defects and atopic dermatitis. Most of the studies were on western European populations, but three case-control studies and one family study were on a Japanese population and one case-control study was on a North American population.

Case-control studies

See web table B for details of the case-control studies. The study by Sugiuara et al was the first to describe the association between filaggrin and atopic dermatitis. It found down-regulation of the cornified envelope genes like filaggrin in the skin of people with atopic dermatitis (P=0.035). This study was not included in the meta-analysis because it was not possible to calculate an odds ratio and 95% confidence interval. Four other case-control studies and one family study were also not included in the meta-analysis. Three studies were on a Japanese population and the most common European filaggrin gene mutations R501X and 2282del4 were absent in 253 participants. Novel filaggrin gene mutations were, however, noted in the Japanese population and a statistical association was found.
### Combined genotype

| Case-control studies | Odds ratio (random) (95% CI) | Odds ratio (95% CI) |
|----------------------|-----------------------------|---------------------|
| Marenholz 2006*12    | 3.73 (1.98 to 7.02)         | 3.73 (1.98 to 7.02) |
| Palmer (Denmark) 2006*11 | 2.49 (1.26 to 4.93)         | 2.49 (1.26 to 4.93) |
| Palmer (Ireland) 2006*11 | 13.40 (6.20 to 27.50)       | 13.40 (6.20 to 27.50) |
| Barker 2007*10       | 7.70 (5.30 to 10.90)        | 7.70 (5.30 to 10.90) |
| Sandilands 2007*3     | 5.00 (3.01 to 8.79)         | 5.00 (3.01 to 8.79) |
| Steffler 2007*17      | 4.10 (2.30 to 7.59)         | 4.10 (2.30 to 7.59) |
| Weidinger 2007*16     | 4.60 (4.98 to 9.32)         | 4.60 (4.98 to 9.32) |
| Brown 2008*19        | 4.56 (3.08 to 6.74)         | 4.56 (3.08 to 6.74) |
| Weidinger 2008*23     | 1.53 (0.98 to 2.37)         | 1.53 (0.98 to 2.37) |
| Brown 2008*22       | 4.78 (3.31 to 6.92)         | 4.78 (3.31 to 6.92) |
| Total (95% CI)       |                             |                     |
| Test for heterogeneity: df(Q)=10, P=0.031, I²=83% |                     |

### Case-control studies: good and high quality

| Case-control studies | Odds ratio (random) (95% CI) | Odds ratio (95% CI) |
|----------------------|-----------------------------|---------------------|
| Marenholz 2006*12    | 3.73 (1.98 to 7.02)         | 3.73 (1.98 to 7.02) |
| Palmer (Denmark) 2006*11 | 2.49 (1.26 to 4.93)         | 2.49 (1.26 to 4.93) |
| Palmer (Ireland) 2006*11 | 13.40 (6.20 to 27.50)       | 13.40 (6.20 to 27.50) |
| Barker 2007*10       | 7.70 (5.30 to 10.90)        | 7.70 (5.30 to 10.90) |
| Sandilands 2007*3     | 5.00 (3.01 to 8.79)         | 5.00 (3.01 to 8.79) |
| Steffler 2007*17      | 4.10 (2.30 to 7.59)         | 4.10 (2.30 to 7.59) |
| Weidinger 2007*16     | 4.60 (4.98 to 9.32)         | 4.60 (4.98 to 9.32) |
| Total (95% CI)       |                             |                     |
| Test for heterogeneity: df(Q)=8, P=0.031, I²=86% |                     |

### Case-control studies: hospital based cases

| Case-control studies | Odds ratio (random) (95% CI) | Odds ratio (95% CI) |
|----------------------|-----------------------------|---------------------|
| Palmer (Ireland) 2006*11 | 13.40 (6.20 to 27.50)       | 13.40 (6.20 to 27.50) |
| Barker 2007*10       | 7.70 (5.30 to 10.90)        | 7.70 (5.30 to 10.90) |
| Sandilands 2007*3     | 10.02 (6.75 to 14.89)       | 10.02 (6.75 to 14.89) |
| Weidinger 2007*16     | 4.18 (2.60 to 7.63)         | 4.18 (2.60 to 7.63) |
| Total (95% CI)       |                             |                     |
| Test for heterogeneity: df(Q)=3, P<0.05, I²=61.5% |                     |

