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WHAT IS THE EVIDENCE FOR INTERACTIONS BETWEEN FILAGGRIN NULL MUTATIONS AND ENVIRONMENTAL EXPOSURES IN THE AETIOLOGY OF ATOPIC DERMATITIS? - A SYSTEMATIC REVIEW

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Authors' contributions

HB contributed to the design of the study, was the guarantor, developed the search strategy and the PROSPERO protocol, drafted the published protocol, led data extraction and critical appraisal and drafted the final manuscript.

VV, VA and GK contributed to the abstract screening, data extraction and critical appraisal and critically reviewed the manuscript

MJP developed the search strategy and critically reviewed the manuscript

LuP contributed to the design of the study interpretation of analysed data and statistical calculations and critically reviewed the manuscript

CF contributed to the design of the study and critically reviewed the manuscript

RW contributed to the design of the study and critically reviewed the manuscript

ADI refereed the data extraction and critically reviewed the manuscript

TP, AR, HCW and NR critically reviewed the manuscript

SJB, LaP and SML contributed to the design of the study, developed the search strategy and the PROSPERO protocol, performed, supervised and refereed data extraction, reviewed data analysis, contributed to drafting the manuscript and critically reviewed the final manuscript.

All authors approved the final manuscript as submitted.

Competing interests statement

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and have declared any relevant competing interests.

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Data Access, Responsibility, and Analysis

Helena Blakeway (Faculty of Health Sciences, University of Bristol, Bristol Medical School, Oakfield House, Oakfield Grove, Bristol, BS8 2BN, UK) and Sinéad Langan (Department of Non-Communicable Disease

Epidemiology, London School of Hygiene and Tropical Medicine, London, WC1E 7HT, UK) had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The corresponding author and guarantor (HB) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Ethics approval

Ethics approval was not required as this is a systematic review and meta-analysis.

Data sharing

Our OVID MEDLINE search strategy has been registered on PROSPERO (<https://www.crd.york.ac.uk/prospero/>) and all data are publicly available.

Abstract

Background: Epidemiological studies indicate that gene-environment interactions play a role in atopic dermatitis.

Objective: To review the evidence for gene-environment interactions in atopic dermatitis aetiology, focusing on *FLG* loss-of-function mutations.

Methods: Systematic search from inception to September 2018 in EMBASE, MEDLINE and BIOSIS. Search terms included all synonyms for atopic dermatitis (AD) and filaggrin/*FLG*; any genetic or epidemiological study design using any statistical methods were included. Quality assessment using criteria modified from guidance - ROBINS-I and Human Genome Epidemiology Network (HuGENET)- for non-randomised and genetic studies was completed, including consideration of power. Heterogeneity of study design and analyses precluded the use of meta-analysis.

Results: Of 1,817 papers identified, 12 fulfilled the inclusion criteria required and performed formal interaction testing. There was some evidence for *FLG*-environment interactions in six of the studies (P value for interaction ≤ 0.05) including early-life cat ownership, older siblings, water hardness, phthalate exposure, higher urinary phthalate metabolite levels (which all increased AD risk additional to *FLG* null genotype) and prolonged breastfeeding (which decreased AD risk in the context of *FLG* null genotype). Major limitations of published studies were low numbers of individuals with AD and *FLG* loss-of-function mutations and exposure to specific environmental factors (n=5 to 94), and variation in exposure definitions.

Conclusions and relevance: Evidence on *FLG*-environment interactions in atopic dermatitis aetiology is limited. However, many of the studies lacked large enough sample sizes to fully assess these interactions. Further research is needed with larger sample sizes and clearly defined exposure assessment.

Review registration: PROSPERO CRD42017057818

WHAT'S KNOWN STATEMENT

- Gene-environment interactions are considered important in the aetiology of atopic dermatitis.
- Loss of function mutations in the gene coding are the most consistently reported genetic variants for atopic dermatitis.
- Studies have reported evidence for gene-environment interaction involving filaggrin and a range of different environmental exposures.

WHAT'S NEW STATEMENT

- There is some evidence for *FLG*-environment interactions in the aetiology of atopic dermatitis, however the evidence is limited.
- Studies lack large enough sample sizes to achieve adequate power in order to fully assess these interactions.

CLINICAL IMPLICATIONS

Understanding the role of *FLG*-environment interactions in the aetiology of atopic dermatitis would be helpful to tailor preventative strategies, but published evidence is currently lacking.

KEY WORDS

Atopic dermatitis, atopic eczema, filaggrin, *FLG*, exposure, gene-environment interaction.

ABBREVIATIONS

<i>FLG</i>	Gene encoding filaggrin
GEI	Gene-Environment Interaction
HR	Hazard Ratio
PROSPERO	Prospective Register of Systematic Reviews
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analysis
ORs	Odds Ratios
CI	Confidence Interval
PINT	P value for the interaction
MEP	Monoethyl phthalate
MBP	Monobutyl phthalate
MBzP	Monobenzyl phthalate
5OHMEHP	Mono(2-ethyl-5-hydroxyhexyl)phthalate

Introduction

Atopic dermatitis (AD), also known as eczema or atopic eczema, is a complex, multifactorial often debilitating disease.¹ The prevalence of AD has risen rapidly, suggesting that environmental factors might be responsible for such changes.² It is estimated that up to 20% of children and 3% of adults suffer from AD in high income countries.³ It is crucial to better understand the aetiology of AD to discover ways to reduce the personal and public health burden.

Considerable phenotypic heterogeneity, evidence for multiple genetic risk mechanisms⁴ and incomplete penetrance have led to complexities understanding the genetic basis of AD.⁵ There have been 31 risk loci identified for AD to date.⁴ Loss-of-function mutations in the gene encoding filaggrin (*FLG*) are the strongest and most significantly associated genetic variants for AD.⁶

Profilaggrin is an insoluble protein found in the outer epidermis; monomeric filaggrin has multiple functions including aggregation of keratin filaments.⁷ *FLG* is essential for normal epidermal barrier function and formation,⁸ contributing to the skin water-holding capacity and pH balance.⁹ The two most prevalent loss-of-function mutations in *FLG* in white European populations are R510X and 2282del4, present in approximately 9% of healthy people in northern European populations; these are strongly associated with AD risk, particularly early onset and severe disease.⁶ Other, less prevalent loss-of-function mutations in *FLG* have been identified.¹⁰⁻¹² Despite increasing understanding of the importance of genetic factors, the rising AD prevalence has been too substantial and rapid to be explained purely by genetic

factors.¹³ Environmental factors have been implicated in the rising AD prevalence; exposure to such factors *in utero* or later life may play a role in AD aetiology.¹⁴

Gene-environment interaction (GEI) may be defined as occurring when individuals with different genotypes respond to an environmental exposure in different ways; this interaction contributes to many common phenotypes and complex genetic traits. There is evidence that GEI plays a role in atopic diseases, such as asthma, in which genotype interacts with environmental factors, including maternal smoking and house dust mite.^{15,16} A recent review on allergic diseases highlighted that several studies exploring GEI in AD exist, yet findings have not been synthesised (e.g. in a systematic review).¹⁷ Our objective was to systematically review the evidence for GEI in AD, focusing on interactions with *FLG*-null genotype because this is the strongest and most widely replicated AD genetic risk factor, and because the role of filaggrin in skin barrier function provides *a priori* support for a hypothetical GEI effect.

Methods

This systematic review was prospectively registered (PROSPERO ID CRD42017057818)¹⁸. A detailed electronic search of MEDLINE and EMBASE via Ovid and BIOSIS via Web of Science was undertaken from inception of each database to September 2018 identifying manuscripts in any language. To define AD, the search terms 'atopic dermatitis', 'atopic eczema' and 'eczema' were used, and to define *FLG* mutations, filaggrin, *FLG*, possible mis-spellings and previously reported *FLG* mutation names were included in the strategy (Supplementary material methods 1). We focussed on incident AD cases, including studies looking at interactions for AD development rather than interactions for established AD. To avoid *a priori* defining an exhaustive list of environmental factors, the inclusion of any environmental factor was assessed during the title and abstract screening. We defined environmental exposures as proposed by Rothman.¹⁹ The primary outcome measure was evidence of a statistically significant (defined as $p < 0.05$) *FLG*-environment interaction in the aetiology of AD and the secondary outcome was the strength of the association of the interaction (evidence of a dose response relationship) and AD severity. For details of abstract screening, inclusion and exclusion criteria and data extraction, see Supplementary material methods 2 and Supplementary table 1.

Quality and bias assessment was performed using criteria modified from guidance for non-randomised studies to determine quality of studies, ROBINS-I: to assess risk of bias in non-randomised studies of interventions, and HuGENet for genetic studies, including assessing if confounders were considered.^{20,21} These two tools were combined to determine bias in genetic and environmental studies, as neither tool was designed for GEI studies.

Post hoc sample size calculations were undertaken to estimate the sample size required to detect a GEI effect varying between 1.2 and 2⁶ in a case-control/cohort study for a binary SNP and binary exposure under a series of assumptions for model parameters (Supplementary table 4), using R 3.5.0 (R package powerGWASinteraction).

Results

The search identified 1817 papers of possible relevance (Figure 1); 12 met our inclusion criteria (Supplementary Table 1). Papers tested various environmental exposures (Table 1).

Table 1: Environmental exposures assessed in included studies

Environmental exposures assessed in one paper*	Environmental exposures assessed in two or more papers *
Older siblings and day-care attendance ³⁰ , Sex ²⁴ , Maternal parity ²⁴ , Maternal AD ²⁴ , Maternal smoking ²⁴ , Environmental tobacco smoke exposure in early life ²⁴ , Birth-year ³¹ , Serum vitamin D levels ³² , Maternal IgE sensitization ³³	Early life cat exposure ^{22,23} , Breastfeeding ^{24,25} , Phthalate exposure in urine metabolites and household dust ^{26,27} , Water hardness ^{28,29}

*Environmental exposures where significant interactions with *FLG* were reported (P<0.05)

Study designs were cohort (10), case-control (1) and a family-based study (1) (Supplementary Table 3). Study populations ranged from 296 to 5188 individuals and participant ages were from one month to 69 years. The number of participants in each study with *FLG* loss-of-function mutations ranged from 27 (9.1%) to 459 (10.2%). The AD definition and method of ascertainment varied between studies (supplementary table 2).³⁴⁻³⁶ None of the included studies investigated the strength of interaction or AD severity.