### Fig 3
Association between filaggrin combined genotype (≥1 mutation), filaggrin gene mutation R501X, and filaggrin gene mutation 2282del4 and atopic dermatitis in case-control studies, including those of good and high quality and with hospital based cases, family studies

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**Table 1**: Odds ratios and 95% CIs for association between filaggrin combined genotype (≥1 mutation), filaggrin gene mutation R501X, and filaggrin gene mutation 2282del4 and atopic dermatitis in case-control studies, including those of good and high quality and with hospital based cases, family studies

**Table 2**: Odds ratios and 95% CIs for association between filaggrin combined genotype (≥1 mutation), filaggrin gene mutation R501X, and filaggrin gene mutation 2282del4 and atopic dermatitis in family studies
investigated the association between the filaggrin combined genotype and atopic dermatitis in hospital based cases.\textsuperscript{10} Our assumption was that cases recruited from hospital based dermatology clinics probably had more severe atopic dermatitis than those selected from the population. The overall odds ratio for the combined genotype was 7.98 (5.35 to 11.89; fig 3); although heterogeneity was reduced it was still significant ($I^2=61.5\%$; $P<0.05$).

Two studies\textsuperscript{10,19} investigated the association between the filaggrin combined genotype and persistent atopic dermatitis. Pooled data from these studies gave an overall odds ratio of 7.01 (5.42 to 9.07; fig 4). Heterogeneity in this subgroup analysis was not significant ($P=0.54$). Three case-control studies\textsuperscript{16,17,19} showed data for the association between filaggrin combined genotype and atopic dermatitis at age less than two years at onset. One study\textsuperscript{20} was not included in the subgroup analysis as it reported no original data for early onset atopic dermatitis. Pooling the data for the combined genotype from the two other studies\textsuperscript{17,19} gave an overall odds ratio of 6.31 (4.68 to 8.49; fig 4). Heterogeneity was not significant ($P=0.83$).

### Family studies

Seven familial analyses were on western European populations\textsuperscript{4,8,15,20} and one on a Japanese population (see web extra table C for details).\textsuperscript{21} One of the studies\textsuperscript{8} analysed two different family panels and presented results for the panels separately as well as combined. As the study on a Japanese population reported $P$ values only it was not included in the meta-analysis.\textsuperscript{21}

The overall odds ratio for the combined genotype using random effects modelling was 1.99 (1.72 to 2.31; fig 4). Heterogeneity was not significant ($P=0.21$). The funnel plot did not suggest publication bias. The overall odds ratio for R501X was 2.44 (1.98 to 3.02; fig 3) and for 2282del4 was 2.27 (1.91 to 2.69; fig 3). Heterogeneity was not significant ($P=0.46$ and $P=0.83$, respectively).

### Cohort study

One cohort study\textsuperscript{20} investigated the interaction between filaggrin loss of function mutations and environmental exposures in the development of eczema. The data were from two independent birth cohorts (Denmark and the United Kingdom). This study found that filaggrin mutations increased the risk of eczema during the first year of life: hazard ratios 2.26 (95\% confidence interval 1.27 to 4.00) and 1.95 (1.13 to 3.36), respectively. The risk of eczema further increased with exposure to cats at birth among children with filaggrin gene mutations (hazard ratios 11.11 and 3.82, respectively). This study found that exposure to dogs was moderately protective (hazard ratios 0.49 and 0.59, respectively).