Out of 12 publications, including 15 studies (Supplementary Table 2), six studies showed evidence for GEI (P <0.05) (Table 1). Most used regression models to calculate P values and some presented hazard ratios.

Heterogeneity in study design and exposures precluded formal meta-analysis.

Cat exposure

Two studies assessed the *FLG**cat interaction^{22,23}. Bisgaard *et al.* tested for an interaction in the Copenhagen Prospective study on Asthma in Childhood (COPSAC) (n=379) and reported an increased risk related to cat exposure at birth amongst children aged 0-5 years with *FLG* null mutation (HR=11.11, 95% CI 3.79-32.60, P_{interaction} = 0.0008); findings were replicated in the Manchester Asthma and Allergy Study (MAAS)(n=503), with an increased risk due to interaction of cat exposure at birth and *FLG* null genotype (HR=3.82, 95% CI:1.35-10.81, P_{interaction}=0.011). Schuttelaar *et al.* (n=934) reported no overall interaction (P=0.85) between *FLG* null genotype (one/two *FLG* loss-of-function mutations) and cat exposure at home (OR with *FLG* loss-of-function mutation(s) and cat exposure=1.9; OR for *FLG* wild-type individuals and cat exposure=2.1). However, Schuttelaar *et al.* reported an interaction when examining the 2282del4

mutation only ($P=0.003$), with a stronger effect in children aged 0-8 years with a cat at home ($OR=6.0$, $95\% CI:3.2-11.3$) compared to those without ($OR=2.2$, $95\% CI:1.4-3.7$).²³ All those with 2282del4 mutations were heterozygous.²³ As all *FLG* loss-of-function mutations have biological equivalence on filaggrin protein expression, there is no clear biological plausibility for an interaction with one mutation and not another. Evidence for GEI comes from small numbers of individuals with *FLG* mutation, cat exposure and development of AD: in Bisgaard *et al* ($N=5$), Schuttelaar *et al.* did not provide the number but it can be inferred that $N<84$ for the overall interaction and $N<50$ for the 2282del4 interaction.^{22,23}

Dog exposure

Bisgaard *et al* tested for an interaction between *FLG* loss-of-function mutations and dog ownership in the first year of life. There was no evidence for an interaction in COPSAC ($n=379$) (result statistics not reported) or MAAS ($n=503$) ($HR=0.59$, $95\% CI:0.16-2.20$, $P=0.43$).²²

Siblings

One study reported an interaction between *FLG* genotype and presence of older siblings amongst both children attending or not attending day-care at 2 years in two separate studies –LISApplus cohort (aged 6-72 months, $n=1037$, interaction $OR=3.27$, $95\% CI:1.14-9.36$, $P<0.05$) and GINIplus cohort (12-72 months, $n=1828$, interaction $OR=2.41$, $95\% CI:1.06-5.48$, $P<0.05$).²⁹ This interaction increased the risk of AD.³⁰

Parity, maternal atopy and child's sex

Henderson *et al* found no evidence of an interaction between *FLG* genotype with parity ($P=0.802$) ($n=4463$), maternal asthma or AD ($n=5188$), ($P=0.486$ and $P=0.884$ respectively) or the child's sex ($P=0.959$)²⁴ in children aged 6 months to 11 years who were part of a prospective cohort study.

Maternal IgE sensitisation

Esparza-Gordillo *et al.* conducted a parent-of-origin analysis investigating the effect of a child's *FLG* genotype and maternal and paternal *FLG* genotypes on the child's AD risk ($n=1209$ families). Although interactions were not the focus of this study, results were stratified by maternal IgE sensitization status, allowing us to compare the effect of child's *FLG* genotype in those whose mothers were or were not IgE sensitized. The child's *FLG* genotype had a stronger risk effect when mothers had normal IgE levels (Relative Risk for 1 *FLG* loss-of-function mutation= 2.30 ($95\% CI:1.64-3.22$); RR for 2 *FLG* loss-of-function mutations= 7.19 ($95\% CI:3.77-13.7$)) compared to those with sensitized mothers (RR1= 1.37 ($95\% CI:0.97-1.94$); RR2= 2.98 ($95\% CI:1.19-7.45$)). However, confidence intervals were wide and overlapping. Esparza-

Gordillo *et al.* reported the opposite for effect of maternal genotype, so it is unclear if this evidence points to a true interaction with exposure to mothers with elevated IgE or whether the observation results from maternal genetic effects *in utero* or imprinting effects of *FLG* genotype.³³

Smoking

One study tested for possible interactions with *FLG* mutations and maternal smoking during pregnancy (n=5140) or childhood environmental tobacco smoke exposure (n=4874). Their results showed no evidence for either interaction (maternal smoking P=0.362, child environmental tobacco smoke exposure P=0.742).²⁴

Breastfeeding

Ziyab *et al.* found evidence for a protective association between breastfeeding duration and AD in children aged 1 or 2 years old (n=885) carrying one or more *FLG* loss-of-function mutations (P=0.02), with no evidence in those without a *FLG* null mutation (P=0.64).²⁵ However, this was stratified analysis and formal interaction testing was not undertaken. Henderson *et al.* found no evidence for this interaction (P=0.952) in their earlier, larger study (n=5158).²⁴

Birth year

Thyssen *et al.* investigated *FLG* and year of birth in adults aged 18-69 years (n=3202) but did not report any evidence of interaction (P=0.19) on AD risk.³¹

Water hardness

Interaction of *FLG* genotype and water hardness has been investigated in two papers within the same cohort. Perkin *et al.* investigated calcium and chlorine levels in water and AD during the first three months of life in the Enquiring About Tolerance study, a UK population-based cohort of 1,303 infants. Interaction tests did not show a statistically significant interaction between *FLG* genotype and: high calcium-low chlorine concentration in water; low calcium-high chlorine; or high calcium-high chlorine concentrations.²⁹ They subsequently reported evidence of an interaction between *FLG* loss-of-function mutations and water hardness increasing AD risk, when studying high calcium (>256mg/L CaCO₃) from three months of age in this cohort (n=1,303 total; n=75 with *FLG* null mutation exposed to high CaCO₃ levels, P=0.0008).³⁷

Vitamin D

A possible interaction between serum vitamin D levels and *FLG* genotype was investigated by Berents *et al.* They measured serum vitamin D levels in 558 participants at 1-13 months and 2 years alongside interviews assessing Vitamin D intake. They did not find any evidence for an interaction ($P>0.13$).³²

Urine phthalate metabolites and household dust phthalate

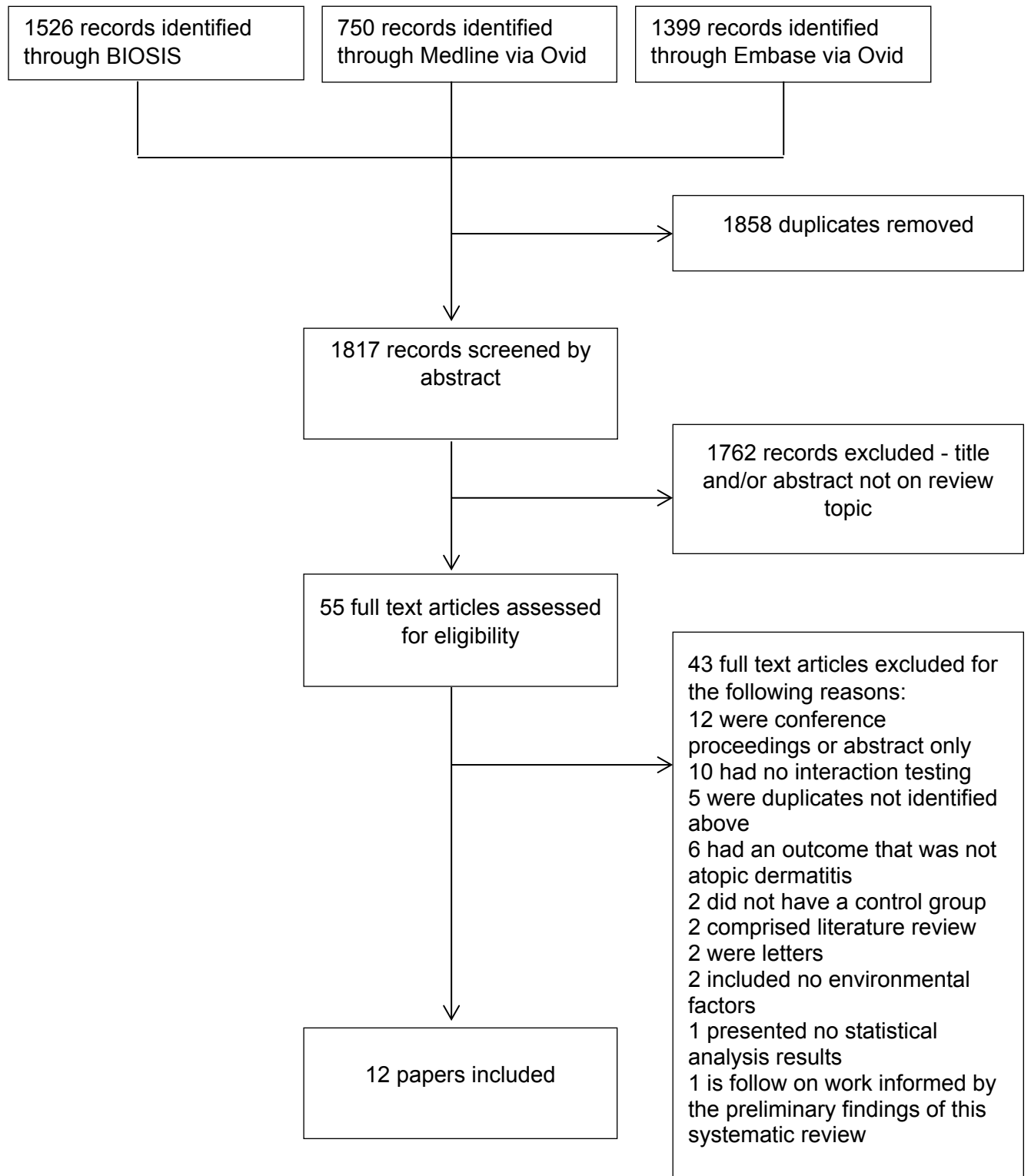
Phthalates are added to plastic to increase flexibility. They have been reported to be associated with childhood AD³⁸. Wang *et al.* investigated whether there was an interaction between urine phthalate metabolite levels and *FLG* genotype in children aged 3 years in the aetiology of AD ($n=453$). They studied four phthalates, monoethyl phthalate (MEP), monobutyl phthalate (MBP), monobenzyl phthalate (MBzP) and mono(2-ethyl-5-hydroxyhexyl)phthalate (5OHMEHP) which they classified as lower or higher levels in relation to the median. They reported evidence for an interaction between the P478S genotype TT and phthalates MBP ($P=0.015$) and MBzP ($P=0.018$)²⁷ and increased AD risk; however, they did not replicate their findings or perform corrections for multiple testing.²⁷ A similar interaction was investigated by Bamai *et al.* who assessed seven phthalates found in household dust, and eleven phosphorus flame retardants. They found evidence for an interaction between *FLG* loss-of-functions and diisononyl phthalate (DiNP) ($P=0.039$)²⁶. The group also reported a non-significant negative dose-response relationship among children with *FLG* loss-of-function mutation(s) in a categorical model (first quartile compared with fourth quartile, P for trend = 0.087). This analysis was undertaken on a sample size of 5 children with AD and *FLG* null genotypes and the researchers did not correct for multiple testing.²⁶