### Allergic rhinitis

**People without atopic dermatitis or eczema**

Three case-control studies investigated the association between filaggrin gene defects and the risk of...
combined genotype

Case-control studies: no atopic eczema

Marenholz 2006

Weidinger 2008

Total (95% CI)

Odds ratio (random)

0.01

0.1

1

10

100

1.03 (0.30 to 3.20)

1.93 (1.12 to 3.05)

1.78 (1.16 to 2.73)

Test for heterogeneity: df(Q)=1,
P=0.009, z=2.61

Case-control studies: atopic eczema

Marenholz 2006

Weidinger 2008

Total (95% CI)

Odds ratio (random)

0.01

0.1

1

10

100

4.79 (2.00 to 11.60)

2.64 (1.76 to 4.00)

2.84 (2.08 to 3.88)

Test for heterogeneity: df(Q)=1,
P=0.006, z=6.56

Family studies: atopic eczema

Marenholz 2006

Ekelund 2008

Total (95% CI)

Odds ratio (random)

0.01

0.1

1

10

100

3.14 (1.94 to 5.07)

2.03 (1.39 to 2.97)

2.46 (1.61 to 3.76)

Test for heterogeneity: df(Q)=1,
P=0, I^2=0%, z=4.16

Fig 5 | Association between filaggrin combined genotype (≥1 mutations) and allergic rhinitis in people without and with atopic eczema in case-control studies and in people with atopic eczema in family studies

developing allergic rhinitis in people without atopic dermatitis (see web extra table D). One of these studies reported a P value (0.66) for the combined genotype only (not detailing any original data to allow calculation of odds ratios). Data were pooled from the other two studies, on German populations. Using a random effects model the overall odds ratio for the combined genotype was 1.78 (1.16 to 2.73; fig 5).

People with atopic dermatitis or eczema

Three case-control studies and two family studies investigated the association between filaggrin gene defects and the risk of developing allergic rhinitis in people with atopic dermatitis (see web extra table E).

All three case-control studies were on German populations. One study reported no original data for allergic rhinitis but only odds ratios adjusted for age and sex (4.04, 95% confidence 2.11 to 7.72); this study was not included in the meta-analysis. Data were pooled from the other two case-control studies. The overall estimated odds ratio for the combined genotype was 2.84 (2.08 to 3.88; fig 5).

One family study was on a German population and another on a Swedish population. Pooled data for the combined genotype from these two studies gave an overall odds ratio of 2.46 (1.61 to 3.76).

Asthma

People without atopic dermatitis or eczema

Five case-control studies and one family study investigated the association between filaggrin gene defects and asthma in people without atopic dermatitis. The five case-control studies were on western European populations, whereas the family study was on a North American population (see web extra table F). None of these studies showed a significant association between filaggrin gene defects and asthma. The family study reported an odds ratio for the combined genotype of 1.0 (0.51 to 1.96; P=1.00).

Data were pooled from the case-control studies to estimate an overall odds ratio. One study was not included in this meta-analysis because the population comprised affected and unaffected offspring from family panels in a case-control setting. Another study was also not included because it reported a P value (0.15) for the combined genotype only. After pooling the data from the three remaining studies, the overall odds ratio for the combined genotype was 1.30 (0.73 to 2.30; fig 6). Heterogeneity was not significant (P=0.37).

People with atopic dermatitis or eczema

Six case-control studies and seven family studies investigated the association between filaggrin gene defects and the risk of developing asthma in people with atopic dermatitis. All the studies except one, were on western European populations; the remaining study was on a North American population.

Case-control studies

Web extra table G provides details of the included six case-control studies. One study was not included in the meta-analysis because the researchers reported no original data for asthma, only odds ratios adjusted for age and sex. Another study reported a P value of <0.001 for the combined genotype only. Pooled data for the combined genotype from the other four case-control studies gave an overall odds ratio of 2.79 (1.77 to 4.41; fig 6). Heterogeneity was significant (P<0.001). The funnel plot did not suggest publication bias (see web extra fig C).