Quality of studies

Most studies included unselected cases from the general population or cohorts, and controls were selected from the same population as those with AD. *FLG* genotype was assessed using accepted methodology (Supplementary table 3). The timing and method of assessment of environmental exposures was variable; studies may be vulnerable to reverse causality due to exposure status being assessed after AD onset. Participants in each study were of homogenous ethnicity and analyses were adjusted for age. Details of confounder adjustment was missing in five of 12 included studies (Supplementary Table 3). Studies varied in their presentation of interaction results, with some providing only a P value or statement of statistical significance and others also providing effect estimates and confidence intervals across strata and a P value for the interaction term or statement of statistical significance (Supplementary Table 2). None of the included studies adjusted for multiple testing and only Berents *et al.* reported power calculations.³² The results of the studies supported their conclusions, however they were underpowered - results of these studies must be interpreted with caution. Under reasonable assumptions on the

magnitude of main effects, prevalence of AD, environmental exposure and SNP allele frequency, *post hoc* power calculations (Supplementary Table 4) indicate that the sample size required to detect an interaction with OR ~2.0 is approximately 5,000 individuals, whereas a sample size of ~63,000 is required to detect a more modest GEI effect with OR 1.2.

Figure 1: Flow chart showing systematic review



Discussion

Our results highlight important challenges when studying GEIs in the aetiology of AD. We identified only 12 articles that reported *FLG*-environmental interactions in AD. Our initial search strategy returned many results, but the majority were excluded because they lacked information essential to the review. Evidence was found for interactions between *FLG* genotype and: breastfeeding duration, older siblings, phthalate exposure in household dust and urine phthalate metabolite levels, early-life exposure to cat and water hardness. All interactions increased the risk of AD apart from prolonged breastfeeding which decreased the risk. Because of very limited evidence to support these interactions, small numbers and lack of replication (one study undertook replication) it is difficult to interpret the results and findings must be interpreted with caution. Table 2 shows our suggestions of the components required for a rigorous GEI study which may improve conclusions from future studies.

Table 2: Components required for rigorous GEI study

Suggestion	Reason
Large sample size with mutation and environmental exposure (see <i>supplementary table 4</i>)	Sample size must be large enough to detect true GbyE interaction effect
Design the study basing the sample size on power to detect interaction effect	Design the study and basing the sample size on power to detect a main effect will likely result in insufficient sample size
Use accepted diagnostic criteria for AD	This reduces the possibility of introducing heterogeneity into the results
Use robust methods of exposure measurement	Questionnaires or indirect measurements of exposure can introduce variation and recall bias into the results. Using validated tools will help reproducibility and reduce information bias
Collect measurements of exposure at defined time periods across the study population	This avoids variation in the timing of exposure measurements influencing disease risk
Correct for multiple testing and publication bias	This reduces the possibility of interpreting chance results as positive findings
Tailor studies to different ethnic groups currently not covered by research.	Increasing diversity in genetic research will enable us to understand the importance of GEI in populations of different ethnicities

Our review has several strengths. A detailed search strategy was used to identify all relevant papers. Screening and data extraction were carried out in duplicate, with secondary resolution of conflicts, reducing the possibility of introducing bias by systematically selecting certain papers. The majority (10/12, 83%) of studies used data from cohort studies, thus they were able to consider temporality, and researchers mostly measured the outcome 'AD' using validated criteria.