Family studies

Seven family studies investigated the association between filaggrin gene defects and the risk of developing asthma in people with atopic dermatitis (see web extra table H). One study analysed two different family panels and presented results for each panel separately as well as combined.

Pooled data for the combined genotype from the family studies gave an overall odds ratio of 2.30 (1.66 to 3.18; fig 6). Heterogeneity was significant (P<0.001). Publication bias was judged to be unlikely (see web extra fig D).

An overall odds ratio for R501X was 2.30 (1.72 to 3.09; fig 6) and for 2282del4 was 2.82 (2.19 to 3.64; fig 6).

DISCUSSION

In this systematic review and meta-analysis we found that filaggrin gene defects increase the risk of
developing sensitisation, atopic eczema or atopic dermatitis, and allergic rhinitis. The risk of those with coexistent atopic eczema developing asthma was also increased, but not those without eczema. These findings provide strong supporting evidence that, at least in a subset of those with allergic problems, the filaggrin gene defect may be the fundamental predisposing factor not only for the development of eczema but also for initial sensitisation and progression of allergic disease.

The key strengths of this work include the comprehensiveness of the searches and our assessment for a range of clinical and immunological outcome measures. This approach does, in theory at least, increase the possibility of type 1 errors, although we focused on the pooled odds ratios and 95% confidence intervals of data obtained from the meta-analyses thereby allowing readers to judge for themselves the strength of the associations identified. As with all systematic reviews, we may have failed to identify some studies, particularly those with negative findings, and this might have influenced our findings. Although we attempted to assess for publication bias, we recognise that funnel plots have relatively low power to detect such bias, particularly when there are relatively few studies included in these plots, and so these need to be interpreted with caution.17

Overall this work underscores the importance of filaggrin gene defects in increasing the risk of sensitisation and the development of a range of allergic clinical phenotypes, most probably through exposure to allergens through the skin. Our findings suggest that filaggrin is a robust biomarker for allergic conditions. Given the consistency of the genetic data from epidemiological studies, we suggest that further confirmatory studies in eczema serve little purpose. A need remains, however, to understand better the possible role of filaggrin gene defects in other systemic atopic allergic disorders such as food allergy and anaphylaxis. Future population based epidemiological studies should use prospective designs categorising people on the basis of genotype; we are aware of at least one such study under way in the UK (S Mukhopadhyay, personal communication, 2009). Although family based designs are often more costly than population based studies, greater emphasis should be placed on using these as they have the advantage of being less influenced by population substructure.16 Further work also needs to focus on the mechanisms through which defective skin function impacts on the presentation of antigens and the possible associated immune modulation. Useful insights into disease pathophysiology may also be gained by studying people with filaggrin gene defects who do not develop atopy or atopic allergic conditions. A need also exists to investigate whether the filaggrin biomarker can be used to identify those at high risk so that preventive measures can be introduced such as interventions to restore the barrier function of the skin or measures to avoid allergens in filaggrin defective infants.

**Fig 6** | Association between filaggrin combined genotype (carriage of ≥1 mutation) and asthma in people without and with atopic dermatitis in case-control studies and with atopic dermatitis in family studies, filaggrin R501X mutation and asthma in people with atopic dermatitis in family studies, and filaggrin 2282del4 mutation and asthma in people with atopic dermatitis in family studies
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Ethical approval: Not required.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Atopic allergic disorders affect up to a third of people worldwide

Eczema is often the herald condition in those with allergic conditions, typically beginning in the first year of life

Flaggrin gene defects, present in up to one in 10 western Europeans and North Americans, are possible predisposing factors in the development of atopic eczema

WHAT THIS STUDY ADDS

Flaggrin gene defects increase the risk of allergic sensitisation, suggesting that defective function of the skin barrier may be fundamental in people with allergic disorders

Flaggrin gene defects are associated with a significantly increased risk of atopic eczema, allergic rhinitis, and asthma in people with eczema

Interventions to restore the barrier function of the skin or measures to avoid allergens in filaggrin defective infants need investigation

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