Our findings should be considered in light of limitations. Many studies were excluded from the review as they did not specifically test for GEI in their analyses. Studies were also excluded if they measured indirect outcomes of AD by looking at IgE levels, transepidermal water loss, or skin-prick tests, which are not

measures of the outcome (AD), but responses to exposures. Many of the included studies performed GEI analysis as a secondary analysis, for example, Berents *et al.*³² meaning they did not aim to have sufficient power to assess GEIs, hence, the importance of measuring GEIs as primary outcome (Table 2). We were unable to formally evaluate the risk of reporting biases, so cannot rule out the possibility that studies which found non-significant interactions yet failed to report such results are missing from our review. Many of the studies included in this review relied on population data and there may be heterogeneity in outcome definition (Table 2).³⁹ The pre-defined scope of this review was to look at *FLG*-environmental interactions, which by definition has excluded the study of effects within populations where *FLG* null mutations are not prevalent or have not been identified.^{7,40} Many large genetic studies have been within populations of European ancestry, where *FLG* null mutations are prevalent. On-going work to increase diversity in genetic research⁴¹ will allow future investigations of GEI in populations of all ethnicities (Table 2).

Heterogeneity in methodology between published studies and the limited number of studies assessing the same exposure precluded meta-analysis or formal assessment of publication bias. None of the included studies reported correcting for multiple testing; hence, interaction effects could be by chance. It is unclear how many studies had predefined hypotheses which risks introducing reporting bias. Replication of findings was limited and in some cases where two studies investigated the same interaction, discordant findings were seen, such as Ziyab *et al.* (*FLG* genotype and breastfeeding) and Henderson *et al.*^{24,25}

One reason for the limited evidence for GEI and lack of replication is lack of statistical power. Detailed review showed the number of individuals on whom the interaction analysis was based (i.e. cases with both exposure and *FLG*-null genotype) was small, hence, included studies were likely to be underpowered. The number of individuals with both a *FLG* loss-of-function mutation and exposure to the specific environmental factor was not always specified, but of those that did, it ranged from N=5 to N=167.^{22,24}

In complex diseases such as AD, where the main genetic effect sizes are small, a large sample size is necessary to detect small interaction effects.⁴² Researchers need to utilise sufficiently large sample sizes to detect GEIs, and generally investigators should demonstrate that their sample has adequate power to detect an interaction effect.⁴³ Even in cases where meta-analysis across studies is possible, results are not always meaningful due to variable measurement of environmental exposures.⁴²

Studies of GEI face inherent challenges in attempting to gain full understanding of interactions because of the difficulty in uniformly measuring the environmental parameter, which in turn limits the understanding of the underlying disease mechanism.⁴⁴ The difficulty in measuring exposure in GEI studies in AD is shown in the Wang *et al.* study that tested for an interaction between phthalate exposure and *FLG* genotype. To measure exposure, phthalate metabolite levels were measured from urine samples.²⁷ This is not a direct measure of the exposure, therefore we questioned whether it should be included in the review. It only provides a moderate prediction of exposure due to the short half-life and rapid excretion of phthalates leading to considerable day-to-day variation.²⁷ Other studies used different methods, such as questionnaires, to derive environmental exposure data retrospectively; this could introduce recall bias.³⁰

Variation in the timing of environmental exposure is important in terms of influencing subsequent disease risk, as timing of exposure may not be accurately measured with methods such as infrequent questionnaires.⁴⁵ Using robust validated measures of exposure reduces variation and aids reproducibility of results (Table 2). For some of these exposures it is easy to hypothesise a biological explanation as to why people with *FLG* haploinsufficiency might have different responses for example; pet exposure and older siblings could act via microbial exposure, as proposed by the hygiene hypothesis.⁴⁶ With other possible interactions such as urine phthalate metabolites, it is harder to hypothesise plausible mechanisms. Cohort studies may be vulnerable to reverse causality when assessing early life exposures, as, although outcomes were measured after the exposure in the majority of studies, there remains a possibility that early signs of AD, or the presence of older siblings with AD, influenced the behaviour of parents who subsequently modified the exposure.

GEI are widely viewed as important in the aetiology of AD. However, the limited evidence and lack of power of published studies to detect GEI effects as indicated by the sample size calculations we carried out highlights the importance of further research. This is needed to test for replication of interactions reported to date (Table 1) using larger sample sizes. Further, unexplored GEI may also warrant investigation, including genetic risk variants in addition to *FLG* loss-of-function mutations. The Early Genetics and Lifecourse Epidemiology (EAGLE) consortium is investigating possible GEIs with selected SNPs associated with AD. Our recommendations for future studies of GbyE can be shown in Table 2 which would improve the quality of evidence and enable us to draw more robust conclusions about the nature of GEIs. Together this work will improve understanding of GEI in the aetiology of AD, to inform both public health and individual lifestyle decisions.

References

- 1 Weidinger S, Novak N. Atopic dermatitis. *The Lancet* 2016; **387**: 1109-22.
- 2 Odhiambo JA, Williams HC, Clayton TO *et al*. Global variations in prevalence of eczema symptoms in children from ISAAC Phase Three. *J Allergy Clin Immunol* 2009; **124**: 1251-8 e23.
- 3 Asher MI, Montefort S, Björkstén B *et al*. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *The Lancet* 2006; **368**: 733-43.
- 4 Paternoster L, Standl M, Waage J *et al*. Multi-ethnic genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nature genetics* 2015; **47**: 1449-56.
- 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**: 214-9.
- 6 Rodríguez E, Baurecht H, Herberich E *et al*. Meta-analysis of filaggrin polymorphisms in eczema and asthma: Robust risk factors in atopic disease. *Journal of Allergy and Clinical Immunology* 2009; **123**: 1361-70.e7.
- 7 Brown SJ, McLean WHI. One remarkable molecule: Filaggrin. *The Journal of investigative dermatology* 2012; **132**: 751-62.
- 8 van den Oord RAHM, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. *BMJ* 2009; **339**: b2433.
- 9 McAleer MA, Irvine AD. The multifunctional role of filaggrin in allergic skin disease. *Journal of Allergy and Clinical Immunology* 2013; **131**: 280-91.
- 10 Weidinger S, O'Sullivan M, Illig T *et al*. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. *J Allergy Clin Immunol* 2008; **121**: 1203-9 e1.
- 11 Barnes KC. An update on the genetics of atopic dermatitis: scratching the surface in 2009. *J Allergy Clin Immunol* 2010; **125**: 16-29 e1-11; quiz 30-1.
- 12 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.
- 13 Pesce G, Marcon A, Carosso A *et al*. Adult eczema in Italy: prevalence and associations with environmental factors. *J Eur Acad Dermatol Venereol* 2015; **29**: 1180-7.

- 14 Bauer SM. Atopic Eczema: Genetic Associations and Potential Links to Developmental
Exposures. *International Journal of Toxicology* 2017; **36**: 187-98.
- 15 Rava M, Smit LAM, Nadif R. Gene–environment interactions in the study of asthma in the
postgenomewide association studies era. *Current Opinion in Allergy and Clinical Immunology*
2015; **15**: 70 - 8.
- 16 Sordillo JE, Kelly R, Bunyavanich S *et al.* Genome-wide expression profiles identify potential
targets for gene-environment interactions in asthma severity. *Journal of Allergy and Clinical
Immunology* 2015; **136**: 885-92.e2.
- 17 Turner S. Gene–Environment Interactions—What Can These Tell Us about the Relationship
between Asthma and Allergy? *Frontiers in Pediatrics* 2017; **5**: 118.
- 18 National Institute for Health Research PROSPERO International prospective register of
systematic reviews CRD42017057818. In. 2017.
- 19 Rothman KJ. *Modern epidemiology / by Kenneth J. Rothman and Sander Greenland*, 2nd ed..
edn. Philadelphia: Philadelphia : Lippincott-Raven. 1998.
- 20 Sterne JAC, Hernán MA, Reeves BC *et al.* ROBINS-I: a tool for assessing risk of bias in non-
randomised studies of interventions. *BMJ* 2016; **355**: i4919.
- 21 Little J, Higgins J. The HuGENet™ HuGE Review Handbook, version 1.0. Centers for Disease
Control and Prevention. 2006.
- 22 Bisgaard H, Simpson A, Palmer CN *et al.* Gene-environment interaction in the onset of eczema
in infancy: filaggrin loss-of-function mutations enhanced by neonatal cat exposure. *PLoS Med*
2008; **5**: e131.
- 23 Schuttelaar ML, Kerkhof M, Jonkman MF *et al.* Filaggrin mutations in the onset of eczema,
sensitization, asthma, hay fever and the interaction with cat exposure. *Allergy* 2009; **64**:
1758-65.
- 24 Henderson J, Northstone K, Lee SP *et al.* The burden of disease associated with filaggrin
mutations: a population-based, longitudinal birth cohort study. *J Allergy Clin Immunol* 2008;
121: 872-7 e9.
- 25 Ziyab AH, Mukherjee N, Ewart S *et al.* Filaggrin gene loss-of-function variants modify the
effect of breast-feeding on eczema risk in early childhood. *Allergy* 2016; **71**: 1371-3.
- 26 Ait Bamai Y, Araki A, Nomura T *et al.* Association of filaggrin gene mutations and childhood
eczema and wheeze with phthalates and phosphorus flame retardants in house dust: The
Hokkaido study on Environment and Children's Health. *Environment International* 2018;
121: 102-10.

- 27 Wang IJ, Karmaus WJ. The effect of phthalate exposure and filaggrin gene variants on atopic dermatitis. *Environ Res* 2015; **136**: 213-8.
- 28 Jabbar-Lopez Z, Perkin M, Strachan D *et al*. Gene-environment interaction between filaggrin and hard water associated with increased risk of atopic eczema. *Clinical and Experimental Allergy* 2017; **47**: 1681.
- 29 Perkin MR, Craven J, Logan K *et al*. Association between domestic water hardness, chlorine, and atopic dermatitis risk in early life: A population-based cross-sectional study. *Journal of Allergy and Clinical Immunology* 2016; **138**: 509-16.
- 30 Cramer C, Link E, Horster M *et al*. Elder siblings enhance the effect of filaggrin mutations on childhood eczema: Results from the 2 birth cohort studies LISaplus and GINIplus. *Journal of Allergy and Clinical Immunology*; **125**: 1254-60.e5.
- 31 Thyssen JP, Linneberg A, Johansen JD *et al*. Atopic diseases by filaggrin mutations and birth year. *Allergy* 2012; **67**: 705-8.
- 32 Berents TL, Lodrup Carlsen KC, Mowinckel P *et al*. Vitamin D levels and atopic eczema in infancy and early childhood in Norway: a cohort study. *Br J Dermatol* 2016; **175**: 95-101.
- 33 Esparza-Gordillo J, Matanovic A, Marenholz I *et al*. Maternal Filaggrin Mutations Increase the Risk of Atopic Dermatitis in Children: An Effect Independent of Mutation Inheritance. *PLOS Genetics* 2015; **11**: e1005076.
- 34 Williams HC, Jburney PG, Hay RJ *et al*. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. *British Journal of Dermatology* 1994; **131**: 383-96.
- 35 Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. In, Vol. 92. Stockholm: Acta Derm Venereol Suppl. 1980; 44-7.
- 36 Asher MI, Keil U, Anderson HR *et al*. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *European Respiratory Journal* 1995; **8**: 483.
- 37 Jabbar-Lopez ZK, Craven J, Logan K *et al*. Longitudinal analysis of the effect of water hardness on atopic eczema: evidence for gene-environment interaction. *British Journal of Dermatology* 2019; **0**.
- 38 Kim E-H, Jeon B-H, Kim J *et al*. Exposure to phthalates and bisphenol A are associated with atopic dermatitis symptoms in children: a time-series analysis. *Environmental health : a global access science source* 2017; **16**: 24-.
- 39 Flohr C, Johansson SGO, Wahlgren C-F *et al*. How atopic is atopic dermatitis? *Journal of Allergy and Clinical Immunology* 2004; **114**: 150-8.

- 40 Thawer-Esmail F, Jakasa I, Todd G *et al.* South African amaXhosa patients with atopic
dermatitis have decreased levels of filaggrin breakdown products but no loss-of-function
mutations in filaggrin. *The Journal of allergy and clinical immunology* 2014; **133**: 280-2.e22.
- 41 Sirugo G, Williams SM, Tishkoff SA. The Missing Diversity in Human Genetic Studies. *Cell*
2019; **177**: 26-31.
- 42 Palla L, Higgins JPT, Wareham NJ *et al.* Challenges in the Use of Literature-based Meta-
Analysis to Examine Gene-Environment Interactions. *American Journal of Epidemiology* 2010;
171: 1225-32.
- 43 Danielle MD, Arpana A, Matthew CK *et al.* Candidate Gene-Environment Interaction Research:
Reflections and Recommendations. *Perspectives on Psychological Science* 2015; **10**: 37-59.
- 44 von Mutius E. Gene-environment interactions in asthma. *Journal of Allergy and Clinical*
Immunology; **123**: 3-11.
- 45 Khoury MJ, Wacholder S. Invited Commentary: From Genome-Wide Association Studies to
Gene-Environment-Wide Interaction Studies—Challenges and Opportunities. *American*
Journal of Epidemiology 2009; **169**: 227-30.
- 46 Strachan DP. Hay fever, hygiene, and household size. *BMJ (Clinical research ed.)* 1989; **299**:
1259-60.
- 47 Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect modification
and interaction. *International Journal of Epidemiology* 2012; **41**: 514-20.
- 48 Brown SJ, Sandilands A, Zhao Y *et al.* Prevalent and Low-Frequency Null Mutations in the
Filaggrin Gene Are Associated with Early-Onset and Persistent Atopic Eczema. *Journal of*
Investigative Dermatology 2008; **128**: 1591-4.
- 49 Kooperberg C, Hsu L. Package 'powerGWASinteraction'. In, n 1.1.3 edn., Vol. 2018. 2015;
Analytical power calculations for GxE and GxG interactions for case-control studies of
candidate genes and genome-wide association studies (GWAS). This includes power
calculation for four two-step screening and testing procedures. It can also calculate power for
GxE and GxG without any screening.